Sampling of Ostreopsis cf. ovata using artificial substrates: Optimization of methods for the monitoring of benthic harmful algal blooms

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Abstract

In the framework of monitoring of benthic harmful algal blooms (BHABs), the most commonly reported sampling strategy is based on the collection of macrophytes. However, this methodology has some inherent problems. A potential alternative method uses artificial substrates that collect resuspended benthic cells. The current study examines some improvements in this technique, through the use of fiberglass screens during a bloom of Ostreopsis cf. ovata. A novel set-up for the deployment of artificial substrates in the field was tested, using an easy clip-in system that helped restrain substrates perpendicular to the water flow. An experiment was run in order to compare the cell collection efficiency of different mesh sizes of fiberglass screens and results suggested an optimal porosity of 1–3 mm. The present study goes further on showing artificial substrates, such as fiberglass screens, as efficient tools for the monitoring and mitigation of BHABs.

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1. Introduction

Reports of benthic harmful algal blooms (BHABs) have increased during the last decade (Rhodes, 2011). Toxins produced during these bloom events can induce mass mortalities of aquatic organisms (e.g. Shears and Ross, 2009) and can represent risks for human health (Parsons et al., 2012; Ciminiello et al., 2014). Benthic environments where BHABs develop are various and complex. Substrates colonized by BHABs species include macroalgae, seagrasses, sand, rocks and corals (Faust, 1995, 2009). This habitat complexity, coupled with the patchy distribution of BHABs, creates real challenges for design and execution of sampling in the framework of BHAB monitoring (GEOHAB, 2012). As BHABs have not been extensively investigated yet, several sampling methodologies are currently used and tested. However, sampling precision and data analysis would benefit from a better standardization of sampling protocols.

The most commonly reported sampling strategy is based on the collection of macrophytes, mostly macroalgae. This technique has inherent problems, however, making comparison of BHAB cell abundances between sites and studies potentially problematic (Tester et al., 2014). These issues are partly due to variations in composition and distribution of macroalgal substrates in time and space and to choices for standardization of cell abundances (per weight or surface area). As an alternative, some researchers have used methods independent of macroagal substrates, including the use of suction devices, such as a vacuum apparatus (Parsons et al., 2010) or syringes (Abbate et al., 2012). Other studies reported the use of artificial substrates, such as nylon ropes (Faust, 2009) or pieces of fiberglass screens (Tester et al., 2014). Abundances of cells collected on artificial substrates can be easily standardized to a known surface area, allowing for meaningful comparisons among different sites and studies. The study of Tester et al. (2014) recently described an efficient set-up for incubation and collection of artificial substrates in order to collect harmful benthic dinoflagellates. Authors also clarified that this method may be optimized for the BHAB species of interest and the range of abundances encountered. The present study presents some important improvements in this technique, supporting the view that artificial substrates may be an efficient tool for the monitoring of BHABs, and in particular of Ostreopsis cf. ovata blooms.

2. Material and methods

2.1. Study site

Artificial substrates were tested in a small creek of the Villefranche Bay, French Mediterranean coast (43°41′34.83″ N and 7°18′31.66″ E). This site has experienced recurrent blooms of O. cf. ovata that periodically threaten recreational activities during the summer season. The area corresponds to a sheltered rocky coast, characterized by calm weather conditions during summer months.
2.2. Set-up of artificial substrates

Artificial substrates used in the present study consisted of rectangular pieces (2.5 cm × 27 cm) of plankton net or fiberglass screen that were fixed on a rigid frame, held on both sides by an easy clip-in system (SuperFrame® 21 cm × 21 cm). Each frame was attached to a weight and a small subsurface float, holding the devices at about 50 cm depth (Fig. 1). With this assembly, each frame (with or without pieces of substrate fixed on it) is naturally positioned perpendicularly to the water flow, acting as a kite in the current (Supplementary video).

2.3. Deployment and porosity test

Artificial substrates were deployed during a bloom of O. cf. ovata that lasted from mid-June to the end of August 2014. Three stations, a dozen meters apart, were sampled weekly in order to monitor this bloom event. The whole survey involved the use of classical methods based on collection of macrophytes and seawater (data not shown) and was carried out from early June to the end of September 2014. Artificial substrates were deployed in each station as soon as the O. cf. ovata bloom started; the deployment was done weekly, using new substrates each time, and was carried out over eight subsequent weeks.

A specific set-up was defined in order to test the influence of artificial substrate porosity on the efficiency of benthic dinoflagellate collections in the water column. For this purpose, up to four different types of substrates were positioned on the same frame with two potential combinations: either a set of mesh sizes of 50 μm, 200 μm, 450 μm and 1.15 mm or a set combining mesh sizes of 1.15 mm, 1.4 mm and 3.2 mm.

The first set of porosities, ranging from 50 μm to 1.15 mm, was tested over the 2 month-period, using two replicated frames per station: one frame was sampled after 24 h of incubation while the other was incubated for 48 h in the field. During five out of these eight weeks, an additional frame was deployed per station in order to test two additional porosities of 1.4 mm and 3.2 mm. These additional frames hold a replicated piece of 1.15 mm substrate and were deployed for 24 h only.

Fig. 1. Description of the set-up used for deployment of artificial substrates in the field. A picture showing the deployment of artificial substrates during an Ostreopsis bloom in Villefranche Bay (France) is given on the left side. This picture shows the incubation of four types of substrates, characterized by four different porosities (from 50 μm to 1.15 mm), that were hold on a unique rigid frame. It is schematized on the right side in order to detail each piece of the assembly.

2.4. Sampling procedure

Plastic bottles of 250 mL were used for sampling. A sample of surrounding seawater was taken before collection of artificial substrates, at 50 cm depth and about 30 cm apart from artificial substrate devices. Artificial substrates were collected using scissors. For retrieval, the substrate was cut on one side and carefully put into a plastic bottle filled with ambient seawater (Supplementary video). Then, the other side of the substrate was cut and the sampling bottle was capped under water. All samples were brought back to the laboratory for processing in less than an hour and fixed with acidic lugol’s solution (1% final concentration).

2.5. Treatment of samples

The method used for detachment of epiphytic cells from substrate was similar to the processing of a macroalgal sample: bottles containing a piece of artificial substrate were vigorously shaken during 10 s in order to dislodge O. cf. ovata cells, then the substrate was rinsed two times with 100 mL of FSW (Filtered Sea Water). Water collected after agitation and washing was mixed, the total volume was recorded and a 50 mL-subsample was taken and stored at 6 °C until counting of O. cf. ovata cells. Cells in subsamples were counted using a 1 μL Sedgwick Rafter Counting Cell examined with an Axiovert 40 CFL Zeiss microscope.

Abundances of O. cf. ovata cells located in the water that surrounded artificial substrates were estimated using the Utermöhl method, after settling 50 mL of seawater in sedimentation columns. These planktonic cell counts were performed using an inverted microscope (Axiovert 40 CFL Zeiss) and were used as blank values: they were subtracted from abundances of O. cf. ovata cells evaluated for artificial substrate samples. This allowed for precise enumerations of cells collected on each piece of net or screen during the bloom. Final data of cell abundances collected on artificial substrates were expressed as number of cells per cm² using two types of standardization: either (i) by the cutting surface area of substrate (2.5 cm × 16.5 cm) or (ii) by the surface area of filaments composing the net or screen, taking into account intersections as defined by Tester et al. (2014) and Weisstein (2013).

2.6. Estimation of detachment efficiency

The detachment of cells from artificial substrates was analyzed on 6 samples of fiberglass screens (porosity of 1.15 mm). For each piece of substrate, subsamples were taken during different steps of the isolation and collection of epiphytic cells: one subsample was taken after agitation of the substrate in ambient seawater and subsamples were taken at the end of the first and second washing steps (after the agitation of substrate in FSW for rinsing). Cells of O. cf. ovata were enumerated in each subsample. This allowed for an estimation of the contribution of a unique agitation and washing steps in the detachment of cells collected on pieces of artificial substrates.

2.7. Statistical analysis

Statistical analyses (t-tests and regressions) were performed using the Statgraphics Centurion software (Manugistics, Inc.). When data were not showing normal distributions and/or equal variances, they were log transformed before running statistical analyses in order to fulfill required assumptions. For the porosity test, estimations of relative cell abundances were expressed as percentages and analyzed by one-sample t-tests in order to define if these values were significantly different from their associated control; their distributions were compared to the fixed value of 100%. Comparison of cell collection efficiency after one or two days of incubation was done using paired t-tests.
3. Results

3.1. Efficiency of cell collection using artificial substrates

Every type of substrates tested in the present study collected efficiently O. cf. ovata cells. After 24 h of deployment in the field, the contribution of planktonic cells (blank values) in the samples was low, representing between 0.1% and 5.4% of the whole sample (0.3% ± 0.6% on average); for porosities of 450 μm and higher, this proportion dropped under 0.8% (0.1% ± 0.1% on average).

The quantity of cells collected on artificial substrates showed a strong dependence on mesh size. To observe this relationship in detail, densities of O. cf. ovata cells were expressed as percentages of the abundance measured on the 1.15 mm mesh that was held on the same frame during 24 h of incubation. Both ways of standardization, by the cutting surface area or by the surface area of net/screen filaments, lead to similar patterns of variations as a function of substrate porosity (Fig. 2A and B). At low porosities, the quantity of cells collected on 50 μm and 200 μm was significantly lower than abundances collected on 1.15 mm substrate (percentages were significantly lower than 100%, one-sample t-test, p < 0.01, n = 23). The number of collected cells per cm² showed a strong linear increase with mesh size in the range of porosity 50 μm–1.15 mm: linear regressions of the data (p < 0.05) were characterized by R² values of 0.96 and 0.98, for data standardized by the cutting area and the surface of filaments, respectively. No significant differences (one-sample t-test, n = 15) were observed between quantity of cells collected on substrates of 1.15 mm and 1.4 mm (p = 0.42 and p = 0.36 for data standardized by the cutting area (Fig. 2A) and the surface of filaments (Fig. 2B), respectively). The highest porosity of 3.2 mm showed a lower collection efficiency than the 1.15 mm mesh size when considering data per cm² of cutting surface (one-sample t-test, p < 0.01, n = 15), but this difference was not significant when referring to the surface of filaments (p = 0.10).

These differences in collection efficiency with porosity induced differences in precision of cell counts. For porosity of 450 μm or higher, the number of collected cells after 24 h allowed for counting more than 200 cells in a 1 mL Sedgewick Rafter Counting Cell for most of the samples; this proportion was similar to analysis of macroalgal samples (Fig. 3) and validated the efficiency of direct counting of samples, without a need for pre-concentration. For lower mesh sizes, counting using a Sedgewick Rafter Counting Cell was not well adapted to levels of abundances of collected cells. In particular for the 50 μm mesh size net, less than 75 cells were enumerated per mL for more of 80% of the samples collected during the bloom survey (Fig. 3); these measurements would have benefited from the use of sedimentation columns in order to concentrate the samples and get more precise estimations of cell concentrations.

3.2. Influence of incubation duration

Two incubation durations were tested on four types of substrates (mesh sizes of 50 μm, 200 μm, 450 μm and 1.15 mm). The difference between the quantity of cells collected after 24 h and 48 h of deployment in the field was not significant (paired t-tests, n = 20, p values ranging from 0.07 to 0.98) for any of the four types of artificial substrates tested.

3.3. Detachment of O. cf. ovata cells from artificial substrates

Analysis of subsamples taken during the separation step, between epiphytic cells and artificial substrates, showed that most of the O. cf. ovata cells were dislodged after 10 s of agitation of the sampling bottle. Compared to the total number of cells collected after agitation and wash- ing of the substrate, this agitation step allowed for collection of 90% ± 2% of the cells. Similarly, the first washing step dislodged 7% ± 3% of cells and the second washing step released 3% ± 2% of additional cells from the artificial substrate.

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monitoring, but studies reporting so are rare. Such materials include test tube brushes and plastic plates (Bomber and Aikman, 1989) or nylon ropes (Faust, 2009). The study of Tester et al. (2014) recently described a set-up for the use of fiberglass screens that allow for an easy and precise standardization of cell abundances to a known surface area. This study shows that deployment of pieces of fiberglass screens in the field can efficiently collect benthic toxic dinoflagellate cells, including Ostreopsis cells, after 24 h of incubation. Present results showed that O. cf. ovata cells are also easily retrieved from fiberglass screens: 90% ± 2% of the O. cf. ovata cells were detached from screens after 10 s of agitation. This loose attachment of O. cf. ovata cells represents an advantage for the processing of samples as only few cells will be lost during the separation of epiphytic cells from the substrate. However, for the monitoring of Ostreopsis blooms, this also shows that pieces of fiberglass screens have to be incubated and sampled with caution, to avoid any unexpected disturbance that could resuspend collected cells.

The present study defines substantial optimizations of the protocol defined by Tester et al. (2014), starting with the set-up of fiberglass screens in the field. When Tester and collaborators used large pieces of screens (rectangles of 10.2 cm × 15.2 cm, Fig. 2 in Tester et al. (2014)) fixed only on one side, we incubated small pieces of screens (2.5 cm × 16.5 cm) fixed on both sides. Our set-up reduces the risk of losing cells during incubation and sampling; such criteria are particularly interesting for the monitoring of BHAB cells loosely attached to their substrate, such as Ostreopsis cells. A fixation on both sides avoids any physical disturbance during the incubation that could be due to a twist or a rubbing of the substrate on the fixation devices. Furthermore, with the set-up described in the present study, artificial substrates are hold perpendicularly to the water flow and not moving freely with the current as described in Tester et al. (2014): this helps for optimization of cell collection efficiency. The use of small pieces of screens allowed for and easy sampling with 250 mL plastic bottles, without having to roll or touch pieces of substrate. Even if the surface of cut used in the present study was four times lower than the one used in the study of Tester et al. (2014), the number of collected cells was adapted to the monitoring of Ostreopsis blooms in coastal waters of the Mediterranean Sea: during our two months of survey, artificial substrates of high porosity (higher than 450 μm) allowed to reach a similar counting precision than the classical method based on macroalgal samples, using directly a 1 mL-counting chamber. However, it is important to note that larger screens may also be useful in environments characterized by low BHAB cell abundances or to follow specifically conditions at bloom initiation. Finally, the specific set-up described in the present study also helps for carrying out replicated measurements per station as up to four pieces of artificial substrates can easily be fixed on a unique rigid frame.

Experiments were run using this optimized set-up, to assess the influence of mesh size and incubation duration on collection efficiency. A strong variation of collection efficiency was noted with porosity of artificial substrates and allowed for the definition of an optimal mesh size that ranges from 1 to 3 mm. For substrates of porosity comprised between 50 μm and 1 mm, the collection efficiency increased linearly with mesh size. However, for all these substrates, an incubation of 24 h or 48 h led to a similar number of collected cells per cm². Recruitment and colonization of artificial substrates by benthic microalgal cells are indirect consequences of immigration, disturbance and emigration of cells from the surrounding seawater on a short timescale (hours or days), but also of cell division, death and grazing when substrates are incubated for weeks or months (Dalu et al., 2014 and references herein). With only one or two days of incubation tested in the present study, present results showed that abundances of O. cf. ovata cells collected on net and screens were proportional to ambient cell densities after 24 h, when an equilibrium between immigration and emigration rates was reached. This shows that incubation time was not limiting the collection efficiency for low porosity substrates during the experiment. The linear increase in collection efficiency noted with increasing porosity probably comes from physical constrains: low porosity substrates might represent an obstacle to the water flow that circumvents them more than goes through the mesh, limiting potentiality for microalgal cell collection.

The use of artificial substrates for BHAB monitoring shows various advantages in comparison with traditional macroalgae collection (Tester et al., 2014). At the sampling step and thanks to its independence from macroalgae, this method (i) reduces difficulties associated with patchy distribution of BHAB cells on macroalgal substrates, (ii) eliminates issues due to variations in macrophytes distribution and the preference of microalgae cells for specific macroalgal substrates and (iii) avoids destruction of the habitat due to macrophyte collection. Furthermore, if the treatment of samples from artificial substrates is similar to the treatment of macroalgal samples, it allows for an easy and more accurate standardization of BHAB cell abundances per unit of surface area (cell·cm⁻²), allowing for efficient comparison of data sets between studies. Concerning potential disadvantages, the main one is associated with field work constraints because each sampling site must be visited twice, once to deploy the substrates and again to retrieve them (Tester et al., 2014). Some may also have reservations over the use of artificial substrates for BHAB monitoring because this method collects resuspended microalgal cells, without being directly in contact with the benthic stock of the populations. However, in particular for Ostreopsis blooms, several studies reported significant and positive correlations between Ostreopsis spp. cell concentrations in the water column and cell densities on macrophytes (Vila et al., 2001; Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2011). This is coherent with the good correlation found by Tester et al. (2014) when comparing abundances of benthic toxic dinoflagellates (Gambierdiscus, Prorocentrum and Ostreopsis) recruited on artificial substrates and collected from macroalgal samples.

While the need to standardize collection methods for benthic toxic dinoflagellates has long been recognized, the methodologies currently used to do so are still diverse and challenging (GEOHAB, 2012). In this context, the present study defines important optimizations and steps for standardization of the use of artificial substrates. It goes further on
showing artificial substrates, such as fiberglass screens, as efficient tools for the monitoring and mitigation of BHBs.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.03.047.

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