Lab 3: Particle absorption June 14, 2023

1.1 Introduction and Lab Goals

Particulate absorption measurements provide the second part of the determination of absorption by aquatic systems, dissolved measurements in Lab 2 being the first. Suspended particulate absorption is obtained in two ways: first, by difference between paired spectrophotometric measurements of total and filtered sample in cuvettes (Slade et al. 2010), and second by filtration onto glass fiber filters and direct spectrophotometric analysis. This lab builds upon Lab 2 to demonstrate three ways of measuring particulate absorption spectra, with ac-meters, in cuvettes placed inside an integrating sphere, and on filters placed inside an integrating sphere.

The major absorbers in seawater are water itself, CDOM, and absorbing particles. These two components are separated easily by filtration. The total particulate absorption (designated a_{part} or a_p), consists of phytoplankton, other living organic particles such as viruses, bacteria, zooplankton, dead or detrital organic particles, and inorganic minerals or sediments. The particulate absorption component is separated operationally via extraction with a strong polar solvent such as methanol (Kishino et al. 1985) into phytoplankton (designated as a_{phyt}, a_{phi}, a_{ph}, or a_{ϕ}), and non-algal particles (a_{NAP}, capitalized because it is an acronym like CDOM). The non-algal particle term was introduced by Babin et al. (2003), replacing the designation a_d, detrital absorption, because *detrital* has different meanings in geology and biology, neither of which captures the true nature of particles in this fraction. The solvent extraction step effectively removes the phytoplankton pigments while leaving all other particles (organic and inorganic, living and non-living) and the non-pigmented portion of phytoplankton cellular matter intact; this material is identified as NAP. While a_{NAP} consists of this large range of materials, the grouping is about more than extractive techniques. The living and dead organic and inorganic constituents within NAP all have a generally exponentially-decaying absorption coefficient which is very different from the absorption spectra of the *in vivo* phytoplankton pigments, which exhibit strong features associated with distinct pigments.

Absorption coefficients are additive, hence:

$$a_{part}(\lambda) = a_{phyt}(\lambda) + a_{NAP}(\lambda) \quad (1)$$

The phytoplankton absorption is determined by difference between the measured $a_{part}(\lambda)$ and $a_{NAP}(\lambda)$, *after* computation of absorption from absorbance. This method provides the best estimate of absorption by phytoplankton pigments, as they were *in vivo* (specifically, as they were packaged in the chloroplasts of living cells). Phycobilipigments associated with cyanobacteria are not extractable with solvents and must be extracted with hot water or phosphate buffer, otherwise a_{NAP} will contain pigment features and be overestimated, leaving a_{phyt} underestimated. These extractions, when gently performed, do not disrupt the particulate matter, including cells.

The outcomes of this lab are for students to gain experience in measuring particulate absorption in the ac meters and in the benchtop spectrophotometer with the integrating sphere accessory (in cuvettes and on filter pads). One aspect for the filter pad measurement is gaining some intuition for how much sample to collect on the filter pad. Because there are a lot of time-consuming aspects to this lab, samples will be prepared for students. Students will learn how to filter samples on Friday in lab 5.

1.2 Activities

Rotations: As with Lab 2, the class will be divided into two sections, each spending 90 minutes with the ac meters and 90 minutes with the spectrophotometer.

Samples: We will perform particulate absorption analysis on Harpswell Sound (HS) water (whole water and filtrate for the ac-meters, filter pad samples for the spectrophotometer) and phytoplankton cultures (for ac-meters and cuvette and filter pad samples in the spectrophotometer). *Ivona will collect samples to measure chlorophyll via extractive fluorescence analysis on cultures and HS samples. Students will learn this technique in Lab 5.*

Each group will be responsible for a *specific set of measurements* during their rotation. Each group will be responsible for *processing their measurements*. Data synthesis requires the results from all of groups combined. In this way, everyone has the opportunity to make one set of each type of measurement but does not have to make all of the observations nor process all of the data. This is a *jigsaw approach* to collaborative learning. The analysis and data synthesis will be completed after dinner.

Answer the following questions using the data you collected:

1. *How do the absorption spectra of the phytoplankton culture measured in suspension in the spectrophotometer, on the filter pad, and with the acs compare*? Describe similarities and differences in magnitude and spectral shape (i.e., location of peaks, shape of peaks, differences between cultures).
2. *How does the particulate absorption measured on filter pads vary as a function of volume filtered*? Select the values of a_p(440 nm) and plot versus filter volume. How does it relate to your visible observations of filter pad sample loading (i.e., can you see visually the "right" range of filter volumes)?
3. *What is the standard deviation spectrum for the triplicate sample filter pad absorption as a function of volume filtered*?
4. *What is the standard deviation spectrum for the triplicate blank filter pad absorption as a function of volume filtered*? What conclusions can you draw concerning the optimum volume filtered? What is larger, the standard deviation in sample or blank triplicates?
5. *How does the particulate absorption measured in the spectrophotometer compare to that measured in the acs*? Describe similarities and differences in spectral shape. Think about what might be responsible for any differences.
6. *Benchtop spectrophotometers and in situ ac meters both provide estimates of absorption but involve different processing steps, make sure you can articulate the differences in processing steps and the underlying reasons for each step*.

1.3 The instruments

The **benchtop spectrophotometer** from Lab 2 now has an integrating sphere accessory which provides estimates of absorbance, A. Samples are placed inside the sphere so that radiant power scattered by particles or the filter are detected in the transmitted beam, leaving the only loss of radiant power due to absorption. Note that running samples in the integrating sphere is much trickier because the sphere is fragile with respect to alignment, due to the fact that the PMT detector sits directly underneath the samples (who designed this??), and the PMT detector can easily be fried if exposed to outside illumination (so follow the checklist of steps we will talk about in lab). We will discuss the sample and reference beam configuration and how that allows the scattered light from the particulate sample to be collected.

The **in situ absorption meters** provide estimates of the particulate absorption coefficient by difference between measurements of whole water absorption minus measurements of the filtrate of that whole water:

$$a_{part}(\lambda) = a_{whole}(\lambda) - a_{filt}(\lambda)$$

We will discuss how the acs configuration optimizes scattered light collection and some correct schemes for the portion of scattered light that is not collected.

1.4 Sample preparation and processing

Particulate absorption analysis should be performed as soon as possible after water collection. Filtered samples for spectrophotometry can be stored in liquid nitrogen if it is not possible to measure them immediately. However, once thawed, analysis should be immediate. Filter pads for NAP absorption can then be extracted without immediacy but should not be allowed to dry out. All samples should be kept in the dark and at cool temperatures. *Think about each of these steps and consider why they are recommended. What factors should you consider in sample collection, processing and analysis to retrieve the most robust estimates of* a_{part}, a_{phyt} *and* a_{NAP}*?*

1.5 What to do at each station

We have three absorption meters and one spectrophotometer. Students will be broken up into two sections, each group spending approximately 90 minutes in spectrophotometry and 90 minutes with absorption meters, with a break in between. Each section will be broken into 3 subgroups (A, B, C).

1.5.1 **Benchtop Spectrophotometer**

For each 90-minute interval, students will be divided into three subgroups (A, B, C) to give every student the opportunity to get hands on a sample in the integrating sphere. In 30-minute rotations, one group works on the spec (Collin), one group will examine the set of prepared filtered sample and perform sample extraction (Ivona), one group will be discussing strategies for data processing (Meg). Each group will measure the absorption of their culture suspension in a cuvette and filtered onto a glass fiber filter as well as a Harpswell Sound water sample filtered onto a glass fiber filter (each group will have a different volume filtered). Details are found in Table 1.

Table 1. Particulate absorption samples to be run by each of the four groups.

Spec Group	Sample	V_{filt} (ml)‡	Sample	V_{filt} (ml)†	cuvette
1A	HS	50	*Thallassiosira sp.*	3.9	1-cm
1B	HS	100	*Thallassiosira sp.*	3.9	1-cm
1C	HS	200	*Thallassiosira sp.*	3.9	1-cm
2A	HS	500	*Dunaliella sp.*	3.9	1-cm
2B	HS	750	*Dunaliella sp.*	3.9	1-cm
2C	HS	1000	*Dunaliella sp.*	3.9	1-cm

‡These volumes are approximate until we see what the concentration of of particulate matter in HS today.
†This volume is equivalent to a column of water whose diameter is equal to the effective filter diameter (1.115 cm ± 0.08 cm for Collin's filter rig, see Kostakis et al. 2021) and whose height is 1 cm to demonstrate equivalence with the 1-cm cuvette measurement geometric pathlength.

Spectrophotometer Protocols

- Spectrophotometer Setup: Menu: **wavelength scan**; Mode: **Abs**; scan from **750 nm** to **350 nm** at **1 nm intervals** with **slow** scan speed. Automatically subtract Baseline.
- The photomultiplier tube (PMT) is very sensitive to light. If exposed to room light levels while scanning, the PMT will be destroyed, and we are done for the duration of the course. **Never** click *scan* without the sphere cover in place and the box cover closed. The PMT also sits directly beneath the sample. Thus, it is imperative that no liquid is spilled from the cuvette nor any water drips from the filter. If you have any questions, please ask for help.
- Place the 1-cm cuvette with filtered culture water in the cuvette. Collect **Baseline scan**.
- Collect **Zero scan**, press Start to run the Milli-Q water as a baseline-corrected scan (this is the minimum resolution achievable).
- Collect **Sample scan** with the phytoplankton culture.
- Remove the cuvette and replace with the Plexiglas filter holder.
- Place a blank filter in the holder, top towards the beam. Collect **Baseline scan**.
- Collect **Zero scan**, press *Start* to run the baseline filter as a baseline-corrected scan (this is the minimum resolution achievable).
- Collect **3 blank scans**, press *Start* to run the blank filters as a baseline-corrected scan (this provides uncertainty associated with filter pads).
- Collect **Sample scan**, press *Start* to run the sample as a baseline-corrected scan
- One of the sample filters will be extracted with hot methanol as explained by the rotation through Ivona's group. (Filter placed back into the filter cup, extracted with 10 ml hot methanol -may need repeated extractions. Rinse with MQ.) Collect as **Sample scan** as above.

Compute spectral absorption coefficients from spectral absorbance for each scan. Make sure you use the correct equations for cuvette and filter pad samples, section 1.6. For the blank filter scans, assume the volume filtered is the same as for your samples. Compute the mean and standard deviation of the triplicate blank scans and the triplicate sample scans.

1.5.2 **In situ absorption meters**

As in Lab 2, each of the two sections that rotate though this lab will break into 3 subgroups (A, B, C) of 2-3 students and will collect absorbance spectra of the samples in Table 2. Make sure you record the temperature and salinity of the whole water and filtrate readings.

Table 2. Samples to be run by each subgroup and the instrument to use. Fill in the associated temperature and salinity.

Subgroup	Water sample	Instrument	Temperature (C)		Salinity (ppt)	
			Whole	Filtrate	Whole	Filtrate
1A	HS	acs S/N_____				
1B	HS	acs S/N_____				
1C	*Thallassiosira sp.*	acs S/N_____				
2A	HS	acs S/N_____				
2B	HS	acs S/N_____				
2C	*Dunaliella sp.*	acs S/N_____				

Ac meter Protocols

- Clean the sensor windows and tubes prior to measurements with lens paper and ethanol or isopropyl alcohol. Measure the temperature and salinity of every sample (even those entered as target values).
- Focus on getting good pure water calibrations: each student should run her/his own Milli-Q water calibration (either a-tube or c-tube of the ac-meter, or both). Save files in your group's folder. Note the magnitude and shape of the pure water spectra; they should be repeatable to within the instrumented resolution between calibrations (i.e. 0.005 m^{-1} for absorption; 0.01 m^{-1} for attenuation).
- Run the filtered water sample in both the a-tube AND c-tube of the ac-meter. Measure and record the temperature and salinity.
- Repeat for the whole water sample.
- Collectively you will be able to compare a_{part} of HS water between instruments, a_{part} of HS and culture with an acs and compare $a_{part}(\lambda)$ for HS and culture with the spectrophotometric observations (filter pad and culture suspension). Save the files.
- Instructions and code for processing data will be provided at the lab.

1.6 Data analysis for spectrophotometric filter pad absorption

Recall from lab 2 that absorption, $a(m^{-1})$, is computed from the absorbance, A, obtained from the spectrophotometer, where L is the geometric pathlength of the cuvette (m):

$$a(m^{-1}) = 2.303\ \frac{A}{L} \quad (1)$$

The geometric pathlength is given by the height, $H\ (cm)$, of a cylinder of water whose area is equal to the effective area of the filter pad with radius $r(cm)$ and thus is equivalent to quotient of the volume of sample filtered $V(ml)$ to the effective area of the filter (cm^2):

$$H = \frac{V}{\pi\, r^2} \quad (2)$$

Recognizing that $ml = cm^3$ so the absorption is computed as:

$$a(\lambda)(m^{-1}) = 2.303\ \times 100\ \frac{A(\lambda)}{\left(\frac{V}{\pi\, r^2}\right)} \quad (3)$$

Where 100 is the conversion between *cm* and *m*. Recall that the sample absorbance measured on the filter pad has been corrected for its blank (baseline scan with a blank filter):

$$A_{pad}(\lambda) = A_{sample}(\lambda) - A_{blank}(\lambda) \quad (4)$$

The advantage of the center-mounted integrating sphere technique is that there is no scattering error in the measurement. Thus, any signal observed in the near IR is due to absorption. Note how this might change your interpretation of the ac-meter scattering correction. See Pegau et al. (2002) for an extensive discussion of "issues" with selecting a null wavelength where theoretically absorption is zero or negligible. Also see Babin and Stramski (2002) for a discussion on null wavelength correction for filter pad absorption.

We typically use a high-quality caliper to measure the effective filter diameter. For the filter set up used today, we have determined that $r = 1.115 \pm 0.080\ (cm)$.

In addition to concentrating the particles, the glass fiber filter also provides a highly diffusing light field for the spectrophotometric measurement and increases the optical pathlength that photons travel between the source and detector. The consequence is higher probability of photon absorption and an overestimated absorption coefficient due to "pathlength amplification". The absorption calculation must correct for pathlength amplification (Stramski et al. 2015):

$$A_{pad_{corr}}(\lambda) = 0.323\ \times\ A_{pad}(\lambda)^{1.0867} \quad (5)$$

Where $A_{pad_{corr}}(\lambda)$ is substituted for $A(\lambda)$ in the absorption equation (3) above. Each group will measure triplicate filters; compute the mean absorption spectrum and the standard deviation ($\sigma_{a_{triplicate}}(\lambda)$), which assesses uncertainty associated with (*fill in the blank*):

_____.

You can also compute the standard deviation associated with the triplicate *blank* absorption spectra ($\sigma_{a_{blank}}(\lambda)$), which assesses uncertainty associated with (*fill in the blank*):

_____.

This means you need to compute the absorption coefficient associated with the standard deviation of the blank absorbance scans ($\sigma_{A_{blank}}(\lambda)$). Note that it requires the volume filtered. Protocol is that the blanks are prepared with the volume of MQ water equivalent to the sample volume so that the filters are compressed to the same degree. Use this volume in your calculation. Thus, the standard deviation absorption associated with the filter blanks is:

$$\sigma_{a_{blank}}(\lambda) = 2.303 \times 100 \frac{\left(0.323 \times \sigma_{A_{blank\,pad}}(\lambda)^{1.0867}\right)}{\left(\frac{V}{\pi r^2}\right)}$$

Friday you will learn how to propagate uncertainty. Return to this assignment and consider how to do it for filter pad absorption (example can be found in Kostakis et al. 2018 Supplemental material). For now, which term is larger ($\sigma_{a_p}(\lambda)$or $\sigma_{a_{blank}}(\lambda)$)? Once you have an estimate of standard deviation ($\sigma_{a_{triplicate}}(\lambda)$) or ultimately a total uncertainty ($u_{a_{blank}}(\lambda)$), you would report $a_p(\lambda) \pm \sigma_{a_x}(\lambda)$, where the term after the $\pm$ is your standard deviation or uncertainty and in a figure, you would graph $a_p(\lambda)$ with error bars given by that uncertainty. For this lab, where we are interested in volume filtered impacts, use the $\sigma_{a_{blank}}(\lambda)$term (see Roesler et al. 2018 for an example).

1.7 References:

Babin, M., and D. Stramski. 2002. Light absorption by aquatic particles in the near-infrared spectral region. *Limnol. Oceanogr.,* 47(3), 2002, 911–915.

Babin, M., D. Stramski, G.M. Ferrari, H. Claustre, A. Bricaud, G. Obolensky, and N. Hoepffner. 2003. Variations in the light absorption coefficients of phytoplankton, nonalgal particles, and dissolved organic matter in coastal waters around Europe. Journal of Geophysical Research Ocean 108 (C7): article number 3211 , doi: 10.1029/2001JC000882.

Kishino, M., N. Takahashi, N. Okami, and S. Ichimura, 1985: Estimation of the spectral absorption coefficients of phytoplankton in the sea. *Bulletin of Marine Science.* 37, 634-642.

Kostakis, I., M. Twardowski, C. Roesler, R. Röttgers, D. Stramski, D. McKee, A. Tonizzo, and S. Drapeau. 2021. Hyperspectral optical absoprtion closure experiment in complex coastal waters. Limnol. Oceanogr. Methods, 19: 589-625.

Pegau, S., J.Ronald V. Zaneveld, B. Gregg Mitchell, James L. Mueller, M. Kahru, J. Wieland and M. Stramska. 2002. Ocean Optics Protocols For Satellite Ocean Color Sensor Validation, Revision 4, Volume IV: Inherent Optical Properties: Instruments, Characterizations, Field Measurements and Data Analysis Protocols. Edited by Mueller, J. L., G.S. Fargion and C R. McClain. NASA/TM-2003-211621/Rev4-Vol.IV. Greenbelt, Maryland.

Roesler, C., D. Stramski, E. D'Sa, R. Röttgers, and R.A. Reynolds. 2018. Chapter 5: Spectrophotometric measurements of particulate absorption using filter pads. In Neeley, A. R. and Mannino, A. [eds.], IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 1.0. IOCCG, Dartmouth, NS, Canada. doi: 10.25607/OBP-119

Slade, W.H, E. Boss, G. Dall'Olmo, M.R. Langner, J. Loftin, M.J. Behrenfeld, C. Roesler, and T.K. Westberry, 2010. Underway and moored methods for improving accuray in measurement of spectral particulate absorption and attenuation. Journal of Atmospheric and Oceanic Technology, 27:10, 1733-1746.

Stramski, D., R. A. Reynolds, S. Kaczmarek, J. Uitz and G. Zheng. 2015. Correction of pathlength amplification in the filter-pad technique for measurements of particulate absorption coefficient in the visible spectral region. Applied Optics, 54(22): 6763-6782.