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Termination of a toxic *Alexandrium* bloom with hydrogen peroxide

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ABSTRACT

The dinoflagellate *Alexandrium ostenfeldii* is a well-known harmful algal species that can potentially cause paralytic shellfish poisoning (PSP). Usually *A. ostenfeldii* occurs in low background concentrations only, but in August of 2012 an exceptionally dense bloom of more than 1 million cells L^{-1} occurred in the brackish Ouwerkerkse Kreek in The Netherlands. The *A. ostenfeldii* bloom produced both saxitoxins and spirolides, and is held responsible for the death of a dog with a high saxitoxin stomach content. The Ouwerkerkse Kreek routinely discharges its water into the adjacent Oosterschelde estuary, and an immediate reduction of the bloom was required to avoid contamination of extensive shellfish grounds. Previously, treatment of infected waters with hydrogen peroxide (H_2O_2) successfully suppressed cyanobacterial blooms in lakes. Therefore, we adapted this treatment to eradicate the *Alexandrium* bloom using a three-step approach. First, we investigated the required H_2O_2 dosage in laboratory experiments with *A. ostenfeldii*. Second, we tested the method in a small, isolated canal adjacent to the Ouwerkerkse Kreek. Finally, we brought 50 mg L^{-1} of H_2O_2 into the entire creek system with a special device, called a water harrow, for optimal dispersal of the added H_2O_2. Concentrations of both vegetative cells and pellicle cysts declined by 99.8% within 48 h, and PSP toxin concentrations in the water were reduced below local regulatory levels of 15 $\mu g\ L^{-1}$. Zooplankton were strongly affected by the H_2O_2 treatment, but impacts on macroinvertebrates and fish were minimal. A key advantage of this method is that the added H_2O_2 decays to water and oxygen within a few days, which enables rapid recovery of the system after the treatment. This is the first successful field application of H_2O_2 to suppress a marine harmful algal bloom, although *Alexandrium* spp. reoccurred at lower concentrations in the following year. The results show that H_2O_2 treatment provides an effective emergency management option to mitigate toxic *Alexandrium* blooms, especially when immediate action is required.

1. Introduction

Harmful algal blooms (HABs) have been a consistent problem in coastal regions worldwide (Smayda, 1990; Anderson et al., 2002, 2008; Heisler et al., 2008), and seem to increase in terms of frequency, severity and the geographical expansion of toxic species (Hallegraeff, 1993; Paerl and Huisman, 2008). These blooms can cause devastating impacts on public health, shellfisheries and local wildlife (Ayres, 1975; Paerl, 1988; Scholin et al., 2000; Hoagland et al., 2009).

The dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech and Tangen is a HAB species known to produce saxitoxins associated with paralytic shellfish poisoning (PSP). Saxitoxin is a particularly acute paralytic shellfish toxin with as little as 1 mg fatal to humans (Bates and Rapoport, 1975). *A. ostenfeldii* can also produce spirolides, fast-acting neurotoxins which induce similar symptoms as saxitoxin but are not commonly produced by other species of the *Alexandrium* genus (Cembella et al., 2000; Otero et al., 2010). Usually, *A. ostenfeldii* strains produce either saxitoxins or spirolides (Suikkanen et al., 2013). In only a few strains, including European strains isolated from Danish and Scottish waters, both saxitoxins and spirolides were found simultaneously (Cembella et al., 2000; MacKinnon et al., 2006; Brown et al., 2010). These toxins can accumulate in shellfish and other filter-feeding organisms, often rendering them too toxic for consumption (Bates and Rapoport, 1975; Glibert et al., 2007; Etheridge, 2010). As is common with dinoflagellates, *A. ostenfeldii* has both vegetative and cyst life stages. A pellicle, or temporary cyst, forms directly from the

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vegetative stage as a highly sensitive response to environmental disturbances (Jensen and Moestrup, 1997; Bravo et al., 2010). This is a short-term cyst stage that allows for re-germination between 1 and 17 days (Bravo et al., 2010). In addition, *A. ostenfeldii* can also produce sexual cysts (also known as resting cysts), which form from sexual fission of two vegetative gametes when growth conditions decline and can persist in sediment for several years until optimal environmental conditions return (Jensen and Moestrup, 1997; McQuoid et al., 2002).

Alexandrium ostenfeldii is typically found in marine and brackish waters of temperate and cold regions (Balech and Tangen, 1985; Okolodkov and Dodge, 1996; Mackenzie et al., 1996; Maclean et al., 2003). It is relatively common in Europe, with reports from, e.g., Norway, Denmark, the Baltic Sea, Scotland, Italy and Spain (Jensen and Moestrup, 1997; Scatasta et al., 2003; Gribble et al., 2005; Kremp et al., 2009; Otero et al., 2010). The species usually occurs in low background numbers, but during recent years high concentrations of 10,000–2 million cells L^{-1} have been found in coastal waters of the Baltic Sea (Kremp et al., 2009; Hakanen et al., 2012). Although one of the earliest descriptions of a bloom attributed to this species comes from nearby Belgium (Woloszynska and Conrad, 1939), there are no reported bloom events of *A. ostenfeldii* in The Netherlands.

In early August 2012, however, a toxic *Alexandrium ostenfeldii* bloom developed in the Ouwerkerkse Kreek, in the south-western region of The Netherlands (Fig. 1). The Ouwerkerkse Kreek is a brackish water creek. It functions as a natural recreational area and a drainage basin for agricultural fields and the local village Ouwerkerk. A pumping station regularly discharges water from the creek into the adjacent Oosterschelde estuary (Fig. 1) to prevent flooding of the surrounding lands. The Oosterschelde is a tidal estuary with extensive mussel, cockle and oyster beds (Nienhuis and Smaal, 1994; Troost et al., 2010). Because filter-feeding molluscs are important to the estuary, both financially as a fishery and ecologically to support the vast populations of water birds in the region, protecting these shellfish from harmful algal blooms is a top priority of the regional water management.

The bloom in Ouwerkerkse Kreek was first noticed when it was linked with the death of a dog through the ingestion of contaminated material. Post-mortem analysis of the stomach content of the dog revealed a saxitoxin concentration of 2–4 mg kg^{-1}. Subsequent analysis of the creek revealed a dense bloom of *Alexandrium ostenfeldii*, with 1–2 million cells L^{-1}, producing saxitoxin (STX), saxitoxin analogs (mainly gonyautoxins) and spirolides (SPX) at alarmingly high concentrations (STX and its analogs at 10–20 μg STXeq L^{-1}; SPX at 20–40 μg L^{-1}). The creek, a popular recreational area for nearby campgrounds (Fig. 1), was immediately closed to the public. To add to the crisis, an impending rain event would fill the creek to capacity. To avoid flooding of the agricultural land would therefore require pumping the toxic bloom into the Oosterschelde and directly onto active shellfish beds. Yet, to protect the shellfisheries, pumping of creek water into the Oosterschelde was restricted by local safety regulations, specifically defined for this crisis, to *Alexandrium* concentrations of less than 1000 cells L^{-1} and saxitoxin concentrations less than 15 μg L^{-1}. Thus, a quick and complete mitigation of the bloom was required to protect the Oosterschelde shellfisheries and the local community.

The ultimate prevention of harmful algal blooms in both freshwater and coastal waters is often an overall reduction of nutrient loads, as eutrophication is generally considered the primary cause for the increase in both occurrence and severity of harmful algal blooms (Heisler et al., 2008; Conley et al., 2009; Glibert et al., 2010). Unfortunately a measurable decline of HAB events from nutrient reduction initiatives may take years to materialize. Rapid HAB reduction options are minimal and with mixed effectiveness. These include artificial mixing or flushing of enclosed water bodies (Visser et al., 1996; Huisman et al., 2004; Verspagen et al., 2006). In more open water situations, clay dispersal or clay-bound algicides which aggregate cells and induce

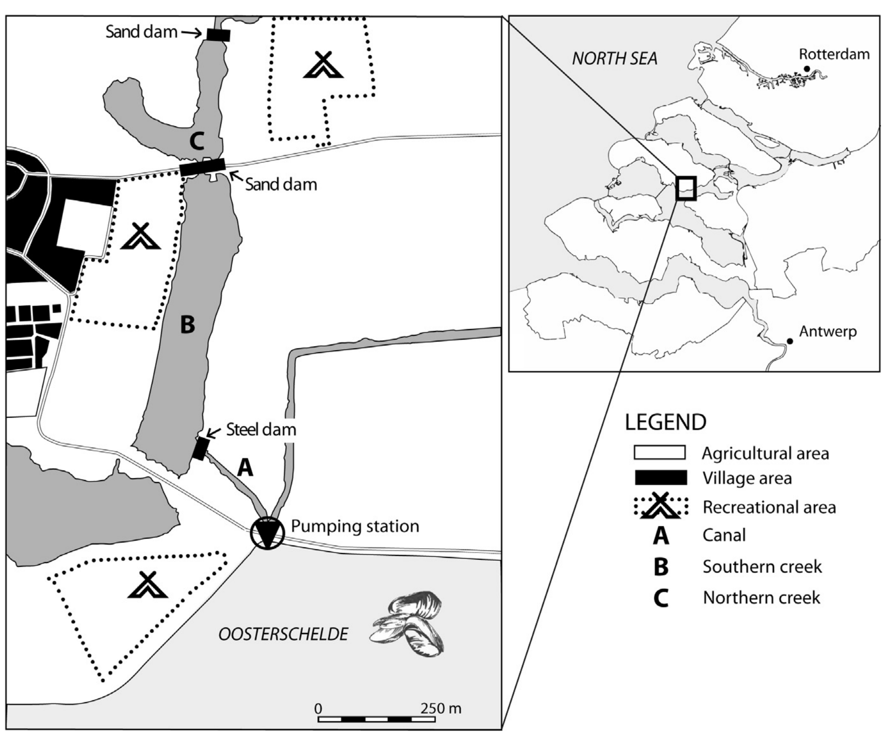

Fig. 1. Map of the Ouwerkerkse Kreek. The letters A, B, and C indicate the three areas where the H_2O_2 treatment was performed. The creek was separated into a southern part (B) and a northern part (C) by two temporary sand dams. The canal (A) served as pilot study area; it was isolated from the Southern creek (B) by a temporary steel dam. The pumping station at the end of the canal was temporarily switched off to prevent flushing of the *A. ostenfeldii* bloom into the Oosterschelde estuary.