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Alexandrium peruvianum (Balech and Mendiola) Balech and Tangen a new toxic species for coastal North Carolina

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ABSTRACT

Routine sampling of the water quality stations in the New River Estuary (Jacksonville, North Carolina, USA) during November 2004 revealed the presence of a previously unidentified dinoflagellate. Preliminary observations of its morphology suggested it to be consistent with that of *Alexandrium peruvianum* (Balech et Mendiola) Balech et Tangen. Observations using brightfield, epifluorescence and scanning electron microscopy confirmed the diagnostic thecal plates to be those of *A. peruvanium*. Clonal cultures established from cells isolated from the New River Estuary samples were also used for further studies of morphology and for the presence of toxins. Thecal morphology was consistent with that described by Balech clearly separating it from the sister species *Alexandrium ostenfeldii*. Three classes of toxins were detected from these cultures. An erythrocyte lysis assay (ELA) was used to confirm the presence of hemolytic toxins in *A. peruvianum* cultures. A cellular EC_{50} for lysis was 1.418×10^4 cells, well within the range the maximal cells densities found in the New River and more potent when compared on a cellular basis with *Prymnesium parvum*. Another toxin class detected in *A. peruvianum* cultures was the fast acting 13-desmethy C and D spirolides also produced by the sister species *A. ostenfeldii*. The last toxin type detected in the *A. peruvianum* cultures was the paralytic shellfish toxins, GTX 2, 3, B1, STX and C1,2. These findings expand the geographic range of occurrence for *A. peruvianum* in the U.S. to be much greater than previously considered. The morphological characters agreed with previously reported molecular data in separating *A. peruvianum* from *A. ostenfeldii*. It is also the first confirmed report that this species produces PSP toxins, spirolides and naturally occurring hemolytic substances. In light of these findings additional attention is needed for the detection of *Alexandrium* species in all coastal waters of the U.S. This added effort will enhance the evaluation of the relative impacts of the species to shellfish safety and bloom surveillance.

1. Introduction

Members of the dinoflagellate genus *Alexandrium* are well recognized as producers of potent neurotoxins and causative agents of the human syndrome called paralytic shellfish poisoning (PSP). The global distribution of this genus and its impacts on marine systems and human health were recently reviewed (Anderson et al., 2012) where 31 species were listed along with toxin types. Among them, the two species *Alexandrium peruvianum* (Balech et Mendiola) Balech et Tangen and *Alexandrium ostenfeldii* (Paulsen) Balech et Tangen, were noted. Both appeared similar in general morphology with subtle differences in plate structures but varied in toxin type and production.

These two species produced the novel class of cyclic imine neurotoxins called spirolides. Spirolides were discovered in the digestive glands of shellfish from the southeastern Nova Scotia (Hu et al., 1995, 2001a), as well as in shellfish and water column populations in Norway (Hu et al., 2001b; Aasen et al., 2005). *A. ostenfeldii* was confirmed as the biological source of the seven spirolide congeners isolated from shellfish, natural populations and cultures (Cembella et al., 1998, 1999, 2000). Some forms of the spirolides were bioactive (A–D, G; Hu et al., 2001a) while others (E and F; Hu et al., 1996) were inactive metabolites in shellfish (Richard et al., 2001; Gill et al., 2003). *A. peruvianum* was reported from the

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Mediterranean as vegetative cells and cysts (Figueroa et al., 2008) that were also found to produce spirolides (Franco et al., 2006). The Mediterranean *A. peruvianum* appeared identical to *A. ostenfeldii* based on molecular ITS sequences data (Penna et al., 2008) but had subtle differences in sulcal plates. In Malaysian waters, *A. peruvianum* was reported (Lim et al., 2005) to contain PSP toxins only with no mention of spirolides. Most recently, *A. peruvianum* isolated from North Carolina was shown to have both a novel gymnodimine congener (12-methylgymnodimine) as well as 13-desmethylspirolide C (Van Wagoner et al., 2011).

Originally *A. peruvianum* was first identified from Callao, Peru in 1976 as *Gonyaulax peruviana* (Balech and Rojas de Mendiola, 1977), changed by Taylor (1979) to *Protogonyaulax peruviana* (Balech and Mendiola) and subsequently renamed *A. peruvianum* based on material collected in Oslofjorden, Norway (Balech and Tangen, 1985). Balech (1995) mentions *A. peruvianum* from Mineola, NY, whose location was recently confirmed to be Hempstead Harbor located on the south shore of Long Island, New York, USA (A. Freudenthal, per. comm.). This is the southernmost extent of *A. peruvianum* reported to date. Due to the difficulties of observing thecal plates and quantifying variations among them, identification led to some taxonomic confusion. Recent molecular studies of the of the 18S rDNA gene (SSU, ITS spacers and partial LSU) of several New River clones including AP0411 conducted by Schwarz (2011) of this isolate showed it to be identical to other *A. peruvianum* sequences. The GenBank accession numbers for this clone are JF921179, JF921180 and JF921181. The difficulty of identifying *A. peruvianum* by morphology alone may have resulted in misidentification or ambiguities in routine plankton samplings. The fact that this species produces several types of toxins (Van Wagoner et al., 2011) prompts greater investigations into its presence in local waters.

2. Materials and methods

2.1. Field collections and strain information

Routine monthly samplings during 2004 in the New River, North Carolina, USA (Fig. 1) obtained from the North Carolina Department of Environment and Natural Resources (NC DENR) were observed as live and Lugol's preserved samples using a Nikon Diaphot Inverted Microscope. The toxic strain of *A. peruvianum* used in these studies was isolated from a surface sample (upper 0.5 m) at the Frenchs Creek Station (34.639 N, 77.34 W) of the New River estuary in Southeastern, North Carolina, USA (Fig. 1) on 5 November 2004. This culture is presently deposited at the Center for Marine Science, Toxic Algal Culture Collection identified as TACC AP0411. Isolation were made using an Olympus CK40 (Olympus America, Center Valley, PA, USA) inverted tissue culture microscope and hand held micropipette. Single cells were first grown in filter sterilized Frenchs Creek water at 20 psu and a temperature of 15 °C. Once growth was evident, additions of L1 medium (Guillard and Hargraves, 1993) modified by the elimination of silica, were gradually introduced until a stable culture could be maintained in full strength modified L1. All growth studies were conducted in an EGC 8 (Chargin Falls, OH, USA) temperature regulated incubator having cool white fluorescent light with a fluence rate of 60 μmol photons m^{-2} s^{-1} and a 14:10 h light:dark period.

2.2. Morphology

Morphology of *A. peruvianum* cells from culture and field samples were studied using brightfield, epifluorescence and scanning electron microscopy (SEM). Live and Lugol's preserved cells were observed with a Zeiss Axio Imager II (Carl Zeiss,

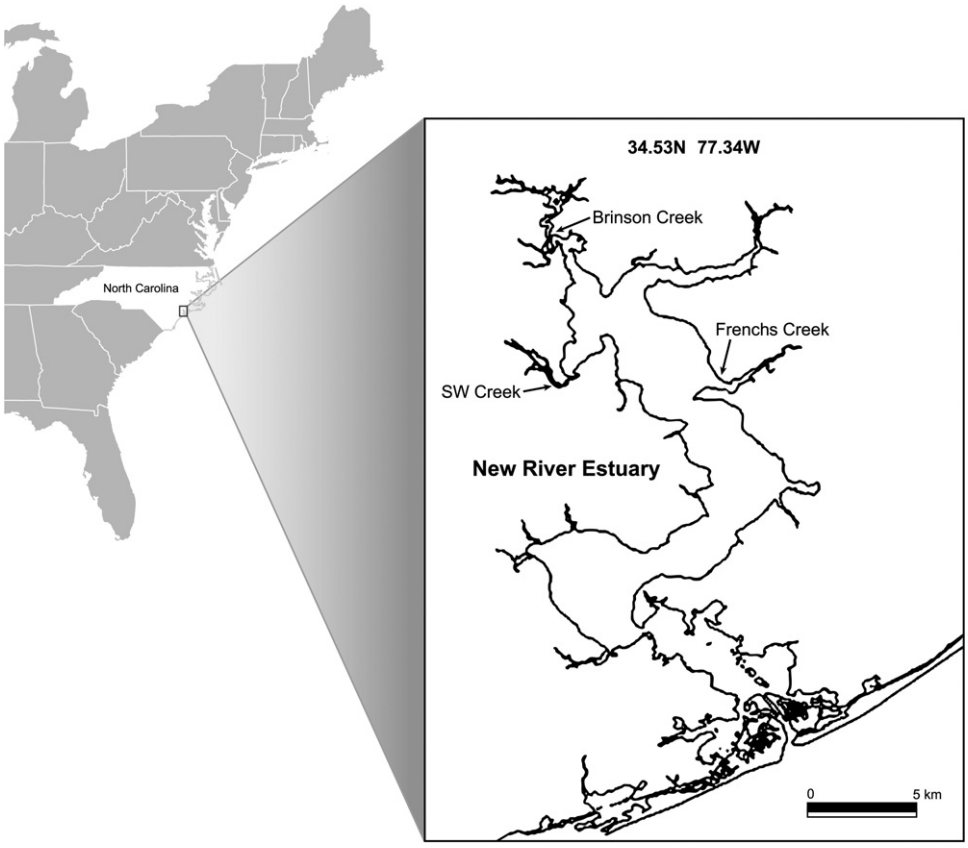

Fig. 1. New River Estuary, North Carolina with the location of Frenchs Creek where *A. peruvianum* cells were initially found in surface samples.