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Note

First record of *Prorocentrum lima* (Dinophyceae) inside harbor areas and along the Abruzzo region coast, W Adriatic

Cristina Ingarao *, Giovanna Lanciani, Carmen Verri, Tommaso Pagliani

Environmental Science Center, Consorzio Mario Negri Sud, Via Nazionale 8/A, 66030 Santa Maria Imbaro, Chieti, Italy

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ABSTRACT

Prorocentrum lima (Ehrenberg) Dodge has been found for the first time during the summer of 2007 inside Ortona harbor and along the coast of the Abruzzo region, a slightly eutrophic area influenced by runoff from a nearby river. The investigations were conducted in two harbors and at six coastal sampling stations. Samplings were conducted using a phytoplankton net and with a pump. Average *P. lima* cellular concentrations were 3.2×10^4 cells L^{-1}. Other well-known toxic and potentially toxic phytoplankton species have been considered. The number of toxic cells from net samples were higher than the numbers of toxic cell samples collected without the net. Occurrences of *P. lima* with abiotic factors revealed that temperature was positively correlated with *P. lima* abundance ($p = 0.01$), while salinity was highly negatively correlated with *P. lima* presence ($p = 0.001$). The total phytoplankton community was studied.

1. Introduction

Prorocentrum lima (Ehrenberg) (Dodge, 1975) is a marine dinoflagellate, epibenthic on sediments or detritus and epiphytic on macroalgae. It is also a neritic and estuarine species, and its distribution ranges from tropical to temperate coastal waters (Faust, 1991). *P. lima* is a small to medium-sized obovate cell with a central pyrenoid and a posterior nucleus. Each valve contains 50–80 regular marginal pores and approximately 60–100 evenly spaced pores on the valve surface (Tomas, 1997).

The attention focused on *P. lima* is due to the toxicity of this species. As a matter of fact, this species produces DSP toxins (diarrhetic shellfish poisoning) that can contaminate filter-feeding shellfish and subsequently cause a gastrointestinal illness in humans (Quilliam and Wright, 1995). The complex of DSP toxins produced by *P. lima* include okadaic acid (OA), dinophysistoxins (DTX-1, DTX-2), prorocentrolide and a fast acting toxin (Moestrup et al., 2004).

The first *P. lima* appearance along the Adriatic Sea coasts of Italy can be traced back to 2006 along Emilia Romagna Region coasts (NW Adriatic) (Pompei pers. comm.) and Ancona coast (Congestri et al., 2006) to the West coast of the Abruzzo region during the summer of 2007.

P. lima has not previously been listed among the dinoflagellates of the Abruzzo coasts (W Adriatic Sea), in particular in the confined harbor waters of this region. This paper underlines and lists the new presence of *P. lima* and other well-known toxic phytoplanktonic species. These species were collected throughout the Abruzzo coastal slightly eutrophic water column of the W Adriatic Sea (Mediterranean Sea), an area influenced by river runoff.

Phytoplanktonic toxic and potentially toxic species just recognized in the Adriatic Sea and studied in this work included: *Alexandrium minutum*; many toxic species of *Dinophysis* genus; the potentially toxic genus *Pseudo-nitzschia*; *Gonyaulax spinifera*; *Lingulodinium polyedrum*; *Prorocentrum minimum* and *Protoceratium reticulatum*.

2. Materials and method

The study was conducted at six sampling stations located at 500 and 3000 m from the coastal cities of Pescara, Ortona and Francavilla during the period of June through August 2007. The sampling stations were located using a Garmin GPS45 GPS (Global Position System). In addition, we sampled monthly inside Pescara and Ortona harbors (Fig. 1). The harbors are considered as potential habitats for the proliferation of epibenthic toxic species like *P. lima* due to the absence of water movement and the stagnant environments (Paerl, 1988).

At each station, one seawater sample was taken from the water 0.5 m from the surface by means of a pump. At each station, net samples were also collected with a 10 μm mesh phytoplankton net, both at the surface and through the water column, in order to observe the presence/absence and abundance of toxic and potentially toxic species. In the harbor area, pump samples were taken 0.5 m from the surface.

Cell abundance concentrations are expressed as cells L^{-1}. Sample counts collected 0.5 m from the surface followed the Utermöhl

* Corresponding author. Tel.: +39 0872 570425; fax: +39 0872 570416.
E-mail address: ingarao@negrisud.it (C. Ingarao).

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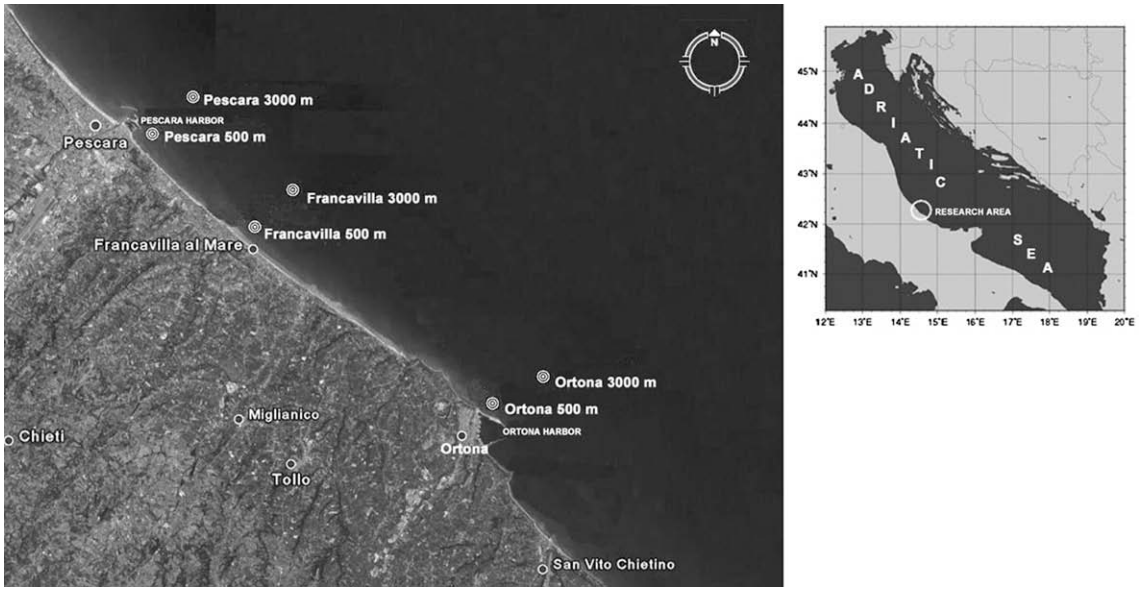

Fig. 1. Research area.

method (Utermöhl, 1958) using a volume of 50 mL, while net samples were counted by reading the entire well using a volume of 2 mL.

Algae observations were conducted using a light microscope (ausJENA Telaval 3 model) at 400× and 1000× magnifications.

For SEM analysis of *P. lima* isolated cultures, cells were fixed in 4% formaldehyde. After storage for three days at 4 °C, cells were washed three times with distilled water, eliminating the supernatant at each washing. They were successively dehydrated in alcohol series up to 100%, dried with CO_2 critical point (CPD), sputter coated with Au/Pd and examined with a Philips XL–30–CP electron microscope according to Zingone et al., (1990).

3. Results and discussion

P. lima cells isolated in culture measured 36.61 μm in length and 25.19 μm in width for a length to width ratio of 1.45. There were 51 ± 1 regular marginal pores and 72 ± 1 small spaced pores per valve (averaged values) (Fig. 2a and b). The right valve had a shallow V-shaped similar to a triangular excavation with a protruding flange (Fig. 2c and d). Finally, in Fig. 3a and b, it is clearly possible to observe the central pyrenoid. Cells were fixed with Lugol's solution and 0.4% formalin, respectively (Zingone et al., 1990).

The first appearance of *P. lima* was on June 13th inside Ortona harbor, specifically on macroalgae *Ulva lactuca,* over the reef around the marginal harbor perimeter. The cellular abundance in this month was 4.7×10^5 cells L^{-1} only in the sample collected at 0.5 m from the surface. This slightly harmful algal bloom phenomenon was verified only inside the harbor area (Table 1). The chemical–physical conditions were: pH 8.19; 24 ± 1 °C water temperature; 33.28 psu salinity; 7.7 mg L^{-1}dissolved oxygen.

Along the coast of study, *P. lima* was absent, but other toxic and potentially toxic species were present in this monthly sample. *Dinophysis caudata* was the most abundant species among the six coastal sampling stations (2.0×10^3 cells L^{-1} in the net sample along the water column; 40 cells L^{-1} in the sample collected 0.5 m from the surface), followed by *Lingulodinium polyedrum* (1.5×10^3 cells L^{-1} in the net sample along the water column; 40 cells L^{-1} in the sample collected 0.5 m from the surface) both collected at Ortona station 500 m from the coast (referred to as 'Ortona 500') (Fig. 4).

On July 9th and August 2nd, *P. lima* cellular concentrations inside the Ortona harbor area were 2.4×10^5 and 5.0×10^4 cells L^{-1}, respectively (only the sample collected at 0.5 m from the surface). These values were lower than those observed during the previous monthly sampling. The presence of *P. lima* was revealed by the observation of the cellular life cycle with a light microscope. On the contrary, this species has been never observed in Pescara harbor.

Chemical–physical parameters measured on July and August at Ortona harbor were, respectively: pH 8.19 and 8.22; 25 and 22 ± 1 °C temperature; 33.18 and 34.52 psu salinity; 7.9 and 7.6 mg L^{-1} dissolved oxygen.

A periodic decrease in *P. lima* cellular abundances inside Ortona harbor corresponded with a monthly increase outside the harbor itself, specifically at 500 m from the coast of this station during July and August. The cellular concentrations were 1.0×10^3 and 5.0×10^2 cells L^{-1} in net samples along the water column, respectively; and 40 cells L^{-1} in July and 20 cells L^{-1} in August in samples collected at 0.5 m from the surface. Chemical–physical parameters measured in this sampling point (Ortona 500) during these two last months were, respectively: pH 8.18 and 8.21; 25 and 23 ± 1 °C water temperature; 33.38 and 34.81 psu salinity; 8.0 and 7.5 mg L^{-1} dissolved oxygen.

Statistical analyses were carried out on the monthly trends of *P. lima* cellular concentrations and chemical–physical parameters during the period of monitoring at all eight sampling stations in order to address the relationships between the occurrence of *P. lima* and abiotic factors (Table 2). Temperature trends were positively correlated with *P. lima* abundances ($r = 0.31$, $p < 0.01$, $N = 24$), while salinity trends were highly negatively correlated with *P. lima* content ($r = 0.95$, $p < 0.001$, $N = 24$).

Other toxic and potentially toxic species were present in these two months along the coast being studied (maximum concentrations): *Prorocentrum minimum* (7.5×10^3 cells L^{-1} in net samples along the water column; 60 cells L^{-1} in samples collected 0.5 m from the surface) in July at Pescara station 500 m from the coast; the potentially toxic genus *Pseudo-nitzschia* (1.1×10^6 cells L^{-1} in net samples along the water column; 3.0×10^5 cells L^{-1} in samples collected 0.5 m from the surface) in July at Francavilla station 500 m from the coast; *Dinophysis caudata* (4.5×10^3 cells L^{-1} in net samples along the water column; 60 cells L^{-1} in samples