Yellow-green tides could become a recurrent event along the Ligurian coast (Italy)

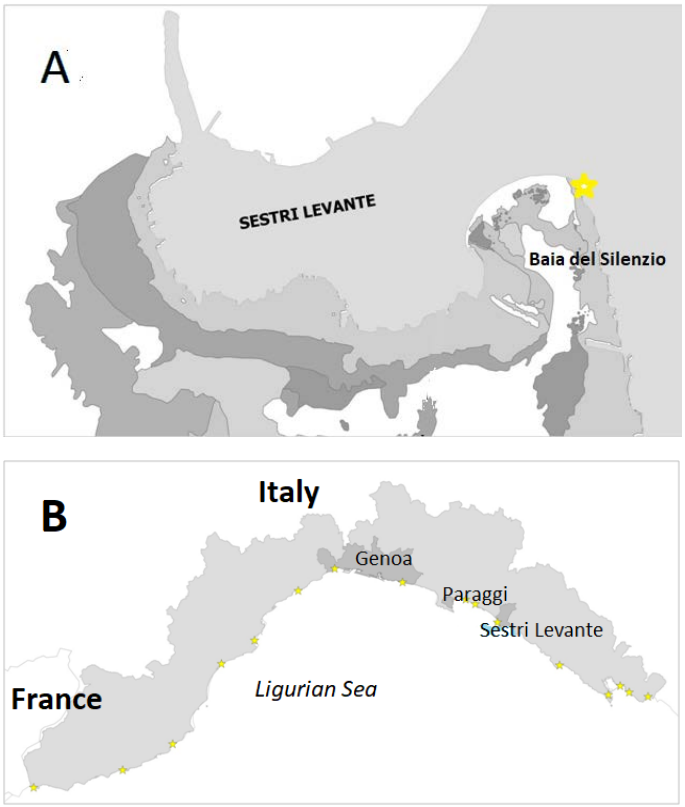

Fig. 1. A) Sestri Levante and Baia del Silenzio bay, characterized by shallow water and mixed rocky-sandy seabed containing the macrophyte, Posidonia oceanica (dark grey) and carpet-like "matte" (light grey) habitat. B) Liguria Region and the sites (yellow stars) included in the harmful algal bloom monitoring program

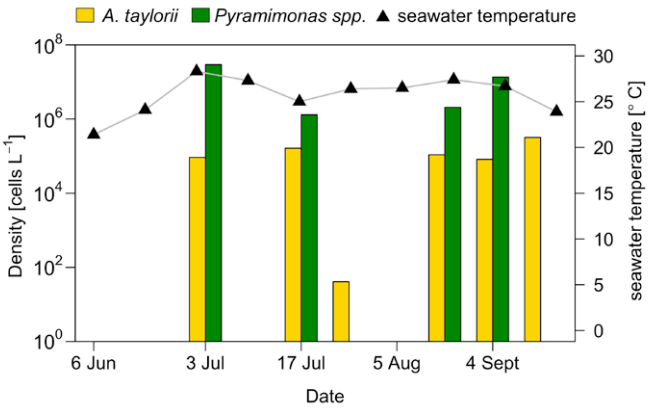

Fig. 2. Cell densities of Alexandrium taylorii and Pyramimonas spp. and seawater temperature (°C) from Sestri Levante Bay (Genoa, Italy), summer 2019.

Alexandrium blooms are becoming an increasing phenomenon in the Mediterranean Sea. These blooms mainly occur in semi-enclosed areas exposed to anthropogenic pressures from aquaculture, tourism and nutrient enrichment [1]. *Alexandrium taylorii* Balech is a potentially toxic dinoflagellate that causes a yellow-green water discoloration. Some published studies suggest that four western Mediterranean areas are affected by blooms of this species: the Catalan Coast (NE Spain), the Balearic Islands, northern Sardinia and Sicily (Vulcano Island, Italy) [2].

During the summer of 2019, blooms of *A. taylorii* impacted the eastern Ligurian coast (north-western Italy) causing significant long lasting yellow-green water discolorations. These were especially noticeable along the Sestri Levante area (Fig. 1) causing concern from both the tourism industry and local authorities.

The first bloom of *A. taylorii* in Ligurian coastal waters, with a maximum density of 1.4 x10^6 cells L^{-1}, was reported by the Regional Environmental Protection Agency of Liguria (ARPAL) in the summer of 2010. Although samples from this bloom did not test positive for algal toxins, since then ARPAL have increased monitoring efforts in compliance with the European Bathing Water Directive. Every year, from June to September, ARPAL collects and analyses samples of both seawater and macrophytes according to the Italian monitoring protocols for harmful algal blooms [3].

Significant *A. taylorii* blooms have been reported during the summers of 2012 (up to 6.0 x 10^4 cells L^{-1}), 2013 (> 3.0 x 10^6 cells L^{-1}), 2016 (5.0 x 10^4 cells L^{-1}) and 2019 (> 3.0 x 10^5 cells L^{-1}) (Fig.2). This suggests an increased frequency in the occurrence of *A. taylorii* blooms, especially on the eastern Ligurian coast. These blooms often co-occur with high cell densities of *Pyramimonas* spp., a nano-phytoplankton taxon belonging to the *Chlorophyta* group that may cause "bright green tides".

During the summer of 2019, additional water discoloration events at different sites along the eastern Ligurian coast (e.g. Paraggi, S. Margherita Ligure) with more than 6.5 x 10^4 cells L^{-1} were recorded by the local sanitary agency and Genoa University. A study was performed isolating that causative organisms into laboratory culture to confirm species identification by molecular analysis, thecal plate morphology using epifluorescence microscopy, and toxin analysis. Unfortunately, it was not possible to maintain the algal culture for the toxin characterization of the strain.

In general, *A. taylorii* is considered a high biomass HAB species that negatively impacts the use of coastal waters for recreational purposes. This species also has the potential to produce Paralytic Shellfish Toxins (PSTs) and other unknown harmful compounds [4]. To date, there have never

Continued on page 12

Table 1. Physico-chemical parameters of P. quadridentatum bloom area in Cochin Estuary

Parameters	
Water Temperature (°C)	28
Salinity (psu)	20
Dissolved Oxygen (ml L^{-1})	2.01
Nitrate (µmol L^{-1})	10.6
Silicate (µmol L^{-1})	23.3
Phosphate (µmol L^{-1})	1.3
Chlorophyll a (mg m^{-3})	27.5

higher turbidity, lower planktonic species richness and discolouration of water [7-9]. Even though *P. quadridentatum* has been reported from different parts of the world, unil now there have been no records of blooms or harmful effects of this species in Indian waters. The study reports the first bloom of *P. quadridentatum* along the tropical waters of Cochin estuary (Southwest coast of India).

Cochin backwater is a productive estuarine system with a high plankton diversity. It is highly influenced by the monsoon run off and inputs from anthropogenic activities which results in a dynamic change in the overall functioning of the system between seasons [10,11]. In November 2018, a sudden discolouration of water was observed near the Marine Science Jetty (Lat. 09°57'51''N; Long. 76°16'56'' E) in Cochin estuary (Fig.1). Phytoplankton samples were collected from the bloom area by filtering ~50 liters of surface water through 20µm mesh bolting silk and analysed using a Leica DM2000 microscope. Hydrobiological parameters such as temperature, salinity and dissolved oxygen were measured immediately and dissolved nutrients (nitrate and silicate) were estimated according to standard protocols. Chlorophyll a was measured spectrophotometrically using a Hitachi U-2900 UV/Visible spectrophotometer following the acetone extraction method.

Microscopic examination of samples collected from the bloom event revealed that the discoloration was caused by the dinoflagellate *P. quadridentatum* (Fig. 2). The cells were solitary, small in size and little longer than wide. The cell was overall ovoid in shape and divided by a cingulum in the middle. The epitheca was conical with pointed apex and the hypotheca was round in shape with four antapical spines, which varied in length. The cells included numerous chloroplasts which were small and somewhat yellow-greenish in colour. A number of *P. quadridentatum* cells undergoing encystment were also observed (Fig. 2).

During the bloom event, the surface chlorophyll a concentration was relatively high (27.5 mg m^{-3}) and *P. quadridentatum* contributed more than 70% of the overall phytoplankton population with a cell density 6.2 x 10^4 cells L^{-1}. As well as *P. quadridentatum*, the dinoflagellate community included *Noctiluca scintillans, Tripos muelleri* and *Protoperidinium* sp. *Skeletonema costatum* and *Nitzschia sigma* were the dominant diatom species.

The *P. quadridentatum* bloom occurred when physical and chemical conditions during the bloom revealed the surface water temperature was 28°C, salinity 20 psu, and nitrate and silicate concentrations were 10.6 µmol L^{-1} and 23.3 µmol L^{-1} respectively. A low dissolved oxygen concentration (2.01 mg L^{-1}) was recorded during the bloom event, but there were no reports of fish mortalities from the bloom area.

This observation is the first bloom report of *P. quadridentatum* along Cochin estuary and further investigation on the bloom dynamics is required. This information will be useful in planning for potential HAB events along Cochin estuary.

References

1. *Gárate-Lizárraga I & MS Muñetón-Gómez 2008. Acta Bot Mex 83: 33–47*
2. *Alkawri A et al 2016. Plankton Benthos Res 11: 75-78*
3. *Okolodkov Y et al 2016. Mar Pollut Bull 108: 289-296*
4. *Stein FRv 1883. In: VvW Engelmann (eds), II Halfte: Die Naturgesichte der Arthrodelen Flagellaten. Leipzig, pp 1–30*
5. *Abé TH 1927. In: Peridiniales. Rep Tôhoku Imper Univer, 4th Ser Biol, Sendai, Japan 2: 383–438*
6. *Hansen G 1995. Phycologia 34: 166–170*
7. *Al-Hashmi K et al 2013. J Fish Aquat Sci 8: 595–606*
8. *Gárate-Lizárraga I & MS Muñetón-Gómez 2008. Acta Bot Mex 83: 33–47*
9. *de Madariaga I et al 1989. Bot Mar 32: 159-165*
10. *Menon NN et al 2000. Hydrobiologia 4: 149-183*
11. *Qasim SZ 2003. Indian Estuaries 259 pp*

Authors

Twinkle Sathish, Lathika Cicily Thomas & Kunnatholickal B Padmakumar, Department of Marine Biology, Microbiology & Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (CUSAT), Kochi-16, Kerala, India

Email corresponding author:
kbpadmakumar@gmail.com

Continued from page 10

been any reports of toxicity associated with Mediterranean strains of *A. taylorii* [5]. For these reasons, further studies will be planned to better understand the potential risks associated with this microalga and its co-occurrence with other phytoplankton species along the Ligurian coast.

Acknowledgements

We are grateful to the ARPAL colleagues involved in the bathing water monitoring program and to Susanna Alloisio (ETT S.p.A., Genoa) for her support. This work was funded by EU project "@lgaWarning" (EuroTransBio-Program ID: ETB-2017-028).

References

1. *Penna A 2002. Mar Ecol 23: 320-328*
2. *Basterretxea G et al 2005. Estuar Coast Shelf Sci 62 (1-2): 1-12*
3. *ISPRA, Quaderni - Ricerca Marina n. 5/2012, ISBN 978-88-448-05586.*
4. *Katsuo et al 2007. Harmful Algae, 6(6), 790-798.*
5. *Satta et al 2010. Adv Ocean Limn 1(2): 259-269*

Authors

Valentina Giussani, Fabrizia Colonna & Rosella Bertolotto, Agenzia Regionale per la Protezione dell'Ambiente Ligure (ARPAL), Genova, Italy

Monica Cangini, Centro Ricerche Marine, Cesenatico (FC), Italy

Stefano Accoroni & Cecilia Totti, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy

Mariachiara Chiantore, Department for Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy

Silvia Casabianca & Antonella Penna, Department of Biomolecular Sciences, University of Urbino, Urbino, Italy

Email corresponding author:
valentina.giussani@arpal.liguria.it