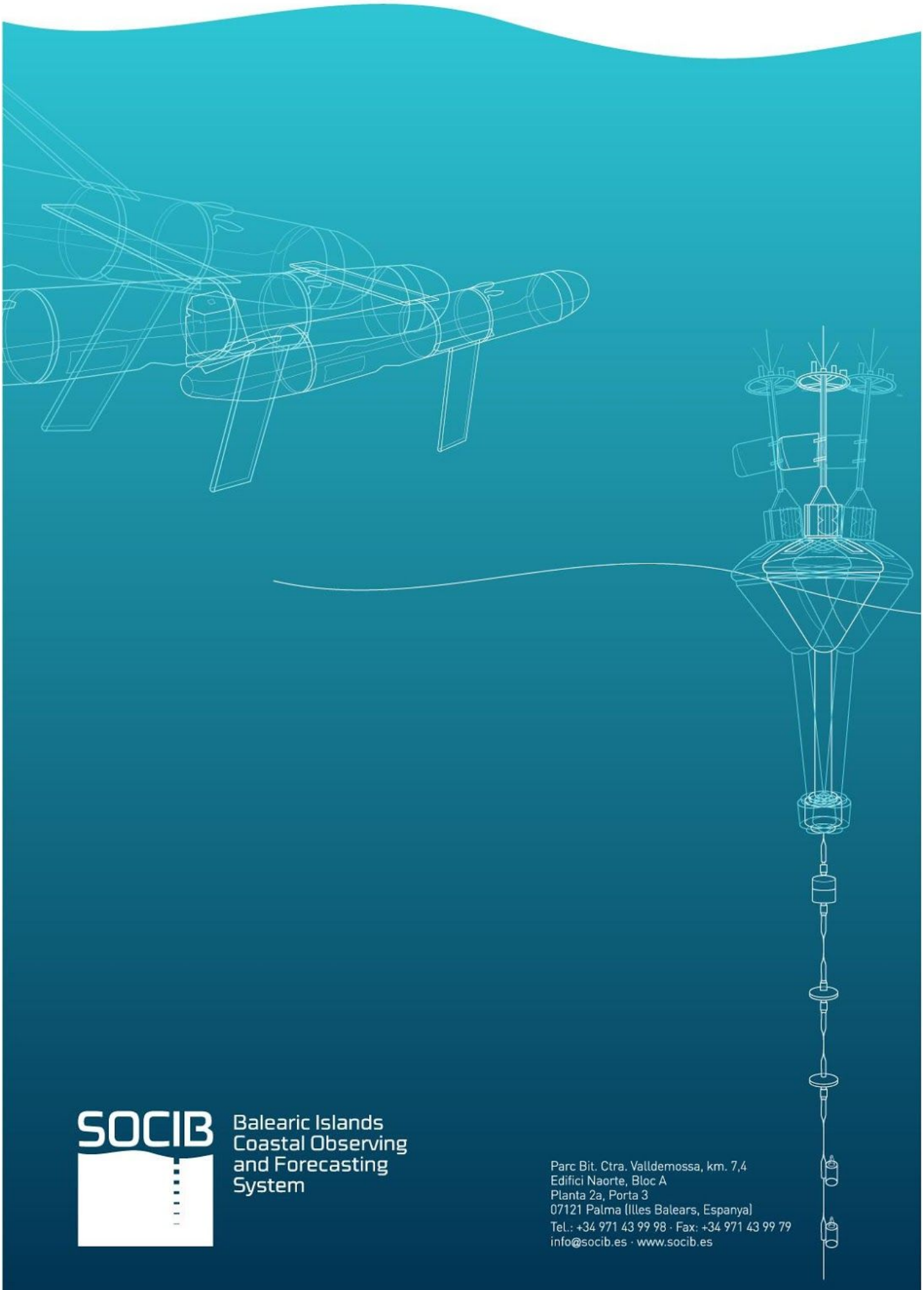

SOCIB

Balearic Islands
Coastal Observing
and Forecasting
System

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FLUROMETRIC DETERMINATION OF TOTAL CLOROPHYLL *a* (chl *a*) CONCENTRATION

Determination of total chl *a*: 24 h 90 % acetone extraction without acidification.

The samples have been previously obtained from R/V SOCIB cruises and SOCIB fixed stations following the SOCIB established protocols (see monthly and R-V water sampling documents).

Material

Glass amber tubes and caps.
90 % acetone with a dosing device.
Single fluorometer vials.
Graduated cylinder.
Glass beaker.
Trilogy Laboratory Fluorometer (Turner Designs).

Laboratory analysis procedure

Extraction

1. Check the volume of acetone 90 % dispensed by the dosing device (15 ml, use a graduate cylinder for this). Discard the first dose, it is always under the required volume.
2. Dispense 15 ml of acetone 90 % to each amber tube and place the cap firmly (acetone is a highly volatile compound). Write down the volume of the extraction for each sample (15 ml, V_e).
3. Take the filters out of their storage in the freezer and when they are thawed, introduce each filter into an amber tube using tweezers. The filter has to be completely immersed in the acetone.
4. Write down in the logbook which tube (numbers are located in its cap) corresponds to each sample.
5. Keep in the dark the tubes containing the filters and store them at cold temperature (-4ºC) for a period of 24h.

Extracted chl *a* measurements

1. After 24 h, shake the tubes gently, making sure the filter is still completely immersed in the acetone - always remember to keep the tubes in the dark.
2. Keep the samples at room temperature for 1h before the fluorometric determination.
3. Turn on the fluorometer 30 minutes before reading the samples.

Blanks

1. Fill a vial with 90 % acetone using a pipette. Wipe the vial with a tissue.
2. Introduce the vial into the fluorometer and read the signal by selecting: Chl-NA, ok, measure fluorescence raw.
3. Use the software supplied by Turner (Trilogy) to read the determination and store the data. If the software is not used, note the data (Relative Fluorescence Units, RFU) displayed in the screen of the fluorometer.

This reading will be your first blank reading; you should do at least 3 blanks.

In situ water samples

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1. After the blanks, start with the chl *a* determination of the first sample to analyze, pipetting the extract from the amber tube into a vial.
2. Wipe the vial with a tissue and proceed with reading the sample (as described for the blanks, measure fluorescence raw).
3. If you are not using the software supplied by Turner, please write in the logbook the readings displayed (R_b, RFU).
4. Use part of the extracted volume of the sample to rinse the vial 3 times before reading. Do this with each sample.

Precautions

Use a pencil to take notes and write down the data of the analysis if necessary (acetone would erase ink).
Work in the dark as much as possible.

After the analysis

Rinse (4 to 5 times) the amber tubes with distilled water and once with Milli-Q.
NEVER use acids to clean the chl *a* tubes.
Clean the single fluorometer vials with acetone 90 % and leave them upside down to dry.

Calculations and expression of the results

Generate an excel file with the following data: filtrated volume, volume used for extraction (volume of acetone), blank readings and sample readings.

The formula used for the final chl *a* concentration of the *in situ* water samples collected is:

$$\text{Chl } a \text{ (mg m}^{-3}) = F*(R_b-B)*V_e/V_m$$

F= fluorometer factor.
R_b= fluorescence reading.
B= blank readings using acetone 90%.
V_e= acetone volume used for the extraction (15 ml).
V_m= volume filtrated in liters (1l).

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