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Sampling harmful benthic dinoflagellates: Comparison of artificial and natural substrate methods

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ABSTRACT

This study compared two collection methods for *Gambierdiscus* and other benthic harmful algal bloom (BHAB) dinoflagellates, an artificial substrate method and the traditional macrophyte substrate method. Specifically, we report the results of a series of field experiments in tropical environments designed to address the correlation of benthic dinoflagellate abundance on artificial substrate and those on adjacent macrophytes. The data indicated abundance of BHAB dinoflagellates associated with new, artificial substrate was directly related to the overall abundance of BHAB cells on macrophytes in the surrounding environment. There was no difference in sample variability among the natural and artificial substrates. BHAB dinoflagellate abundance on artificial substrates reached equilibrium with the surrounding population within 24 h. Calculating cell abundance normalized to surface area of artificial substrate, rather than to the wet weight of macrophytes, eliminates complications related to the mass of different macrophyte species, problems of macrophyte preference by BHAB dinoflagellates and allows data to be compared across studies. The protocols outlined in this study are the first steps to a standardized sampling method for BHAB dinoflagellates that can support a cell-based monitoring program for ciguatera fish poisoning. While this study is primarily concerned with the ciguatera-associated genus *Gambierdiscus*, we also include data on the abundance of benthic *Prorocentrum* and *Ostreopsis* cells.

1. Introduction

The resurgence of interest in the biodiversity of harmful benthic dinoflagellates, most notably the ciguatera-associated genus *Gambierdiscus* (Fraga et al., 2011; Nishimura et al., 2013; Tester et al., 2013) has been facilitated by recent advances in taxonomy (Litaker et al., 2009) and molecular detection and quantification methods (Murray et al., 2009; Penna et al., 2010; Nagahama et al., 2011; Perini et al., 2011; Accoroni et al., 2012; Pfannkuchen et al., 2012; Vandersea et al., 2012). However, before the full potential of molecular assays can be utilized, especially species-specific quantitative polymerase chain reaction assays (qPCR), problems inherent to sampling benthic harmful algal bloom (BHAB) dinoflagellates need to be addressed (GEOHAB, 2012). As a group, BHAB dinoflagellate species co-occur globally in shallow, tropical and subtropical environments where they are typically associated with benthic substrates. The most common substrates colonized by BHAB dinoflagellates include macroalgae, algal turf, seagrasses,

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coral rubble, rocks and sediments (Bomber and Aikman, 1989; Aligizaki et al., 2009; Cohu et al., 2011). The abundance of BHAB dinoflagellates is most commonly quantified by collection of macrophytes, which are shaken in ambient seawater to suspend the attached cells. The BHAB dinoflagellate cells are then concentrated and enumerated using standard microscopy methods (Litaker et al., 2010 and references therein). BHAB dinoflagellate abundances are generally expressed as cells g^{-1} wet weight (=fresh weight) of macrophyte (e.g., Yasumoto et al., 1979; Chinain et al., 1999; Mangialajo et al., 2011). However, colonized substrates often possess complex morphologies with a wide range of surface area to mass ratios, making comparison of BHAB dinoflagellate cell abundances among different substrates problematic. The advantages of normalizing cell abundance to algal surface area (cells cm^{-2}) rather than algal mass (cells g^{-1} wet weight) was identified by Bomber et al. (1985) and Lobel et al. (1988), although methods for measuring algal surface areas are difficult and often impractical. Other problems inherent to the macrophyte method include inconsistent distribution of macroalgae in time and space, scarcity or lack of the targeted macroalgal species among different environments, unequal dinoflagellate abundances among different macrophytes and discontinuous or patchy distribution of BHAB dinoflagellate cells. This variability means that a relatively large number of replicate samples may be required for a statistically robust measure of cell abundance (Lobel et al., 1988).

As an alternative to macrophytes, some researchers have used artificial substrates to assess BHAB dinoflagellate abundance. Caire et al. (1985) employed fabric strips suspended in the water column to monitor the *Gambierdiscus* population at an atoll in French Polynesia. Similarly, artificial materials (test tube brushes, plastic plates) have been used to compare the abundance of *Prorocentrum lima* on substrates with different surface areas in the Florida Keys, USA (see Bomber and Aikman, 1989). Kibler et al. (2010), Tester et al. (2010) and Tan et al. (2013) used measured pieces of fiberglass screen and Ishikawa et al. (2011) deployed fabric tubes (cotton 65%, synthetic 35%) as substrates to collect BHAB dinoflagellate cells in other tropical and subtropical ecosystems.

Artificial substrates offer numerous advantages over macrophytes. The most important advantage is that dinoflagellate cell abundances can be more easily normalized to a known surface area (cells cm^{-2}, cells 100 cm^{-2}) for comparison among studies. Artificial substrates can be readily deployed across multiple spatial and temporal scales in any environment independent of the availability of macroalgae or other natural substrates. They can be easily randomized, allowing the design of statistically rigorous field studies. Significantly, artificial substrates also eliminate dinoflagellate-macroalgae preference effects, grazing by fish or other fauna and algal palatability considerations (see Cruz-Rivera and Villareal, 2006). Another advantage of samples collected from artificial substrate is that the samples tend to be cleaner than those from natural substrates with fewer contaminating biota or particulates. This is likely a consequence of the short incubation time. A disadvantage of the artificial substrate method is that each sampling site must be visited twice, once to deploy the substrates and again to retrieve them.

In this study we compare two collection methods for measuring BHAB dinoflagellate abundances, an artificial substrate method and the traditional macrophyte method. The objective was to develop a widely applicable, statistically robust sampling method whereby cell abundances can be normalized across different studies. While this effort was primarily concerned with quantifying *Gambierdiscus* abundance as a cell-based monitoring protocol for ciguatera fish poisoning (CFP), we also report data on the abundance of benthic *Ostreopsis* and *Prorocentrum* cells. Development of a universally adopted, fully validated sampling protocol will help resolve long-standing questions such as the potential environmental triggers for species-specific bloom formation, species toxicity, seasonality of abundance and environmental risks of BHAB events.

2. Methods

2.1. Sample sites

The feasibility of using artificial substrate (fiberglass screen) for quantifying the abundance of benthic dinoflagellates was tested in a range of tropical marine coastal environments in the Caribbean (Belize, Central America) as well as in the Indo-Pacific (Malaysia). Screen and comparative macrophyte (algae and seagrass) samples were collected in an array of habitats from the central lagoon system of Belize, Central America near Carrie Bow Cay (16.8025° N, 88.0820° W) during May of 2009 and January of 2012 (Fig. 1A). This portion of the Belizean central lagoon is the type locale for a number of BHAB *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* species (Faust, 1993, 1994, 1999; Faust and Morton, 1995; Faust et al., 2008; Litaker et al., 2009) and was an ideal location to test the new sampling method. Malaysian screen and macrophyte samples were collected in May 2012 along the eastern and western coasts of Pulau Sibu (2.2133° N, 104.0676° E) and on the west coast of Pulau Tinggi (2.2943° N, 104.1177° E) as part of the International Training Workshop on the Ecology and Taxonomy of Benthic Marine Dinoflagellates held 21–31 May 2012 in Pulau Sibu and the Universiti Kebangsaan, Malaysia. The field sites were protected islands located on the southeast coast of the Malay Peninsula, 30–33 km SSE of the city of Mersing (2.4357° N, 103.8308° E, Fig. 1B). Habitats sampled included protected mangrove embayments, island fringe environments, lagoonal patch reefs, seagrass beds, coral fore and back reef sites, as well as rocky hard bottom areas (Tables 1 and 2). Screen and macrophyte samples were collected from 0.2 to 20 m in relatively pristine environments as well as those heavily impacted by humans.

2.2. Screen sampling method

In order to test the screen method for characterizing BHAB dinoflagellate abundance, this study was designed to address four main topics: (1) How long does it take for BHAB cells on the artificial substrate to achieve equilibrium with the surrounding cell abundances? (i.e., incubation or soak time); (2) How does the size of the sampling screen (artificial substrate) affect cell abundance estimates?; (3) How well does the abundance of BHAB cells associated with screens correlate with cell abundances from macrophytes (natural substrate)?; and (4) How many replicate screens are needed to assess BHAB dinoflagellate abundances for monitoring purposes?

The artificial substrate used in this study consisted of pieces of black fiberglass screen (window screen) cut into rectangles measuring 10.2 cm × 15.2 cm (Fig. 2A). Each screen was attached to monofilament fishing line and suspended in the water column within ~20 cm of the seabed using a weight and small subsurface float (Fig. 2B). The subsurface floats were used to limit the length of monofilament line and avoid disturbance to the screen. After placement, the screens were allowed to incubate for a defined period of time before being retrieved. For retrieval, a 775 ml plastic wide-mouth jar filled with ambient seawater was positioned beside each screen before the screen was gently removed from the monofilament line and transferred to the jar (underwater) without being folded. The jar was then capped and returned to the laboratory for processing.