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<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AOP</td>
<td>Apparent Optical Properties</td>
</tr>
<tr>
<td>AOT</td>
<td>Aerosol Optical Thickness</td>
</tr>
<tr>
<td>BC</td>
<td>Brockmann Consult</td>
</tr>
<tr>
<td>CDOM</td>
<td>Coloured Dissolved Organic Matter</td>
</tr>
<tr>
<td>CERTO</td>
<td>Copernicus Evolution: Research for harmonised Transitional water Observation</td>
</tr>
<tr>
<td>Chl-a</td>
<td>Chlorophyll-a</td>
</tr>
<tr>
<td>CNR</td>
<td>The National Research Council of Italy</td>
</tr>
<tr>
<td>CTD</td>
<td>Conductivity, Temperature, and Depth</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved Organic Carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved Organic Matter</td>
</tr>
<tr>
<td>EO</td>
<td>Earth Observation</td>
</tr>
<tr>
<td>GeoEcoMar</td>
<td>The National Institute for Research and Development of Marine Geology and Geoecology, Romania</td>
</tr>
<tr>
<td>GNSS</td>
<td>Global Navigation Satellite System</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
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<td>HZG</td>
<td>Helmholtz-Zentrum Geesthacht</td>
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<td>ICAM</td>
<td>Integrating Cavity Absorption Meter</td>
</tr>
<tr>
<td>IOCCG</td>
<td>International Ocean-Colour Coordinating Group</td>
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<td>IOP</td>
<td>Inherent Optical Properties</td>
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<td>IS</td>
<td>Integrating Sphere</td>
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<tr>
<td>ISM</td>
<td>Inorganic Suspended Matter</td>
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<tr>
<td>LWCC</td>
<td>Liquid Waveguide Capillary Cell</td>
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<tr>
<td>NAP</td>
<td>Non-algal Particles</td>
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<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
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<tr>
<td>NIR</td>
<td>Near-infrared</td>
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<tr>
<td>OD</td>
<td>Optical Density</td>
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<td>OSM</td>
<td>Organic Suspended Matter</td>
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<tr>
<td>PC</td>
<td>Phycocyanin</td>
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<tr>
<td>PML</td>
<td>Plymouth Marine Laboratory</td>
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<td>POC</td>
<td>Particulate Organic Carbon</td>
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<td>PSICAM</td>
<td>Point-source Integrating Cavity Absorption Meter</td>
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<td>SRFA</td>
<td>Suwanee River Fulvic Acid</td>
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<td>Short-Wave Infrared</td>
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<td>TSM</td>
<td>Total Suspended Matter</td>
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<tr>
<td>UL</td>
<td>University of Lisbon</td>
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<tr>
<td>UTC</td>
<td>Coordinated Universal Time</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VSF</td>
<td>Volume Scattering Function</td>
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<tr>
<td>WGS</td>
<td>World Geodetic System</td>
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<td>WP</td>
<td>Work Package</td>
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## List of Symbols

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<thead>
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<td>$a$</td>
<td>total absorption coefficient</td>
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<tr>
<td>$\tilde{a}_{\text{CDOM}}$</td>
<td>absorption coefficient of CDOM</td>
</tr>
<tr>
<td>$\tilde{a}_{\text{NAP}}$</td>
<td>absorption coefficient of non-algal particles</td>
</tr>
<tr>
<td>$a_p$</td>
<td>particulate absorption coefficient</td>
</tr>
<tr>
<td>$\tilde{a}_{\text{ph}}$</td>
<td>absorption coefficient of phytoplankton pigment</td>
</tr>
<tr>
<td>$a_w$</td>
<td>absorption coefficient of pure water</td>
</tr>
<tr>
<td>$A_p$</td>
<td>area of chromatographic peak of pigment</td>
</tr>
<tr>
<td>$A_s$</td>
<td>area of chromatographic peak of internal standard</td>
</tr>
<tr>
<td>$b$</td>
<td>total scattering coefficient</td>
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<tr>
<td>$b_b$</td>
<td>backscattering coefficient</td>
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<tr>
<td>$b_{bp}$</td>
<td>particulate backscattering coefficient</td>
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<tr>
<td>$b_{bw}$</td>
<td>backscattering coefficient of pure water</td>
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<tr>
<td>$c$</td>
<td>beam attenuation coefficient</td>
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<tr>
<td>$C_p$</td>
<td>concentration of pigment</td>
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<tr>
<td>$C_s$</td>
<td>concentration of internal standard</td>
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<tr>
<td>$E_d$</td>
<td>downwelling irradiance just above water surface</td>
</tr>
<tr>
<td>$E_d(0^-)$</td>
<td>downwelling irradiance just below water surface</td>
</tr>
<tr>
<td>$E_d(z)$</td>
<td>downwelling irradiance at depth $z$ below water surface</td>
</tr>
<tr>
<td>$E_u(0^-)$</td>
<td>upwelling irradiance just below water surface</td>
</tr>
<tr>
<td>$E_u(z)$</td>
<td>upwelling irradiance at depth $z$ below water surface</td>
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<tr>
<td>$K_d$</td>
<td>attenuation coefficient of downwelling irradiance</td>
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<tr>
<td>$K_u$</td>
<td>attenuation coefficient of upwelling irradiance</td>
</tr>
<tr>
<td>$L_{\text{sky}}$</td>
<td>sky radiance</td>
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<tr>
<td>$L_t$</td>
<td>total radiance from the water</td>
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<tr>
<td>$L_u(0^-)$</td>
<td>upwelling radiance just below water surface</td>
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<tr>
<td>$L_u(z)$</td>
<td>upwelling radiance at depth $z$ below water surface</td>
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<tr>
<td>$L_w$</td>
<td>water-leaving radiance</td>
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<tr>
<td>$R_{rs}$</td>
<td>remote-sensing reflectance</td>
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<tr>
<td>$z$</td>
<td>depth</td>
</tr>
<tr>
<td>$Z_{\text{SD}}$</td>
<td>Secchi disk depth</td>
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<tr>
<td>$\theta$</td>
<td>zenith angle</td>
</tr>
<tr>
<td>$\phi$</td>
<td>azimuth angle between sensor and sun</td>
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<tr>
<td>$\beta$</td>
<td>volume scattering function</td>
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<tr>
<td>$\beta_p$</td>
<td>volume scattering of particles</td>
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<td>volume scattering of pure water</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>wavelength</td>
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<tr>
<td>$\rho$</td>
<td>water surface reflectance factor</td>
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<td>$\Delta$</td>
<td>residual reflected skylight effects</td>
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1 Executive Summary

This report presents a review and gap analysis of state-of-the-art protocols for measuring bio-optical properties of oceanic and inland water. Specifically, the protocols from International Ocean-Colour Coordinating Group (IOCCG) and the protocols adopted in NERC-funded GloboLakes project are reviewed and analysed, key needs for carrying out bio-optical measurements in transitional waters are identified and, finally, recommendations are made for measuring high quality bio-optical properties in transitional waters. The main findings of this report are:

- IOCCG Ocean Optics and Biogeochemistry Protocols, which include methods for measuring absorption, beam attenuation, remote-sensing reflectance, coloured dissolved organic matter and particulate organic carbon, as well as best-practices to use in-line systems, are mainly designed for ocean waters, but some methods in the protocols are also applicable to transitional waters.

- The Protocols used in GloboLakes for inland waters, which include methods for measuring chlorophyll-a concentration, total suspended matter, coloured dissolved organic matter, and absorption coefficients, are applicable to transitional waters, but some specific sample processing procedures need to be reconsidered.

- Key needs identified for transitional waters include: choosing appropriate sampling location to cover bio-optical gradients and to characterise adjacency effects in satellite products validation, adopting the same method in different study sites to standardise all bio-optical measurements, carrying out temperature and salinity correction for absorption coefficient, and extending the wavelength range of radiometric measurements to the near-infrared to support total suspended matter and turbidity algorithm development in turbid water bodies.

- All measurements are classified into five categories: biogeochemistry (chlorophyll-a and phycocyanin concentration, total suspended matter), apparent optical properties (remote-sensing reflectance, diffuse attenuation coefficient of downwelling irradiance, and Secchi disk depth), inherent optical properties (total absorption, absorption coefficient of phytoplankton pigments, absorption coefficient of non-algal particles, absorption coefficient of coloured dissolved organic matter, beam attenuation coefficient, and backscattering coefficients), physical parameters (water temperature, salinity, turbidity, water depth, wind speed, and aerosol optical thickness), and additional information (location, date, time, cloud and surface water condition).

- Based on the review of protocols from IOCCG, protocols adopted in GloboLakes and CERTO partner organisations, recommendations are made for measuring bio-optical and biogeochemical parameters in transitional waters.

The gap analysis and protocol recommendations of D3.2 support Task 3.3 to develop open web-based community processing approaches for bio-optical data, and field surveys which are going to be carried out in the six study sites. WP3 will work closely with all other work packages throughout the project to provide in situ bio-optical measurements from transitional waters to support the research on, for example, the optical water type classification (WP4), atmospheric correction (WP5) and environmental indicators (WP6).
2 Introduction

The Copernicus programme services provide scientific data for water quality observation. However, these services have largely evolved independently and split across three services: Copernicus Marine, Copernicus Climate Change and Copernicus Land. As a result, there are methodological differences and gaps in data provision. Most notably, there is a lack of data covering complex near-shore and inshore environments, including estuaries, lagoons, bays and large rivers (hereafter called “transitional waters”). The lack of Earth Observation (EO) data products for transitional waters mainly due to the challenges in observing these systems: non-covarying biogeochemical parameters, vicinity of land (adjacency effect), the complexity of the atmosphere (e.g., aerosols), and the influence of bottom reflectance on the signal in optically shallow waters.

Therefore, the overall objective of the CERTO project (Copernicus Evolution: Research for harmonised Transitional water Observation) is to provide a system to harmonise water quality products from the three Copernicus services across transitional waters to support industry, policy-makers and academia. This system will incorporate research on harmonised water optical classification approaches, improved atmospheric correction in optically complex waters and environmental indicators that can be applied to all these waters.

As part of WP3 (In situ characterisation of case study sites), this report fulfils Task 3.2, to improve the standardisation and accessibility of protocols for the collection and processing of validation data. A widely accepted set of operational protocols for in situ data collection is critical in ensuring consistency, comparability, traceability and quality of in situ calibration/validation (cal/val) data. Specifically, in this report, protocols for ocean/marine waters (IOCCG) and those for inland waters (e.g., as adopted in GloboLakes project) are reviewed and analysed to identify areas that lack consensus on appropriate methods. This will ultimately highlight where more research is needed to address methods for transitional water bodies. Based on the review and analysis, recommendations of protocols are made for field surveys in transitional waters. This report will then feed into Task 3.3 to develop open web-based community processing approaches for bio-optical data for end users, which will benefit the water colour remote sensing community. The recommended protocols in this report will support field surveys in the six case study sites (Curonian lagoon, Elbe estuary, Plymouth sound, Razelm-Sinoe lagoon, Tagus estuary and Venice lagoon) in Europe, and thus provide ground biogeochemical and bio-optical data to support algorithm calibration/validation activities in other work packages, such as characterising and validating of optical water type classification (WP4), understanding the atmospheric and adjacency effects and facilitating atmospheric correction method development (WP5), and development of environmental indicators (WP6). The recommendations will also contribute to the development of relevant commons for the pan-European research infrastructure DANUBIUS-RI.
3 Gap Analysis of Existing Protocols for Ocean Waters Published by IOCCG

The protocols published by the International Ocean Colour Coordinating Group (IOCCG) are focused on measurement of bio-optical properties for ocean waters. Below, we examine each protocol volume from the IOCCG and determine gaps in knowledge for transitional waters, and provide suggestions for improvement.

3.1 Volume 1. Inherent Optical Property Measurements and Protocols: Absorption Coefficient (November 2018)

3.1.1 Reflective Tube Absorption Meters

The reflective tube absorption meter is the main approach for in situ absorption measurement in the past 25 years, with typical commercial instruments such as Wetlabs ac-9 and ac-s. The basic concept of the reflective tube approach is retention of most of the forward scattered light in the detected signal when passing a collimated light beam through a particle suspension, by using a highly reflective cuvette that redirects scattered light toward a diffuser in front of a detector. The resulting (collimated and diffuse) beam attenuation coefficients are corrected for (1) pure water absorption by subtracting a purified-water blank, (2) water temperature and salinity correction, and (3) a scattering correction.

3.1.1.1 Temperature and salinity corrections

The absorption by pure water exhibits linear dependencies on temperature and salinity, therefore the IOCCG protocols recommend to perform a temperature and salinity correction on measurements with a reflective tube absorption meter (e.g. ac-s, ac-9). The temperature correction is significant for near-infrared (NIR) wavelengths whereas scattering from dissolved salts acts more strongly in the blue. Moreover, absorption coefficients at NIR are often used for scattering correction methods, thus an accurate correction for temperature and salinity is critical for reliable absorption measurements.

Temperature and salinity corrections are also important for measurements in transitional waters as rapid changes and strong gradients of temperature and salinity can occur, especially at the boundaries between water masses and the presence of steep thermoclines and/or chemoclines. Therefore, co-registering temperature and salinity alongside the ac measurement is important to carry out the correction in transitional waters.

3.1.1.2 Scattering correction

The main problem of reflective tube absorption meter is the scattering error caused by the loss of light which does not reach the absorption detector. The scattering error needs to be corrected after blank, temperature and salinity corrections for absorption measurement. Several methods were detailed in the IOCCG protocol according to Zaneveld et al., 1994:

- The baseline correction method, which assumes that the absorption at NIR is negligible, thus the measured absorption at NIR is due to scattering errors, and then the measured absorption at a NIR wavelength is subtracted from the entire absorption spectrum to correct the scattering errors. However, the NIR wavelength of negligible absorption assumption may be different as the increasing concentration of phytoplankton pigments and non-algal particles in transitional waters will lead to higher absorption in NIR.
- The constant percentage error method, which assumes that the scattering error is a constant percentage of the total scattering and this constant is wavelength independent in a given water mass. The priori assumption of particle composition and phase function...
may not be suitable in transitional waters, because the bio-optical properties change widely from near-shore to ocean.

- The proportional correction method, which accounts for the spectral variation in scattering errors as a constant proportion of the total scattering, and the constant proportion is determined by dividing the absorption by the total scattering at a NIR wavelength (assuming the absorption equal to the scattering error). 715 nm is widely used but this is problematic in turbid coastal waters (e.g. Tassan and Ferrari, 2003; Röttgers et al., 2013). The wavelength independence assumption of the scattering phase function, which has been shown to break down in some extreme cases such as very high turbidity and algal bloom conditions (McKee et al., 2009; Chami et al., 2006; Werdell et al., 2018). There are two important considerations when this method is used in transitional waters. First, to determine which NIR wavelength should be used for the assumption of negligible absorption. Second, the validity of wavelength independence assumption of the scattering phase function.

- The semi-empirical scattering correction method (Röttgers et al. 2013), which is updated based on proportional correction method. This method still retains the assumption of a wavelength-independent scattering function, and Stockley et al. (2017) found the semi-empirical scattering correction to be only applicable in turbid conditions.

- The iterative scattering correction method (McKee et al., 2013), which does not make assumptions about spectral independence of the scattering phase function or negligible absorption in the NIR. However, this method is limited by the unknown variability in the cuvette wall reflectance, and its applicability in transitional, inland or near-shore waters needs further validation.

Since the amount of scattered light varies in different water types depending on the composition of water constituents, the scattering correction should be considered important for transitional waters. Study on developing a general applicable method for scattering correction in various water types may be needed in the future.

### 3.1.2 Integrating Cavity Absorption Meters and Point Source Integrating Cavity Absorption Meters

An integrating cavity absorption meter (ICAM) is a closed cavity with Lambertian reflecting walls to achieve isotropic illumination of the sample held within. Because the light lost from the sphere (absorbed by the walls) is negligible and the light field within is fully diffuse, scattering by the sample does not influence the diffuse transmissivity measurement. The ICAM can enhance sensitivity to weak absorption compared to collimated beam attenuation methods because the light passes multiple times through a sample, but to achieve this it requires a bright light source.

Because the integrating cavity designs are closed systems, they only allow discrete sampling of water. Some modifications were made to the original integrating cavity system to make it applicable for flow-through sampling (Gray et al., 2006; Musser et al., 2009). These alterations consist of a central cylindrical quartz tube with open ends to allow water to flow through the cavity. However, this modification allows light to enter and leave the system, which alters the spatial distribution of radiant energy in the water sample and the sphere so that the diffuse transmissivity measurement becomes flawed (Gray et al., 2006).

The point-source integrating cavity absorption meter (PSICAM) follows another modification to avoid scattering effects. The PSICAM fills an integrating sphere with the water sample and arranges a central isotropic light source inside the sphere. As the light inside the system and
the detector spectral responses are not typically monochromatic, some fluorescence from pigments and dissolved substances alters the distribution of spectral light energy in addition to absorption effects. In natural samples, the fluorescence from chlorophyll-a associated with photosystem II which peaks around 685 nm, is particularly worth correction for in addition to temperature and salinity corrections of the pure water absorption.

ICAM and PSICAM only measure the total absorption coefficient. Thus, to separate particulate and dissolved absorption fractions, samples need to be filtered and measured in sequence.

### 3.1.3 Spectrophotometric Measurements of Particulate Absorption using Filter Pads

Particulate absorption can also be measured in the laboratory using the filter pad method to assess absorption spectrophotometrically. Three configurations for determining particulate absorption from filter pads are described in the IOCCG protocol: the transmittance mode (T-mode), the transmittance-reflectance mode (T-R mode) and the internally mounted integrating sphere mode (IS-mode).

- The T-mode approach is likely the least accurate among the three methods, but it has been used extensively for a number of years and thus there are large historical data sets based on this approach. The sample is placed directly in front of the detector and a diffuser, or at the front of an integrating sphere. Absorption data measured using T-mode should be corrected for the pathlength amplification, and the null-point correction. The null-point correction is the subtraction of a spectrally-constant value from the NIR spectral region and is used to account for large scattering losses. However, in transitional waters, samples are likely to contain significant detrital and mineral particles, which may not have negligible absorption in the NIR.

- The T-R mode can measure a large fraction of both forward- and backward-scattered light, which will largely minimize the issues of scattering error and null-point correction. The sample is sequentially placed at the front and the rear ports of an integrating sphere, characterizing forward and backward transmissivity and scattering. The T-R method is more suitable for optically complex waters when samples contain highly scattering mineral particles. However, the energy conservation law assumed in the T-R method causes uncertainties in actual measurements, and it is more laborious to implement as multiple scans at different filter positions are needed.

- The IOCCG protocols recommend the internally mounted integrating sphere (IS) mode, which places the sample inside an integrating sphere during measurement, and thus can avoid the scattering errors. The IS mode does not need to apply the null-point correction, and it does not require multiple scans of the same filter at different positions. However, the sphere should be relatively large compared to sample size (150 mm sphere diameter for a 25 mm filter).

Therefore, the spectrophotometric method with the IS mode is recommended in transitional waters.

In all cases, the filter pad method allows for the separation of particulate and dissolved absorption. In addition, filters can be bleached using dilute NaOCl or a suitable organic solvent to remove pigments and thus isolate the non-algal pigment fraction of the suspended matter. A combination of bleaching methods may be required to accommodate bleaching in all samples.

Another issue contributing to measurement uncertainty is that the filters introduce a pathlength amplification which has some dependency on the material positioned on the filter. This is
important as the water constituents can vary widely in transitional waters. The IS method is sufficiently sensitive to use low sample concentrations, which allows the measurement to be carried out in the approximately linear range of pathlength amplification. To achieve this, the optical density measured on the filter should be kept <0.25.

3.2 Volume 2. Beam Transmission and Attenuation Coefficients: Instruments, Characterization, Field Measurements and Data Analysis Protocols (April 2019)

A beam transmissiometer (or attenuation meter by approximation) is suggested to measure the attenuation coefficient in the IOCCG protocols. The transmissiometer should be calibrated with, ideally, pure water, cleaned and checked before deployment. It can be used to estimate depth profiles or horizontal transects of the beam attenuation coefficient.

This method is indeed useful for measuring beam attenuation coefficient in transitional waters, but attention should be paid to three aspects:

- Because the transmissiometer has a finite acceptance angle, a portion of forward scattered light will be measured by the detector, which will then lead to the problem of scattering error. In transitional waters, the scattering error measured by the acceptance angle may vary, because the volume-scattering function changes with the constituents of the water (Boss et al., 2009). For the scattering error correction, an iterative scattering correction method for ac-9 and ac-s was proposed by McKee et al. (2013). However, this method still needs further validation. Another commonly used method is not to apply any scattering corrections to the measured attenuation, but simply report the acceptance angle characteristics of the transmissionmeter used to make the measurements and leave all considerations of how to handle scattering artifacts to the user of the data (Voss and Austin, 1993; Pegau et al., 1995).

- Similar to the absorption measurement, the temperature offsets due to difference in water temperature between in situ water sample and reference should be corrected for the measured attenuation coefficients at red and NIR wavelengths. The correction method suggested by IOCCG protocols is the one of Sullivan et al., (2006).

- For extremely turbid waters a saturation problem has been reported for the ac-9 instrument (Röttgers et al., 2013).

Therefore, studies on how to accurately remove the scattering errors for attenuation measurements in transitional waters may be required in the future.


3.3.1 In-water radiometric measurements

In-water radiometric measurements are commonly performed with profiling systems such as free-falling systems or profiling floats to measure the spectral upwelling radiance \(L_u(z)\), downwelling irradiance \(E_d(z)\) and upwelling irradiance \(E_u(z)\) at different depths \(z\) or just below the water surface. The target radiometric values of \(L_u(0^-)\), \(E_d(0^-)\) and \(E_u(0^-)\) at just below the water surface \((z=0^-)\), and their corresponding attenuation coefficients \(K_u\), \(K_d\) and \(K_u\) are determined by extrapolation based on profile measurements from some specific near-surface
layers. Radiometric measurements should be carried out during clear sky conditions and far from land if data is used for satellite product validation.

The accuracy of in-water radiometry is influenced by several aspects:

- The specific near-surface layers selected for extrapolation. The IOCCG protocol suggests to visualize the profile at various bands, and to attempt linear regressions of the log-transformed data as a function of depth to determine suitable upper (risking near-surface wave focusing effects) and lower boundaries (low-light conditions) for extrapolation. This is subjective and quite laborious. For transitional waters, the issue of extrapolation is even more complex, because the attenuation of light at certain wavelengths can happen over relatively short depth interval. Using the same water layers for all bands for extrapolation, as suggested in the IOCCG protocol, in coastal optically complex waters may lead to fitting errors at long wavelengths, because most of the light has been attenuated in the upper layers, which results in a non-linear log-transformed depth profile. Many studies have shown that reflectance at red and NIR wavelengths is important for water quality retrieval in turbid waters (such as Chl-a, TSM). Moreover, the number of near-surface layers observations for extrapolation may be limited (particularly in a free-falling system), leading to more significant effects of wave-induced outliers in profile data (Ruddick et al., 2019).
- Self-shading from radiometers or superstructure is another problem, and the error is much greater at NIR bands in transitional waters as the increase of concentration of absorbing particles and coloured dissolved organic matters lead to higher absorptions.
- The conversion of water-leaving radiance from just below the water surface to above the water surface is dependent on knowledge of the angular radiance distribution, which introduces a measurement uncertainty when translating the measurement into above-water surface radiance or remote-sensing reflectance.
- Other influences such as inelastic scattering varying with depth, strongly changing absorption within a wide bandwidth band (e.g. >5 nm), possibility of sensor tilt particularly near the inflow of rivers, and tidal currents causing vertical stratification, may also influence or invalidate the in-water radiometry measurement.

Alternative in-water methods include performing measurements at fixed depths (Clark et al., 1997; Antoine et al., 2008), this method can produce a larger data volume at each depth, but it need corrections to minimize the impact of inelastic scattering (Li et al., 2016), and data from only two depths does not allow using regression methods to minimize the impact of a non-exact exponential decay with depth. Another in-water method by using one single \( L_d \) sensor at a small depth and several \( E_d \) sensors at various depths to determine the near-surface attenuation coefficient (Zibordi et al., 2012), however, the self-shading effects of the floating system need to be quantified.

With all in-water radiometric measurements, changing illumination conditions need to be monitored and corrected for by using a reference sensor above the water surface. In addition, sensors require inter-calibration prior to deployment.

### 3.3.2 Above-water radiometry measurements

The above-water radiometry measurement method in the IOCCG protocol is mainly based on Mobley (1999), which is carried out through measuring the total radiance from the water \( (L) \), the sky radiance \( (L_{sky}) \), and the downwelling irradiance \( (E_d) \) at given observation geometries, i.e. the viewing angle \( \theta \) and relative azimuth angle between sensor and sun \( \phi \). The water-leaving radiance is calculated as \( L_w=L_{r}\rho L_{sky} \), where \( \rho \) is the water surface reflectance factor. The observation geometry of \( \theta=40^\circ \) and \( \phi=135^\circ \) was proposed as the most appropriate to
minimize the sun-glint perturbations (Mobley, 1999). However, the protocol reported that the use of $\phi=135^\circ$ may easily become the source of perturbations in $L_1$ measurements because the radiometer necessarily looks at the sea close to the deployment structure or at its shadow. Therefore, a $\phi=90^\circ$ has been suggested as a better solution (Zibordi et al., 2009) in such cases. The use of telescopic poles to avoid the influence of superstructure on $E_d$ measurement, and a viewed area at a distance greater than the superstructure height from the bow of a ship to minimize the influence of deployment structures on $L_{\text{sky}}$ and $L_1$ measurements, were also suggested in the protocol.

The above-water radiometry measurement method does not have the extrapolation problem in in-water method, but it has some other challenges:

- As with in-water radiometry, $L_1$, $L_{\text{sky}}$, and $E_d$ measurements can be affected by excessive sensor tilt, clouds or cloud shadows. In addition, sunglint, foam, spray, and non-Cox-Munk waves apply to above-water radiometry.
- The most critical part for measuring water-leaving radiance by the above-water method is determining the water surface reflectance factor ($\rho$). The most basic approximation of $\rho$ is determined from viewing and illumination geometries and wind speed based on simulations from Mobley (1999), in which polarization effects were neglected but later were reported to be non-negligible (e.g. Harmel et al., 2012; Gilerson et al., 2018). New values of $\rho$ were proposed accounting for polarization effects, as well as wave height and slope variance (Mobley, 2015). However, experimental assessments on these two $\rho$ sets showed that those neglecting polarization effects (i.e. Mobley, 1999) have better performance (Zibordi, 2016). Therefore, $\rho$ values computed neglecting polarization effects are suggested for operational processing of above-water radiometry data in the IOCCG protocol.
- The field-of-view is an important factor for the above-water approach as wind-induced wave may cause spatial variation of radiance and influence the measured $L_1$. Studies may be needed on how to select an appropriate field-of-view in the future.
- Correction of non-nadir view should also be carried out for the water-leaving radiance to remove the viewing angle dependence. A chlorophyll-a (Chl-a) based correction approach (Morel et al., 2002) has good performance for Case-1 waters. For optically complex waters, an IOP-based approach (Lee et al., 2011) shows good performance (Gleason et al., 2012). Further studies on developing a general applicable method to correct non-nadir view for a wide range of water types are needed.

Alternative above-water radiometry measurement methods are also described in the IOCCG protocols. These include the use of a reflectance plaque (Carder and Steward, 1985; Rhea and Davis, 1997; Sydor and Arnone, 1997), which needs to be held perfectly horizontal and unobstructed (similar to the $E_8$ sensor). Another alternative method uses a polarization filter to minimize the measured reflectance from the water surface (Fougnie et al., 1999). However, the accuracy of this method is largely depending on the capability of accurately modeling the residual sky-glint radiance and accounting for the non-zero polarization of $L_w$. A skylight-blocked approach leads to the direct measurement of $L_w$ by equipping with a screen blocking the skylight around the sensor is also proposed (Lee et al., 2010). However, this approach suffers from the self-shading problem, and more assessments on this approach are still needed.

For above-water method, the measured reflectance spectra sometimes remain residual reflected-skylight effects due to inaccurate $\rho$ value or glint. In those cases, further quality control or correction may be needed using recent proposed methods (e.g. Ruddick et al., 2005; Jiang et al., 2020). In transitional waters, especially enclosed bays, non-Cox-Munk wave
makes it more difficult to accurately estimate $\rho$ value from wind speed. Thus, more studies on how to accurately determine $\rho$ values can be carried out in the future.


To increase the spatial resolution and sampling frequency of optical data, the IOCCG protocols shared experiences with using an "in-line" or "underway" system, which provides the opportunity to continuously collect optical data and obtain data at sub-pixel resolutions. Flow-through systems installed on research vessels or merchant ships pump the sea water into the vessels from a fixed depth, and have capacity for sequential sensors to observe absorption, attenuation, scattering, CDOM and Chl-a fluorescence. Sampled water enters a vessel from a rectangular or cylindrical recess in the hull of the vessel, the water is pumped into a de-bubbling system, an (optional but recommended) coarse particle filter and then the measuring system. GNSS records are needed to annotate location and time of each measurement, a thermostalinograph is needed to measure the temperature and salinity which may be used for temperature and salinity corrections, and a flow meter which provides means to compute the system residence time is required. According to the protocols, diaphragm and peristaltic pumps are recommended as they can minimize artifacts introduced by the pump such as bubbles. Opaque plumbing entering and exiting the instruments is recommended to minimize contamination by ambient light for instruments which are sensitive to light (e.g. ac-meter). Laboratory analysis of discrete water samples taken along the route is recommended to calibrate and validate the in-line sensor data.

The in-line system is useful for long cruises and clear open ocean waters. However, for transitional waters, especially for near-shore and river mouth sampling activities, there will be occasions where field surveys are carried out by smaller boats which require dedicated flow-through systems. As water constituents may change rapidly within a very short distance in transitional waters, such as along fronts, the time lag correction in the flow-through system becomes critical for adequate location accuracy. Another issue is that the in-line system usually takes samples from a single depth, while transitional waters can have steep vertical profiles. In addition, as salinity also changes in transitional waters, salinity corrections for measurements such as absorption are essential. For turbid waters, high particle concentrations can easily clog filters and accumulate in tubing, saturate instruments, or cause biofouling in the tubing and on optical surface. Thus, frequent cleaning and calibration are needed.

3.5 Volume 5. Measurement Protocol of Absorption by Chromophoric Dissolved Organic Matter (CDOM) and Other Dissolved Materials (DRAFT – March 2020)

The IOCCG protocols detail methods for measuring CDOM using liquid capillary waveguide spectroscopy, double-beam spectroscopy, Sea-Bird Scientific absorption-attenuation (ac) meters, and integrating cavity absorption instrument. Brown glass bottles with Teflon-lined caps are recommended to store the sample filtrates to mitigate UV exposure and thus potential photooxidation of samples. Measurement of the absorption spectrum of solutions of Suwanee
River Fulvic Acid (SRFA) dissolved in ultrapure water is also recommended to verify the instrument performance and measurement procedures before sample measurements.

Liquid capillary waveguide spectroscopy offers pathlengths up to 200 or even 500 cm for more sensitive CDOM measurements in oligotrophic waters. This method is especially useful for low CDOM waters such as open oceans. Attention should be paid to the following: (1) In addition to normal photometric calibration, liquid waveguide instruments should have their effective pathlength determined based on Cartisano et al. (2018). (2) For transitional waters, salinity correction is important because liquid waveguide cells are sensitive to refractive index changes. The IOCCG protocols recommend to generate a correction curve by measuring NaCl solutions across a range of salinities, and then to subtract the NaCl interpolated curve from the CDOM absorbance spectra to produce a value close to zero at 685 nm.

For CDOM measured using a double beam ultraviolet-visible scanning spectrophotometer and quartz cells, salinity correction is not necessary, but the length of quartz cells should be considered. For clear water samples, a 10 cm cuvette is required, while for transitional waters with higher CDOM, typically are visible coloured to the human eye, a 1 cm or 5 cm cuvette is required.

For CDOM measured using ac meters, two ways of measurements are introduced in the protocols. The first one is to measure discrete samples in the laboratory using a gravity-fed system. The second one is to measure in situ CDOM using flow-through systems as detailed in Boss et al. (2019). CDOM measurements using integrating cavity absorption instruments is the same as absorption measurements described in Röttgers (2018). The problems of these methods are described in section 3.1.

For all the aforementioned methods, a null point correction is recommended for samples which exceeds the noise threshold of the instrument in excess of $\pm 0.001$ AU between 650-700 nm. The NASA Ocean Optics Protocols recommended to subtract the average value in the range of 590-600 nm from the entire spectrum. While the IOCCG protocol recommended the average absorbance between 650-680 nm, which is more suitable for transitional waters. However, its applicability for CDOM-rich waters, such as rivers or estuaries, need more validations.


#### 3.6.1 Elemental analysis

Elemental analyzers are commonly used for measuring particulate organic carbon (POC). The sample is first combusted in an oxygen atmosphere, the C in the sample will be oxidized to CO$_2$, and the CO$_2$ is then separated by gas chromatography or temperature-controlled desorption and measured by non-specific thermal conductivity detection. For transitional waters, attention should be paid to the oxygen dosage in the combustion phase according to the IOCCG protocol. As coastal ocean waters out to the continental slope contain a combination of young and old organic C, enhanced combustion conditions could be necessary and the amount of O$_2$ and the time of combustion phase can be increased to ensure complete oxidation.
Ash within the combustion tube, which can disrupt the flow of gases and lead to incomplete combustion should be prevented, as the non-algal particle concentration is higher in transitional waters compared with open oceanic waters.

### 3.6.2 Filtrate blank correction

POC measurements can have a significant filtrate blank, mainly because of adsorption of DOC onto the filter matrix, as well as other processes such as sample manipulation and processing. A simple approach to minimize the filtrate blank is to increase the volume of sample filtered, however, this method may be impractical due to tight water budget and filter saturation, and increasing particle load on filters has the potential to induce cell breakage and leakage thus decrease the measured POC value (Collos et al., 2014). The IOCCG protocol lists three commonly used approaches: regression-based correction, filtrate blank filters and dipped blanks.

- A regression-based correction method is carried out by measuring multiple replicate samples filtered from different volumes, and plotting the measured C content of multiple replicate sample filters against the filtered volume with a linear regression (or two-order polynomial regression), the positive y-axis intercept is interpreted as the amount of dissolved carbon adsorbed onto the filter (Menzel, 1966; Moran, et al., 1999; Turnewitsch et al., 2007), then the true POC can be obtained by subtracting the intercept. Other studies have also reported that the DOC adsorption onto the filter is not a constant, which is a function of volume filtered with a saturation point (Turnewitsch et al., 2007; Novak et al., 2018), an exponential model can be used to fit this relationship, but more validations are still needed. In addition, the capacity of DOM to bind onto glass fiber filters may be different due to the different nature of DOM in transitional waters.

- Filtrate blank filters is another method for filtrate blank correction, after filtering the water sample using the first filter, the filtrate is re-filtered on a separate filter, and the filtrate blank is directly measured from the second filter. This method holds the advantage that it provides the DOM sorption characteristics of different water masse (Goñi et al., 2019), but this method will increase the sample processing time.

- For in-line systems, dipped blank is an alternative method for filtrate blank correction. The filters are prefiltered to exclude particles and exposed to seawater during the pump operation for each in situ pump cast, and they are then expected to represent saturated sorption blanks (Lam et al., 2015). However, this method is less practical for surveys in transitional waters, especially for surveys which are not carried out using in-line system and the discrete samples are taken to the laboratory for further analysis.

### 3.7 Noteworthy and Supplemental Topics on Ocean Colour Radiometry Protocols (DRAFT)

A skylight-blocked approach for radiometry measurement is provided in the IOCCG protocol, and this approach is referred to as "on-water" radiometry. Different to the standard in-water and above-water approaches, the skylight-blocked approach attaches an open-ended apparatus to the front of a downward-looking radiance radiometer. The apparatus penetrates a few centimeters through the water surface but keeping the radiometer in air, and it will block the surface-reflected light (from both sky and sun) from entering the field-of-view of the radiometer. Thus, the radiance sensor can directly measure the water-leaving radiance. Compared with in-water and above-water approaches, the skylight-blocked approach can avoid the post-processing procedures such as the extrapolation of upwelling radiance at
different depth to the radiance just below water surface for in-water approach, and the surface-reflected light correction for the above-water approach, it is also applicable under variable sky conditions and to all aquatic environments. However, skylight-blocked approach still suffers from the problem of self-shading, thus the spectrum measured by the skylight-blocked approach needs correcting. A self-shading correction method was proposed by Shang et al. (2017), which need the concurrent solar zenith angle, diameter of the cone, and the $R_s$ spectrum for the correction. The accuracy of this self-shading correction method still needs more validation. Another important point is, the cone-shaped apparatus in front of the downward radiance sensor in the skylight-blocked system is about 15 cm, which means the distance between the window of the radiance sensor and water surface is <15 cm and the radiance sensor is easily influenced by waves during the measurement. With the skylight-blocked system either floating on the water surface or held manually, waves or movement of the boat will lead to water droplets on the lens of the radiance sensor, and thus produce uncertainties for the measured spectrum (Ruddick et al., 2019). This issue is important for surveys carried out using small boats and under strong wave conditions.

3.8 Summary

The IOCCG protocols provide detailed options and recommendations on measurements of absorption coefficients, beam attenuation coefficient, remote-sensing reflectance, coloured dissolved organic matters, particulate organic carbon, as well as experiences with using in-line systems. Some of the methods described in IOCCG protocols are useful for transitional waters. The main focus of the IOCCG protocols is on marine water, which are relatively clear and deep, and phytoplankton-dominated. Transitional water bodies are comparatively shallow, turbid and complex in terms of constituents, thus some of the detailed methods in IOCCG protocols are not necessarily applicable to transitional waters, especially turbid systems. For example, the in-water approach for measuring $R_s$ may be difficult to apply in turbid shallow waters near river mouths because of water depth, sensor tilt, and vertical stratification or bottom reflection. Above-water approaches can be widely applied to transitional waters but still registration of bottom effects and provide no insight into stratification status. In addition, to accurately determine the $\rho$ value to exclude the influence of reflected skylight remains a prominent challenge. Because of the presence of highly turbid waters, and wide variability of bio-optical properties in transitional waters, other challenges such as how to accurately remove scattering errors in absorption and attenuation measurements using ac meters, how to select appropriate wavelength for null-point correction for CDOM measurement using spectrophotometry method, and how to thoroughly removing the phytoplankton pigments in $a_{NAP}$ measurement using filter pad method also exist when applying those methods in transitional waters.
4 Gap Analysis of Existing Protocols for Inland Waters Adopted in GloboLakes

4.1 Pigment concentration

The central method for measuring pigment concentration in the GloboLakes protocols is a modified High Performance Liquid Chromatography (HPLC) method based on Van Heukelem and Thomas (2001) method (VHT). Water samples are filtered using 25 mm GF/F filters (0.7 μm nominal pore size), and then pigments are extracted using 90% acetone. Pigments are separated on a C8 column using a two-solvent gradient system, solvent A: 70:30 (vol:vol) mixture of 28mM TBAA (6.5 pH) and methanol; solvent B: 100% methanol. The gradient used is as follows: 0 min=95% A, 11 min=45% A, 15 min=45% A, 22 min=5% A, 29 min=5% A, 31 min=95% A. Flow rate is 1.1 mL/min, and column temperature is kept at 55°C. Pigments are verified by the retention time and absorption spectra of each chromatographic peak and quantified by the detector signal at 436 nm.

For use in transitional waters, HPLC method is the only approach to resolve rich pigments profiles, while uncertainties regarding extraction yield and pigment degradation varies.

4.2 Chlorophyll-a concentration

The spectrophotometric method with a dual-beam spectrophotometer is detailed in the protocols used in GloboLakes for measuring Chl-a concentration, which is the ISO-10260 (1992) standard method. Water samples are filtered using 0.7 μm pore size GF/F filters, and pigments are extracted using boiling ethanol. The absorbance of pigments at 665 and 750 nm are measured using the spectrophotometer. Then, 0.01 mL of 1 mol L^-1 HCl is added to sample in cuvette and agitate gently for 1 minute, and absorbance at 665 nm and 750 nm are measured again. The measured absorbance at 750 nm is subtracted from absorbance at 665 nm measured before and after adding HCl, and finally Chl-a concentration is calculated based on the corrected absorbance at 665 nm.

Spectrophotometric method is quick in determining Chl-a concentration, although it can separate less number of pigments than HPLC analysis. Acidification is needed to confirm whether degradation forms were present, and if they were then this can influence the Chl-a (or total Chl-a) estimate quite a lot. In the presence of chlorophylls b and c, Chl-a concentration measurement may be affected using the monochromatic method. In addition, there are different procedures and solvents used for pigment extraction in Chl-a measurement, studies on comparing the difference of using different extraction solvents and procedures, and to standardize the pigment extraction may be needed in the future.

4.3 Phycocyanin concentration

In the protocols used in GloboLakes, the phycocyanin concentration is determined by extraction in 50 nM sodium phosphate buffer at pH 6.8 according to Sarada et al., (1999). Water samples are firstly filtered using 0.7 μm pore size GF/F filters, then phycocyanin is extracted by freeze-and-thaw and sonication, and the extractions are measured using spectrophotometric method to determine the absorbance at 615 nm, 652 nm and 750 nm. The absorbance at 750 nm is subtracted from that measured at 615 nm and 652 nm. Finally, the concentration of phycocyanin is calculated using equations from Bennett and Bogorad (1973).
This method could be useful for transitional waters where cyanobacteria occur. But as turbidity varies significantly in transitional waters, the volume of water samples filtered for phycocyanin measurements should be changed and determined according to the turbidity of the water, a larger filter can also be used to solve this problem. More studies on how to efficiently extract phycocyanin from water samples can be carried out in the future.

4.4 Total suspended matter

As detailed in the protocols used in the GloboLakes project, Total Suspended Matter (TSM) and Inorganic Suspended Matter (ISM) are measured using a high precision electro-balance. First, the water sample is filtered using a 0.7 μm (nominal pore size) GF/F filter. The filter should be pre-washed, pre-combusted (450°C for 1 hours), dried and pre-weighed. The filter with sample is then dried in an oven at 65°C until a constant weight occurs, and weighed when the filter cools down to room temperature using a high precision balance. TSM concentration is calculated by dividing the weight difference (between filter weight and loaded filter) by the volume of filtered water. The filters are then combusted at 450°C for 5 hours to remove organic matter, cooled and weighed again. ISM concentration is calculated by dividing the weight difference between filter weight and 450°C combusted weight by the volume of filtered water.

This method is the standard approach for measuring TSM concentration in waters. Some attention should be paid to the following when applying this method in transitional waters. First, as particle concentrations vary widely, an appropriate volume of water sample should be determined and filtered. For clear waters, insufficient filtering volume may reduce the TSM measurement accuracy. For turbid waters, too much filtering volume may increase the filtration time. Second, it is important to wash the filtration cup using distilled water after filtering each water sample, to ensure all particles attached on the filtration cup are filtered on the filter. Third, during transportation, special attention should be paid to avoid the loss of particles from the filter. Finally, salt retention in the filter leads to overestimation of TSM, and this uncertainty is highly related to salinity. Therefore, distilled purified water should be used to dissolve any salts from the filter after concentrating the water sample onto them.

4.5 Coloured dissolved organic matter

The method of using a dual beam spectrophotometer is detailed in the protocols used in the GloboLakes project to measure CDOM. Water samples are collected from Niskin bottles directly and stored in pre-washed dark bottles. Filtering at least 250 mL of water sample using 0.2 μm polycarbonate filters (Whatman Nucleopore is recommended in the protocol), storing the filtrate in dark bottles. CDOM is then analysed using a dual beam spectrophotometer against a reference of MilliQ water.

This method is applicable in transitional waters, but as CDOM can vary in a wide range in transitional waters, choosing appropriate wavelengths (usually at red wavelengths) for null-point correction is necessary. Moreover, the cuvette size should be considered before analysis. For example, for clear waters, a 10 cm cell can enhance the signal-to-noise ratio, but can also lead to instrument saturation in CDOM rich waters. Therefore, for CDOM-rich waters, a 1 cm or 5 cm cuvette is more appropriate. Multiple cuvette lengths can be used to arrive at a high signal-to-noise spectrum from the UV-B up to the NIR spectrum.
4.6 Absorption coefficients

The filter-pad method, carried out by using a UV-Visible scanning spectrophotometer equipped with an integrating sphere (IS mode), is described in the protocols used in GloboLakes project for measuring absorption coefficients of algal and non-algal particles.

Water samples are filtered using 25 mm GF/F filters (nominal pore size 0.7μm). A pair of blank filters are also prepared through which 0.22 μm pre-filtered seawater has been passed. First, blank filter is placed inside the integrating sphere chamber and scanned, and this blank scan is subtracted in data processing. Then, the sample filter is measured two times at 0 and 90 degrees by mounting it inside the integrating sphere chamber, these two scans are averaged in data processing. After correcting blank, amplification factor correction is done using the method from Stramski et al., 2015, and the absorptance is finally transformed to absorption coefficients. Absorption of phytoplankton pigments are not directly measured. The total particulate absorption coefficients are firstly measured, the filters are then bleached using NaOCl to remove the pigments, and in the end the absorption coefficients of non-algal particles are measured. Finally, the absorption of phytoplankton pigments is calculated by subtracting non-algal particle absorption from the total particulate absorption.

As the integrating sphere mode (IS mode) is used in the protocols used in GloboLakes project, scattering errors correction can be avoided. This method is certainly suitable for transitional waters, but appropriate sample filtration volume should be considered with balance between enough signal from filter pads and the filtration time. And cares should be taken to thoroughly extract pigments before measuring non-algal particulate absorption.

4.7 Summary

The protocols used in GloboLakes project detailed the methods for measuring the concentrations of pigments, Chl-a concentration, phycocyanin concentration, total suspended matter, coloured dissolved organic matter, and absorption coefficients. All these methods are based on benchtop systems, and for discrete water samples. The protocols used in GloboLakes were selected for use in fresh inland water bodies. There are some differences between inland waters and transitional water, such as phytoplankton species and components, vertical and horizontal variability, and the most typically change of salinity. Therefore, although these methods used for inland waters are generally applicable for transitional waters, some procedures or settings of sample processing should be reconsidered to improve the accuracy of measurements when applying to transitional waters. For example, salt retention in the filter will lead to overestimations of TSM and ISM so that marine protocols which purge salts from the filter, are preferred. Incompletely removing phytoplankton pigments in anAP measurement due to an inappropriate choice of organic solvent is likely due to the diversity of phytoplankton communities in transitional waters. To address these issues in successful field campaigns, it is recommended to organise a pilot campaign to test the efficacy of these methods, and to obtain additional samples for spot checks of methodological differences.
5 Key Needs Identified for Transitional Water Protocols

5.1 Choosing appropriate sampling locations and time

*In situ* data should be representative of daily and seasonal ecosystem dynamics for meaningful satellite product validation. The choice of a sampling location generally needs to consider its representability for a specific range of bio-optical properties, and its potential to match up with satellite images. In transitional waters, because bio-optical properties may vary significantly across the freshwater-marine boundary, it is usually necessary to collect data over a environmental and spatial gradients to cover a representative range of bio-optical properties present in the totality of the study area.

For coastal, inland and inshore areas, another important issue is the effect of adjacent land, which may lead to uncertainties in the atmospheric correction of satellite products (Martinez-Vicente *et al.*, 2013). To reduce adjacency effects, sampling locations should be on open water. On the other hand, to characterise the satellite product uncertainty in the presence of land, transect measurements from shoreline to open water are of equal importance. If the objective of study is to demonstrate the best-case capabilities of satellite observations, radiometric measurements have been recommended to keep a distance of at least five nautical miles from any coast (Zibordi *et al.*, 2019). However, this distance is not appropriate for surveys in coastal waters such as bays or estuaries. Moreover, enclosed waters will have land adjacency effects amplified from land masses surrounding the target, and it is therefore more appropriate to target capturing the adjacency effect in field campaigns than to try to avoid it. More datasets that characterise these effects are needed to ultimately improve atmospheric correction methods. These efforts should aim to characterise *in situ* water-leaving reflectance, bathymetry and atmospheric properties in parallel.

*In situ* data collected from locations with abrupt changes of bio-optical properties, such as those associated with sediment plumes, may lead to uncertainties in satellite product validation, because sub-pixel variations of bio-optical properties may not correspond with *in situ* data collected from individual sampling stations. Therefore, sampling locations with sharp horizontal gradients should be avoided (Mueller, 2003a), or characterised by transect sampling.

For transitional waters, especially for coastal turbid waters, vertical stratification of the water column often affects the vertical structure of bio-optical properties. To minimize the disruption of vertical stratification caused by the research vessel when collecting stationary data samples, it is recommended to reduce the speed of the boat before approaching the sampling site (Mueller, 2003a).

The time of sampling is also important, particularly in shallow coastal waters, as strong tidal currents may influence the mixture of particles, and vertical gradient formed in the slack water tidal phase may lead to biases in relating water-leaving radiance to total suspended matter. Therefore, water samples and satellite data should be temporally concurrent within the same tidal phase. Accurate recording of sampling time is needed to match-up *in situ* data with satellite overpass. Timing the sampling of the most dynamic *in situ* stations to be coincident with the satellite overpass can be beneficial to gain the most representative *in situ* data from a single sampling day.
5.2 Determining appropriate volume of water sample for filtration

As the particle composition and concentration vary widely in transitional waters, sometimes even within a short distance, a fixed volume of water sample for filtration may be not practical. Too much water for filtration will overload the filter, resulting in potential breakage of cells, and is also time consuming. Filtering a small volume of water sample may be not enough for gravimetric measurement as the signal-to-noise ratio may be too low. Therefore, it is recommended to change the filtration volume according to the observed turbidity and also according to the objective of the measurement. For example, for absorption measurement, a suggested method is filtering water samples until the colour on the filter is just visible but pale. For concentration measurements of Chl-a or TSM, the volume of water sample for filtration should be then increased in regular steps until filtration slows down markedly. A dosing pump calibrated to a set volume is a convenient way to gradually increase the amount of sample passed through the filter. The concurrently measured Secchi disk depth and turbidity value can be used to estimate an appropriate volume of water samples for filtration, but the type of particulate matter will determine how rapidly the filter blocks. It should be noted that for Chl-a measurement, attention should be paid to non-algal particle dominated turbid waters, e.g. river mouth with strong sediment loading, as there may be very low concentration of phytoplankton pigments even a large volume of water is filtered.

5.3 Adopting the same method in different study sites

The bio-optical properties and physical conditions may vary widely in different transitional water bodies, such as from clear to extremely turbid waters, and from optically deep to shallow waters. Each research group may have their own protocols for bio-optical measurements. However, data collected or processed using different methods may have systematic uncertainties when compared to another, because different methods may need different post-processing and correction procedures. For example, the in-water approach for measuring remote-sensing reflectance needs extrapolation procedure and conversion from below water surface to above water surface. It has high accuracy in clear water columns but it is not always applicable in near-shore turbid shallow waters because of tidal current, disturbing any vertical stratification or presence of near surface phytoplankton blooms. Differently, the above-water approach has no such processing procedures but need to remove reflected skylight from water surface. It can be selected and used to harmonise the measurement in different study sites even though it is not always as accurate as in-water approach for open oceanic waters, while it does yield a larger observation volume to select from.

Therefore, in the absence of a true reference, it is recommended that field campaigns across all study sites follow the same protocols for sensor calibration, deployment, data collection and data processing, reporting the details of methodology, parameters, and data processing steps. Consistent approaches will ensure all observation data have similar uncertainties associated with the method itself, thus data collected from different study sites will be most comparable and provide the largest benefit to the earth observation community.

5.4 Correcting for temperature and salinity variations in pure water absorption

In transitional waters, located in between open oceanic water and inland fresh water, the salinity can vary and be higher than that in fresh waters for some locations. The salt
concentration influences the refractive index, which further influence the absorption coefficient of pure water. Therefore, salinity correction should be carried out for absorption coefficients and beam attenuation coefficients measured using spectral absorption and attenuation meters (ac meters) in transitional waters to account for the difference in salinity between the water samples and the standard pure water. Similar correction of water temperature should be also carried out to account for the difference in temperature between the water samples and the standard pure water. Therefore, temperature and salinity should be measured concurrently in the field. A halocline or thermocline may also exist in coastal areas, thus temperature and salinity should be measured simultaneously (e.g. using a CTD) to do corrections for profile data.

Following temperature and salinity correction, correction for scattering errors should be carried out for absorption coefficients measured by ac meters. Although the proportional correction method is recommended in NASA’s and IOCCG protocols (Pegau et al., 2003; Twardowski et al., 2018), more research on which NIR wavelength is most appropriate for this method is needed in future, as the wavelength of negligible absorption assumption varies in different water types. For example, 715 nm is used for clear waters (Twardowski et al., 2018), while 870 nm is used in turbid waters, but not included in all ac meters (Doxaran et al., 2016).

5.5 Extending wavelength coverage to NIR range

The wavelength coverage of radiometric measurements is critical in transitional waters. For clear oceanic waters the signal at long wavelength can be assumed to deviate negligibly from zero, due to low signal from particles in oligotrophic waters and a high absorption coefficient of pure water. With increasing particle concentrations, particularly of non-algal particles (also referred as non-pigmented particles), absorption and backscattering increase significantly in NIR region, which cannot be ignored as in open oceanic waters.

The remote sensing reflectance, or water-leaving reflectance, at NIR bands is of great significance for post-processing of reflectance and water quality retrievals. For example, to remove the residual reflected-sky light from the above-water remote-sensing reflectance measurement, wavelengths across 720 nm to 865 nm are needed in the methods of Ruddick et al. (2005) and Jiang et al. (2020). Knaeps et al. (2018) subtracted the reflectance at 1305 nm to account for the residual reflected skylight in above-water measured reflectance in three turbid estuaries. Another example for water quality retrieval is the reflectance at short wavelengths which tends to saturate in high TSM waters. In this case, the reflectance at NIR or short-wave infrared (SWIR) bands (e.g. 865 nm, 1020 nm) is suitable for TSM retrieval (Kneps et al., 2015; Novoa et al., 2017). This is extremely important for estuaries, where high TSM loading from rivers occur. Therefore, the wavelength coverage of radiometric data up to 800 nm, as some instruments’ figuration, may not be sufficient for research in transitional waters. It is important to extend the wavelength of radiometry measurements to the long NIR wavelength region, to ensure wavelength availability for water quality retrieval algorithm development in transitional waters. Sensors calibrated to a minimum of 950 nm are recommended.

In addition to remote-sensing reflectance, spectral features in water radiance ($L_i$) and sky radiance ($L_{sky}$) in UV and NIR range are useful in determining accurate remote sensing reflectance (Simis and Olsson, 2013; Groetsch et al., 2017). Therefore, it is recommended that the sensor should cover the spectral range of both UV and NIR (e.g., 350-950 nm, if possible) for the use in transitional waters.
6 Recommendations for Transitional Waters

Transitional waters are influenced by marine currents, tides and the flow of water from the catchment. Thus, transitional waters are often characterised by highly dynamic shallow, turbid, partly saline, productive waters close to land (Zaldivar et al., 2008). The IOCCG and GloboLakes protocols will only be partially applicable in such water bodies. Here we consider and recommend data collection protocols for transitional waters by considering these special characteristics, logistical practicability of sampling, and comparability of in situ data.

The recommendations that follow are based on IOCCG, GloboLakes, NASA protocols and those already adopted by CERTO partner organisations. Our aim is to derive a consistent measurement approach for bio-optical and biogeochemical properties of transitional waters. In this chapter, all measurements are classified into five categories: (i) biogeochemical parameters, (ii) apparent optical properties (AOP), (iii) inherent optical properties (IOP), (iv) physical parameters and (v) additional information.

The following sections provide the details of the recommended methods for parameters in each category. Table 1 provides a brief summary of the recommended methods and is given at the end of this chapter.

6.1 Sampling strategy

For stationary data collection, when approaching the sampling location, the speed of research vessel should be reduced to reduce the disruption of vertical structure of water properties. It is recommended to collect surface water samples using clean Niskin bottles, and to transfer the sample to a sample bottle or carboy immediately. Care should be taken to ensure large particles do not settle in the Niskin bottle. Water samples should be filtered on board or as soon as possible afterwards (same day) if the bottles can be stored dark and at zero degrees Celsius. Samples concentrated onto filters should be stored in a dry shipper or liquid nitrogen dewar until lab analysis. It is important to ensure the particles are uniformly distributed in the water sample when taking subsamples for filtration to avoid bias between subsamples caused by particles sinking, especially for turbid waters.

Low sun zenith angle should be avoided when carrying out radiometric measurements in the field, campaigns should be undertaken under clear sky conditions where possible. Information on sky conditions should also be recorded. Measurements over environmental gradients are needed in the study area, but be careful when using data collected from steep gradients for satellite product validation because of the sub-pixel problem. For stations where data will be used for satellite product validation, it is important to check the overpass of satellite when planning sampling schedule to decrease the time gaps between in situ measurement and satellite images.

6.2 Biogeochemical parameters

6.2.1 Chlorophyll-a concentration

For measuring Chl-a concentration, the HPLC method is recommended because of its high accuracy in determining multiple pigment concentrations. Alternatively, the recommended spectrophotometry is also described in case the HPLC is not available.
The recommended HPLC method is based on Van Heukelem and Thomas (2001) and Hooker et al. (2009). This method is applicable to all water types including freshwater, estuarine, coastal and open ocean waters.

1. Filter water samples using an appropriate filter (25 mm, 0.7 μm pore size) as soon as possible after sample collection. It is important to concentrate as large a volume of water sample as possible to accurately quantify minor pigment groups. The reference of filtration volume for different water types are: 3 L to 4 L for oligotrophic waters, 1 L to 2 L for mesotrophic waters, and 0.5 L to 1 L for eutrophic waters (or less for extremely turbid waters) (Trees et al., 2003). Sample filters are recommended to be stored at -80°C for no more than one year until laboratory analysis.

2. To extract the pigment prior to quantification, place each sample filter in a 2 mL Eppendorf vial, and add 1.5 mL 95% Methanol (pre-made stock solvent – 95% Methanol+α Tocopheryl acetate as an internal standard), which is then sealed. Samples are kept dark throughout the procedure.

3. Samples are initially extracted for 1 hour in the freezer at -40°C (preferred, if not available can be -20°C) before being agitated on a mixer for 15 seconds and then sonicate for 2 minutes. The vials are then returned to the freezer to complete extraction for a further 3 hours at -40°C.

4. After extraction is complete, samples are filtered through Teflon syringe filters (25 mm, 0.45 μm pore size) into amber glass vials for HPLC analysis. Samples are then kept in the freezer at -40 °C until analysis. For a total of no longer than 24 hours since starting the extraction procedure.

5. For HPLC analysis, a 150 x 2.1mm C18 column with 1.8 μm pore size is used to separate pigments. Gradient analysis is performed using a two solvent system. Solvent A is 70:30 Methanol:TBAA solution (28mM, pH 6.5) and Solvent B is 100% Methanol. Both solvents are filtered through Teflon (45mm, 0.45 μm). Seal wash and needle wash are 10% IPA in Di H2O. Flow rate is 0.5 mL/min and column temperature is 57.5°C. Pigments are detected at 445 nm, 665 nm, and the internal standard is detected at 222 nm. Absorption spectra by the Diode Array Detector (DAD) is used for peak identification where needed. Calibration of pigments is carried out with certified standards (available from DHI) and it is recommended to include a standard pigment blend at the start and end of each analysis run to establish drift in elution times. The gradient used is as follows: 0.0 min=95% A, 0.2 min=95% A, 22.0 min=5% A, 23.0 min=1% A, 30.5 min=1% A, 31.0 min=5% A. Post time=2.0 min (returns to conditions at start of gradient ready for next injection).

6. Final concentrations are calculated using relative response factors and a filtration volume adjustment calculation:

\[
Response \ Factor \ (RF) = \frac{Peak \ Area}{Concentration \ in \ mg/L} \tag{1}
\]

\[
Relative \ Response \ Factor \ (RRF) = \frac{Response \ Factor \ of \ pigment}{Response \ Factor \ of \ internal \ standard} \tag{2}
\]

\[
C_p = \frac{V_e}{V_f} \times \frac{A_p}{RRF(A_d/C_s)} \times 1000 \tag{3}
\]

where, \(C_p\) is the pigment concentration in μg/L (or mg/m³), \(V_e\) is extraction volume, \(V_f\) is volume of water filtered, \(A_p\) is the area of chromatographic peak of pigment, \(A_d\) is the area of chromatographic peak of internal standard, \(C_s\) is the concentration of internal standard.

In the case that HPLC is not available, spectrophotometry method can be used to measure Chl-a concentration alternatively.
(1) Filter a known volume of sample over 47 mm GF/F filters (0.7 μm pore size) as soon as possible after sample collection, and place the filter in a centrifuge tube stored at -20°C, in a dark environment, until extraction.
(2) Place the filter in a centrifuge tube and add 10 mL boiling ethanol, extract for 24 hours in darkness at room temperature (approximately 20 °C).
(3) Pigment extracts are centrifuged at about 3000-5000 g for 10 minutes.
(4) Prior to sample analysis, warm up the spectrophotometer until lamp and detector outputs are stable.
(5) Autozero the spectrophotometer with nothing in the compartment.
(6) Decant the clear supernatant to a 1 cm cuvette cell, and measure the absorbance of sample against 90% ethanol blank at 630, 647, 664, and 750 nm.
(7) Correct the absorbance by subtracting the measured absorbance at 750 nm from the measured absorbance at 664, 647 and 630 nm.
(8) Finally, Chl-a concentration (C<sub>chla</sub>, μg/L or mg/m³) can be calculated using the following equation (Jeffrey and Humphrey, 1975):

\[
C_{\text{chla}} = \frac{(11.85A_{664} - 1.54A_{647} - 0.08A_{630})v}{V_L} 
\]

(4)

where, \(A_{664}\), \(A_{647}\) and \(A_{630}\) are the corrected absorbance at 664, 647 and 630 nm, respectively, \(v\) is the volume of extraction solution in mL, \(V\) is the volume of filtered water sample in liters, \(L\) is the length of cuvette cell in cm.

It is recommended to triplicate samples to measure Chl-a concentration for each station.

### 6.2.2 Phycocyanin concentration

It is recommended to measure phycocyanin concentration (PC) using the spectrophotometric method, because the water-soluble pigment will not separate well on pigment HPLC gradients of organic solvents.

(1) Filter a known volume of water (adjust for turbidity) using GF/F filters (0.7 μm pore size) following sample collection. Filters can then be stored frozen prior to analysis within several weeks, if stored at -20°C, or up to one year at -80°C.
(2) Prior to analysis, filters are placed in a centrifuge tube and suspended in a solution of 10 mL 50 mM phosphate buffer (pH=6.8).
(3) Phycocyanin is extracted using the freeze-thaw and sonication combined method (Horváth et al., 2013; Riddick et al., 2019): the filter in the buffer solution is frozen at -20°C and thawed at 9±1°C in a water bath (Sarada et al., 1999), and then sonicated (tip sonicator) over ice for 90 seconds.
(4) After sonication, centrifuge for 10 minutes at 5000 rpm to clarify the extract.
(5) Decant the supernatant to a 1 or 4 cm path length cell, and measure the absorbance at 615, 652 and 750 nm against the blank of 50 mM phosphate buffer using a spectrophotometer.
(6) Subtract the absorbance at 750 nm from that measured at 615 nm and 652 nm, and the phycocyanin concentration (mg/m³) is calculated using the following equation according to Bennett and Bogorad (1973):

\[
PC = \frac{(A_{615} - 0.474A_{652})v}{V_L} 
\]

(5)

Where, \(A_{615}\) and \(A_{652}\) are the corrected absorbance at 615 nm and 652 nm respectively, \(v\) is the volume of extraction solution in mL, \(V\) is the volume of filtered water sample in liters, \(L\) is the length of cuvette cell in cm.
It is recommended to analyse samples in triplicate to measure phycocyanin concentration for each station. For additional quality control, obtain the absorbance spectrum over the 400-800 nm range and ensure that no chlorophyll-a absorption peaks are visible. Additionally, pellets resulting from centrifugation can be spectrophotometrically scanned to ensure no PC absorption remains in these fractions. Finally, if foam is seen to form in any part of the procedure, sonication steps should be reduced because these will easily break down the pigment.

### 6.2.3 Total suspended matter

It is recommended to measure TSM concentration using the gravimetric method (e.g., Strickland and Parsons, 1972).

1. First, a known volume of water samples is filtered through a pre-washed, pre-ashed and pre-weighed 47 mm GF/F filter (0.7 μm pore size) (weight A, mg). It is important to rinse the filtration cup after filtering each sample and to rinse the filter with Milli-Q water following the sample to remove salt crystals in the filter.
2. Filters should be stored dry and stable to avoid material dislodging from the filter. Freezing on-board can help to stabilize the material on the filter.
3. The filter is weighed again after drying at 60°C for 24 hours (weight B, mg). Then TSM concentration (mg/L) is calculated as:

   \[
   \text{TSM} = \frac{\text{weight } B - \text{weight } A}{V}
   \]

   Where, \( V \) is the volume of filtered water sample in littres and TSM will be specified in mg/L or g/m³.
4. After measuring TSM, combust the filter at 450°C for 4 hours, and weigh again (Weight C, mg) to measure the concentration of inorganic suspended matter (ISM, mg/L):

   \[
   \text{ISM} = \frac{\text{weight } C - \text{weight } A}{V}
   \]

5. Finally, organic suspended matter (OSM) can be calculated by subtracting ISM from TSM, i.e. OSM=TSM-ISM.

It should be noted that a high precision electrobalance should be used to weigh the sample. Care should be taken while transporting the filters to avoid any losses of particles on the filter. It is recommended to collect samples in triplicate with different filtering volume of water sample for each station so that linearity between the samples can be observed and filter weight is obtained from the slope of the concentration curve, instead of individual weights (Röttgers et al., 2014).

### 6.3 Apparent optical properties

#### 6.3.1 Remote-sensing reflectance

Considering the turbidity, water depth and high dynamics of transitional waters, the above-water approach is recommended for measuring remote-sensing reflectance \((R_s)\). This approach can be used for various water types, and is more useful in relatively shallow waters where the in-water approach cannot be used, either because boats cannot reach these areas or because the sensors itself cannot be deployed at depth. The measurement should be made from a location where shading and reflections from superstructure are avoided, and where the
sensor can be pointed at the water surface where it is not disrupted by ship movement, excessive whitecaps, spray, foam or floating materials appear. The best position to collect $L_t$ on a ship is from the bow, whereas $E_d$ is collected from a high point with unobstructed view of the sky.

(1) The upwelling radiance from water surface ($L_t$), radiance of sky ($L_{sky}$) and downwelling irradiance ($E_d$) are ideally measured at the same time with a 3-sensor system, or in the order $E_d$, $L_{sky}$, $L_t$ when using a single spectrometer system. To avoid reflected sky light and direct sun glint at the water surface, the relative azimuth angle between sensor and sun should be close to $\varphi=135^\circ$. $L_t$ and $L_{sky}$ are obtained at oblique forward angles of $\theta=40^\circ$ away from the ship to avoid nadir and hull reflections or shading. $E_d$ should be measured level with the sensor pointing straight upward. The average and range of sensor tilt should be recorded during the measurement sequence. The geometry of $L_t$ and $L_{sky}$ measurements is shown in Figure 1 and Figure 2.

(2) $R_{rs}$ with a unit of sr$^{-1}$ is then obtained using the following equation (Mobley, 1999).

$$R_{rs}(\lambda) = \left( L_t(\lambda) - \rho L_{sky}(\lambda) \right) / E_d(\lambda)$$

(8)

The water surface reflectance factor ($\rho$) can be estimated using wind speed based on the approach in Ruddick et al. (2006) if the value of wind speed can be recorded, or it can be determined using the method from Simis and Olsson (2013).

Figure 1. Geometry of $L_t$ and $L_{sky}$ measurements for above-water approach
Figure 2. HyperSAS for above-water radiometric measurement (PML)

It is recommended to repeat the measurement at least 10 times within a short time period, removing measurements which are obviously affected by sunglint, floating material and foam. Changing illumination conditions can also be evaluated by comparing the $E_d$ measured throughout the repeated measurement sequence, and the ratio of $L_{sky}/E_d$ is useful to evaluate presence of non-homogeneous cloud cover. A visual validation of $R_{rs}$ is recommended. In cases where an inappropriate $\rho$ value is used or glint effect exists, residual reflected skylight effects ($\Delta$ effects) may show as features in $R_{rs}$ corresponding to absorption by atmospheric gases, or high $R_{rs}$ in NIR wavelengths and/or a more gradual slope towards shorter wavelengths indicating under-correction of Rayleigh scatter. If needed, $\Delta$ effects can be compensated (but not always removed, e.g. if they are not spectrally neutral) based on methods proposed by Ruddick et al. (2005) or Jiang et al. (2020).

### 6.3.2 Diffuse attenuation coefficient

The spectral diffuse attenuation coefficient can be measured with the method of in-water profiling.

1. Measure downwelling irradiance ($E_d(z)$) at different depth ($z$) of the water using an upward irradiance sensor and a depth sensor. Record $E_d(0^*)$ throughout the cast to compensate for changing illumination.
2. Correct each $E_d(z)$ measurement against the reference sensor on deck and subsequently select $E_d(z)$ profile data at a suitable depth range up to the water surface but away from near-surface effects.
3. Apply a natural logarithm to the selected $E_d(z)$ profile data, and apply a linear regression analysis for log-transformed $E_d(z)$ against depth for each band.
4. The spectral diffuse attenuation coefficient ($K_d$, m$^{-1}$) is as the slope of the regression.
Eq. (9) illustrates the linear relationship between $K_d$ and log-transformed $E_d$. It should be noted that $z$ is depth range between $z_1$ and $z_2$, and all measured $E_d$ and depth between $z_1$ and $z_2$ are used for regression analysis.

$$\ln(E_{d,z2}(\lambda)) = -K_d(\lambda) \cdot z + \ln(E_{d,z1}(\lambda))$$  \hspace{1cm} (9)

A visual check of the light profile is useful to help select the specific depth range which is used for regression. Noisy data caused by wind-induced water surface turbulence which cannot be avoid, can be treated with a smoothing to the $E_d(z)$ profile data. Special attention should be paid to red and NIR wavebands, because light refraction at the water-air interface is a function of wavelength. Ship shadow, change of illumination should be avoided when measuring the $E_d(z)$ profile (Mueller et al., 2003b).

### 6.3.3 Secchi disk depth

For measuring Secchi disk depth, an approximately 30 cm diameter white disk is recommended.

1. Vertically lower the Secchi disk into the water until the disk is invisible from the observer’s view.
2. Secchi disk depth is then recorded as the distance from the Secchi disk to the water surface in units of meters (Figure 3).

The measurement of Secchi disk depth should be carried out at the shady side of the ship to avoid the influence of surface glint (Pitarch, 2020). Secchi disk depth is a proxy of water transparency, which can be used as information to decide the water volume for filtration, and is also helpful for quick check of the radiometric measurements in the field. In the later stage of data processing, the value of Secchi disk depth can be used to roughly assess the quality of calculated $K_d$ because Secchi disk shows strong negative correlation with $K_d$ (Lee et al., 2015a; Jiang et al., 2019). In strong currents or high winds, the problem of tilt of the rope can be problematic and circumvented by adding weights to the disk. If the Secchi disk reaches the bottom while still visible, this should be recorded, and transparency tube can be used to estimate Secchi disk depth instead.

![Figure 3](image_url)

**Figure 3.** Illustration of Secchi disk and Secchi disk depth measurement.
6.4 Inherent optical properties

6.4.1 Absorption coefficient

The total absorption coefficient is recommended to be measured using the filter pad method by a spectrophotometer equipped with a 150-mm integrating sphere (IS mode, Figure 4) (Röttgers and Gehnke, 2012; Stramski et al., 2015). Using the filter pad method with a pigment extraction or bleaching approach, the absorption coefficients of phytoplankton pigments ($a_{ph}$) and non-algal particles (also referred as non-pigmented particles) ($a_{NAP}$) can be separated.

![Illustration of the IS mode for measuring absorption coefficients](image)

Figure 4. Illustration of the IS mode for measuring absorption coefficients (Stramski et al., 2015)

1. Prepare three to five blank filters and prepare the sample filters by filtering a known volume of water over a 25 mm GF/F filter (0.7 μm pore size) under low vacuum. Place each filter in a labeled petri dish and analyse immediately. Alternatively, if analysis is delayed, keep the sample filters and blank filters flat and store at -80°C until they are to be measured.
2. In the laboratory, warm up the spectrophotometer until lamp and detector outputs are stable.
3. The recommended scan wavelength is 350 – 850 nm with 1 nm interval, slit bandwidth 2 nm, scan speed is adjusted based on detector sensitivity.
4. Perform an air-vs-air scan to autozero the spectrophotometer with the filter holder in the instrument but without a filter inserted. Repeat the scan and save the instrument baseline to a data file. The baseline should be spectrally flat and noise should be less than ±0.0005 optical density (OD) units. Adjust scan speed if required to achieve sufficient signal. The air-vs-air scan should be regularly performed to ensure no significant instrument drift.
5. When measuring absorbance of filters, place the filter on a drop of purified water (in case of blanks) or sample filtrate to moisten it. Subsequently place the filter on a tissue paper to remove excess moisture and finally place the filter in the filter holder at the center of the sphere at a slight angle (10° is sufficient) to reduce reflections in the direction of the entrance port of the sphere.
6. Measure the absorbance of the blank filter (i.e. blank-filter baseline), save the scan to a data file. Repeat the above measurement for all blank filters, and average the scans of all the blank filters to determine the average blank-filter baseline.
7. Place the sample filter on the filter holder within the center of the sphere, scan the sample filter and save the scan to a data file. Rotate the sample filter 90 degree and repeat the scan to check the homogeneity of sample distribution on the filter, and save to a data file.
(8) For each sample filter, subtract the blank-filter baseline from the measured absorbance of the sample filter, and average the two repeat scans at 0 and 90 degree to get the corrected absorbance (OD\(_{\lambda}\)).

(9) Confirm that the peak OD\(_{\lambda}\) is below 0.3 to avoid non-linear pathlength amplification (e.g. Simis and Kauko, 2012).

(10) The corrected absorbance can be converted to the absorption coefficient using the following equation:

\[
a_s(\lambda) = \ln(10) \cdot 0.323 \left[ OD_f(\lambda) \right]^{1.0867} / (V/A) \tag{10}
\]

Where, \(V\) is filtration volume in \(m^3\), \(A\) is measured interception area of filtration in \(m^2\). \(a_s\) can be either particulate absorption coefficient \((a_p, \ m^{-1})\) or absorption coefficient of non-algal particle \((a_{NAP}, \ m^{-1})\). It should be noted that a power function is used to correct the path-length amplification in the above equation (coefficients used here are from Stramski et al., 2015).

(11) After measuring the \(a_p\), return the sample filter to the filtration system, gently add a few drops of sodium hypochlorite (NaOCl) to the filter, and leave the filter to bleach for 5-10 minutes (time depends on filter load). The filtration cup should be covered to avoid contamination during bleaching.

(12) After bleaching, rinse the sample filter by filtering approximate 50 mL Milli-Q water to remove bleach.

(13) Measure the absorbance of the depigmented filter following the same procedure as above, to obtain \(a_{NAP}\).

(14) The absorption coefficient of phytoplankton pigments \((a_{ph}, \ m^{-1})\) is defined as:

\[
a_{ph}(\lambda) = a_p(\lambda) - a_{NAP}(\lambda) \tag{11}
\]

The total absorption coefficient \((a, \ m^{-1})\) can be calculated by adding the absorption coefficients of all water components as follows:

\[
a(\lambda) = a_{ph}(\lambda) + a_{NAP}(\lambda) + a_{CDOM}(\lambda) + a_w(\lambda) \tag{12}
\]

Where, \(a_w\) is the absorption coefficient of pure water, e.g. based on Lee et al. (2015b), Pope and Fry (1997) and Kou et al. (1993). \(a_{CDOM}\) is the absorption of coloured dissolved organic matter. It should be noted that if a Chl-a absorption peak remains near 675 nm when measuring \(a_{NAP}\), then the bleaching should be repeated or combined with an organic solvent extraction (e.g., Simis et al., 2017) to remove the residual pigments remaining on the filter until the absence of Chl-a absorption peak at near 675 nm.

In addition to the method described above, the total absorption coefficient can also be measured in the field by using reflective tube absorption meter such as ac-9 and ac-s. If the ac meter is used to measure the absorption coefficient in the field, temperature and salinity corrections should be applied to the observations as follows:

\[
a_{m}^{TS}(\lambda) = a_m(\lambda) - \frac{\partial a_w(\lambda)}{\partial T} (T - T_r) - \frac{\partial a(\lambda)}{\partial S} S \tag{13}
\]

Where, \(a_m(\lambda)\) is the measured uncorrected absorption coefficient, \(a_{m}^{TS}(\lambda)\) is temperature and salinity corrected absorption coefficient, \(\frac{\partial a_w(\lambda)}{\partial T}\) can be found in Pegau et al., (1997). \(\frac{\partial a(\lambda)}{\partial S}\) is provided by the manufacturer. \(T\) and \(S\) are the temperature and salinity measured concurrently in the field. \(T_r\) is the reference temperature of pure water.

After temperature and salinity correction, a further step of scattering error correction should be done as the following recommended method (Pegau et al., 2003; Twardowski et al., 2018):
\[ a(\lambda) - a_w(\lambda) = a_w^{TS}(\lambda) - \left[ \frac{a_m^{TS}(\lambda_{NIR})}{c_m^{TS}(\lambda_{NIR}) - a_m^{TS}(\lambda_{NIR})} \right] \left[ c_m^{TS}(\lambda) - a_m^{TS}(\lambda) \right] \]  

Where, \(a(\lambda)\) is the final absorption coefficient, \(a_w(\lambda)\) is absorption coefficient of pure water, \(a_m^{TS}(\lambda_{NIR})\) and \(c_m^{TS}(\lambda_{NIR})\) is temperature and salinity corrected absorption and beam attenuation coefficients at near-infrared wavelength respectively.

### 6.4.2 Coloured dissolved organic matter

It is recommended to measure CDOM using a dual-beam spectrophotometer.

1. Prepare CDOM samples by filtering water samples using a 0.2 μm pore size filter (e.g., Nuclepore filter), it is noted that filtration should be done as soon as possible after water sample collection. CDOM samples should be kept in brown bottles with Teflon caps in a 4°C refrigerator, and samples should preferably be analysed within 2 weeks to avoid the degradation of the organic matter. For longer storage periods a biocide can be added to prevent bacterial degradation.

2. In the laboratory, warm up the spectrophotometer until lamp and detector outputs are stable.

3. The recommended scan wavelength is 250 – 800 nm with 1 nm interval, slit bandwidth no wider than 4 nm and scan speed adjusted for detector sensitivity.

4. Conduct an air-vs-air baseline scan to zero the instrument across the full spectral range. Repeat an air-vs-air scan to evaluate the performance of the instrument, and save the spectrum to a data file. This spectrum should be spectrally flat, and the noise should be less than ±0.0005 OD units.

5. Fill a pair of 10 cm cuvette cells with ultrapure water. Place the reference cuvette in the sample compartment and conduct a water-vs-air scan. Place the sample cuvette in the sample compartment and conduct a water-vs-air scan, save the spectrum. Compare the water-vs-air scans of reference cuvette and sample cuvette to ensure these two cuvettes are well matched optically.

6. Discard the ultrapure water in the sample cuvette, and rinse the cell with CDOM sample three times, then fill CDOM sample in the sample cuvette. Dry the exterior of the sample cuvette carefully, and inspect it to ensure there are no particles and bubbles. Place the sample cuvette in the sample compartment, conduct a scan of the CDOM sample and save to a data file.

7. Correct the CDOM absorbance against the pure water baseline and a null value. The null value is determined by averaging the measured absorbance between 700-800 nm.

8. Finally, the absorption coefficient of CDOM with a unit of m⁻¹ is defined as:

\[ a_{CDOM}(\lambda) = \ln (10) A(\lambda)/L \]  

Where, \(A\) is the null-point corrected absorbance, \(L\) is length of the cuvette cell in meters. CDOM spectrum can be fitted using an exponential function with an additional background parameter as the method described in Stedmon et al. (2000).

It should be noted that the ultrapure water reference and sample water should be at the same temperature. If the sample is rich in CDOM, a 1 or 5 cm cuvette cells may be sufficient and a longer cell could saturate the spectrophotometer. The null value and wavelength range used for null-point correction should be reported along with the CDOM data. In addition, it is recommended to check the performance of the spectrophotometer using standard (e.g., neutral density filters) before starting each set of measurement.

Additional CDOM measurements can be carried out in the field using the ac meters (eg. Ac-9, ac-s), detail of the method can be referred to Mannino et al., (2019).
6.4.3 Beam attenuation coefficient

The beam attenuation coefficient \((c)\) measurement can be done in the field by using a beam attenuation meter such as CStar, ac-9 or ac-s (Boss et al., 2019; Figure 5). Before measurement, ensure the window of the beam attenuation meter is clean, and check the instrument performance before and after the field campaign. For transitional waters, it is important to do temperature and salinity correction for the measured beam attenuation coefficient as the temperature and salinity in the field are different with the reference temperature and salinity of pure water. Temperature and salinity correction can be done using the following equation:

\[
c_m^{TS}(\lambda) = c_m(\lambda) - \frac{\partial a_w(\lambda)}{\partial T} (T - T_r) - \frac{\partial c(\lambda)}{\partial S} S
\]

(16)

Where, \(c_m(\lambda)\) is the measured raw beam attenuation, \(c_m^{TS}(\lambda)\) is temperature and salinity corrected beam attenuation, \(\frac{\partial a_w(\lambda)}{\partial T}\) can be found in Pegau et al, (1997). \(\frac{\partial c(\lambda)}{\partial S}\) is provided by the manufacturer. \(T\) and \(S\) are the temperature and salinity measured concurrently in the field. \(T_r\) is the reference temperature of pure water absorption.

Figure 5. Example of the absorption and attenuation meter (SEABIRD ac-s, from ac-s manual)

6.4.4 Backscattering coefficient

The backscattering coefficient is not generally directly measured in the field. Instead, the volume scattering function (VSF), \(\beta(\varphi, \lambda)\), at one or more fixed angles \((\varphi)\) around 120°-140° is measured using a configuration as shown in the following graph:
Figure 6. An illustration of the optical configuration of scattering meter (ECO-BB, from ECO-BB manual)

Figure 7. Examples of backscattering meter, left: SEABIRD ECO-BB9 (from ECO-BB9 manual), right: HOBI Labs Hydroscat-6 (from Hydroscat-6 manual)

The backscattering coefficient can be derived by integrating $\beta(\lambda, \varphi)$ over the backward hemisphere which in the case of a single-angle scattering measurement requires assumptions to be made on the angular shape of the VSF. Practically, sensors such as WET Labs ECO-BB and HOBI Labs Hydroscat (Figure 7) can be used to measure the backscattering coefficient in the field.

1. Collect the uncorrected volume scattering ($\beta_{meas}$) by submerging the sensor away from any obstructions.
2. Correct the $\beta_{meas}$ for absorption and scattering losses to obtain corrected volume scattering ($\beta_{cor}$).
3. Third, subtract the volume scattering of pure water ($\beta_w$) to get the volume scattering of particles ($\beta_p$) (Boss and Pegau, 2001):

$$\beta_p(\lambda, \varphi) = \beta_{cor}(\lambda, \varphi) - \beta_w(\lambda, \varphi)$$  \hspace{1cm} (17)

$\beta_w$ can be obtained from Morel (1974).

4. The particulate backscattering coefficient ($b_{bp}$, m$^{-1}$) can be then calculated as:

$$b_{bp}(\lambda) = 2\pi \chi \beta_p(\lambda, \varphi)$$  \hspace{1cm} (18)

Where $\chi$ is a constant provided by the manufacturer, e.g. $\chi = 1.1$ for ECO-BB.
Finally, the backscattering coefficient \( (b_b, \text{ m}^{-1}) \) is defined as:

\[
b_b(\lambda) = b_{bp}(\lambda) + b_{bw}(\lambda)
\]

Where, \( b_{bw}(\lambda) \) is backscattering coefficient of pure water (Morel, 1974; Zhang et al., 2009).

However, those scattering meters are originally designed for open ocean waters, there are two problems should be taken into consideration when deploy them to transitional waters, especially turbid waters. First, the saturation of the sensor in extremely turbid waters. Second, the procedure for correcting absorption and scattering losses in the second step described above. According to Doxaran et al. (2016), ECO-BB is less sensitive to absorption losses as it has short pathlength, but it may face saturation problem in turbid waters. Hydroscat has five adaptive gains to deal with saturation problem in turbid waters, but the absorption and scattering losses correction (sigma correction) is a main problem as it has longer pathlength compared to ECO-BB. Accordingly, a new method was proposed in Doxaran et al. (2016) to correct the absorption and scattering losses for Hydroscat, which can be used for transitional waters.

It is noted that the instrument should be cleaned and calibrated regularly to avoid any uncertainties caused by scaling factor changes, as about 10% changes of scaling factor per year was observed for ECO-BB sensor at blue channel (Sullivan et al., 2013).

### 6.5 Physical parameters

#### 6.5.1 Water temperature and salinity

Water temperature and salinity can be measured by a calibrated conductivity, temperature, and depth (CTD) device (Mueller, 2003a). The water temperature and salinity are needed to correct for water absorption in IOP measurements.

#### 6.5.2 Turbidity

The nephelometric method is recommended to measure water turbidity. This method is capable of measuring a wide turbidity range. The principle of nephelometry is to measure the scattered light at 90° to the incident beam (APHA, 1998; Davies-Colley and Smith, 2001). Turbidity measurements should be carried out as soon as possible after the sample collection, if the measurement is delayed, water samples should be stored at 4°C. Care should be taken to avoid bubbles in the sample cell. Portable turbidimeters are available, such as HACH 2100P.

#### 6.5.3 Sensor and water depth

Depth information includes the deployment depth of sensors used in the water column and the maximum water depth at a given location. Sensor depth can be measured simultaneously by a CTD device or a separate pressure-based (‘D’ in CTD) sensor. Sensor depth is a key parameter for determining diffuse attenuation coefficient and studies of vertical biogeochemical profiles. The maximum water depth can be measured using a portable water depth sounder gauge held just below the water surface. This information should be recorded to inform satellite cal/val in relatively shallow (including tidal) areas. The maximum water depth can be used to guide the in-water instrument deployment, and can also help to roughly check the problem of sensor depth of in-water measurements (a marked rope with depth can also
be helpful). For example, if sensor depth larger than the maximum water depth, which means there may be a problem of the depth sensor of the in-water instrument.

6.5.4 Wind speed
Wind speed and wind direction can be measured using an anemometer. Wind speed can be used to estimate surface reflectance factor in radiometric measurements and to explain sediment suspension in shallow areas.

6.5.5 Aerosol optical thickness
Aerosol optical thickness (AOT) is a critical parameter for atmospheric correction algorithm development, used to validate the satellite-derived aerosol product, or to determine the suitability of aerosol models used in atmospheric correction. AOT is important for studies in transitional waters as the aerosol is complex compared to open oceans. AOT can be measured in situ by using a sun photometer such as CIMEL or PREDE, or a portable sun photometer such as MicroTops or SIMBAD. The lens of the instrument should be kept clean. The instrument is then pointed at the sun and kept stable throughout the measurement. When a portable sun photometer is used to measure AOT manually on the boat, the major uncertainty is the radiometer off the sun under rough conditions such as strong wind and wave. In this case, repeat measurements are recommended assuming that smaller AOT values are more accurate while higher AOT values are indicative of sampling errors, either by obstruction of the sun beam or by missing the sun spot entirely (Frouin et al., 2003).

6.6 Additional information

6.6.1 Location
The geographic location of each sample is essential information to match up in situ data with satellite images, and should thus be included in the metadata. The location of sampling site is generally measured using a GNSS receiver which provides records of UTC time, latitude, longitude, altitude and satellite fix quality. It is recommended to include the latitude and longitude using the World Geodetic System (WGS84) coordinate system. In satellite product validation activities, it is recommended to use with high accuracy (quality, e.g., fix quality 3) coordinates to avoid mismatching between in situ data and satellite product, especially where steep spatial gradient occurs.

6.6.2 Date and time
Date and time are important to record to determine the offset between satellite acquisition time and in situ data collection time, and thus to get more representative matchup selection for satellite product validation within an acceptable time window. To make it easier for processing data collected from different time zone across all the study sites, it is recommended to record the time as UTC.

6.6.3 Cloud and surface water condition
Recording cloud coverage helps to understand the illumination conditions during radiometric measurements, and to assess the quality of radiometric data or remove questionable results. Cloud condition (sunny, cloudy or overcast) can be expressed as the percent of cloud coverage in the sky, it is also recommended to record the cloud condition by wide angle vertical photographs or videos.
In addition, it is recommended to take some photos or video records of the water at each sampling site, this is helpful during data processing and screening when questionable data appear, such as surfacing phytoplankton blooms, visibility of water bottom, or other possible contamination sources.
### Table 1. Recommendations for sampling in transitional water bodies

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>Method/instrument</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biogeochemical data</strong></td>
<td>Chlorophyll a, Chl-a</td>
<td>HPLC, spectrophotometry</td>
<td>Van Heukelem and Thomas, 2001; Hooker et al., 2009; Jeffrey and Humphrey, 1975</td>
</tr>
<tr>
<td></td>
<td>Phycocyanin concentration, PC</td>
<td>Spectrophotometry</td>
<td>Bennett and Bogorad, 1973; Sarada et al., 1999; Horváth et al., 2013</td>
</tr>
<tr>
<td></td>
<td>Total suspended matter, TSM</td>
<td>Gravimetry</td>
<td>Strickland and Parsons, 1972; Röttgers et al., 2014</td>
</tr>
<tr>
<td><strong>Apparent optical properties (AOP)</strong></td>
<td>Remote sensing reflectance, Rs (include $L_s$, $L_{sky}$ and $E_d$)</td>
<td>Above-water approach</td>
<td>Mobley, 1999; Zibordi et al., 2019; Ruddick et al., 2006; Simis and Olsson, 2013</td>
</tr>
<tr>
<td></td>
<td>Diffuse attenuation coefficient, $K_d$</td>
<td>In-water approach</td>
<td>Mueller et al., 2003b; Zibordi et al., 2019</td>
</tr>
<tr>
<td></td>
<td>Secchi disk depth, $Z_{SD}$</td>
<td>30 cm diameter white Secchi disk</td>
<td>Wernand, 2010; Pitarch, 2020</td>
</tr>
<tr>
<td><strong>Inherent optical properties (IOP)</strong></td>
<td>Total absorption coefficient, $a$</td>
<td>absorption meter</td>
<td>Twardowski et al., 2018</td>
</tr>
<tr>
<td></td>
<td>Absorption coefficient of phytoplankton pigments, $a_{ph}$</td>
<td>Filter pad method (IS mode)</td>
<td>Roesler et al., 2018</td>
</tr>
<tr>
<td></td>
<td>Absorption coefficient of non-algal particles, $a_{NAP}$</td>
<td>Filter pad method (IS mode)</td>
<td>Roesler et al., 2018</td>
</tr>
<tr>
<td></td>
<td>Coloured dissolved organic matter, CDOM</td>
<td>Spectrophotometry</td>
<td>Mannino et al., 2019</td>
</tr>
<tr>
<td></td>
<td>Backscattering coefficient, $b_b$</td>
<td>Backscattering meter</td>
<td>Twardowski et al., 2007; Sullivan et al., 2013; Doxaran et al., 2016</td>
</tr>
<tr>
<td><strong>Physical parameter</strong></td>
<td>Water temperature</td>
<td>CTD</td>
<td>Mueller, 2003a</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>CTD</td>
<td>Mueller, 2003a</td>
</tr>
<tr>
<td></td>
<td>Turbidity</td>
<td>Nephelometry method</td>
<td>APHA, 1998</td>
</tr>
<tr>
<td></td>
<td>Water depth</td>
<td>CTD, or water depth sounder gauge</td>
<td>Mueller, 2003a</td>
</tr>
<tr>
<td></td>
<td>Wind speed</td>
<td>Anemometer</td>
<td>Mueller, 2003a</td>
</tr>
<tr>
<td><strong>Additional data</strong></td>
<td>Aerosol optical thickness, AOT</td>
<td>Sun photometer</td>
<td>Frouin et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Location (WGS84)</td>
<td>GNSS</td>
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</tr>
<tr>
<td></td>
<td>Date and time (UTC)</td>
<td>GNSS</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Cloud and surface water condition</td>
<td>Notes, photograph or video</td>
<td>Mueller, 2003a</td>
</tr>
</tbody>
</table>
7 Protocols Comparison

7.1 Comparison of methods

In this section, the proposed protocols for transitional waters are compared against the previous protocols from IOCCG and GloboLakes, as well as those protocols that CERTO partner organisations are currently using (Table 2). Generally, the IOCCG protocols mainly focus on IOP and AOP measurements and protocols used in GloboLakes focus on biogeochemical measurements. A main difference between the proposed protocols in this report and the IOCCG reports is the method for measuring CDOM.

Compared to the protocols that are currently in use in partner organisations, the methods for measuring TSM, \( R_{1s} \), \( Z_{SD} \), \( a \), \( b_b \), \( c \) and CDOM are the same across all organisations, except where different instruments are used. For example, backscattering meter is used to measure \( b_b \), but HydroScat-6 is used in Curonian Lagoon and Venice Lagoon, Wetlabs ECO-BB3 is used in Plymouth Sound. The method for measuring Chl-\( a \) is either HPLC or spectrophotometry in the various organisations, and in this report we allow the use of either of these methods according to instrument availability and best-practices. \( a_{ph} \) and \( a_{NAP} \) are all measured using filter pad method, but with IS mode, \( T \) mode or \( T-R \) mode in different organisations.

In summary, most of the bio-optical parameters are measured using the same methods across partner organisations and these conform to the methods proposed for transitional waters in this report.

However, there are some differences in sample (data) processing procedures or configurations even when the same method is used to measure the parameter, such as the procedure for drying filters to measure TSM, the \( \rho \) value for \( R_{1s} \) measurement, the wavelength coverage of radiometric data, the method for pigment extraction in absorption of non-algal particles measurement, and the wavelength chosen for null-point correction of CDOM measurement. These issues will be discussed in the next section of this chapter.
Table 2. Comparison between CERTO recommended protocols and other protocols (IOCCG, GloboLakes, partner organisations)

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>IOCCG</th>
<th>GloboLakes</th>
<th>Curonian Lagoon (CNR)</th>
<th>Venice Lagoon (CNR)</th>
<th>Razelm-Sniee Lagoon (GeoEcoMar)</th>
<th>Plymouth Sound (PML)</th>
<th>Tagus Estuary (UL)</th>
<th>Elbe Estuary (DG)</th>
<th>CERTO recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biogeochemical data</strong></td>
<td>Chl-a</td>
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<td>HPLC, spectrophotometry</td>
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<td>spectrophotometry</td>
<td>fluorimetry</td>
<td>HPLC, spectrophotometry</td>
<td>fluorimetry</td>
<td>HPLC, spectrophotometry</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>--</td>
<td>spectrophotometry</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>spectrophotometry</td>
</tr>
<tr>
<td></td>
<td>TSM</td>
<td>--</td>
<td>gravimetry</td>
<td>--</td>
<td>--</td>
<td>gravimetry</td>
<td>gravimetry</td>
<td>--</td>
<td>--</td>
<td>gravimetry</td>
</tr>
<tr>
<td><strong>Apparent optical properties (AOP)</strong></td>
<td>$K_{pr}$</td>
<td>in-water approach, above-water approach</td>
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<td>above-water approach</td>
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<td>above-water approach</td>
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<td>above-water approach</td>
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<tr>
<td></td>
<td>$K_{s}$</td>
<td>in-water approach</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>in-water approach (Satlantic)</td>
<td>in-water approach (TrOs)</td>
<td>--</td>
<td>--</td>
<td>in-water approach</td>
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<td></td>
<td>$Z_{SD}$</td>
<td>--</td>
<td>30 cm diameter white Secchi disk</td>
<td>30 cm diameter white Secchi disk</td>
<td>20 cm diameter white Secchi disk</td>
<td>white Secchi disk</td>
<td>30 cm diameter white Secchi disk</td>
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<td>30 cm diameter white Secchi disk</td>
<td></td>
</tr>
<tr>
<td><strong>Inherent optical properties (IOP)</strong></td>
<td>$a$</td>
<td>absorption meter, ICAM, PSICAM</td>
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<td>--</td>
<td>--</td>
<td>absorption meter (Wetlabs ac-9)</td>
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<td>--</td>
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<td>absorption meter</td>
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<tr>
<td></td>
<td>$a_{ph}$</td>
<td>filter pad (IS mode)</td>
<td>filter pad (T mode)</td>
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<td>filter pad (IS mode)</td>
<td>filter pad (T-R mode)</td>
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<td>filter pad method (IS mode)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>$a_{NAP}$</td>
<td>filter pad (IS mode)</td>
<td>filter pad (T mode)</td>
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<td>filter pad (IS mode)</td>
<td>filter pad (T-R mode)</td>
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<td>filter pad method (IS mode)</td>
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<td>CDOM</td>
<td>LWCC</td>
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<td>spectrophotometry</td>
</tr>
<tr>
<td></td>
<td>$b_{p}$</td>
<td>--</td>
<td>backscattering meter (HydroScat-6)</td>
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<td>backscattering meter (HydroScat-6)</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>backscattering meter</td>
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<tr>
<td></td>
<td>$c$</td>
<td>transmission meter (attenuation meter)</td>
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<td>--</td>
<td>attenuation meter (Wetlabs ac-9)</td>
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<td>--</td>
<td>--</td>
<td>attenuation meter</td>
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<tr>
<td><strong>Physical parameter</strong></td>
<td>Water temperature</td>
<td>CTD</td>
<td>CTD90</td>
<td>Seabird or Idronaut CTD</td>
<td>multiparameter kit</td>
<td>Seabird CTD</td>
<td>Multi parameter sonde</td>
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<td>--</td>
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<tr>
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<td>Salinity</td>
<td>CTD</td>
<td>CTD90</td>
<td>Seabird or Idronaut CTD</td>
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<td>Seabird CTD</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>Turbidity</td>
<td>--</td>
<td>--</td>
<td>Sea Point turbidimeter</td>
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<td>--</td>
<td>fluorimeter</td>
<td>Nephelometry method</td>
<td></td>
<td></td>
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<td>Water depth</td>
<td>CTD</td>
<td>echo sounder from the boat</td>
<td>echo sounder from the boat</td>
<td>boat sonar</td>
<td>Seabird CTD</td>
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<td>--</td>
<td>--</td>
<td>CTD, or water depth sounder gauge</td>
</tr>
<tr>
<td></td>
<td>Wind speed</td>
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<td>--</td>
<td>weather station</td>
<td>weather station</td>
<td>Airmar unit</td>
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<td>--</td>
<td>--</td>
<td>anemometer</td>
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<tr>
<td></td>
<td>AOT</td>
<td>--</td>
<td>sun photometer (Microtops)</td>
<td>--</td>
<td>--</td>
<td>sun photometer (Microtops)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>sun photometer</td>
</tr>
</tbody>
</table>
7.2 Comparison of parameters and settings

7.2.1 Temperature and drying time for TSM measurement

Table 3 lists the settings of temperature and time for drying and combusting filters to measure TSM and ISM across partner organisations as well as selected literature. The most widely adopted setting for measuring TSM is drying the filter at 60°C for 24 hours. The most widely adopted setting for measuring ISM is combusting filters at 450°C for 4 hours. Thus, the settings of drying filters at 60°C for 24 hours is recommended to measure TSM, and combusting filter at 450°C for 4 hours is recommended to measure ISM in this deliverable.

Table 3. Comparison of temperature and time settings for measuring TSM and ISM

<table>
<thead>
<tr>
<th>Reference</th>
<th>TSM</th>
<th>ISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>protocols used in GloboLakes</td>
<td>65 °C, until a constant weight occurs</td>
<td>450 °C, 5 h</td>
</tr>
<tr>
<td>Curonia Lagoon (CNR)</td>
<td>60 °C, 24 h</td>
<td>550 °C, 4 h</td>
</tr>
<tr>
<td>Venice Lagoon (CNR)</td>
<td>75 °C, 24 h</td>
<td>450 °C, 4 h</td>
</tr>
<tr>
<td>Razelm-Sinoe Lagoon (GeoEcoMar)</td>
<td>60 °C, 24 h</td>
<td>480 °C, 4 h</td>
</tr>
<tr>
<td>Tagus Estuary (UL)</td>
<td>60 °C, 24 h</td>
<td>450 °C, 4 h</td>
</tr>
<tr>
<td>Groom et al., 2009</td>
<td>75 °C, 24 h</td>
<td>450 °C, 4 h</td>
</tr>
<tr>
<td>Ondrusek et al., 2012</td>
<td>60 °C, 12 h</td>
<td>--</td>
</tr>
<tr>
<td>Knaeps et al., 2018</td>
<td>60 °C, 24 h</td>
<td>--</td>
</tr>
<tr>
<td>Dorji et al., 2016</td>
<td>60 °C, 24 h (repeated until change &lt;0.001 mg/L)</td>
<td>--</td>
</tr>
<tr>
<td>Yu et al., 2019</td>
<td>60 °C, 24 h</td>
<td>--</td>
</tr>
<tr>
<td>Oyama et al., 2009</td>
<td>110 °C, 2 h</td>
<td>500 °C, 4 h</td>
</tr>
</tbody>
</table>

7.2.2 The value of $\rho$ for above-water $R_s$ measurement

Table 4 lists the value of $\rho$ (or procedure to determine the value of $\rho$) used in partner organisations and some selected literature for calculating the remote-sensing reflectance measured though the above-water approach. Generally, the determination of $\rho$ value can be categorized into three methods:

- using a fixed $\rho$ value for all cases, and the used $\rho$ valued ranges from 0.021 to 0.028. The value of 0.028 is recommended by Mobley (1999) for the cases that radiometric data are measured with viewing geometry of ($\theta=40^\circ$, $\varphi=135^\circ$) and wind speed < 5m/s, or the sky is heavily overcast at all wind speeds;
estimating $\rho$ value based on wind speed and sky condition. These estimations are generally also based on the simulations in Mobley (1999), i.e. when the sky is not completely overcast, $\rho$ value is estimated according to wind speed. When the sky is completely overcast, a fixed $\rho$ value is then used (0.0256 or 0.028);

- optimizing $\rho$ value based on the method from Simis and Olsson (2013) or Groetsch et al. (2017).

In this report, the $\rho$ value is recommended to be estimated from wind speed or determined based on Simis and Olsson (2013). It is also recommended to report the $\rho$ value, along with the $L_i$, $L_{\text{sky}}$, $E_d$ and $R_{\text{rs}}$ data.

Table 4. Comparison of $\rho$ used in above-water remote-sensing reflectance measurement

<table>
<thead>
<tr>
<th>Reference</th>
<th>$\rho$ value</th>
<th>Study water</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNR</td>
<td>0.028</td>
<td>Curonia Lagoon</td>
</tr>
<tr>
<td>CNR</td>
<td>0.028</td>
<td>Venice Lagoon</td>
</tr>
<tr>
<td>Mueller et al., 2003b</td>
<td>$\rho= f(w)$ for clear sky, 0.028 for completely overcast sky</td>
<td>Ocean, coastal</td>
</tr>
<tr>
<td>Zibordi et al., 2019</td>
<td>Reply on Mobley (1999)</td>
<td>Ocean, coastal</td>
</tr>
<tr>
<td>Ondrusek et al., 2012</td>
<td>0.021</td>
<td>Chesapeake bay</td>
</tr>
<tr>
<td>Knaeps et al., 2018</td>
<td>0.0256</td>
<td>Scheldt Estuary (Belgium), Gironde Estuary (France), Río de La Plata Estuary (Argentina)</td>
</tr>
<tr>
<td>Ruddick et al., 2006</td>
<td>$\rho= f(w)$ for clear sky, 0.0256 for completely overcast sky</td>
<td>southern North Sea</td>
</tr>
<tr>
<td>Dorji et al., 2016</td>
<td>0.022</td>
<td>Australian coastal water</td>
</tr>
<tr>
<td>Yu et al., 2019</td>
<td>$\rho= f(w)$ for clear sky, 0.0256 for completely overcast sky</td>
<td>Changjiang Estuary</td>
</tr>
<tr>
<td>Simis and Olsson, 2013</td>
<td>variable, determined through spectral optimization</td>
<td>Baltic Sea, Plymouth sound (PML)</td>
</tr>
<tr>
<td>Groetsch et al., 2017</td>
<td>variable, determined by modelling sky reflectance against the Gregg &amp; Carder 1990 model</td>
<td>Baltic Sea, Lake Paterswoldsemeer</td>
</tr>
<tr>
<td>Jiang et al., 2019;</td>
<td>0.028</td>
<td>Japanese lakes</td>
</tr>
</tbody>
</table>

Note: $\rho= f(w)$ means $\rho$ is estimated from wind speed.

### 7.2.3 Spectral range of $R_{\text{rs}}$ measurements

Table 5 presents the comparison of spectral range of $R_{\text{rs}}$ measured by partner organisations and some research papers in transitional waters around the world. Most of the $R_{\text{rs}}$ measurements cover wavelengths up to 900 nm. In practical, the wavelength range depends
on the hyperspectral radiometer, its calibration and the requirements stemming from research objectives.

In this report, it is recommended to use a hyperspectrophotometer which covers the wavelengths of 350-950 nm (if possible) for radiometric measurement in transitional waters. For research on turbidity or TSM concentration in extremely turbid waters (e.g., TSM > 100 g/m$^3$), wavelength covering longer than 1000 nm may be needed.

### Table 5. Comparison of spectral coverage of remote-sensing reflectance measured in transitional waters

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Wavelength range</th>
<th>Reference</th>
<th>Study area</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD at CNR</td>
<td>until 2500 nm</td>
<td>--</td>
<td>Curonian Lagoon</td>
</tr>
<tr>
<td>WISP-3 at CNR</td>
<td>400 – 800 nm</td>
<td>--</td>
<td>Venice Lagoon</td>
</tr>
<tr>
<td>HyperSAS at PML</td>
<td>until 897.7 nm</td>
<td>--</td>
<td>Plymouth Sound</td>
</tr>
<tr>
<td>TriOS at HZG</td>
<td>until 950 nm</td>
<td>--</td>
<td>Elbe Estuary</td>
</tr>
<tr>
<td>ASD (literature)</td>
<td>350 – 2500 nm</td>
<td>Knaeps et al., 2018</td>
<td>Scheldt Estuary (Belgium), Gironde Estuary (France), Río de La Plata Estuary (Argentina)</td>
</tr>
<tr>
<td>TriOS on So-Rad (PML)</td>
<td>350 – 950 nm</td>
<td>Simis and Olsson, 2013</td>
<td>Western Channel, Tagus, Danube Delta, (previously Baltic Sea, Lake Balaton)</td>
</tr>
<tr>
<td>TriOS (literature)</td>
<td>350 – 950 nm</td>
<td>Ruddick et al., 2006</td>
<td>southern North Sea</td>
</tr>
<tr>
<td>HyperOCR (literature)</td>
<td>350 – 900 nm</td>
<td>Ondrusek et al., 2012</td>
<td>Chesapeake bay</td>
</tr>
<tr>
<td>HyperSAS (literature)</td>
<td>350 – 858 nm</td>
<td>Yu et al., 2019</td>
<td>Changjiang Estuary</td>
</tr>
<tr>
<td>DALEC (literature)</td>
<td>380 – 900 nm</td>
<td>Dorji et al., 2016</td>
<td>Australian coastal water</td>
</tr>
</tbody>
</table>

### 7.2.4 Pigment extraction in absorption measurements

For measuring the absorption coefficient of non-algal particles ($\bar{a}_{\text{NAP}}$, also referred as non-pigmented particles), the phytoplankton pigments on the filter should be removed. Table 6 lists methods used for extracting phytoplankton pigments in $\bar{a}_{\text{NAP}}$ measurement. Generally, bleaching using NaOCl or extracting using methanol is used for removing the phytoplankton pigments in $a_{\text{NAP}}$ measurement. As discussed in Pegau et al. (2003), Roesler et al. (2018) and Stramski et al. (2019):

- **Bleaching method using NaOCl** is preferred for cyanobacteria or chlorophytes dominant in inland waters because of the presence of phycobilins and of extraction resistant algae, but the use of a strong bleaching agent can introduce unwanted artifacts especially in the short visible wavelength and in the UV.
- **Methanol extraction** remains the most widely used method, especially for coastal waters, as there is no particular advantage to using the bleaching method. Using methanol can ensure the results to be comparable with previously published results. However, there are possibilities that pigments are not completely extracted using methanol and thus 675nm peak occurs in $a_{\text{NAP}}$.

In addition to NaOCl and methanol, acetone and (hot) ethanol are another two widely used solvents to extract phytoplankton pigments in Chl-$a$ measurement (Jeffrey & Humphrey, 1975; ISO-10260, 1992). 90% acetone, combined NaOCl and hot ethanol are also used in $\bar{a}_{\text{NAP}}$ measurement (table 6).

In this report, NaOCl bleaching method is recommended to remove phytoplankton pigments when measuring $\bar{a}_{\text{NAP}}$ in transitional waters to avoid the incomplete extraction of pigments using methanol method.
Table 6. Comparison of pigment extraction method in absorption of non-algal particles measurement

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curonian Lagoon (CNR)</td>
<td>90% Acetone</td>
</tr>
<tr>
<td>Tagus Estuary (UL)</td>
<td>NaOCl</td>
</tr>
<tr>
<td>Pegau et al., 2003</td>
<td>NaOCl or Methanol</td>
</tr>
<tr>
<td>Roesler et al., 2018</td>
<td>Methanol</td>
</tr>
<tr>
<td>Simis et al., 2017</td>
<td>NaOCl, followed by hot ethanol if necessary</td>
</tr>
</tbody>
</table>

7.2.5 Waveband for CDOM null-point correction

Null-point correction is important for CDOM measurements because of scattering errors included in the spectrophotometric method. Table 7 presents the comparison of wavebands used for null-point correction in CDOM measurement at partner organisations as well as in the literature. As discussed in Mitchell et al., (2002), the wavelength used for null-point correction depends on the CDOM concentration, shorter wavebands may be used for low CDOM waters (e.g., oceanic samples), while longer wavebands should be used in CDOM-rich water bodies (e.g. estuaries).

Because CDOM concentration can vary widely across transitional waters bodies, it is recommended to use the average of 700-800 nm for the null-point correction for CDOM measured in transitional waters. In addition, CDOM spectrum can be fitted as an exponential function with an additional background parameter to address any baseline shift or attenuation not due to CDOM using the method in Stedmon et al. (2000).

Table 7. Comparison of wavelength used for null-point correction for CDOM measurement

<table>
<thead>
<tr>
<th>Reference</th>
<th>Wavelength used for null-point correction</th>
<th>Study water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell et al., 2002</td>
<td>590 – 600 nm</td>
<td>Ocean</td>
</tr>
<tr>
<td>Mannino et al., 2019</td>
<td>650 – 680 nm</td>
<td>Ocean, coastal waters</td>
</tr>
<tr>
<td>NR</td>
<td>750 nm</td>
<td>Curonian Lagoon</td>
</tr>
<tr>
<td>UL</td>
<td>700 – 800 nm</td>
<td>Tagus Estuary</td>
</tr>
<tr>
<td>Vodacek et al., 1997</td>
<td>700 – 800 nm</td>
<td>Middle Atlantic Bight</td>
</tr>
<tr>
<td>Miller et al., 2002</td>
<td>700 nm</td>
<td>Eight locations in inland, coastal and oceanic waters</td>
</tr>
<tr>
<td>Chen et al., 2004</td>
<td>700 – 800 nm</td>
<td>Mississippi River plume</td>
</tr>
<tr>
<td>Kowalczuk et al., 2006</td>
<td>650 – 700 nm</td>
<td>Baltic Sea</td>
</tr>
<tr>
<td>Andrew et al., 2013</td>
<td>650 – 700 nm</td>
<td>Equatorial Atlantic Ocean</td>
</tr>
<tr>
<td>Mannino et al., 2014</td>
<td>695 – 700 nm</td>
<td>Northeastern US coast</td>
</tr>
</tbody>
</table>
8 Conclusions

Existing protocols for bio-optical measurements either focus on the open ocean or fresh inland waters. Therefore, it is warranted to revisit these protocols for transitional waters by reviewing the suitability of the existing protocols. By doing so, it is hoped to harmonise in situ data quality, and make in situ data more comparable.

This report reviewed the IOCCG Ocean Optics and Biogeochemistry Protocols for oceanic waters, which include methods for measuring absorption, beam attenuation, remote-sensing reflectance, coloured dissolved organic matter and particulate organic carbon, and best-practices for in-line systems. From the available protocols, we selected filter pad methodology for the absorption coefficient, and the above-water approach for remote-sensing reflectance in transitional waters. Protocols for inland waters (as adopted in GloboLakes) were generally considered applicable to determine biogeochemical variables in transitional waters, whilst taking care to include steps to correct for salinity effects.

Further key sampling requirements for transitional water bodies were identified, which include choosing appropriate sampling locations to cover bio-optical gradients and to characterise adjacency effects in satellite product validation, determining appropriate volumes for water sample concentration, adopting the same method in different study sites to standardise all bio-optical measurements, correcting for temperature and salinity effect on water absorption, and ensuring spectral coverage of radiometric measurements include the NIR range to support algorithm development for TSM and turbidity and to validate atmospheric correction. Field measurements were classified into five categories: biogeochemical data, apparent optical properties, inherent optical properties, physical parameters and additional support information. Recommendations were made for each of parameter group.

CERTO project offers a unique opportunity to review the existing protocols, identify the key needs for field sampling and standardise the methods for bio-optical measurements in transitional water bodies. The methods for measuring bio-optical properties of waters continue to be developed, and we will continue to explore and review the best ways for collecting high quality in situ data in transitional water bodies.
9 References


A.R. Neeley and and A. Mannino, IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 1.0, IOCCG, Dartmouth, NS, Canada.


