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Assessment of the efficacy of clay flocculation in Korean fish farm waters: *Cochlodinium* cell removal and mitigation of ichthyotoxicity

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ABSTRACT

The ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* poses a significant threat to the Korean finfish aquaculture industry, thus calling for effective mitigation strategies. We here report for the first time on investigations to assess the efficacy of routine application of clay to a naturally occurring *Cochlodinium polykrikoides* bloom off Namhae Island in South Korea during August-September 2015. Applications of conventional Korean loess reduced *Cochlodinium* cell concentrations from a maximum of 4600 mL^{-1} to levels considered safe for aquaculture operations (<1000 cells mL^{-1}). However, at the same time, two clay application episodes increased ichthyotoxicity in water samples (measured by the gill cell line RTgill-W1 assay) by up to 32% compared to non-treated areas. Simulated laboratory culture experiments identified clay mediated *Cochlodinium* cell lysis as the probable cause. Lysed cells maintained cytotoxicity for up to 48 h (gill cell viability reduced to 39 ± 7% after 2.5 h exposure), but application of a finely-milled Korean loess completely eliminated ichthyotoxicity. Our results suggest that the fish-killing mechanism of *Cochlodinium* is comprised of both a stable (up to 48 h) and a transient fraction (decay within minutes). The latter indicates potential involvement of reactive oxygen species (ROS). We offer recommendations to fine-tune existing clay application regimes in Korean waters by focusing not on *Cochlodinium* cell removal, but on the adsorptive properties of clays to eliminate ichthyotoxicity.

1. Introduction

Harmful algal blooms pose a significant threat to an ever expanding finfish aquaculture industry attempting to meet increased global consumer demand. The fish-killing dinoflagellate *Cochlodinium polykrikoides* is of particular concern, due to the production of highly potent, yet uncharacterised ichthyotoxins and its apparent geographic range extension across Asia, North America, Europe and the Middle East (Kudela and Gobler, 2012; Kudela et al., 2008). Nowhere has the economic and cultural impact of *C. polykrikoides* been as devastating as in Korea, where in 1995 almost 10% of the total annual finfish production equivalent to $US100 M was lost (Kim et al., 2010; Lee et al., 2013a; Park et al., 2013). Since then, annually recurrent blooms of this species have increased in frequency and continue to threaten extensive fish farm operations in the region. Starting in 1996, the Korean government implemented Korean loess dispersal (active ingredients are montmorillonite and kaolinite clay minerals; Imai et al., 2006; Kim et al., 2010; Lee, 2008; Park et al., 2013) to attempt to reduce economic damages.

First pioneered by Shirota (1989) in Japan and Yu et al. (1994a) in China, clay dispersal aims to promote the generation of rapidly sinking clay-algal aggregates that entrain additional cells during their descent. Both laboratory and field trials investigating the efficacy of this approach have reported excellent cell removal efficiencies (up to 100%), dependent upon clay type and target species, algal concentration, turbulence and clay loading (Archambault et al., 2003, 2004; Beaulieu et al., 2005; Hagström and Granéli, 2005; Padilla et al., 2010; Sengco and Anderson, 2004; Sengco et al., 2001; Sun and Choi, 2004; Yu et al., 1994b).

Clay flocculation traditionally has targeted the removal of HAB cells through flocculation, but recent work has highlighted the effective adsorption of dissolved, extracellular phycotoxins to clay particles (Pierce et al., 2004; Prochazka et al., 2013; Seger et al., 2015; Sengco et al., 2005). This property of clay deserves more attention, since physical contact between clay particles and algal cells during flocculation can cause algal cell lysis and concomitant release of intracellular ichthyotoxins (Archambault et al., 2003; Lee et al., 2013b; Rivera et al., 2014; Seger et al., 2015; Sengco, 2001), which can amplify fish-killing effects (Deeds et al., 2002; Dorantes-

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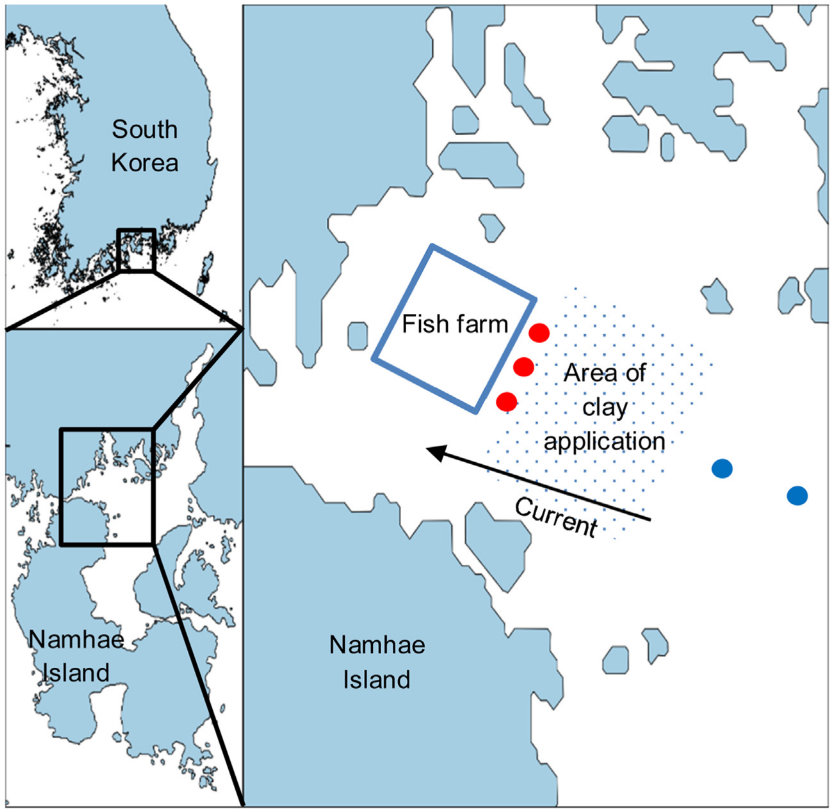

Fig. 1. Location of the Namhae Island fish farm (blue square) and zone of clay application (dotted area). Locations of clay treated (red circles), control sampling sites (blue circles) and direction of current (arrow) are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Aranda et al., 2015, 2011). To capitalise on this property of clay requires critical knowledge of the fish killing mechanism of different harmful algal species. Most fish-killing HABs, such as *Cochlodinium,* comprise fragile cells that upon cell lysis release reactive oxygen species (ROS) that react with free fatty acids to produce highly ichthyotoxic lipid peroxidation products (Dorantes-Aranda et al., 2015; Kim et al., 1999; Marshall et al., 2003).

Working with Korean scientists during the 2015 *Cochlodinium* bloom season in the Korean South-East Sea, we here report for the first time in detail on the routine application of clay to a naturally occurring *Cochlodinium polykrikoides* bloom to assess implications for ichthyotoxicity. Cytotoxicity of field samples was newly assessed with the RTgill-W1 gill cell line assay and field observations interpreted through a series of complementary laboratory culture experiments simulating field conditions.

2. Methods

2.1. Field application of clay

2.1.1. Sampling location

The Namhae Island fish farm area is located in South Gyeongsang Province, South Korea (34.95°N 127.92° E, Fig. 1) and focuses on the cultivation of red sea bream (*Pagrus major*) in deep water (10 m) pens. In early August 2015, this fish farm was threatened by a *C. polykrikoides* bloom moving towards the farm in a South-Easterly current. The Korean Government commenced clay spraying operations on the 3rd of August and continued until 20th of September, covering an approximate area of 1 km^2 upstream of the farm. Locally sourced Korean loess (from Namhae Island) was continually dispersed in a rectangular pattern from a boat fitted with an internal clay dispenser (on-board mixing of clay with seawater) and additionally hosed off the back of a barge with seawater (Fig. 2a and b, respectively). Several smaller boats crisscrossed the wake of the clay plume to induce turbulence and thereby facilitate dispersal.

2.1.2. Field sampling

On day 27 and 33 during the ongoing clay application, 1 L water samples were collected using a Van Dorn water sampler at 1 m depth intervals (0.5–9 m) within the clay treated area during continuous dispersal (~50 m upstream of fish farm; 3 replicates) and in the untreated area (~1.5 km upstream; 2 replicates; see Fig. 1 for sampling locations). Control samples were collected 30–60 min after sampling in the clay treated area was completed. Samples were stored overnight at 25 °C in the dark, pooled across replicates for each site (treated and control) and the next morning tested on the RTgill-W1 cell line following the protocols of Dorantes-Aranda et al. (2011) (see 2.4). Seawater collected from a depth of 9 m in the clay treated area served as the non-toxic control (containing 51 ± 41 *Cochlodinium* cells mL^{-1}). Dinoflagellate cell concentration was determined through microscopic cell counts in 0.1–1 mL of Lugol's (2%) preserved sample the following day (Nikon Eclipse TS 100; Sedgewick-Rafter chamber). Depth profiles of temperature and salinity were collected at both sites (Seabird 19plus, USA).

2.2. Ichthyotoxic principle

2.2.1. Laboratory cultures

To interpret field observations, *Cochlodinium polykrikoides* strain CP-YD-1409, isolated from Yeongdeok-gun, Korea on 16/09/2014 was grown in GSe medium prepared from filtered (0.22 μm) coastal seawater. Salinity was adjusted to 32 with