The first recorded bloom of *Protoperidinium quinquecorne* and its link to a massive fish kill in Yemeni coastal waters, Southern Red Sea

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**Abstract:** As part of an ongoing monitoring study of phytoplankton in Yemeni coastal waters we report, for the first time, a dense bloom (14.3x10⁶ cell L⁻¹) of the marine dinoflagellate species *Protoperidinium quinquecorne* (Abé) Balech on June 6, 2012 from the coastal water of Al Hodeidah and Khor Al-Khateeb lagoon, southern Red Sea. Water temperature was 34°C and salinity was 36 during the bloom, indicating its tropical and subtropical nature. This bloom was accompanied by a massive kill of small pelagic fish, which were mostly sardines. Among the phytoplankton species reported during the *P. quinquecorne* bloom, the red tide-forming species, *Trichodesmium erythraeum* (cyanobacteria), *Gonyaulax verior*, and *Prorocentrum micans*, and the known toxic species, *Dinophysysis acuminata*, were notable.

**Key words:** *Protoperidinium quinquecorne*, HABs, Dinoflagellate, fish kill, Red Sea

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*Protoperidinium quinquecorne* (Abé) Balech is one of the most notable species of the genus *Protoperidinium* and is considered a significant component of the coastal and estuarine phytoplankton, causing blooms in different regions. This species can occur in very high concentrations in coastal waters, which change colour as a result, and are sometimes associated with fish kills (Gárate-Lizárraga & Muñetón-Gómez 2008). While some phytoplankton species are known to produce toxins that can be accumulated by filter-feeding organisms, making them hazardous for humans, blooms of the other (nontoxic) species can result in high fish mortalities caused by the development of low oxygen conditions or gill clogging and damage due to mucus secretion and asphyxiation (Claereboudt et al. 2001). Previous plankton surveys from the Red Sea have been sporadic and have reported only a few potential bloom-forming species (Mohamed and Al-Shehri 2012). In June 2012, a dense bloom of a dinoflagellate species was found in the coastal region of Al-Hodeidah city in western Yemen. This bloom occurred concurrently with massive deaths of fish. The aim of this study was to report this bloom and to describe and identify the species that formed the bloom.

Sampling was carried out along the coast off Al Hodeidah city, southern Red Sea, where a sudden water discoloration and fish kills were observed. Sampling sites were close to the shoreline of Al Hodeidah city (14°49′09.11″N, 42°55′13.48″E) and in Khor Al-Khateeb lagoon (Fig. 1). Khor Al-Khateeb is an important lagoon in Yemen, and is located between 42°52′41″ and 42°57′09″E, and 14°48′46″ and 14°55′35″N, north Al Hodeidah city. This lagoon is elongated and extends about 9 km. It is discontinuous and its shallow lagoon ranges between 1–10 m in depth. The lagoon is generally protected from open sea circulation by the Ras Khateeb sand spit (Fig. 1). In general, lagoons are considered potential hotspots for the proliferation of epibenthic toxic species due to the absence of water movement forming a stagnant environment. Phytoplankton samples were collected in triplicate on 6th June 2012 and immediately fixed with Lugol’s iodine solution. They were counted in a Sedgewick-Rafter Cell. A live phytoplankton sample (200 mL) was also taken on the same day and observed within 6 h using a compound light microscope (Novex B-Range, Holland) at X200 and X400 in order to verify species identification. Observations were made concerning the affected fish when and where collection of the water samples were made, on 6th June and three days later, on 9th June 2012. There were many fish that were observed dead on the shoreline or destined for death on the water surface of the lagoon. Only the overall outer appearance of the fish and status of their gills were observed. Temperature and salinity were measured using a thermometer and refractometer, respectively. Using the Winkler titration method, dissolved oxygen (DO)
was also determined.

As part of an ongoing monitoring study of phytoplankton in Yemeni coastal waters, brownish patches were directly observed on 6 June, 2012 on the coast of Al Hodeidah and in Khor Al-Khateeb lagoon (Fig. 1), extending for several kilometers. Microscopic examination of samples collected from this bloom revealed that the discoloration was caused by an armoured dinoflagellate, *Protoperidinium quinquecorne*. Cells of this species were solitary and small in size, a little longer than wide (Fig. 2), with lengths ranging from 21–38 µm and widths ranging from 17–30 µm. The overall shape of the cell body was ovoid, and divided near the middle by the cingulum (Fig. 2A). The epitheca was conical with a pointed apex (Fig. 2B and C), whereas the hypotheca was rounded and bore four antapical spines, which were variable in length (Fig. 2D). The spines on the hypotheca were prominent and straight, but in some specimens one or two spines were curved (Fig. 2). The numerous chloroplasts were small and yellow-greenish. In general, the dimensions of *P. quinquecorne* reported from Yemeni coastal waters were similar to those recorded by Gárate-Lizárraga & Muñetón-Gómez (2008). The number of spines, four in specimens found in the Yemeni coastal waters is similar to the typical four of *P. quinquecorne* (Abé 1927, Horiguchi & Piennar 1991), but different from those specimens collected by Aké-Castillo and Vázquez (2011) from the Sontecomapan lagoon, Mexico, which described *P. quinquecorne* with three spines.

The average cell abundance of *Protoperidinium quinquecorne* collected during this bloom was $14.3 \times 10^6$ cell L$^{-1}$, contributing more than 99% of the overall phytoplankton population. A list of co-occurring phytoplankton is provided (Table 1). A total of 16 taxa, belonging to 3 phytoplankton groups: dinoflagellates, Bacillariophyceae and Cyanobacteriae, were identified. Dinoflagellates were by far the most important group in terms of abundance, but diatoms, with 9 species, were more diverse (Table 1). The *P. quinquecorne* bloom was accompanied by a massive kill of small pelagic fish, which extended along the coast of Al Hodeidah with higher numbers of dead fish being observed in the surface water of the lagoon. The affected fishes were mostly sardine (*Sardinella* sp). During the bloom, fish were not visible on the water surface, but after 3 days (on 9th June), right after the end of the bloom, many fish were observed dead on the surface of the lagoon and along the coast of Al Hodeidah. At the same time, many fish were still gasping for breath, especially in the surface waters of the lagoon. They swam on the surface of the water and some even jumped out of the water. Hours later, the fish were found floating already dead. Dissolved oxygen...
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Concentrations recorded during this bloom were generally low, ranging from 1.90 mg L$^{-1}$ (on 9th June; three days after the bloom) to 4.20 mg L$^{-1}$ (on 6th June; during the *Protoperidinium* bloom). *P. quinquecorne* can reduce water quality by depleting oxygen, leading to fish kills when cell numbers are very high. In several parts of the world, fish kills have been attributed to low dissolved oxygen levels generated by high biomass blooms and not necessarily due to toxicity (Fukuyo et al. 1990, Murray et al. 2015). There has been much research on this phenomenon of high biomass phytoplankton blooms leading to hypoxia in water bodies, due to the large respiratory oxygen demands of phytoplankton and accompanying bacterial communities (Diaz 2001, Diaz & Rosenberg 2008, Murray et al. 2015).

Water temperature recorded during the *P. quinquecorne* bloom was generally high, ranging between 33°C (on 9th June; three days after the bloom) to 34°C (on 6th June; during the *Protoperidinium* bloom), but salinity was 36 both during and after the bloom. The dominance of *P. quinquecorne* appears to be related to its unique ability to thrive under tropical conditions (Al-Hashmi et al. 2013). Horstmann (1980) states that *P. quinquecorne* has a high water temperature tolerance (42°C), and tends to bloom in hot environments; it has caused HABs in Maribago Bay in Malaysia (Horstmann 1980) and South Africa (Horiguchi & Pienaar 1991). The *P. quinquecorne* bloom observed during the present study occurred when air temperature was rather high (35°C) and water temperature had reached 34°C. Similar to our findings, Gárate-Lizárraga et al. (2006) and Gárate-Lizárraga and Muñetón-Gómez (2008) reported frequent blooms in Bahía de La Paz, Baja California Sur, between May and July. Faust et al. (2005) identified *P. quinquecorne* in floating detritus at Douglas Cay and the Lair in Belize also forming red tides. On the other hand, many studies have reported that *P. quinquecorne* can tolerate a wide range of salinities, growing best at relatively high salinities when they are capable of forming red tide blooms under high light and high temperature conditions (Horstmann 1980, Gárate-Lizárraga & Muñetón-Gómez 2008).

To the best of our knowledge, this is the first HAB event...
associated with *P. quinquecorne* and responsible for fish kills that has been reported in Yemeni waters and, indeed, in the entire Red Sea. The presence of harmful phytoplankton species in our waters is not well documented. Further investigations of these species and the mechanisms leading to mass fish mortalities are needed. Furthermore, detailed studies are required to understand the dynamics of these blooms and causal factors initiating such blooms. Such information and knowledge would be beneficial for the prevention and management of future occurrences of HABs in Yemen.

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**Table 1.** List of phytoplankton species, with their cell counts L$^{-1}$ and their relative contribution to total phytoplankton abundance (%) recorded along with the bloom of *Protoperidinium quinquecorne* in the coastal waters of Al Hodeidah, Southern Red Sea

| Phytoplankton Species | No. of Cells (cell L$^{-1}$) | % |
|-----------------------|-------------------------------|---|
| Dinoflagellates       |                               |   |
| 1 *Dinophysis acuminata* | 53                            | 0.0004 |
| 2 Gonyaulax verior     | 3040                          | 0.0213 |
| 4 Procerocentrum micans | 640                           | 0.0044 |
| 5 Protoperidinium oceanicum (Vanhöffen) Balech | 80 | 0.0006 |
| 3 Protoperidinium quinquecorne (Abé) Balech | 14300000 | 99.936 |
| 6 Protoperidinium steinii (Jørgensen) Balech | 160 | 0.0011 |
| Diatoms               |                               |   |
| 7 Amphora sp.         | 27                            | 0.0002 |
| 9 Ceratoneis closterium Ehrenberg | 250 | 0.0017 |
| 8 Coscinodiscus centralis Ehrenberg | 107 | 0.0007 |
| 10 Diploneis sp.       | 27                            | 0.0002 |
| 11 Lithodesmioides polymorphum Stosch | 630 | 0.0044 |
| 12 Lyrella lyra (Ehrenberg) Karajeva | 27 | 0.0002 |
| 13 Mastogloia arabica Hendey | 160 | 0.0011 |
| 14 Pleurosigma sp.     | 27                            | 0.0002 |
| 15 Thalassionema nitzschioides (Grunow) Mereschkowsky | 53 | 0.0004 |
| Cyanobacteria         |                               |   |
| 16 Trichodesmium erythraeum Ehrenberg ex Gomont | 3800 | 0.0266 |