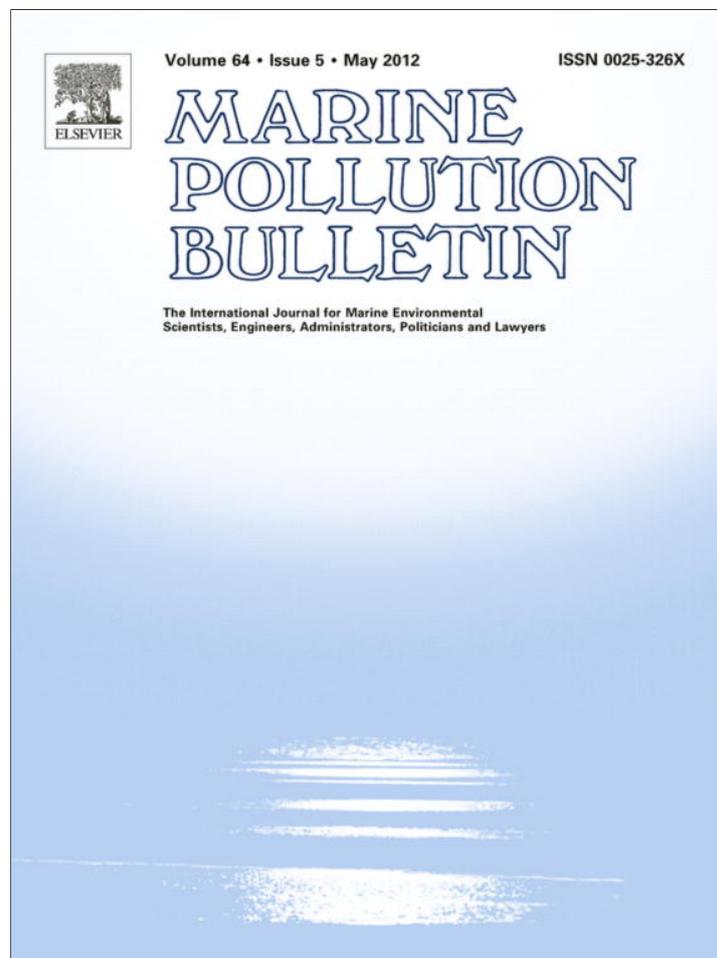


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Note

Ostreopsis cf. *ovata* (Dinophyta) bloom in an equatorial island of the Atlantic OceanSilvia Mattos Nascimento^{a,*}, Julia Vitor França^a, José E.A. Gonçalves^b, Carlos E.L. Ferreira^b^a Departamento de Ecologia e Recursos Marinhos, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Av. Pasteur, 458, Rio de Janeiro 22290-240, Brazil^b Departamento de Biologia Marinha, Universidade Federal Fluminense, Campus do Valonguinho, Niteroi, Rio de Janeiro 24001-970, Brazil

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ABSTRACT

The epi-benthic dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo has an increasingly global distribution. In Brazil there are reports of *O. cf. ovata* along the coast from 8°S to 27°S latitude and blooms have been registered on the Rio de Janeiro coastline. In the current study, an *O. cf. ovata* bloom is reported at Saint Paul's Rocks (0°55'10"N; 29°20'33"W), between the southern and northern hemispheres. The Archipelago is not inhabited and not subjected to eutrophication, due to isolation, and sustains a number of endemic species. Therefore, blooms of *O. cf. ovata* may potentially cause demise to trophic chains by affecting marine invertebrates and vertebrates.

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1. Introduction

Ostreopsis Schmidt is a genus of epiphytic-benthic dinoflagellate living associated to a variety of substrata, including macroalgae, rocks, soft sediments and invertebrates (Totti et al., 2010). Species of *Ostreopsis* are frequently found in assemblages with other dinoflagellates from the genus *Prorocentrum*, *Coolia*, *Gambierdiscus* and *Amphidinium*. The species *Ostreopsis* cf. *ovata* was described by Fukuyo (1981) and since then has been found to be widely distributed with new locations being identified recently (Rhodes, 2011). Since the end of the 1990s, massive blooms of *Ostreopsis* cf. *ovata* have been reported particularly in the Mediterranean Sea, where the species forms mats and has been a recurrent problem annually (Ciminiello et al., 2010).

O. cf. ovata has been found to produce palytoxin analogues, the ovatoxins-a, -b, -c, -d, -e (Ciminiello et al., 2010; Nascimento et al., 2012). In the Mediterranean Sea *O. cf. ovata* blooms were associated to deleterious effects on the benthic community as well as occasional human distress (Ciminiello et al., 2006). Human health is mostly affected through air spray inhalation and in an extensive bloom event in Italy (close to Genoa) in summer 2005, hundreds of people required medical care after exposure to marine aerosols (Ciminiello et al., 2006). At the same time, various adverse effects were observed in benthic organisms, both sessile (cirripeds, bivalves, gastropods) and mobile (echinoderms, cephalopods, fish). Other important human inhalation intoxications occurred thereafter in Italy, Spain, France and Algeria (Tichadou et al., 2010). Respiratory intoxications seem to be due to inhalation of seawater droplets containing *Ostreopsis* cells or fragments of cells

and/or aerosolized toxins (Mangialajo et al., 2011). More recently, the presence of ovatoxin-a and palytoxin was confirmed in mollusks and sea-urchins from the Mediterranean Sea (Aligizaki et al., 2008). The mode of action of palytoxin is by the compound binding to the Na⁺/K⁺ ATPase pump in a configuration that permits the ions to flow in both directions and thus, altering cell permeability to monovalent cations (Artigas and Gadsby, 2003).

In Brazil, *O. cf. ovata* blooms were reported at Arraial do Cabo (22°59'S, 42°00'W), Rio de Janeiro State in summer of 1998 and 2002 (Granéli et al., 2002; Ferreira, 2006). In both events, a biofilm of *O. cf. ovata* cells was seen covering epilithic macroalgae and the bloom was associated with sea urchins (*Echinometra lucunter*) death. The 1998 summer event was later confirmed to be widespread along the southeastern Brazilian coast, causing in some embayment habitats mortality of urchins and fishes (Ferreira, personal communication). Since then, the distribution of epi-benthic dinoflagellates has been investigated along the Rio de Janeiro coast and the species *O. cf. ovata* has been found to be a common component of the epi-benthic microflora associated to macroalgae, with frequent bloom events recorded (Nascimento et al., 2010). Furthermore, there are reports of *O. cf. ovata* along the Brazilian coast from 8°S to 27°S latitude (Nascimento et al., 2012).

The Archipelago of Saint Paul's Rocks consists of a remote group of ten small islands located close to the axis of the Mid-Atlantic Ridge (0°55'10"N; 29°20'33"W). It corresponds to the emerged summits of a meso-oceanic traverse chain with an emerged area of 17,000 m² and maximum altitude of 18 m. Saint Paul's Rocks is located approximately 960 km off the Brazilian coast and 1890 km south-west of Senegal, West Africa, between the southern and northern hemisphere (Luiz and Edwards, 2011). Climate in the area is influenced by the Intertropical Convergence Zone with southeast trade wind velocities between 4 and 7 m s⁻¹. Annual air temperature amplitude is less than 1 °C, around 27 °C and

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seawater temperature is reported to vary between 24.0 and 28.5 °C (Soares et al., 2009). Maximum monthly accumulated precipitation of 370 mm occurs in April while lowest values occur between August and November (Soares et al., 2009).

Only the biggest island has low vegetation and the area is subjected to severe sea and wind conditions. The area provides shelter for many species of sea birds, fish, crustaceans as well as insects and is important as feeding and reproductive area for various migratory species. The waters in the area are oligotrophic but upwelling events caused by the interaction between oceanic currents and the submarine relief may happen. The region is considered strategic to the development of industrial fisheries, although there are recent reports of negative impacts of this activity (overfishing) in the area (Luiz and Edwards, 2011). Koenig and Oliveira (2009) reported that dinoflagellates represent 82% of the total number of microphytoplankton species in the area and the cyanobacterium *Trichodesmium thiebautii* is distinguished by its frequency and dominance.

The current study aimed to describe a bloom of *O. cf. ovata* at the Archipelago of Saint Paul's Rocks, Brazil, during a scientific expedition in June–July 2009. The name *O. cf. ovata* was used in this study because of the lack of molecular data associated with strains from the type locality, following recommendations of Penna et al. (2010).

2. Materials and methods

Macroalgae samples (*Laurencia* sp.) were collected from depths between 3 and 5 m by scuba diving along shallow habitats of the main embayment habitat at Saint Paul's Rocks (Fig. 1) on the 5th July, 2009. Macroalgae specimens ($n = 10$) were collected with the adjacent seawater and placed in plastic bags. After collection, macroalgae samples were shaken vigorously for 2 min to dislodge epiphytic organisms. The epiphyte suspension and macroalgae were fixed with formaldehyde 4%. Once in the laboratory, the volume of the epiphyte suspension was measured and passed through a 255 µm mesh sieve to remove large particles and sand. Macroalgae samples were weighted to obtain fresh weight (FW). Epibenthic dinoflagellates were identified and quantified under light microscopy. Due to the high cell abundance, quantification was performed in two 50 µl aliquots from each sample.

Cell dimensions from the *O. cf. ovata* population were measured under 400× magnification and using the software Axio Vision 4.8.2 (Carl Zeiss) in an inverted microscope (Primo Vert, Zeiss). The dorsoventral diameter (DV) and transdiameter (W) of 77 cells were measured.

Seawater temperature was measured continuously every hour with *ibutton* sensors for two weeks.

3. Results and discussion

A monospecific bloom of *Ostreopsis cf. ovata* was observed in macroalgae samples from Saint Paul's Rocks and a yellowish-brown biofilm was visible covering the macroalgae (Fig. 2). Average ($n = 10$) *O. cf. ovata* abundance was 4.6×10^4 cells g fresh weight (FW) *Laurencia*⁻¹ and varied between 6.9×10^3 cells gFW *Laurencia*⁻¹ and 9.9×10^4 cells gFW *Laurencia*⁻¹. High variability was observed in *O. cf. ovata* abundance between different *Laurencia* sp. specimens. Rare *Amphidinium* sp. cells were observed. Other cells registered were the diatom *Licmophora* and pennate diatom species.

Along the Brazilian coast, epi-benthic dinoflagellates have been studied mainly in the Rio de Janeiro coast, where maximum reported *O. cf. ovata* abundance was 2.2×10^5 cells gFW *Sargassum vulgare*⁻¹ in June 2006 at Tartaruga, Armação dos Búzios

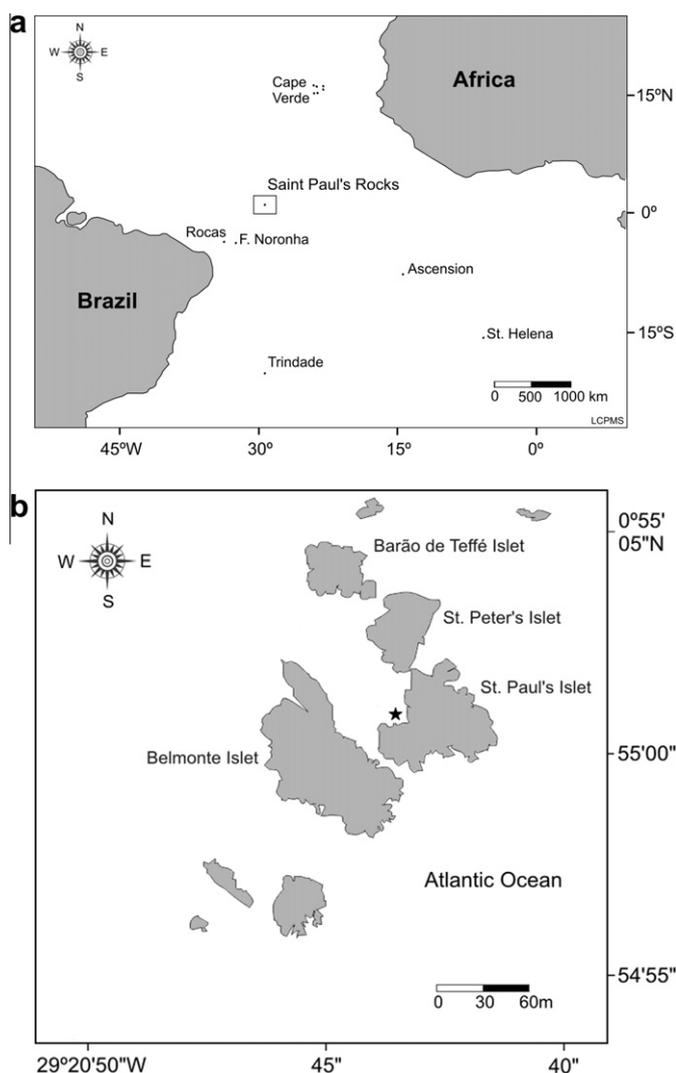


Fig. 1. Location of Saint Paul's Rocks (a) and details of the archipelago showing the embayment (★) where the sampling was performed (b).



Fig. 2. *O. cf. ovata* biofilm covering the epilithic algae at the embayment of Saint Paul's Rocks.

(22°45' S, 41°53' W). At Arraial do Cabo, peak *O. cf. ovata* abundances reached 2.1×10^4 cells gFW *Amphiroa* spp.⁻¹ in December 2006. Four *O. cf. ovata* bloom events have been recorded along

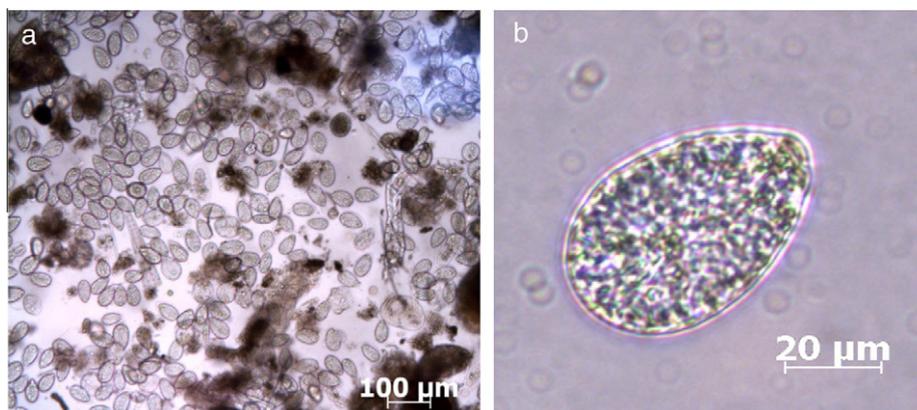


Fig. 3. Light micrographs of *O. cf. ovata* from Saint Paul's Rocks. (a) Overview of *O. cf. ovata* bloom densities (10×), (b) *O. cf. ovata* cell in dorsal view (40×).

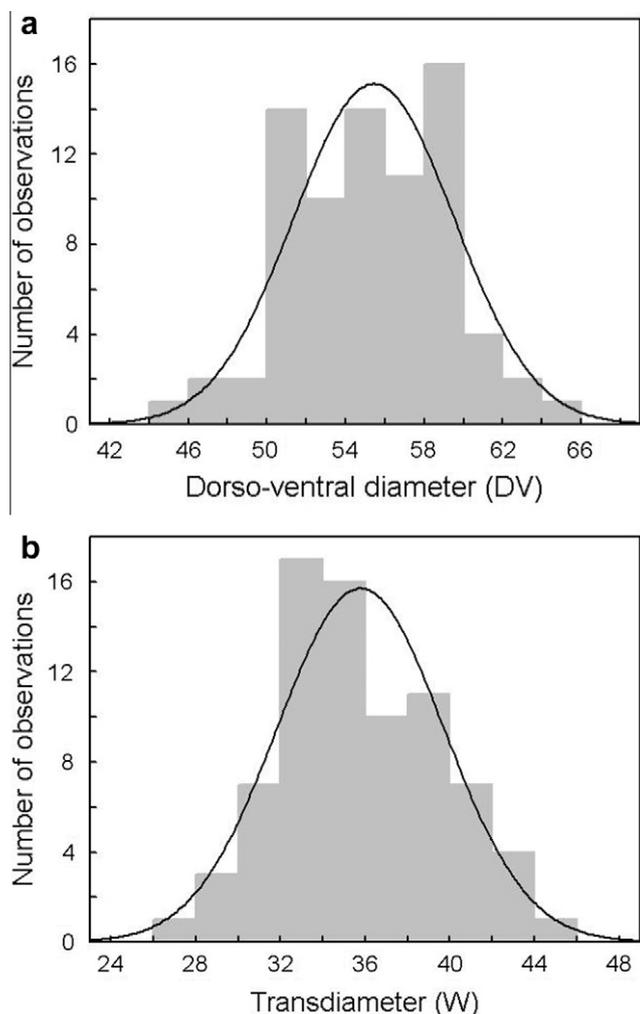


Fig. 4. Size variability in dorsoventral diameter (DV) (a) and transdiameter (W) (b) of *Ostreopsis cf. ovata* population from Saint Paul's Rocks.

the Rio de Janeiro coastline between 2006 and 2007 (Nascimento et al., 2012).

In the Mediterranean Sea, in 2003 and 2004, *O. cf. ovata* maximum abundance was 1.6×10^4 cells L^{-1} in the water column and 4.0×10^5 cells gFW macroalgae $^{-1}$ in *Ulva* sp. and *Gracilaria* sp. samples at the North Aegean Sea, Greece (Aligizaki and Nikolaidis, 2006). Along de Genoa coastline, Italy, in July 2006, *O. cf. ovata* attained abundances of 2.5×10^6 (Mangialajo et al., 2008) and of 1.7×10^6 cells gFW *Hypnea musciformis* $^{-1}$ at the North Adriatic Sea, Italy in September 2007 (Totti et al., 2010). *Ostreopsis ovata* was described by Fukuyo (1981) in samples from the French Polynesia and Japan. *Ostreopsis cf. ovata* cells from Saint Paul's Rocks were ovoid to oblong (Fig. 3), pointed towards the ventral area in apical view, with many golden chloroplasts. The dorso-ventral diameter (DV) varied between 45.9 and 65.6 μm (average 55.4 μm , Fig. 4a, $n = 77$) while the transdiameter (W) ranged between 27.5 and 45.6 μm (average 35.7 μm , Fig. 4b, $n = 77$, Table 1). The dimensions of the *O. cf. ovata* field population from Arraial do Cabo, Rio de Janeiro are similar, but with a wider transdiameter (Nascimento et al., 2012). The morphometrics of the Saint Paul's Rocks *O. cf. ovata* population agree well with the larger cell size class reported for the North Aegean Sea, Greece (Aligizaki and Nikolaidis, 2006) and the Gulf of Naples, Italy (Rossi et al., 2010). More recently, Sato et al. (2011) reported *O. cf. ovata* cell dimensions smaller than elsewhere and unveiled a high diversity of *Ostreopsis* species along Japan using molecular techniques.

Harmful algal blooms (HABs) are a natural phenomenon that has been reported in many areas around the world. However, there is an apparent global increase in the occurrence and intensity of HABs in the last decades (Hallegraeff, 1993) and the related impacts they may cause. The eutrophication of coastal waters is considered a major cause of the increase in HABs occurrence (Hallegraeff, 1993; Anderson et al., 2002; Heisler et al., 2008). Other factors that are thought to contribute to this expansion include the increased scientific awareness of these events through more monitoring programs, global climate change, coastal developments like the construction of harbors, as well as the transport of cysts and vegetative cells in ship's ballast water or associated with the movement of shellfish (Hallegraeff, 1993).

Table 1
Dorsoventral (μm) and transdiameter range (μm) of *O. cf. ovata* cells from Saint Paul's Rocks ($n = 77$) and elsewhere.

Place	Dorso-ventral diameter (DV)	Transdiameter (W)	Reference
Saint Paul's Rocks, Brazil	45.9–65.6	27.5–45.6	Current study
Arraial do Cabo, Rio de Janeiro, Brazil	40–65	18–45	Nascimento et al. (2012)
North Aegen Sea, Greece (larger cell population)	45.2–61.9	28.6–47.6	Aligizaki and Nikolaidis (2006)
Gulf of Naples, Italy	40–64	–	Rossi et al. (2010)
Japan	28.1 ± 2.6	20.8 ± 3.3	Sato et al. (2011)

In the present study, a bloom of *O. cf. ovata* is reported in an oceanic area where the only identifiable anthropogenic impact would be apparently the industrial fishing activity (Luiz and Edwards, 2011). The area is not inhabited and is not eutrophic, but upwelling events do occur. As a comparison, along Rio de Janeiro, at the southeastern subtropical Brazilian coast, *O. cf. ovata* has been found in bloom densities in an area subjected to coastal upwelling, distant from heavy freshwater discharge from rivers and not eutrophic. At other more eutrophic areas, subjected to anthropogenic impacts (such as treated sewage discharge), the species has not been found in bloom densities. It is acknowledged, however, that the coastal areas of Rio de Janeiro are subjected to more diverse impact sources than the oceanic area described in the current study. The question raised is if *O. cf. ovata* blooms are singular for not being associated to eutrophic conditions, in contrast to most other harmful species.

Moreover, studies with *O. cf. ovata* laboratory cultures have shown that the species develops aberrant cell shape when grown in full media (L2), what is reverted when cells are transferred to a less concentrated (L2/2) medium (Nascimento et al., 2012). This same pattern was observed in cultures of *Ostreopsis siamensis* when grown in GSe and f/2 medium (Rhodes et al., 2000; Pearce et al., 2001). According to those authors, increased nitrate and phosphate concentrations impeded the growth of *O. siamensis* and caused aberrant cell shape.

The factors related to *O. cf. ovata* blooms are still poorly understood and these ideas require further examination. Surface seawater temperature has been shown to be an important factor related to *O. cf. ovata* blooms (Mangialajo et al., 2011; Granéli et al., 2011). The Archipelago of Saint Paul's Rocks is located at the equatorial zone and seawater temperature is reported to be higher than 24 °C through the whole year (Soares et al., 2009). During the *O. cf. ovata* bloom in June and July 2009, seawater temperature in the proximity of the macroalgae varied between 24 and 25 °C.

Along the Rio de Janeiro coast, *O. cf. ovata* blooms have been reported to occur when seawater temperature is higher than 22 °C (Granéli et al., 2002; Nascimento et al., 2010). In the Mediterranean Sea similar results were found by Tognetto et al. (1995), Sansoni et al. (2003) and Mangialajo et al. (2008), however, there are reports of high *O. cf. ovata* abundances occurring at temperatures lower than 20 °C in the North Adriatic Sea (Mediterranean Sea, Mangialajo et al., 2011 and references there in).

Culture experiments have shown that *O. cf. ovata* presents higher growth rates at temperatures of 26 °C, considering strains isolated from Rio de Janeiro, Brazil (Nascimento and Corrêa, 2010) and Genoa (Granéli et al., 2011), Trieste, Nancona and Napoli (Zingone et al., 2010), Italy.

Local hydrodynamism through low turbulence is generally related to higher densities of *O. cf. ovata* and in the current study the bloom was present in the more sheltered area, in an embayment habitat at Saint Paul's Rocks. Understanding the factors behind the distribution of *O. cf. ovata* as well as the increase in the frequency of bloom events will require a number of physiological and ecological studies.

HABs impacts include mass mortalities of wild and farmed fish and shellfish, illness and death of mammals, seabirds and other animals, and alteration of marine habitats and trophic structure (Anderson et al., 2002). Events of mass mortality of marine fish have been reported to occur worldwide mostly in coastal regions of continental shelves and nearby offshore islands. In Brazil, an event of reef fish mass mortality occurred at Trindade Island, located 1160 km from the southeastern Brazilian coast in February and March 2007 (Pinheiro et al., 2010). The species *Melichthys niger*, the most abundant omnivorous reef fish of the island was mostly affected and other 24 species were found washed ashore. The causes of the event were not clarified, but HABs is among

the raised possibilities. There is evidence that fish kills have occurred periodically since at least the 1990s in Trindade Island. Moreover, recent events of fish mass mortality have been reported for other oceanic, oligotrophic localities in the Atlantic Ocean and elsewhere (Pinheiro et al., 2010).

A mass mortality of the sea urchin *Diadema mexicanum* was reported in 2009 at the Bahias de Huatulco, on the Pacific coast of Mexico (Benítez-Villalobos et al., 2009). The specimens were described as “possessing spines with mucous attached, showing injuries around the peristomial area” and a disease was suggested as the causative agent. The same symptoms were reported by Ferreira (2006) during the 1998 *O. cf. ovata* bloom event at Arraial do Cabo for *E. lucunter* specimens. Another event of sea urchin mortality (*Diadema antillarum*) was observed in November 2009 at Madeira Island, when the presence of high abundances of *Ostreopsis* sp. was registered (Manfred Kaufmann, personal communication.).

The data presented in the current study for Saint Paul's Rocks raises concerns if the fish and sea urchin mortalities that occurred elsewhere may have been linked to HABs and particularly to *O. cf. ovata* proliferations. The presence of ovatoxin-a, -b, -c, -d, -e (palytoxin analogues) was recently confirmed in two *O. cf. ovata* strains isolated from Rio de Janeiro, Brazil (Nascimento et al., 2012) and *Ostreopsis* blooms have been associated to sea urchins death in Rio de Janeiro (*O. cf. ovata*, Granéli et al., 2002; Ferreira, 2006) and in New Zealand (*O. siamensis*, Shears and Ross, 2009). Palytoxin and analogues have caused human intoxication through the consumption of crabs (Alcala et al., 1988) and fish (Gleibs and Mebs, 1999) and is the cause of clupeotoxism (Onuma et al., 1999).

O. cf. ovata blooms in oceanic islands may potentially cause harmful effects to marine invertebrates and vertebrates. The Saint Paul's Rocks is an oceanic area with a number of endemic species and low functional redundancy relative to coastal sites. As an example, herbivorous fishes such as acanthurids and scarids, which are commonly found in tropical reef areas, are functionally absent there. This role is performed by abundant pomacentrids and balistids (Ferreira et al., 2009). Low redundancy is an indicative of low resilience if acute impacts as those caused by HABs occur, affecting important functional roles on the local trophic chain.

The data presented here raises concerns of the potential deleterious effects of *O. cf. ovata* blooms in this remote and ecologically relevant archipelago. Moreover, the massive abundance of *O. cf. ovata* at Saint Paul's Rocks, located 1000 km away off the main continental land mass and not inhabited is, controversially, an indication that eutrophication is possibly not playing a part in stimulating blooms of this species.

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