## Lab 3: Particle absorption May 2025

### 1.1 Introduction and Lab Goals

Absorption by aquatic systems can be divided into two component parts: dissolved absorption measurements (Lab 2) and particulate absorption measurements (this lab). 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. For detailed information on particulate absorption measurements, see IOCCG report (IOCCG, 2018).

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 $a_{\phi}$), and non-algal particles ($a_{nap}$, a term introduced by Babin et al. 2003 that replaced 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 (Roesler et al., 1995; 1998). These extractions, when gently performed, do not disrupt the particulate matter, including cells.

**1.2 Activities**

*Rotations:* As with Lab 2, the class will be divided into two groups of 9 and 10 students, Group 1 and Group 2. Each group will spend half the lab working with the spectrophotometer (and preparing the filtered samples) and half the lab working with the ac-meters.

*Samples:* We will perform particulate absorption analysis on Damariscotta River Estuary (DRE) water (whole water and filtrate for the ac-meters, filter pad samples for the spectrophotometer) and phytoplankton cultures. We will measure chlorophyll via extractive fluorescence analysis on cultures and DRE samples.

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 the 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.
2. How does the particulate absorption measured on filter pads vary as a function of volume filtered? What is the uncertainty spectrum for each sample based upon volume? What conclusions can you draw concerning the optimum volume filtered?
3. How do the NAP and phytoplankton absorption spectra pads vary as a function of volume filtered? Are there differences in magnitude and spectral shape?
4. How does the particulate absorption measured in the spectrophotometer compare to that measured in the acs? Describe similarities and differences in spectral shape.

**1.3 The instruments**

The **benchtop spectrophotometer we are using in this lab** 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.

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 (the $a_{CDOM}(\lambda)$ samples measured in Lab 2):

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

**1.4 Sample preparation and processing**

Particulate absorption analysis should be performed as soon as possible after water collection. Particles in the sample, prior to filtration or direct measurement (e.g., ac-s) should be kept in suspension by careful swirling of the water container ("*Mary Jane method*"). Filtration should be done with low vacuum (5 mmHg or 0.1 psi) to avoid breakage of the cells. Filters should be placed on a petri dish, containing a moist Kimwipe (*what liquid would you use to moisten that Kimwipe?*). 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 groups, each group spending approximately 90 minutes in spectrophotometry and 90 minutes with absorption meters, with a break in between. World is not a perfect place, so if we are backed up, or you are done earlier, go to a classroom, and read up on absorption measurement or start processing data.

1.5.1 **Benchtop Spectrophotometer** - MEG's LAB

For each 90-minute interval, students will be divided into three groups, one will focus on filtering samples (C), the second on methanol extraction (B), and third on measuring absorption in the integrating sphere (A). Note that some of you will be provided with samples filtered by another group. Please, follow the directions and naming conventions given to you in the group specific sheet - that will help you all when trying to combine the data during processing. Each group will measure the absorption of their culture suspension in a cuvette and filtered onto a glass fiber filter as well as a DRE 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.

| Group | Sample | $V_{filt}$ (mL) | Sample | $V_{filt}$ (mL) | cuvette |
|---|---|---|---|---|---|
| 1A | DRE | 700 | Culture1 | 5 | 1-cm |
| 1B | DRE | 550 | Culture2 | 5 | 1-cm |
| 1C | DRE | 400 | Culture3 | 5 | 1-cm |
| 2A | DRE | 300 | Culture1 | 5 | 1-cm |
| 2B | DRE | 200 | Culture2 | 5 | 1-cm |
| 2C | DRE | 100 | Culture3 | 5 | 1-cm |

To correctly calculate the absorption of the sample, we measure the effective filter diameter using calipers. Make sure you note your uncertainty in the measurement–we will work on including uncertainty in all our measurements as we go through the course (see the group specific handout).

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 it will fry it and we are done for the duration of the course (ask Sasha, who has fried a PMT, if you want to know more about how bad this is). **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
- In the perfect world, you would place the filter back into the filter cup and extract with 10 ml hot methanol as described by your instructor. Rinse with FSW/Milli-Q, and collect as **Sample scan** as above. However, due to the time constraint, you will be taking a different filter (that was used to filter the same volume of sample, and then run through a process of methanol extraction) to do this measurement.

Compute spectral absorption coefficients from spectral absorbance for each scan. For the blank filter scans, assume the volume filtered is the same as for your samples. The equation to compute particulate absorption from filter pad samples is derived in section 1.6.

**1.5.2 In situ absorption meters - Mitchell's lab**

As in Lab 2, each of the two groups that rotate through 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.

| Group | Water sample | Instrument | Temperature (C) | | Salinity (ppt) | |
|---|---|---|---|---|---|---|
| | | | Whole | Filtrate | Whole | Filtrate |
| 1A | DRE | acs | | | | |
| 1B | DRE | acs | | | | |
| 1C | DRE | acs | | | | |
| 2A | Culture 1 | acs | | | | |
| 2B | Culture 2 | acs | | | | |
| 2C | Culture 3 | acs | | | | |

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 their 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 DRE water between different acs, $a_{part}$ of DRE and culture with an acs, and compare $a_{part}(\lambda)$ for DRE and culture with the spectrophotometric observations (filter pad and culture suspension). Remember to save the files in your group's folder.

Instructions and code for processing data will be provided at the lab - please ask Alexander for specific code access if you can't find something you need.

1.6 Data analysis for spectrophotometric filter pad absorption

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

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

The geometric pathlength is equivalent to the quotient of the volume of sample filtered (*ml*) to the effective area of the filter ($cm^2$), essentially a cylinder of water. The volume of this cylinder is the filter volume, $V$, and the geometry of the cylinder is the effective filter area, $\pi r^2$, times the height, $H$; the height is the geometric pathlength:

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

Thus, the absorption is computed as:

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

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.

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.

**1.7 References:**

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