Benthic harmful algal blooms occurrence in the south coast of Madeira Island, Portugal, from 2018 to 2020

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Abstract

Ciguatera fish poisoning episodes have been recently reported in the Madeira Archipelago, requiring actions to protect both public health and economic sectors such as tourism and fisheries. Surveys on the occurrence of benthic harmful algal bloom (bHAB) species were performed during three consecutive years (from 2018 to 2020), at Cais do Carvão Bay, Funchal, Madeira. Samples collected on artificial substrates were analyzed for the presence of bHAB species, the seasonal succession of the bHAB community and the effects of abiotic factors on BHAB dynamics. The bHAB community was characterized by the presence of five genera: *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, and *Amphidinium*. The abundance of the different genera showed marked differences. *Ostreopsis* displayed cell abundances several orders of magnitude higher than the other genera (841.05 x 10^3 cells 100 cm^{-2} (September 2018) and was recorded at all sampling dates. The second most abundant genus was *Coolia*, followed by *Prorocentrum* and *Amphidinium*. *Gambierdiscus* always showed the lowest cell densities (max. 0.43 x 10^3 cells 100 cm^{-2} (September 2018). During summer, *Ostreopsis* abundances exceeded, in several sampling dates the limits of level of concern for respiratory syndrome outbreaks (20 x 10^3 cells 100 cm^{-2}) as referred to in the literature. *In situ* recorded abiotic data suggests that the increase of *Ostreopsis* densities could be linked to an increase in water temperature during summer. These results confirm Cais do Carvão Bay, as a potential high-risk area for bHAB development, especially concerning the genus *Ostreopsis*.

Keywords: Benthic dinoflagellates, bHAB community, ecology, time-series, Madeira Island

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Introduction

Ciguatera fish poisoning (CFP) is a syndrome caused by the bioaccumulation of ciguatoxins in fish and their subsequent consumption by humans (Berdalet *et al*., 2017). These phycotoxins are produced by species of the benthic dinoflagellate genus *Gambierdiscus* and *Fukuyoa*, often associated with the palytoxin-producing genus *Ostreopsis*. Harmful events associated with benthic dinoflagellates, have been reported more frequently over the last decades and are considered a priority for the scientific community and policy-makers, because of bHABs biogeographical expansion from endemic tropical and subtropical areas to temperate areas, namely Macaronesia (Azores, Madeira, Canary Islands, and Cabo Verde) due to natural and anthropogenic causes (GEOHAB, 2012). Both *Gambierdiscus* and *Ostreopsis* genera have been recorded in the Madeira Archipelago (Madeira, Desertas, Porto Santo, and Selvagens islands) and also CFP episodes, affecting several people after ingesting locally caught contaminated fish, were documented in summer 2007 and 2008 from the Selvagens Islands (290 km to SE of Madeira) (Kaufmann and Böhm-Beck, 2013). Thus, actions are required to assess risks and provide baseline information for the implementation of a sustainable monitoring program. The aim of the present study was to characterize the ecology of the bHAB forming species in Madeira Island. This was achieved through: 1) identification of bHAB forming genera in the area, 2) study of the temporal succession of bHABs, and 3) investigating the abiotic factors that could be associated with bHAB dynamics.

Material and Methods

This study was conducted during three consecutive years from 2018 to 2020 at Cais do Carvão Bay, Funchal, south coast of Madeira (Fig. 1). Sampling was performed from June to October in 2018-2019 and from July to December in 2020. Sampling frequency was influenced by sea conditions. Dinoflagellates were collected with an artificial substrate method consisting of the immersion of triplicates of plastic screens in the water column (2-3 m water depth; screens about 20-30 cm above bottom), for 24h (Tester *et al*., 2014). Data loggers were deployed simultaneously next to screens to record patterns of seawater temperature (ST) and light intensity. Seawater samples (~ 250 mL) for quantification of dissolved inorganic nutrients (nitrate, nitrite, phosphate, and silicate) were also collected. The samples were kept frozen (−20 °C) until analysis. Nutrient concentrations were determined following Grasshoff *et al*. (1999) and measured in a UV/VIS spectrophotometer (UVmini1240, Shimadzu).

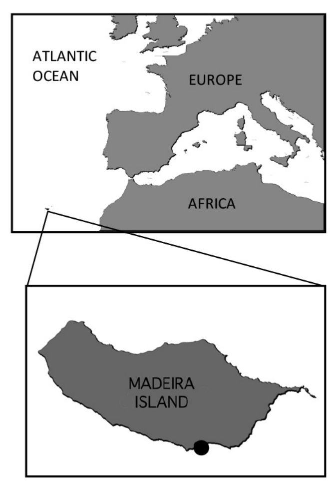

Fig. 1. Sampling site from 2018 to 2020 at the south coast of Madeira Island (black dot), Cais do Carvão Bay.

Screens were retrieved and transferred underwater into plastic bags filled with surrounding seawater and sealed to reduce the chances of losing attached cells. After being transported to the laboratory, plastic bags containing the screens were vigorously shaken for 1 min to detach dinoflagellate cells. Samples were concentrated into a 20 µm sieve and back-washed with pre-filtered seawater into 50 mL tubes. The final volumes (~40 mL) were recorded for further quantification. All samples were immediately fixed with neutral Lugol's solution (Edler and Elbrächter, 2010) and 3.7% formaldehyde and stored in the dark until further analyses. Preserved cells were identified under light microscopy. For the quantification of benthic dinoflagellates, specimens were identified at the genus level. Cell counting was carried out in Sedgewick-Rafter chambers and for each concentrated volume (sample), triplicate counting of 1 mL aliquots was performed. Whenever possible, a minimum of 400 cells were counted for statistical consistency. Screen surface area (A_{Scr}) was determined according to Tester *et al*. (2014) and cell abundances were expressed as cells 100 cm^{-2}.

Regional mean sea surface temperature (SST) and significant wave height for the study period, except 2019, was obtained from the buoy deployed at 2.5 km from the sampling site (Madeira Port Authority - APRAM). In 2019 the buoy was unavailable for maintenance reasons.

Results and Discussion

From 2018 to 2020 the bHAB forming community, was characterized by the presence of five genera: *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, and *Amphidinium*. The abundance of the different genera showed marked differences (Fig. 2). *Ostreopsis* displayed cell abundances several orders of magnitude higher than the other genera and was recorded at all sampling dates. The second most abundant genus was *Coolia*, followed by *Prorocentrum* and *Amphidinium*. *Gambierdiscus* always showed the lowest cell densities.

During the study period, maximum cell abundances for all genera were recorded in 2018, except for *Amphidinium* for which maximum abundances were recorded in 2019. *Ostreopsis* presented maximum cell densities of 841.05 x 10^3 cells 100 cm^{-2}, in September 2018, while *Gambierdiscus,* reached maximum abundances of 4.3 x 10^2 cells 100 cm^{-2}, also in September 2018. *Coolia* and *Prorocentrum* presented maximum cell densities of 6.49 x 10^3 cells 100 cm^{-2} in August 2018, and 7.5 x 10^2 cells 100 cm^{-2} in June 2018, respectively. The highest concentrations of *Amphidinium* (9.9 x 10^2 cells 100 cm^{-2}) were recorded in June 2019.

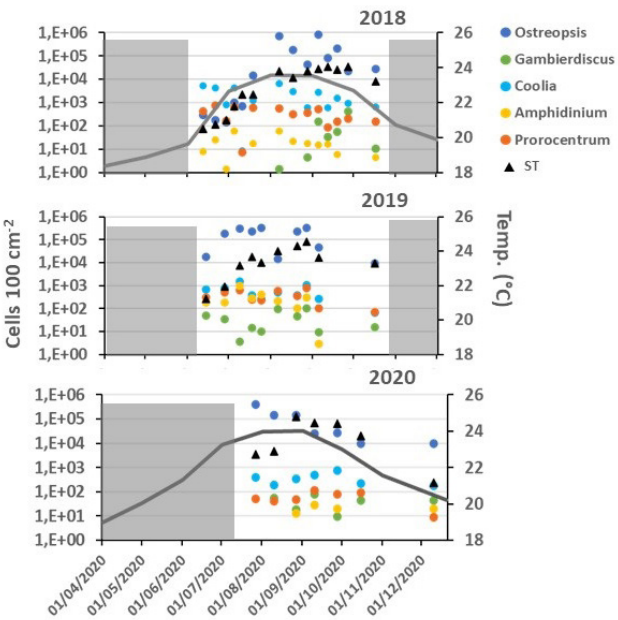

Fig. 2. Distribution of the main bHAB forming genera from 2018-2020 and SST at Cais do Carvão Bay, south Madeira. The grey line represents the mean monthly SST and triangles represent *in situ* ST. Shaded areas, time intervals for which no sampling was performed.

In the three sampled years, *Ostreopsis* abundances increased strongly in July reaching maximum values in August and September (Fig. 2) when higher temperatures (around 24°C) were recorded, suggesting temperature is an important modulator for the development of *Ostreopsis* blooms, while this is not evident for the other genera. Other authors have also found significant positive correlations between *Ostreopsis* spp. densities and temperature (Ciminiello *et al*., 2006; Ben Gharbia *et al*., 2019). Furthermore, low waves height seems to be an important factor contributing to bHAB dynamics, particularly for *Ostreopsis* (Fig. 3), in accordance to what has been reported by Santos *et al*. (2019).

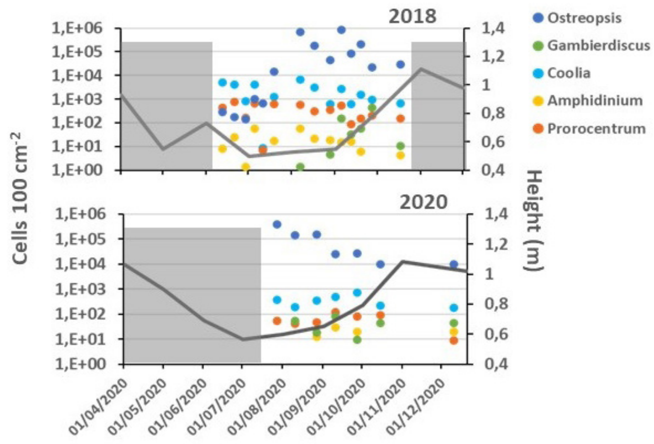

Fig. 3. Distribution of the main bHAB forming genera at Cais do Carvão Bay, south Madeira and significant wave height for 2018 and 2020. Shaded areas, time intervals for which no sampling was performed.

Although 2018 recorded the highest nutrient concentrations and the highest concentrations of *Ostreopsis*, *Gambierdiscus*, *Prorocentrum* and *Coolia*, results indicate that there is no clear relation between bHAB species abundance and nutrients (Figs. 4, 5).

In agreement with what has been described for other Macaronesian islands (Fernández-Zabala *et al*., 2019), in Madeira *Ostreopsis* is the most abundant bHAB forming genus at shallow depths (from 0.5 to 7 m). *Gambierdiscus* was not detected in high concentrations, however, given the reported occurrence of CFP episodes in other islands of the Madeira Archipelago and in the Canary Islands, the presence of *Gambierdiscus* is a reason of concern. On the other hand, in the summer of all sampled years, *Ostreopsis* abundances exceeded, in several sampling dates the limit of level of concern for respiratory syndrome outbreaks of 20 x

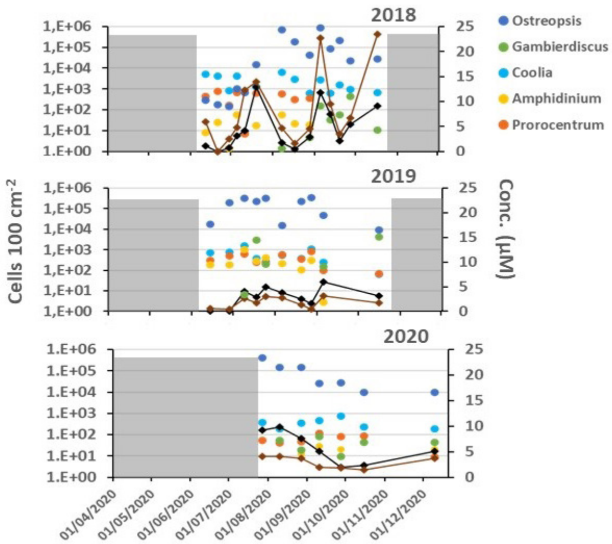

Fig. 4. Distribution of the main bHAB forming genera at Cais do Carvão Bay, south Madeira according to dissolved nutrient concentrations for 2018 and 2020. Black lines represent total dissolved nitrogen (NO_3^- $+NO_2^-$); brown line represents silicate (SiO_3^{2-}). Shaded areas, time intervals for which no sampling was performed.

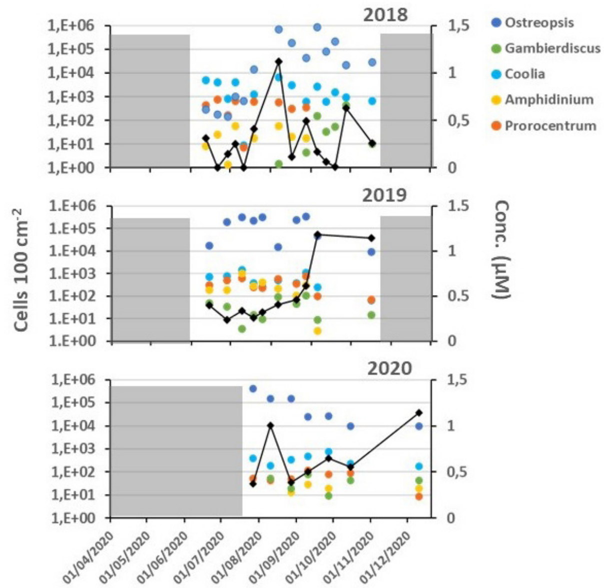

Fig. 5. Distribution of the main bHAB forming genera at Cais do Carvão Bay, south Madeira according to dissolved nutrient concentrations for 2018 and 2020. Black line, total dissolved phosphate (PO_4^{3-}). Shaded areas, time intervals for which no sampling was performed.

10^3 cells 100 cm^{-2} proposed by Tester *et al.* (2014). Overall, these results confirm Madeira (Cais do Carvão Bay), as a potential high-risk area for bHAB development, especially concerning the genus *Ostreopsis*.

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