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First record of the dinoflagellate *Alexandrium catenella* (Dinophyta), a potential producer of paralytic shellfish poisoning, in Italian waters (Sardinia, Tyrrhenian Sea)

Abstract

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Alexandrium catenella (Whedon & Kofoid) Balech, a dinoflagellate known as a potential producer of Paralytic Shellfish Poisoning (PSP), is reported for the first time from a new geographical area of the Mediterranean Sea (Sardinia, Italy). Till now, the distribution of *A. catenella* over the Mediterranean had been limited to Spain and possibly France together with recent, associated events of PSP-toxicity in shellfish. Specimens of *A. catenella* detected in summer 1999 in Sardinia (Gulf of Olbia) exhibited cell size and pattern of thecal plates comparable with those described from other localities, being mostly observed in two to four-celled chains. Despite the existence in literature of some descriptions of this species as a cold-water organism, in Sardinia the environmental scenario during the occurrence of *A. catenella* was typical of the warm season (water temperature: 26 °C), consistent with recent observations from the Catalan coast. The finding of *A. catenella* in the Gulf of Olbia (Tyrrhenian Sea) – a restricted, eutrophic area holding a commercial harbour and an intensive activity of mussel farming – suggests a possible introduction of this species through either the ballast waters discharged from foreign ships or the import-trade of shellfish from other countries. The lack of any previous evidence of this dinoflagellate in stations of the gulf monitored on a routine basis since 1992 further supports the non-indigenous character of *A. catenella* in the affected Tyrrhenian area and its transport from other localities via the above-mentioned means of dispersal. The potential for future bloom events, spreading to new Italian areas and possible constraints to the sustainable development of coastal areas must be considered.

Introduction

Dinoflagellates producing paralytic shellfish poisons (PSP) are receiving an increasing attention due to the public health risk and economic setbacks connected with their occurrence in marine and brackish waters world-wide (Hallegraeff & al. 1991). Many of the causative organisms are small gonyaulacoid species producing smooth-walled resting cysts, often playing a determinant role in the initiation of blooms. A global increase in toxic or harmful algal blooms (HABs) has also involved the Mediterranean Sea, with various species of *Alexandrium* Halim described from different regions (e.g. Garcés 1998; Daly

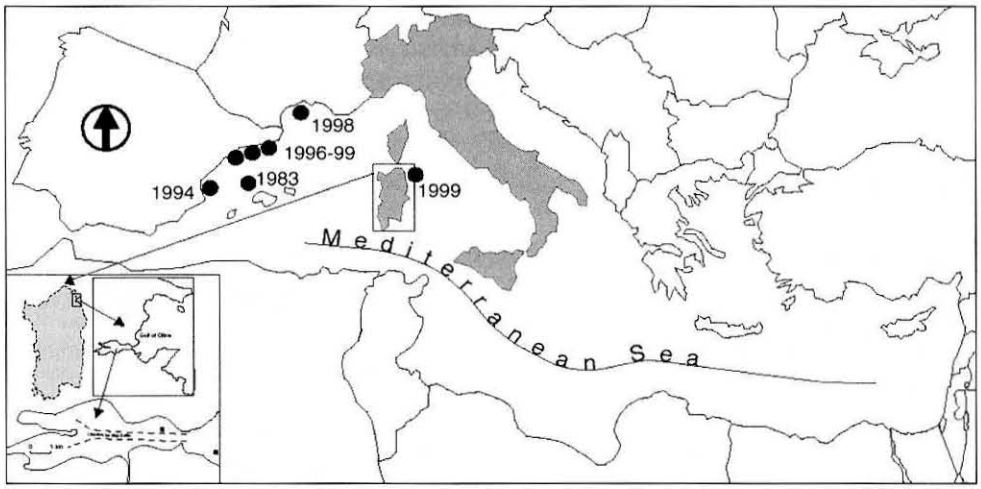

Fig. 1. Map of the Mediterranean area showing the localities affected by *A. catenella* (1983-1999) and detail with the two sampling points within the Olbia Harbour, Sardinia (circle = no cells of *A. catenella*; square = presence of *A. catenella*). Maximum density of this species in Catalonia, Spain (Vila 2001; Vila & al. 2001b).

Yahia-Kefi & al. 2001; Vila 2001). Despite this, only a few records of *Alexandrium catenella* (Whedon & Kofoid) Balech from the Mediterranean can be found in the scientific literature dealing with the biogeography of this species. References include the Balearic Basin (Margalef & Estrada 1987), Valencia harbour (Gomis & al. 1996) and Catalan coast of Spain (Vila 2001; Vila & al. 2001a, 2001b), suggesting a recent, rapid spreading of this dinoflagellate along the NW Spanish coastline with associated cases of toxicity. Toxic events were also reported from the Mediterranean coast of France (Thau Lagoon), where mussels accumulated high amounts of PSP-toxins (Masselin & al. 2001). Gonyautoxins and saxitoxins were also identified in plankton samples containing the *A.* "*tamarense* complex" and suspected cells of *A. catenella*. The occurrence of this species in the French area is further supported by additional characterizations on genetic basis (Lilly & al. 2002).

Here we document the first finding of *A. catenella* in Italian waters, as observed during a monitoring program in coastal areas of Sardinia. The apparent non-indigenous character of *A. catenella* from the Gulf of Olbia (Tyrrhenian Sea) and the possible ways of introduction of this species in the affected area are discussed.

Study area

The Gulf of Olbia (North-Eastern Sardinia, Fig. 1) is a typical "rias", with slow water exchange and significant freshwater inflow from two municipal sewers. Due to these characteristics, it is eutrophic (Sechi & al. 1987; Sannio & al. 1996, 1997). The gulf has an area of 6,5 km^2, a mean depth of about 5 m and a maximum depth of about 10 m, along the channel that allows traffic of big ships. The inner part holds one of the most important commercial ports of the island (with urban, tourist and industrial activities) and the largest

area of mussel (4000 t y^{-1}) and clam farming. Mariculture also involves an import-trade of shellfish from other localities of Italy and Europe, especially in summer when the market demand increases.

Methods

Water samples were taken fortnightly (July-August 1999) at about 0,5 m from the surface, on a transect located in the inner part of the Gulf of Olbia (two stations, 500 m and 3000 m off the coast; Fig. 1). Cell density was determined by using the Utermöhl technique (1931) after fixing the samples with neutralised formaldehyde and settling 100 ml. Microscopical analyses were performed by Axiovert Zeiss microscopes; the counts covered the whole bottom of the settling chamber, using a magnification of 200x.

Fixed specimens of *A. catenella* were isolated from the plankton assemblages, stained with Calcofluor White M2R (Fritz & Triemer 1985), and examined by light microscopy (LM) in epifluorescence under U.V. excitation (Axioplan, filter set Zeiss 487902, 1000x magnification). Tabular formula and morphological features of the thecal plates were studied following the criteria of Balech (1995).

Temperature, salinity and fluorometric chlorophyll *a* were measured with a multiparameter probe Idromar. Total phosphorus and dissolved inorganic nitrogen (sum of nitrate, nitrite and ammonium nitrogen) were determined according to Strickland & Parsons (1972).

Results and discussion

Specimens of *A. catenella* detected in summer 1999 in Sardinia (Gulf of Olbia) exhibited cell size and pattern of thecal plates comparable with those described from other localities, being mostly observed in two to four-celled chains, slightly bent (Fig. 2 a-b). Single cells, as well as chains of six to eight cells, were also found although more rarely (Fig. 2 c-f). Cell size fell within the range reported by Balech (1995) for this species, being 25-34 µm in length and 29-37 µm in width (mean: 28 µm long, 31 µm wide; n=10). In contrast, cell measures somewhat exceeded those of isolates from Australia and Spain (Hallegraeff & al. 1991; Vila & al. 2001b).

The cells examined from Olbia displayed morphological characteristics typical of this species. In the epitheca, the 6" precingular plate was medium-sized (Fig. 2 d), 1' (first apical plate) had no ventral pore and contacted directly the apical pore plate (Po). Po plate was triangular-shaped with an anterior attachment pore (Fig. 2 e). Cingulum displaced of 1 cingular height (Fig. 2 b, h). In the hypotheca, the posterior sulcal plate (Sp) had a large, round posterior attachment pore (pap) linked to the Sp right margin by a small channel (Fig. 2 i).

A. catenella was observed in summer 1999 in the inner part of the gulf at a station located about 500 m off the coast (Fig. 1). Its occurrence was limited in both temporal and spatial terms, based on the following issues:

- cells were detected only in a single occasion (25th August), whereas there was no evidence of *A. catenella* during the fortnightly samplings performed before and after that date;

- the finding involved a single station, although a wider project of coastal monitoring covered a number of points located inside and outside the Gulf of Olbia, as well as nearly the whole coast of Sardinia.

The presence of *A. catenella* at Olbia, with a density of 2,2 x 10^3 cells l^{-1}, coincided with environmental conditions characterised by a temperature of 26 °C and a salinity of 37,8‰ (Fig. 3). The concentration of dissolved inorganic nitrogen was 0,62 µM (NH_4-N = 42%, NO_3-N = 39%) and total phosphorus 0,68 µM (Fig. 3). These values were lower than the annual averages previously indicated (Sannio & al. 1996), as well as than those

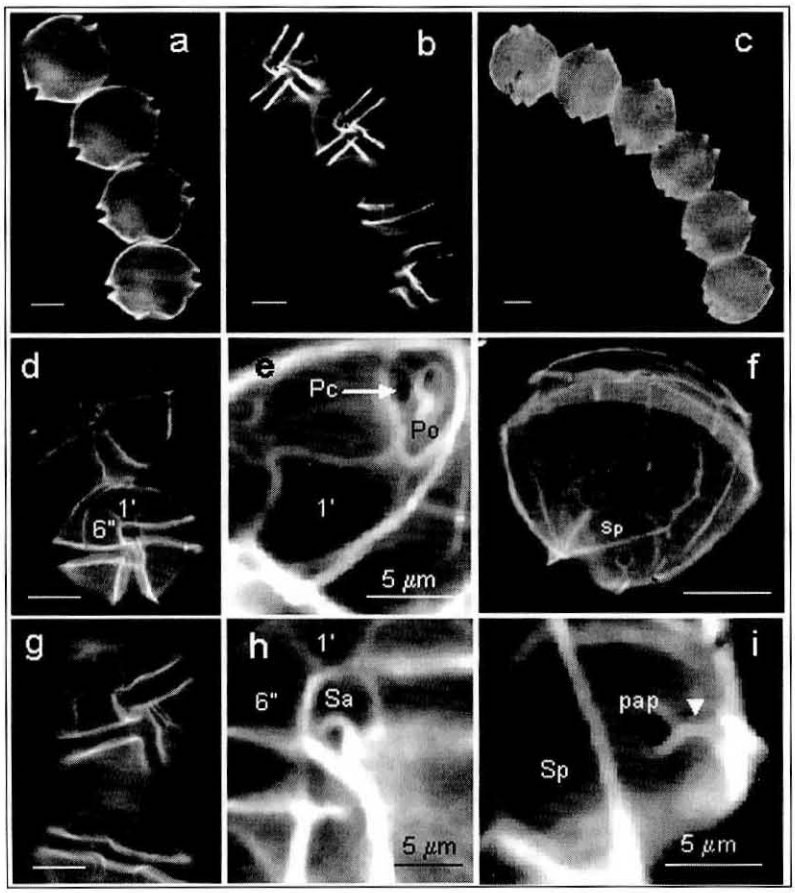

Fig. 2. Specimens of *A. catenella* from the Olbia harbour (Tyrrhenian Sea), stained with Calcofluor White. LM photographs (fluorescence). (a-c) Chains of four to six cells somewhat curved. (d) Chain of two in ventral view. 6" plate of medium-size. (e) 1' plate without a ventral pore, direct contact of 1'-Po; Po triangular with a connecting pore (Pc). (f) Dorsal view and posterior sulcal plate (Sp). (g-h) Cingulum displacement and anterior sulcal plate (Sa). (i) Sp with a large posterior attachment pore (pap). A channel connects pap to Sp right margin (arrowhead). Scale bars = 10 µm, unless indicated.

described from the Mediterranean coast of Spain (Vila & al. 2001a). In the Olbia harbour, a slight increase in chlorophyll *a* and a modest peak of the total phytoplankton density were observed simultaneously to the appearance of *A. catenella* (Fig. 3), being particularly due to an increase in diatoms. In contrast, dinoflagellates (4,2 x 10^3 cells l^{-1}) represented a modest part of the phytoplankton assemblage (Fig. 3), with chain-forming gymnodinioids and other species listed in Figure 3. *A. catenella* was the dominant dinoflagellate at the time of its finding.

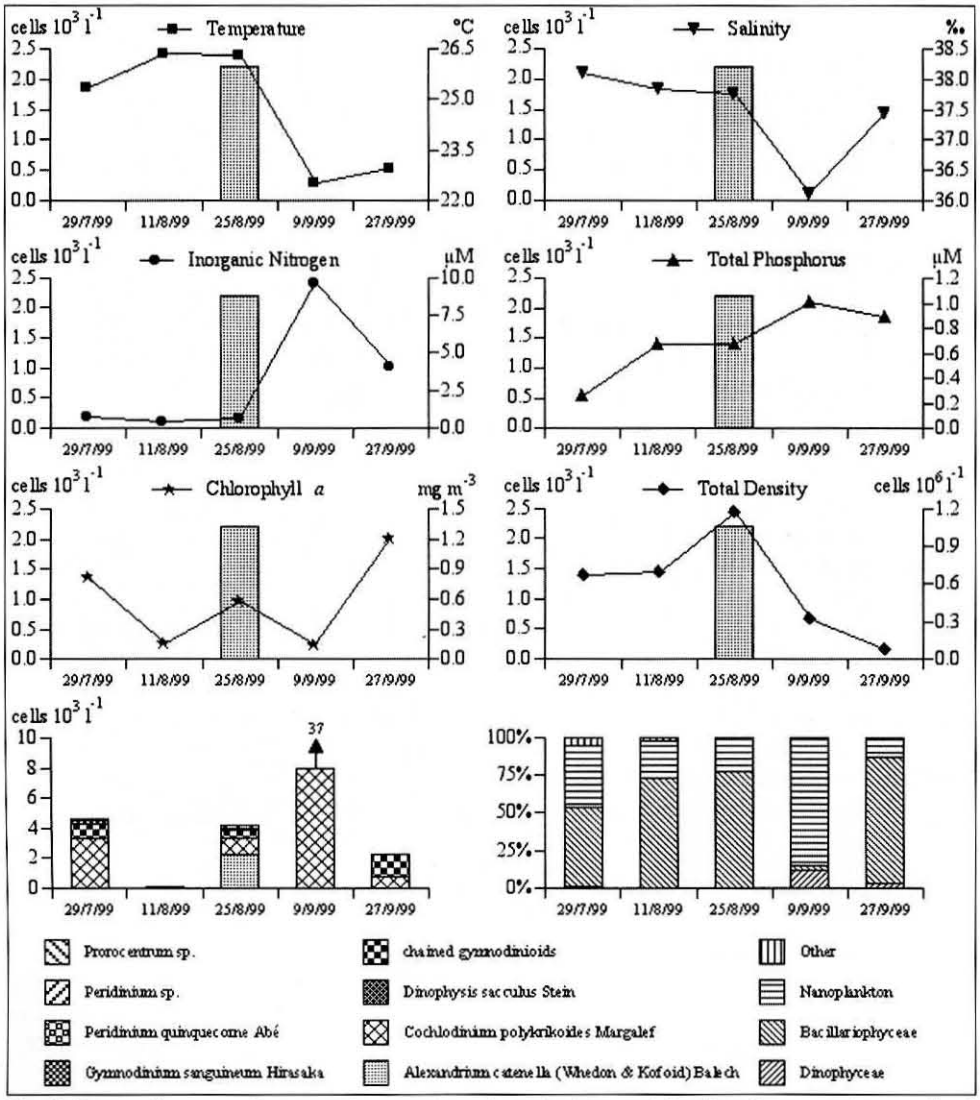

Fig. 3. Status of some environmental parameters, phytoplankton and dinoflagellates species associated to *A. catenella* (Gulf of Olbia, summer 1999).

Conclusion

Despite the existence in literature of some descriptions of *A. catenella* as a cold-water organism, in Sardinia the environmental scenario during the occurrence of this species was typical of the warm season, consistent with recent observations from the Catalan coast. The finding of *A. catenella* in the Gulf of Olbia (Tyrrhenian Sea) - a restricted, eutrophic area - suggests a possible introduction of this species through either ballast waters discharged from foreign ships or the import-trade of shellfish. These two assumptions are based on the presence in the area of an important commercial harbour and an intensive shellfish farming involving, especially in summer, the transfer of mussel stocks from other European countries. Both hypotheses have already been considered as likely ways of introduction and dispersal of species into new areas, via e.g. the transport of viable, resting cysts (see Vila 2001 for a review). Then, the spreading of HAB species along the coastline could be further favoured by the presence of confined environments with low turbulence and low water renewal rates (Vila & al. 2001b).

The lack of any evidence of *A. catenella* in stations of the gulf monitored on a routine basis since 1992 further supports the non-indigenous character of this species in the affected Tyrrhenian area. Its recent appearance in the Olbia harbour, although in very low cell density compared with the peaks achieved in the Barcelona harbour (Spain), may not be negligible on the basis of the possible risks of shellfish contamination by PSP-toxins. The potential for future bloom events, spreading to new Italian areas and eventual constraints to the sustainable development of coastal areas must be considered.

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