

Heterogeneous distribution in sediments and dispersal in waters of *Alexandrium minutum* in a semi-enclosed coastal ecosystem



Z.K. Klouch^{a,b}, F. Caradec^a, M. Plus^a, T. Hernández-Fariñas^{a,b}, L. Pineau-Guillou^c,
A. Chapelle^a, S. Schmitt^a, J. Quéré^a, L. Guillou^b, R. Siano^{a,*}

^a Ifremer – Centre de Brest, DYNECO PELAGOS, F-29280 Plouzané, France

^b Sorbonne Universités, UPMC Univ. Paris 6, CNRS, Adaptation et Diversité en Milieu Marin (UMR 7144), équipe DIPO, Station Biologique de Roscoff, Place Georges Teissier, CS90074, 29688 Roscoff, France

^c Ifremer – Centre de Brest, LOPS OC, F-29280 Plouzané, France

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ABSTRACT

Within the framework of research aimed at using genetic methods to evaluate harmful species distribution and their impact on coastal ecosystems, a portion of the ITS1rDNA of *Alexandrium minutum* was amplified by real-time PCR from DNA extracts of superficial (1–3 cm) sediments of 30 subtidal and intertidal stations of the Bay of Brest (Brittany, France), during the winters of 2013 and 2015. Cell germinations and rDNA amplifications of *A. minutum* were obtained for sediments of all sampled stations, demonstrating that the whole bay is currently contaminated by this toxic species. Coherent estimations of ITS1rDNA copy numbers were obtained for the two sampling cruises, supporting the hypothesis of regular accumulation of *A. minutum* resting stages in the south-eastern, more confined embayments of the study area, where fine-muddy sediments are also more abundant. Higher ITS1rDNA copy numbers were detected in sediments of areas where blooms have been seasonally detected since 2012. This result suggests that specific genetic material estimations in superficial sediments of the bay may be a proxy of the cyst banks of *A. minutum*. The simulation of particle trajectory analyses by a Lagrangian physical model showed that blooms occurring in the south-eastern part of the bay are disconnected from those of the north-eastern zone. The heterogeneous distribution of *A. minutum* inferred from both water and sediment suggests the existence of potential barriers for the dispersal of this species in the Bay of Brest and encourages finer analyses at the population level for this species within semi-enclosed coastal ecosystems.

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1. Introduction

The recovery of resting stages of harmful microalgae in sediment samples and the identification of accumulation sites indicate potential seeding sources for the initiation of blooms (Anderson et al., 2012). Cyst bank mapping is therefore particularly useful for the risk assessment of harmful microalgae, since it enables the prediction of blooming areas and the optimization of the management of coastal economic activities.

The distribution of *Alexandrium* species in coastal and shelf waters is relatively well known; however, comprehensive distributional data, especially for resting stage banks, are still needed (Anderson et al., 2012). On the basis of the available information,

some common features in the distribution of *Alexandrium* cysts can be identified. Previous studies have reported that cyst accumulations are favored in fine-muddy rather than sandy sediments (White and Lewis, 1982; Kremp, 2000; Yamaguchi et al., 1996; Joyce et al., 2005; Gayoso, 2001; Matsuoka et al., 2003; Wang et al., 2004; Anderson et al., 2005; Anglès et al., 2005; Horner et al., 2011; Genovesi et al., 2013; Trikia et al., 2014; Fertouna-Bellakhala et al., 2015), supporting the hypothesis that dinoflagellate resting stages behave physically like fine particles (Dale, 1983). In semi-enclosed, confined ecosystems discrete cyst banks may be found (Anderson et al., 2012). A close link between the local distribution of cyst banks and blooms has been documented for some *Alexandrium* species in estuaries (Cembella et al., 1988; Crespo et al., 2011; Anderson et al., 2014), lagoons (Genovesi et al., 2009, 2013; Trikia et al., 2014; Fertouna-Bellakhala et al., 2015), and harbors (Anglès et al., 2005; Bravo et al., 2008). This local distribution has been associated with the hydrodynamic features of the studied

* Corresponding author.

E-mail address: raffaele.siano@ifremer.fr (R. Siano).

ecosystems. Despite occurring in adjacent waters, local blooms of *A. fundyense* Balech were temporally separated, probably due to water retention in the first site where the blooms occurred (Crespo et al., 2011). Cyst densities of *A. tamarense* (Lebour) Balech were influenced by local hydrodynamics, with wind-induced currents causing cyst dispersal in the shallow ecosystem of the Thau lagoon (Genovesi et al., 2013). These examples prove the interest of characterizing discrete, fine spatial scale cyst distributions to deduce local bloom dynamics in semi-enclosed ecosystems.

Traditionally, hot spots of harmful microalgae accumulation in sediments are identified by microscopy counting of their cysts (Genovesi et al., 2013), a method that is time-consuming and suffers from taxonomical limitations in identification due to the lack of distinctive morphological characteristics for the cysts of some species. In contrast, the analysis of specific genetic material in sediment has proved to be a valuable alternative to infer cyst distribution, enabling a large number of samples to be processed objectively in a relatively short time. The amplification of species-specific marker genes from DNA preserved in sediment samples has been used to infer the presence of dinoflagellate cysts (Godhe et al., 2002; Penna et al., 2010). Real-time PCR amplification to quantify DNA genes from sediments has been shown to be a good proxy for cyst abundances, including some *Alexandrium* species (Kamikawa et al., 2007; Erdner et al., 2010; Park and Park, 2010). Lastly, cyst species have been mapped using the fluorescence in situ hybridization (FISH) technique (Hattenrath-Lehmann et al., 2016). Genetic techniques can therefore be used to provide reliable information on accumulation spots of cysts and to infer ecological patterns. Given the close association between resting cyst abundance and sediment type, high specific DNA abundances should also be found in the corresponding muddy sediments. The DNA extracted from sediments, however, can be of both

intercellular (resting stages) (Godhe et al., 2002; Erdner et al., 2010) and extracellular origin (Pietramellara et al., 2009), making the relationship between specific DNA traces and sediment type not completely predictable and still barely studied.

Although the analysis of genetic material in sediment cores showed that *A. minutum* Halim has been present in the Bay of Brest since at least the 19th century (Klouch et al., 2016), the vegetative form of the species was first identified within the framework of the REPHY (REseau de surveillance et d'observation du PHYtoplankton et des PHYcotoxines: http://envlit.ifremer.fr/surveillance/phytoplankton_phycotoxines/presentation) in 1990. The first cyst abundance survey was carried out in the same year and no cyst of *A. minutum* was found in four estuarine stations of the Bay of Brest (Erard-Le Denn et al., 1993; Erard-Le Denn and Boulay, 1995). The species abundance increased over time in the bay, reaching the record concentration of ca. 42×10^6 cells l^{-1} in July 2012 (Chapelle et al., 2015; Klouch et al., 2016) in the small, enclosed Daoulas estuary, where bloom occurrences were unsuspected and monitoring was not carried out. In parallel, other blooms of the species were observed in other estuaries on the bay (Elorn River, Aulne River), but these were of minor importance ($< 2 \times 10^6$ cells l^{-1}). After the 2012 event, monitoring of Daoulas Bay was initiated and seasonal blooms of *A. minutum* are observed in the Daoulas estuary along with blooms of lower cell abundance in other monitored estuaries. The development of different intensities in the Bay of Brest raises questions about the distribution of the major cyst banks of the species and the potential connectivity between its different adjacent estuarine ecosystems.

In this study, both genetic analyses of sediments and model simulations in the water column were used to try to explain the heterogeneity of *A. minutum* bloom occurrence in the Bay of Brest. With a recently developed real-time PCR assay (Klouch et al.,

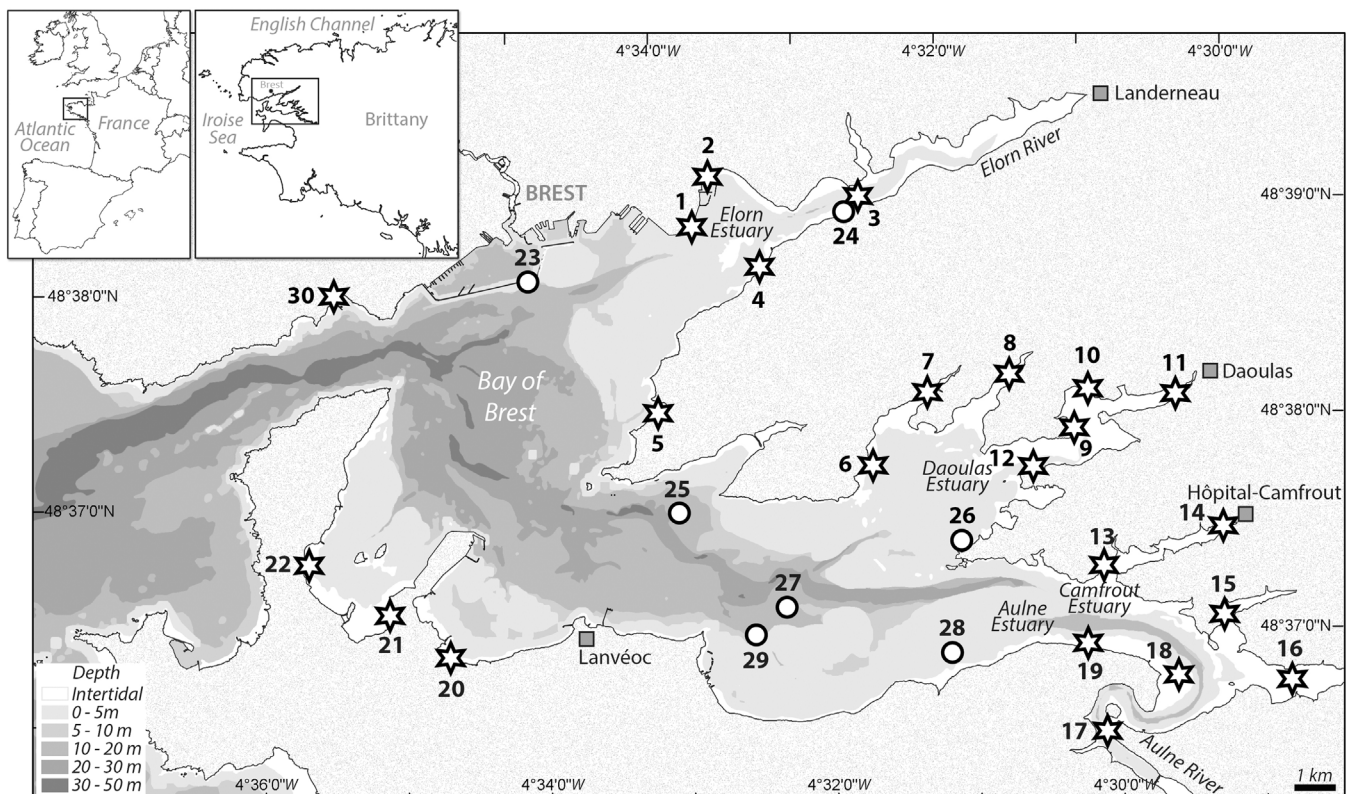


Fig. 1. Map of the Bay of Brest indicating intertidal (stars) and subtidal (circles) sampling stations.

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