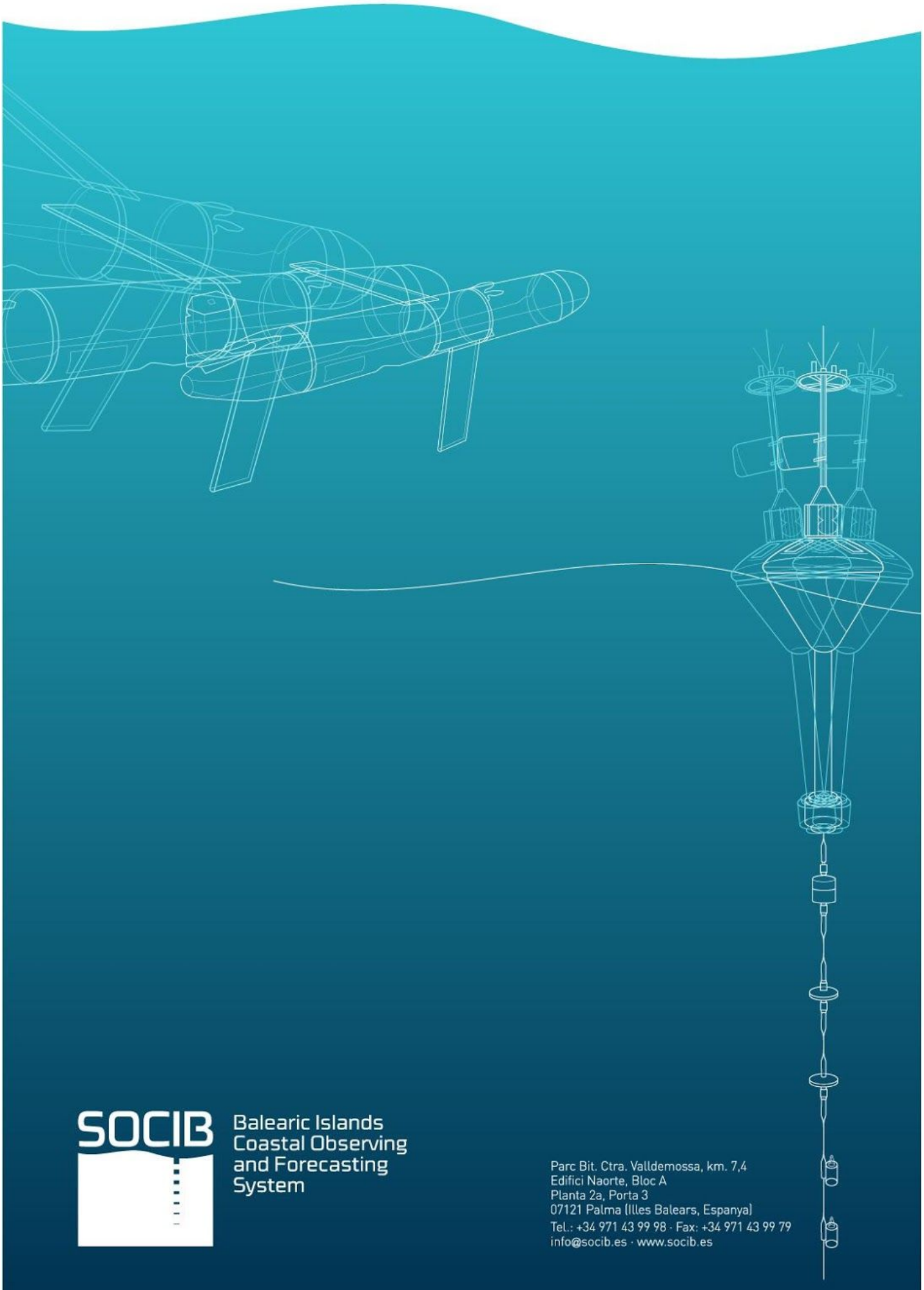

SOCIB

Balearic Islands
Coastal Observing
and Forecasting
System

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WATER SAMPLING PROTOCOL

(CANALES & OTHER R/V SOCIB CRUISES)

This protocol has been used for the Canales April**, July**, November, December, SHE_BEX** and ALMO-FRONT 2015 cruises.

SAMPLING ORDER

Oxygen, nutrients, pigments: high-performance liquid chromatography (HPLC) and total chl *a* (fluorometry), phytoplankton community (lugol), flow cytometry* (glutaraldehyde) and salinity.

SAMPLING DEPTHS

Sample the detailed depths at each station (see cruise plans and logbooks for detailed information).
All the parameters for a specific depth should be sampled from the same Niskin bottle (please, check the number of the bottle).
In the case of 2 bottles closed at the same depth, please collect the samples from the smallest Niskin number (e.g. 5 & 6, sample from the 5th bottle)- if is not possible to proceed this way, you should write the Niskin bottle number sampled in the logbook. This is very important for later comparisons between *in situ* water samples and CTD sensors.

OXYGEN (Winkler)

Water sampling for dissolved oxygen analyses from the Niskin bottle should be done as quickly as possible after the water sample reaches the surface, and before any other samples have been drawn. This is necessary to minimize the exchange of oxygen with the headspace in the Niskin, which typically results in contamination by atmospheric oxygen. Sample first from the deepest Niskin bottle collected and proceed towards the shallowest depth collected from-

Material
Gloves.
Individually numbered sampling glass flasks and a stopper-matched pair.
Tygon tubing 30-50 cm length.
Reagents with dispensers: reagent 1 (R_1, $MnCl_2$) and reagent 2 (R_2, NaI/NaOH).
Digital thermometer.
Oxygen logbook.

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Sampling
Before the oxygen sample is drawn, open the spigot on the sampling bottle while keeping the breather valve closed. If water leaks from the bottle it is likely the Niskin has been contaminated with water from shallower depths, which should be noted on the logbook. If the **Niskin doesn't close note this in the logbook and do not collect this sample.**

Confirm that the flask and stopper are a matched pair.

Take one to two samples (replicates for quality control, QC) from each Niskin and note the Niskin and flask numbers.

Connect the tygon tubing to the Niskin bottle spout. Raise the end of the tube before opening the spout to prevent the trapping of bubbles in the tube. Rinse the sample bottle twice and fill the sample flask smoothly by inserting the drawing tube all the way to the bottom of the flask. With the bottle starting on a roughly 45º angle and as the fill progresses, move the flask to the upright position. While sampling, allow the flask to overflow 2-3 times its volume with the sampling water.

While the flask is filling and overflowing insert the digital thermometer inside the sampling flask and note the sampling temperature.

When full, withdraw slowly the tube from the bottle and add immediately 1 ml of R_1 and 1 ml of R_2 by submerging the tip of the dosing device into the sampled flask.

Place the stopper carefully ensuring that no bubbles are trapped inside the flask. Shake the sample vigorously for several seconds. Re-shake after roughly 20 minutes when the precipitate has settled to the bottom of the flask.

Sample bottles will be stored upright in a cool, dark location and the necks water sealed with distilled water. These samples are analyzed after a period of at least 6 to 8 h but within 48 h (the samples are stable at this stage).
Note: all 2015 cruises' samples have been analyzed after 24 h with the exception of the last day of the ALMO-FRONT cruise (8-12 h).

NUTRIENTS
Water samples for nutrients should be sampled with gloves and filtered (this is an important step for coastal waters or peak bloom conditions). The filtration is directly done with a swinnex filter holder fitted with a Whatman GF/F filter.

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Label the tubes as follows: sample number, field cruise name, month and year, station, depth and replicate number (e.g. 1 CANAL11_15S201_5_1).

Material
Gloves.
Swinnex filter holder with a Whatman GF/F filter (47mm Ø)
5 ml tubes.
Racks.

Sampling
Connect the swinnex filter holder through a tygon tube to the Niskin bottle spout.

Check for any leaking (if this happens, means the GF/F filter is not well fitted into the swinnex and it should be adjusted).

If the filter is clogged, change it. It is better to change the filter to avoid any fibers from broken filters going through the sample (you will notice this if the filtration goes too fast!).

Collect 3 samples (replicates) at each depth.

Rinse the tubes and caps 3 times with the water from the Niskin bottle.

Fill the tubes ¾ full and place the cap on firmly.

Store on board at -20ºC -as soon as possible- after collection.

PIGMENTS
Pigments are light and temperature sensitive therefore the samples should be collected in opaque bottles and protected from warming and direct light exposure. Be especially cautious exposing the water bottles to the direct sun and avoid leaving them on the ship-deck while sampling other parameters!
The water samples should be filtered as quickly as possible. If this is not possible, please keep them in the Niskin bottle or store the bottle samples in a cold and dark environment until filtration.

High-performance liquid chromatography analyses (RP-HPLC)
Material
Nitrile gloves
Swinnex filter holder with a 200 µm mesh fitted (only if there is a high presence of zooplankton).

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2 l opaque water bottles.
Whatman GF/F filters (47mm Ø)
Labeled aluminum foil wrappings as sample containers.
Dry shipper.

Sampling
Collect one water sample at each sampling depth.
Rinse the bottles 3 times with the water from the Niskin bottles.

Fill the bottle and bring the volume up to 2 l.

When possible, collect 2 replicates from the same Niskin bottle (as a QC) and take a sample at 500 m for measuring the "field offset" (see Knap et al. 1996).

Filtration
After sampling, filter the samples proceeding as is detailed below.

Total chl *a* determination (fluorometry)
Material
Gloves
1 l opaque water bottles.
Whatman GF/F filter (47mm Ø).
Plastic tubes containers.
-20ºC freezer.

Sampling
Collect one sample for each sampling depth.
When possible take two replicates from the same Niskin bottle (QC) and take a sample at 500 m for measuring the "field offset".
Rinse the bottles 3 times with the water from the Niskin.
Fill the bottle and bring the volume up to 1 l.
Please store samples waiting for filtration in a light protected & cold environment.

Filtration
After sampling proceed filtering the samples (see protocol below).

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PHYTOPLANKTON (for transmitted light microscopy)
Material
Gloves.
250 ml amber glass bottles with caps.
Plastic Pasteur pipettes.
Lugol.

Sampling
Rinse the bottle 3 times with the water from the Niskin.

Fill the bottle just below the base of the neck. Add 1.5 to 2 ml lugol with a plastic Pasteur pipette.

Gently shake few times up and down to help homogenize the stain through the sample.

SALINITY
Material
200 ml glass bottles.

Sampling
Rinse the bottles 3 times with the water from the Niskin and fill the bottle just below the base of the neck.

Note in the salinity logbook the bottle sampling number and Niskin.

* The flow cytometry sampling has not been done in any of the SOCIB cruises of 2015 (it requires analyst time and another dryshipper to store the samples onboard).

** During these cruises a 200 µm mesh was used for total chl *a* (fluorometric determination) and HPLC sampling.

General note: after each cruise an internal report and a logbook with all the biogeochemistry work done on board are generated.

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FILTRATION PROCEDURE

(both for chl *a* fluorometric and HPLC pigment determination, on board)

1. Place the (whatman GF/F) filter on the filtration system holder using the tweezers.
2. Adjust the filtration funnel into the filter support. Avoid moving the GF/F filter. Make sure the filter stays centered.
3. Shake and mix very gently the seawater bottle sample (in order to avoid cell sedimentation) and fill up the funnel on the filtration system.
4. Turn on the vacuum pump (make sure the pump stays always • 100 mm Hg in order to avoid any potential filter damage)
5. Turn on the switch underneath the funnel in the filtration system.
6. Filter 1 l sample for fluorometry and 2 l for HPLC. Shake very gently the water bottle before filling up the funnel each time (for a more homogeneous sample).
7. Once the filtration is done, turn off the switch underneath the funnel and turn off the pump.
8. Unscrew the funnel and take off the filter with the tweezers, avoid touching the filter inside. Always hold the filter with the tweezers from the outside part of the filter. If no pre-filtration system is used and copepods are present, they should be removed with tweezers (specially for HPLC analyses).
9. Fold the filter in a half and store it in the aluminum wrap (properly labeled for HPLC) and a plastic tube (for chl *a* fluorometric determination).
10. Write down in the logbook and the spreadsheet the water sample volume filtrated (usually 1 l for fluorimetry and 2 l for HPLC).
11. Store the filters in liquid nitrogen immediately (dryshipper, the quickest possible for HPLC) and in the freezer on board at -20ºC (for chl *a* fluorimetric determination).
12. Once all samples are filtered, empty the big plastic bottle and place the GF/F filters on the holder for the next stations.

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