Regional Workshop on Monitoring and Management Strategies for Benthic HABs

Sampling strategies, sampling methods, cell collection and counting

RODOLPHE LEMEE
Sampling of benthic HABs
The main challenge: heterogeneity in the spatial distribution, at different scales

*Ostreopsis* abundances in Villefranche: from 1 to 4 times higher on 1 m² (sampling the same species of macroalgae)

*Ostreopsis* abundances (cells/gFW), in Monaco during summer 2016, in 5 stations. Distances between stations more or less 100 m

Gambierdiscus abundance in Guadeloupe and Martinique
Sampling of benthic HABs
The main challenge: heterogeneity in the spatial distribution, at different scales

Figure 4: A- Ostreopsis proliferation in 2011 between surface and 5 m depth; and B- Toxin concentration and profile of Ostreopsis cf. ovata between surface and 3 m depth. * at 5 m depth, cell number was too low for toxin quantification

Depth distribution of Ostreopsis cf. ovata (Brissard et al. 2014)
Sampling of benthic HABs
The main challenge: heterogeneity in the spatial distribution, at different scales

Depth distribution of benthic HABs in Guadeloupe, Rivière Sens. Wet (left) and dry (right) season (Boisnoir et al., 2018)
Sampling of benthic HABs

Moreover, abundances of benthic HABs depend on:

- Abiotic factors: T, S, nutrients, light, human impacts (?)
- Biotic factors: mainly biotic substrates preferences/associations

Abundances of *Ostreopsis* cf. *ovata* vs biological substrates
Sampling of benthic HABs

How to optimize the sampling strategies?

How to cope with spatial variability issues?

➔ Increase **the number of replicated** samplings

➔ Define **new sampling methods** that could integrate a part of the spatial variability
4 main sampling methods

- Planktonic sampling
  (when official thresholds are in cell/L: *Ostreopsis*)

- Vacuum collection
  Dino-Vac
  Syringe

- Substrate sampling
  Natural substrates
  Artificial substrates

- BEDI: BEthentic Dinoflagellate Integrator
Planktonic sampling

An example with *Ostreopsis* sp. in the Mediterranean (Jauzein et al., 2018)
- Macroalgae at 0.5m depth
- 0.2 m above the chosen macroalgae (0.3 m depth)
- Using 250 ml plastic bottle

**Experiment:** Comparing the efficiency of 3 types of sedimentation columns for the estimation of planktonic cells of *Ostreopsis*.

*Preparation of a planktonic sample fixed with lugol*

*Sedimentation columns used for the Utermöhl method*
**Experiment:**

“100%” represents the counting done with a 50 mL-column.

100 mL-column induces an underestimation of the counts of ~10% (cells stick to the wall of the column?)
Planktonic sampling: also a problem of heterogeneity…
Vaccuum collection

Figure 17. Dino-Vac, the suction-operated sampling device used to collect epiphytes from turf algal substrates (pictures from Parsons et al., 2010, with permission). Skirt of 50 µm mesh and 100 cm² opening/aspiration with a large syringe.

Berdalet et al, GEOHAB 2012
Vaccuum collection

Fig. 1. The modified syringe. On the left: the arrow indicates the cut section on the syringe tip. On the right: lock flange on the syringe cylinder and the stop on the piston rod (see the respective arrows).

Abbate et al, 2012, ICOD (Cryptogamie Algologie)
Substrate sampling

*Left:* Mike Parsons searches for algae to collect at Tennessee Reef in the Florida Keys. *Right:* Algae samples collected include *Acanthophora*, *Dictyota*, *Halimeda*, and *Penicillus*, among others. Algae are shaken and sieved to remove epiphytes and *Gambierdiscus* cells, if found, are isolated to establish cultures.

Photo from Florida Gulf Coast University
Substrate sampling

*Ostreopsis* sp., Mediterranean Sea:

- 0,5m depth
- Dominant macroalgae
- Avoid loss of microalgae
- 250 ml plastic bottle
- between 5 to 10 g of macroalgae
- preserved with 1 % of acidic lugol
Artificial substrate

Photo from Mireille Chinain, French Polynesia

Tester et al. 2014
Artificial substrate

With this set-up, artificial substrates are moving freely in the water, but perpendicularly to the current, as a kite. Artificial substrates are collecting cells, acting as a brush in the water current.

→ Spatial and temporal integration of the water column
Optimal porosity of the screen (for *Ostreopsis*): **from 1 to 3 mm**
Comparison of collection efficiency:

**Tester et al. (2014)**

12h of incubation ⇒ 5% *(Ostreopsis)*

**Jauzein et al. (2016)**

12h of incubation ⇒ 86% *(Ostreopsis)*

⇒ Optimal incubation time: 24 h (saturation of the art. substrate)
**Regional Workshop on Monitoring and Management Strategies for Benthic HABs/ MOW**

Musée océanographique - Monaco, 9th to 12th April

---

**BEDI – BEnthetic Dinoflagellates Integrator**

New **non destructive** method allowing the estimation of cells per surface unit of sea-floor (as much as the potential risk linked to resuspension of cells)

1) Positioning over the seabed

First BEDI device:
open plastic cylinder
(70 cm height, 25 cm diameter)

(Mangialajo et al., 2017)
BEDI – BEenthic Dinoflagellates Integrator

New non destructive method allowing the estimation of cells per surface unit of sea-floor (as much as the potential risk linked to resuspension of cells)

2) Resuspention of cells (mixing for few seconds)
BEDI – BEnthic Dinoflagellates Integrator

New **non destructive** method allowing the estimation of cells per surface unit of sea-floor (as much as the potential risk linked to resuspension of cells)

3) Sampling of seawater

Cells can be quantified as:

- Cells per unit of surface (cells mm$^{-2}$)
- Potentially Resuspended cells per unit of volume (PRcells l$^{-1}$)

(Mangialajo et al., 2017)
BEDI – BEenthic Dinoflagellates Integrator

Second BEDI device (submersible): close plastic cylinder (46 cm height, 35 cm diameter)

- Rubber cross opening for mixing
- Opening for sampling with 50 ml syringe
- Rubber seal
BEDI – Benthic Dinoflagellates Integrator

Third BEDI device
(standardized, submersible and potentially usable from a small boat)
BEDI – Benthic Dinoflagellates Integrator

**BEDI 2**

Not yet available! But very soon.
How to preserve Benthic HABs samples?

Planktonic samples fixed with 1% acidic lugol.
How to separate Benthic HABs from biotic or abiotic samples?

If no lugol in the sample, washing step is needed
Vigorous shaking (10s)
Depose in a sieve (500 µm)
Rinse 2 times with 100 ml FSW each time
Weight Fresh macroalgae
Weight Dry macroalgae
(in stove for 48h at 60/70 °C)
How to measure abundances of Benthic HABs?

Microscope counting (using or not tabulation with calcofluor staining)  
planktonic cells (Utermöhl method)  
benthic cells (Sedgewick Rafter Counting Cell)

Automatic counting: OPR, Optical Recognition Method

Molecular tools: FISH, PCR, qPCR, metabarcoding
Microscope counting, benthic cells

Fill Sedgewick Rafter Counting Cell (1ml) after the homogeneization of the sample
Automatic counting: OPtical Recognition method

Fig. 1 Overview of the processing steps of the OvMeter tool. After the collection of the algae and sample preparation, image acquisition is performed, followed by the presented pipeline of image processing, which culminates in an estimate of the algal concentration.

Vassalli et al., 2018
Automatic counting: OPtical Recognition method

**Fig. 2** Basis for localization scheme. a Optical image of an *O. ovata* cell (left) and a pictorial representation of the averaged pattern shape of a *O. ovata* cell (right). b Example of different values of scale, rotation, and eccentricity chosen as template to localize as many *O. ovata* individuals as possible (considering the inevitable presence of a percentage of false positive results that will be removed during the classification step). c Example of an optical image output from the localization step: the method is able to find almost all *O. ovata* individuals within the image.
Molecular tools

- CARD FISH: with epifluorescence microscope
  Qualitative/quantitative

- Single cell PCR: The Internal Transcribed Spacer (ITS) regions and the Large Subunit (LSU) of the nuclear ribosomal RNA (rRNA) gene complex are commonly used for benthic HABs
  Qualitative

- qPCR: an estimation of the amount of amplified DNA → estimation of number of cells
  Qualitative and quantitative (?)

- Metabarcoding on environmental samples
  Qualitative and quantitative (?)
Sampling Benthic HABs

- Many sampling methods, associated to many sampling strategies

- The method/strategy used will depend on your goal:
  Species isolation for cultures, single cell PCR, electronic microscope
  Species quantification for scientific purpose
  Species quantification for monitoring (threshold)
  Research of « hot spot »

→ Discussion during this meeting for processes optimisation
International intercalibration exercise on benthic HABs sampling methods

In each country/lab (voluntary)
Comparing «natural substrate sampling» with «artificial sampling»
Distribution of a «sampling set» to each partner

We all need to follow the same protocol
(the protocol will be explain on Wednesday morning)
References


Thank you for your attention

Rodolphe Lemée
lemee@obs-vlfr.fr

Regional Workshop on Monitoring and Management Strategies for Benthic HABs/ MOW
Musée océanographique - Monaco, 9th to 12th April