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Studies on dinoflagellate cyst assemblages in two estuarine Mediterranean bays: A useful tool for the discovery and mapping of harmful algal species

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ABSTRACT

The composition and assemblages of living dinoflagellate cysts from two estuarine bays (Alfacs and Fangar bays) in the northwestern Mediterranean Sea were investigated, focusing on the presence and distribution of harmful species. Sediment cores were taken from 10 stations in Alfacs Bay and from 6 stations in Fangar Bay. Sediment samples from the surface (the top 1 cm) and the subsurface profile (from 2 to 5 cm depth) in selected stations, were analyzed. Sixty-two morphotypes were recovered, some of which are new reports for the northwestern Mediterranean area. Few morphotypes dominated in terms of abundance and relative percentage (e.g. the *Scrippsiella trochoidea* complex was the dominant and most widely distributed morphotype in each bay, reaching maxima of 163 cysts cm^{-3} wet sediment (ws) and 102 cysts cm^{-3} ws in Alfacs and Fangar bays, respectively). The assemblage in Alfacs Bay was also characterized by the presence of *Biecheleria cincta* (maximum 116 cysts cm^{-3} ws), whereas the occurrence of *Pentapharsodinium tyrrhenicum* (maximum 37 cysts cm^{-3} ws) was greater in Fangar Bay. Twelve morphotypes belonging to potentially toxic or noxious species were detected, with the genus *Alexandrium* dominating. Among the harmful species, *Gymnodinium litoralis* and *Vulcanodinium rugosum* are reported for the first time from the study areas. Furthermore, cysts of the high biomass bloom-forming species *Kryptoperidinium foliaceum* are reported for the first time in the Mediterranean Sea. All the harmful species, with the exception of *Alexandrium minutum*, showed greatest abundances in subsurface samples. Profile analysis led to the description of a new cyst morphotype belonging to the *Alexandrium* genus (presumably *A. insuetum*). Our results provide information on the presence of harmful species in the studied bays, confirming the usefulness of cyst analysis in assessment of the potential risk of harmful blooms in aquaculture areas.

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1. Introduction

Dinoflagellates are one of the most important microalgal groups, comprising approximately 2000 described extant species (Graham and Wilcox, 2000). The life cycle of many of these is characterized by a planktonic vegetative stage, and at least 10% of the species also have a known benthic stage (Head, 1996). The benthic resting cyst stage is non-motile, and is commonly produced through sexual reproduction (Walker, 1984; Figueroa et al., 2008 and references therein). A unique case of asexual reproduction is also reported (Kremp and Parrow, 2006). Many species are able to produce other types of cysts (e.g. pellicle cysts; Dale, 1977; Bravo et al., 2010). The ability to produce cysts is of fundamental ecological importance to microalgal species, as it ensures their survival, facilitates the recurrence of blooms, and promotes dispersion (Dale, 1977; Anderson and Wall, 1978; Dale, 1983; Anglès et al., 2012).

Once settled, cysts accumulate in the sediments where they remain viable for extended periods (from months up to a century; Lundholm et al., 2011). For those species that produce cysts, this characteristic facilitates assessment of the sediment as a record of their occurrence over time. Thus, cyst studies are a useful supplement to information derived from plankton surveys (Dale, 1983). It is often difficult to define the complex composition of planktonic communities. In particular, the high temporal variability in the cycles of presence/absence of species in the water column (from days to months), the commonly low abundances, and the low frequency of plankton sampling in space and time can lead to

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the under-estimation of, or failure to detect, species (Dale, 1983; Moscatello et al., 2004).

The application of cyst assemblage studies to harmful algal species will extend knowledge of these microalgae. This approach increases the likelihood of detecting the occurrence of species not previously reported from particular areas (e.g. *Alexandrium* species; Montresor et al., 1998; Bravo et al., 2006), and also reveals the potential for the occurrence of blooms in the future (Anderson and Wall, 1978; Joyce et al., 2005; Bravo et al., 2006). Characterizing the distributional patterns of harmful species in both the water column and sediments, and identification of the microalgal community associated with these species, will be useful in enhancing understanding of harmful algal blooms (HABs) (GEOHAB, 2001). Advances in this area are very pertinent to aquaculture localities where HABs can cause major economic losses.

Alfacs and Fangar bays (Ebre River delta, northwestern Mediterranean Sea) are the most important aquaculture sites (fish and shellfish farming) along the northeast coast of Spain (Catalonia). Since 1989, outbreaks of diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP) have been reported (IRTA – Research and Technology, Food and Agriculture Monitoring Program). As a consequence, a monitoring program aimed at detecting toxic phytoplankton species and related toxins in shellfish harvesting areas has been in place since 1989; this has provided historical and practical information on planktonic stages of these species in the monitored areas. Despite the information obtained for these two areas from routine plankton monitoring, there are few data on the presence, abundance, and distribution of cysts. Bravo et al. (2006) presented the first data on resting stages, specifically in Alfacs Bay, but their work was focused only on the toxic genus *Alexandrium*.

The primary objective in this study was the identification and quantification of cysts produced by potentially harmful dinoflagellates in recent sediments from Alfacs and Fangar bays. Samples were obtained from the top 5 cm of sediment to confirm the usefulness of cyst studies in assessment of the potential risk of blooms. The total cyst assemblage was characterized to assess the diversity of dinoflagellates in these shallow and estuarine areas.

2. Materials and methods

2.1. Study areas

Alfacs and Fangar bays are two semi-enclosed water bodies located in the northeast coast of Spain (Catalonia; Fig. 1), separated from the Mediterranean Sea by sandy spit barriers. Alfacs Bay is located in the southern part of the Ebre River delta and it has a surface area of 49 km^2 and an average depth of about 3 m. The mouth of the bay is 2 km wide. The edge of the bay is surrounded by a shallow shelf (18 km^2) that slopes gently from 0 to 1.5 m, with an average depth of 0.6 m. Further out the shelf slopes more steeply, descending to a muddy central basin having a maximum depth of 6.5 m and an area of 31 km^2. Between April and October each year the bay receives freshwater inputs of approximately $275 \times 10^6\ m^3\ yr^{-1}$ (Camp and Delgado, 1987) from its northern edge, but much less during the remainder of the year; this represents a seasonal pattern driven by rice cultivation in the region. Fangar Bay is smaller than Alfacs Bay, and it is located in the northern part of the Ebre River delta. It measures approximately 12 km^2 wide and is very shallow, with a maximum depth of approximately 4 m. The mouth of the bay faces northwest, and consequently the bay is exposed to strong northwesterly winds originating from the Ebre Valley. A shallow shelf delimits the edge of the bay, and slopes gently from 0 to 1.5 m, and then more steeply to the central basin, were the maximum depth is 4 m. The bay receives freshwater inputs of approximately $185 \times 10^6\ m^3\ yr^{-1}$

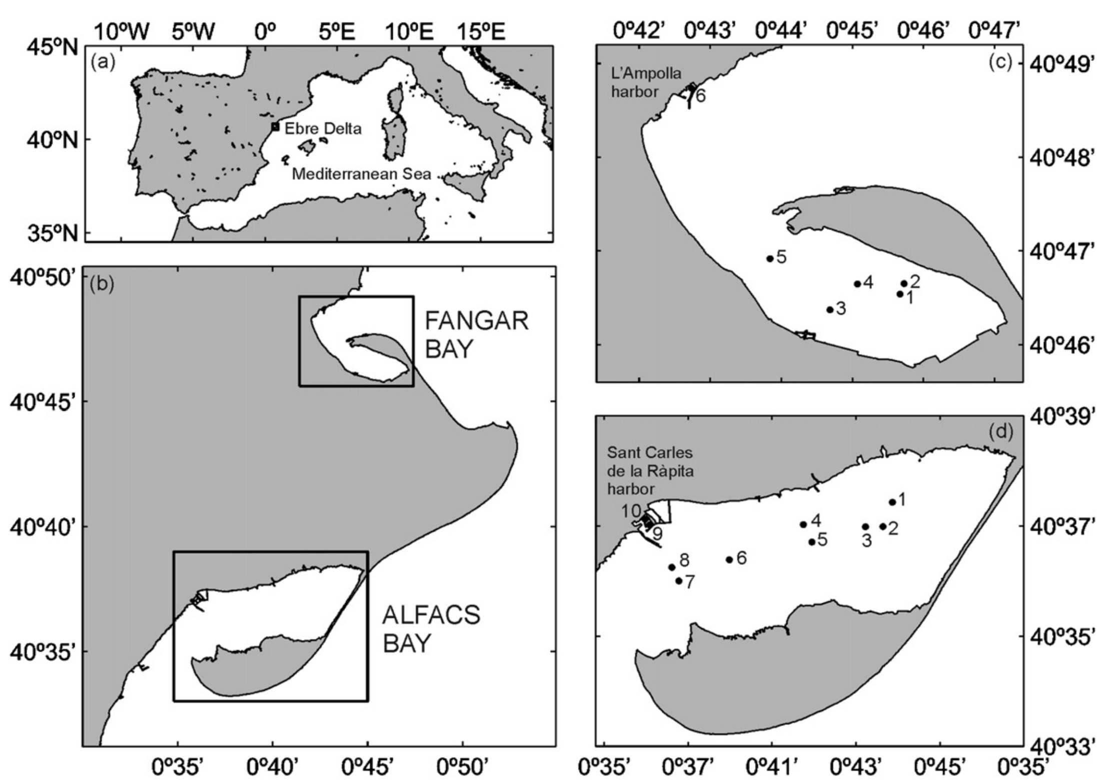

Fig. 1. Locations of the Ebre River delta (a), Alfacs and Fangar bays (b), sampling stations in L'Ampolla harbor and Fangar Bay (c), sampling stations in Sant Carles de la Ràpita harbor and Alfacs Bay (d).

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