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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 defines main improvements in this technique, through the use of fiberglass screens during a bloom of O*streopsis* 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.

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 macroalgal 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 O*streopsis* 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.

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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 dinoflagellates collection 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 was used (Fig. 1) 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.

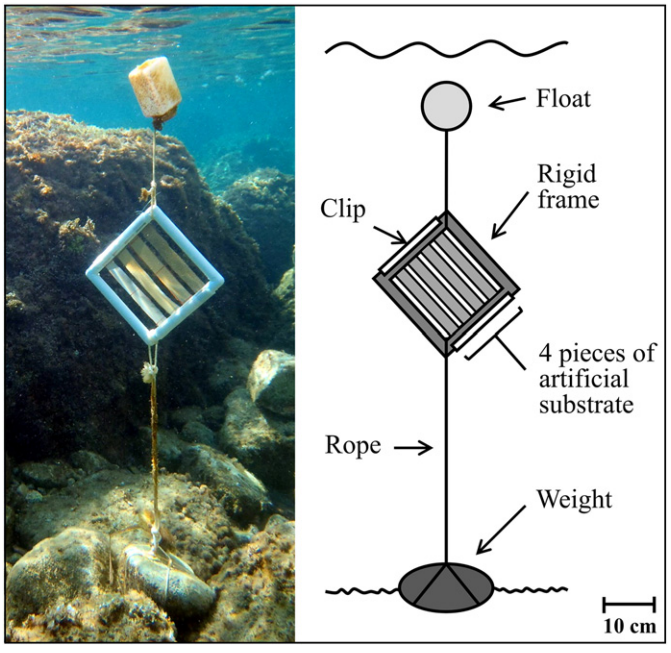

Fig. 1. Description of the set-up used for deployment of artificial substrates in the field. A picture showing the deployment of artificial substrates during an *Ostreopsis* bloom in Villefranche Bay (France) is given on the left side. This picture shows the incubation of four types of substrates, characterized 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 mL Sedgewick Rafter Counting Cell examined with an Axio Scope.A1 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 cm^2 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 agitating 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 step 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.

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