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Distribution of the genus *Alexandrium* (Halim) and paralytic shellfish toxins along the coastline of New South Wales, Australia

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ABSTRACT

Blooms of *Alexandrium* species, in particular the species *Alexandrium catenella*, accounted for more than 50% of algal related, shellfish aquaculture harvest zone closures in New South Wales (NSW) Australia since 2005. While there are indications that species of *Alexandrium* are more abundant than they were formerly, there is little data available on the spatial and temporal distribution and abundance of the genus in NSW. A six and a half year dataset comprising a total of 8649 fortnightly samples from 31 estuaries spread over 2000 km of NSW coastline was analysed. The greatest abundances of *Alexandrium* spp. were observed during the austral Spring and Summer, in estuaries in the mid and southern latitudes of the state. In identifying these high risk zones, we propose variables such as season, temperature, rainfall and estuarine flushing to be targeted in intensive site specific studies, to support the development of predictive tools for resource managers.

1. Introduction

Species of the dinoflagellate genus *Alexandrium* have a cosmopolitan distribution, occurring in sub-arctic, temperate and tropical zones worldwide (Taylor et al., 2003). *Alexandrium* is comprised of 31 species, 13 of which are capable of producing the potentially fatal neurotoxin saxitoxin and its analogues (*A. affine*, *A. andersonii*, *A. angustitabulatum*, *A. catenella*, *A. cohorticula*, *A. fundyense*, *A. minutum*, *A. ostenfeldii*, *A. tamarense*, *A. tamiyavanichii*, *A. taylori* (reviewed by Anderson et al., 2012) and *A. peruvianum* (Tomas et al., 2012)). The accumulation of saxitoxin in shellfish poses a risk to public health, as it may lead to Paralytic Shellfish Poisoning (PSP) (reviewed by Llewellyn et al., 2006; Wiese et al., 2010). Symptoms of PSP range from reports of spreading numbness and tingling sensations, headache and nausea to more extreme fatal cases due to respiratory paralysis (Hallegraeff, 2003).

Species of *Alexandrium* have long been known to be present in the coastal waters of Australia. An observation by Wood (1954) of a chain forming dinoflagellate (*Gonyaulax conjuncta*) in Port Hacking, New South Wales (NSW) is considered the first observation of *A. catenella* in Australia (Hallegraeff et al., 1991). Modern references to Australian blooms of *Alexandrium* began in the 1980s. A toxic bloom of *A. catenella* was reported from Port Phillip Bay in Victoria in 1986 (Hallegraeff, 1992), while *Alexandrium minutum* bloomed in the Port River in South Australia in 1986 and 1987 (Hallegraeff et al., 1988). Up to this time, PSP toxin events were unknown in Australia (Hallegraeff, 1992; Hallegraeff, 2003). Subsequently, the majority of research on *Alexandrium* species in Australia has been carried out in the south-eastern region, in particular, focusing on the suspected introduction of populations through ballast water and threats to local aquaculture (Hallegraeff and Bolch, 1991; Hallegraeff et al., 1998; Hallegraeff, 2003; Bolch and de Salas, 2007).

Eight species of *Alexandrium* have been identified in the south-eastern waters of Australia (*A. affine**, *A. catenella**, *A. fraterculus*, *A. margalefi*, *A. minutum**, *A. ostenfeldii**, *A. pseudogonyaulax*, *A. tamarense** (Hallegraeff et al., 1991; Hallegraeff et al., 2010; Murray et al., 2012; Ajani et al., 2012). Five (*) of these species occur on the list of known toxin-producing *Alexandrium* species stated previously. To date, uptake of saxitoxins in shellfish in Australian coastal waters has been attributed to the species *Gymnodinium catenatum*, *A. minutum*, *A. catenella* Group IV ribotype, and possibly *A. tamarense* Group V (Hallegraeff et al., 1988; Hallegraeff et al., 1991; Negri et al., 2003; Murray et al., 2012). Toxin profile

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characterisation by Murray et al. (2011) on cultures of A. catenella, derived from isolates sourced in NSW and Tasmanian coastal waters, found that the analogs C1,2 and GTX1,4 were the primary STX components, with B2 and C3,4 analogs also being produced by one NSW strain. While as yet, there has been no study published documenting the toxin profile of A. minutum strains isolated from NSW coastal waters, a South Australian strain of this species has been shown to produce GTX1,4 with some GTX2,3 (Lippemeier et al., 2003; Negri et al., 2003). Until recently, it was generally accepted that A. tamarense Group V was non-toxic (Bolch and de Salas, 2007). However, following a PSP event linked to this species in the Hastings River NSW during 2010, Murray et al. (2012) described a toxin profile for the group that was comprised largely of GTX5 with low proportions of STX.

The occurrence of toxic *Alexandrium* events, harmful blooms of other microalgal species and the potential threats from the introduction of non-indigenous phytoplankton species, have prompted many countries to establish monitoring programs to promote shellfish safety, protect local economies and increase consumer confidence in aquaculture products (Shumway et al., 1990; Andersen, 1996). In Australia, monitoring programs are implemented by each state. Located in south-eastern Australia between approximately 28°S and 37°30′S (Fig. 1), NSW hosts 71 commercial shellfish growing areas. During the 2010/11 production season, first sale values exceeded $AUD38 million, based on a harvest of over 65 million oysters sold between 1 July 2010 and 30 June 2011 (Trenaman, 2011). An increasing trend has been observed in *Alexandrium* related PSP events, with a prominent spike in 2010 (NSW Food Authority, 2011a). This has prompted a more in-depth investigation into the distribution of the species, based on data collected as part of the monitoring program.

During the past 20 years, notable incidences of *Alexandrium* (see reviews by Ajani et al., 2001; Todd, 2001; Ajani et al., 2011) have been documented for NSW, including evidence of cyst beds (Hallegraeff et al., 1998). However, the available information is limited, often presenting only a discrete "snapshot" of a particular site. This long-term data set presents a valuable asset whereby historical patterns and emerging trends can be observed for *Alexandrium* spp. all along the NSW coastline.

2. Methods

2.1. Sample collection

Phytoplankton samples were collected on a fortnightly basis from operational shellfish production and harvest sites within NSW estuaries. In total, 31 estuaries along the NSW coast (Fig. 1, Table 1) were included in the sampling regime. In addition, shellfish samples were collected and analysed on a monthly basis for biotoxin content. This process of quality assurance is part of the NSW Food Authority's Shellfish Program and Marine Biotoxin Management Plan (MBMP) (2011b). Samples were collected by sampling officers trained by the NSW Food Authority from sites established as being representative of the water being filtered by the farmed shellfish within each estuary and following the guidelines set out by the MBMP and the Australian Shellfish Quality Assurance Program (NSW Food Authority MBMP, 2011b).

A total of 8649 (Table 1), 500–1000 mL water samples collected from a depth of 50 cm were taken over a period of 78 months (July 2005–December 2011). Samples were preserved with Lugol's Iodine for the purpose of phytoplankton identification and enumeration. Accompanying phytoplankton net haul samples were also preserved with Lugol's Iodine to provide of qualitative species information and additional sample material to aid with species identification. During the same period, 4282 shellfish samples were collected from permanent harvest sites to determine if PSP biotoxins were present.

2.2. Diversity and abundance of Alexandrium spp

Identification of *Alexandrium* species was carried out by Dr. S. Brett and Dr. D. Hill, who have been regularly identifying species of *Alexandrium* for the NSW Shellfish Program since 2003. References consulted for identification purposes included Hallegraeff et al. (1991), Balech (1995) and Taylor et al. (2003). In preparation for analysis, water samples were concentrated by gravity assisted membrane filtration. Phytoplankton cell counts were undertaken in a Sedgwick-Rafter counting chamber using Zeiss Axiolab or Zeiss Standard microscopes, equipped with phase-contrast. Cells were assigned to genus and species when possible and *Alexandrium* spp. were counted to a minimum detection threshold of 50 cells L^{-1}. Where necessary, an aliquot (ca. 0.5 ml) of preserved water sample was placed on a slide and Calcofluor White was utilised to determine the thecal plate morphology of dinoflagellate species under UV epifluorescence (Fritz and Triemer, 1985) using Zeiss Standard or Leitz Diavert microscopes. The Marine Biotoxin Management Plan (NSW Food Authority MBMP, 2011b) outlines "Phytoplankton Action Limits" (PALs) whereby additional shellfish flesh testing and/or harvest zone closures are initiated based on cell concentrations of potentially harmful algae. The minimum PAL for *Alexandrium* spp. is 200 cells L^{-1}. *Alexandrium* is considered a "background" bloom species (Anderson, 1998), capable of producing high level of toxins at low cell concentrations (Hallegraeff, 2003). For the purpose of this study "bloom" refers to *Alexandrium* cell concentrations exceeding the minimum PAL limit of 200 cells L^{-1}.

2.3. PSP toxin analysis

The PSP toxin content of shellfish flesh was determined either by HPLC analysis (AOAC official method 2005.06 for PSP toxins in shellfish, Lawrence et al., 2005) or screened (positive or negative result) with a Jellet test (Jellett Rapid Testing Ltd., Canada). Samples were analysed by laboratories approved by the NSW Food Authority (NSW Food Authority MBMP, 2011b). The regulatory limit for PSP toxins is listed in the MBMP as being greater than or equal to 80 μg of saxitoxin equivalent/100 g of edible shellfish flesh. A positive Jellet screen (http://www.jellett.ca) resulted in a precautionary closure of the harvest zone until additional testing was carried out.

2.4. Site-specific investigations

A review of the available literature indicates that reported toxic blooms of the genus *Alexandrium* in NSW have been predominantly caused by *A. catenella* (Ajani et al., 2001; Todd, 2001; Ajani et al., 2011). All *A. catenella* cultures established to date from the NSW region are of Group IV genotype (i.e. Murray et al. 2011). During the present study, *A. catenella* was the most prevalent toxic species observed. Based on the frequency of blooms of this species, and the related harvest zone closures, three regions were chosen for further examination. These were the Brisbane Water (Fig. 1B), the Hawkesbury River (Fig. 1C) and the Georges River (Fig. 1D). Environmental parameters were not measured concurrently with phytoplankton samples collected for monitoring purposes. However, a limited suite of physical data such as tidal range, water level data and surface temperature and salinity, along with meteorological data (solar exposure and daily rainfall) were examined to gain insight into the distribution of the species in these locations.