Unprecedented *Alexandrium* blooms in a previously low biotoxin risk area of Tasmania, Australia

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Abstract

During October 2012, a shipment of blue mussels (*Mytilus galloprovincialis*) from the poorly monitored east coast of Tasmania, Australia, was tested by Japanese import authorities and found to be contaminated with unacceptable levels of Paralytic Shellfish Toxins (PSTs; 10 mg/kg). Subsequently local oysters, scallops, clams, the viscera of abalone and rock lobsters were also found to be contaminated. This led to a global product recall and loss to the local economy of AUD 23M. Following low toxicity during 2013 and 2014 and implementation of minimal shellfish farm closures, a more severe bloom event occurred during July-November 2015 and again June-September 2016 (up to 300,000 *Alexandrium* cells/L; 24 mg/kg PST in mussels, 6 mg/kg in *Crassostrea gigas* oysters), also causing 4 human illnesses resulting in hospitalization after consumption of wild shellfish. While *Alexandrium tamarense* had been detected in low concentrations in southeastern Australia since 1987, all cultured strains belonged to the mostly non-toxic group 5 (now designated *A. australiense;* detected since 1987) and weakly toxic group 4 (*A. pacificum;* detected in 1997). In contrast, the 2012 to 2016 outbreaks were dominated by highly toxic group 1 (*A. fundyense*) never detected previously in the Australian region. Molecular analyses suggest that *A. fundyense* may have been a cryptic ribotype previously present in Tasmania, but newly stimulated by altered water column stratification conditions driven by changing rainfall and temperature patterns. Increased seafood and plankton monitoring of the area now include the implementation of *Alexandrium* qPCR, routine Neogen™ immunological and HPLC PST tests, but ultimately may also drive change in harvesting strategies and aquaculture species selection by the local seafood industry.

Keywords: Alexandrium tamarense complex; PST; unprecedented novel blooms

Introduction

Starting in 1985, the Tasmanian shellfish industry has become used to annually recurrent closures and public warnings of paralytic shellfish poisoning (PSP) risk inflicted by *Gymnodinium catenatum* blooms (reviewed by Hallegraeff *et al.* 2012). This large chain-forming dinoflagellate can be readily recognised by light microscopy, and, in the past, the affected area was primarily confined to the Huon River and d'Entrecasteaux Channel, near the capital city of Hobart. Over time, mussel farms in the most severely affected Huon River all closed business and an economic decision was made to declare the area unsuitable for shellfish farming with no new leases allowed. Early HAB surveys of other Tasmanian locations since 1987, including the east coast, had detected low concentrations of *Alexandrium tamarense* (Hallegraeff *et al.* 1991; Bolch & Hallegraeff 1990; Bolch & de Salas 2007). However, all cultured strains proved to be non-toxic and belonged to what was initially termed the "Tasmanian ribotype" (now designated group 5 or *Alexandrium australiense*; Scholin *et al.* 1995, John *et al.* 2014). A single small bloom event in Spring Bay in 1997 was caused by toxigenic group 4 (or *Alexandrium pacificum*), also widespread along the New South Wales and Victorian coasts of Australia (Hallegraeff *et al.* 1991; Farrell *et al.* 2013). Despite this event, the Tasmanian east coast continued to be classified as a low biotoxin risk and hence was subject to very limited plankton and biotoxin monitoring.

Unexpectedly, in October 2012, a shipment of blue mussels (*Mytilus galloprovincialis*) from the east coast of Tasmania tested by Japanese import authorities was found to be contaminated with unacceptable levels of Paralytic Shellfish Toxins (PSTs; 10 mg/kg). This incident triggered a recall of all Australian shellfish exported to Japan. Subsequent monitoring of the area confirmed PST in mussels, oysters, scallops, clams and rock lobster. A review of this critical incident (Campbell *et al.* 2013) identified: 1. Failure of plankton monitoring to provide timely results and failure to detect *Alexandrium*; 2. Failure of seafood risk assessment by not recognizing the risk of a new mussel farming venture in a poorly monitored area; 3. Failure of PST monitoring by relying only on plankton monitoring as a first screen rather than including shellfish testing. Here we review the results of increased *Alexandrium* plankton and seafood PST monitoring since the 2012 incident with the aim to identify key regions and seafood species at risk as well as environmental variables driving the blooms.

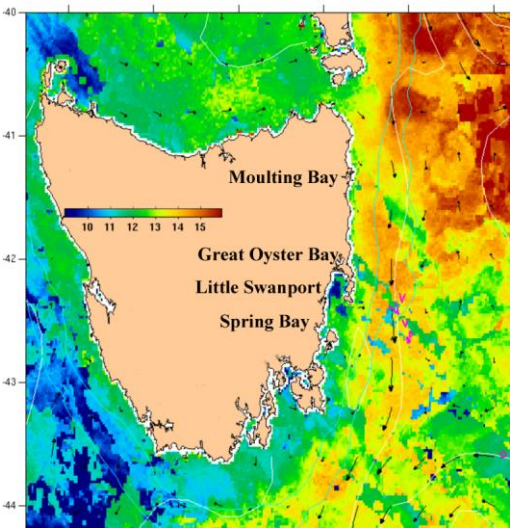

Fig.1. Map of Tasmania, south of the mainland of Australia, showing Sea Surface Temperatures on 27 September 2015 during peak PST, with the East Australian Current (EAC; in red) interacting with the continental shelf. The locations of the main affected shellfish farm areas Moulting Bay, Great Oyster Bay, Little Swanport and Spring Bay are indicated. Source: oceancurrent.imos.org.au.

Material and Methods

Shellfish toxins were monitored at weekly intervals at >20 Tasmanian east coast sites by the Tasmanian Shellfish Quality Assurance Program (TSQAP) using the AOAC approved Liquid Chromatography with fluorescence detection (LC-FLD) method (Lawrence *et al.* 2005). Satellite oceanography of the area was monitored as part of the Integrated Marine Observing System (IMOS; Fig.1). At the height of the August 2016 bloom, additional plankton, toxin and hydrological data were collected along inshore-offshore transects aboard the RV *Southern Cross*. A Seabird SBE 19PlusV2 CTD was used to collect temperature and salinity depth profiles. Plankton counts were obtained by settling 1L Lugol's iodine preserved samples. PST estimates were conducted on 3L of 8µm filtered water using the Neogen™ Reveal 2.0 immunological test kit (modified after Dorantes-Aranda *et al.* 2017). Cyst sediment samples were collected using a Craib corer and processed using primulin staining (Yamaguchi *et al.* 1995).

Results and Discussion

Shellfish toxins

Following low PST detection in 2013 and 2014 (both low rainfall years) with implementation of minimal shellfish farm closures, a more severe bloom event occurred during July-November 2015 (up to 300,000 *Alexandrium* cells/L; 15 mg/kg STX eq. in mussels, 6 mg/kg in *Crassostrea gigas* oysters), also causing 4 human hospitalizations after consumption of wild shellfish. More severe blooms recurred in 2016, following a major flood event in May and blooms lasting until September when up to 24 mg PST/kg was recorded in mussels (Fig. 2). In 2015, the highest PST concentration was measured in the south in Spring Bay, but in 2016 highest PST occurred further north in Little Swanport and Great Oyster Bay. Most shellfish contained high proportions of GTX1&4 (26-88%) and GTX2&3 (8-76%), followed by C1&2 (5-24%) and STX (0-2%) (Dorantes-Aranda *et al.* 2017).

Increased PST flesh testing

The current protocol for sample processing by the Tasmanian Shellfish Quality Assurance Program involves shipping samples to an accredited laboratory in Sydney, leading to frustrating delays (4-12 days) for shellfish growers. The performance of four commercial PST test kits, Abraxis™, Europroxima™, Scotia™ and Neogen™, was compared with the LC-FLD method for contaminated mussels and oysters. Based on their sensitivity, ease of use and performance, the Neogen kit proved the most suitable kit for use with Tasmanian mussels and

oysters. Neogen produced 5% false negatives and 13% false positives when the cut off was altered to 0.5-0.6 mg STX-diHCl eq/kg, whereas the introduction of a hydrolysis conversion step eliminated false negatives. A full single lab and international validation process was conducted (Turnbull *et al*., in press) and once formally approved for regulatory purposes, the Neogen kit will provide shellfish growers with a rapid tool for on-farm harvesting decisions. Rapid screen tests to prevent compliant samples undergoing testing using the expensive LC-FLD method will also result in significant savings (estimated $750k/yr) in analytical costs.

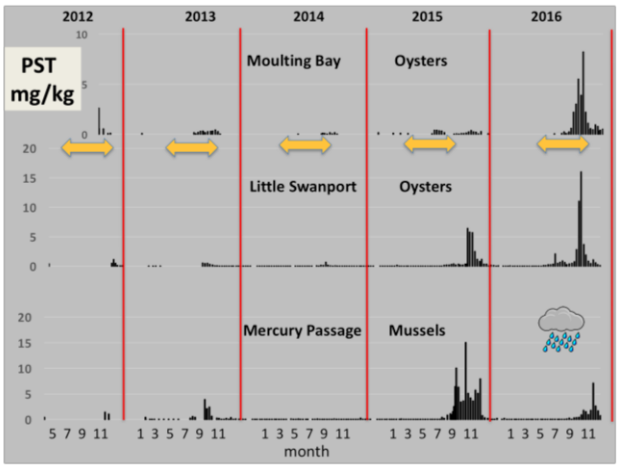

Fig. 2. Shellfish toxicity (mg STX eq./kg) from 2012 to 2016 in Moulting Bay and Little Swanport oysters and Spring Bay mussels. Orange arrows indicate the seasonal 10-15ºC temperature window. The 2016 bloom was preceded by a major rainfall event while anomalously cold water in Great Oyster Bay may explain the 2015 bloom.

Causative dinoflagellates

The causative dinoflagellates morphologically agreed with *Alexandrium fundyense*, possessing a ventral pore in the 1st apical plate (Fig.3a, arrow), and occurring as single cells or division pairs. An unusual feature of field samples was the extreme fragility of cells, ecdysing within 30-60 min after collection (Figs 3 b,c,d). Unexpectedly all cultured strains established during 2012 and 2015 belonged to group 1 never before detected in Australian waters in over 30 years of observations. Unique microsatellite signatures of these cultures (U. John, pers. comm.) suggest an endemic cryptic population being newly stimulated by changing environmental conditions. Paleogenomic research is in progress using dated sediment depth cores from the area to document historic shifts in abundance of *Alexandrium tamarense* ribotypes 1, 4 and 5.

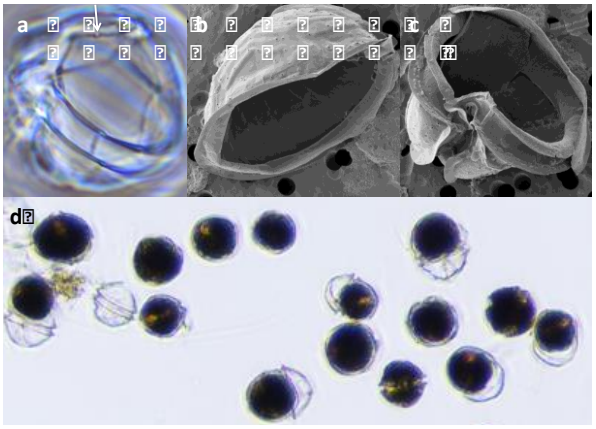

Fig. 3. Light (a,d) and scanning electron micrographs (b,c) of Tasmanian 2015 and 2016 *Alexandrium* field samples. Fig.3a shows ventral pore (arrow) in the first apical plate; Figs.3 b,c,d show the extreme fragility of the cells subject to ecdysis within 30-60 min of collection.

Preliminary views on bloom conditions

The affected Tasmanian coastal region is classified as a climate change "hotspot" resulting from increasing southward movement of the nutrient-poor East Australian Current (Fig.1). These novel *Alexandrium* blooms are not a simple response to increasing water temperatures (2.3ºC increase since the 1940s), as they occur in the cold winter-spring months at water temperatures of 10-15ºC. An observed trend of decreased silica concentrations in these waters would favor dinoflagellates and select against competing diatom blooms (Thompson *et al.* 2009). Preliminary culture growth experiments showed *Alexandrium* growth rates as high as 0.5-0.8 divisions/day, and a preference for low phosphorus and stimulation by humics (R. Quinlan, unpublished). Both culture experiments but notably field estimates using the Neogen test suggest a high cellular toxin content up to 100-500 pg STX eq/cell (Fig. 4, right). In August 2016 *Alexandrium* populations were abundant in inner shelf waters (35-50m deep) (Fig. 4, left) and just inside the sand bars of

the main shellfish growing estuaries of Little Swanport, Great Oyster Bay and Moulting Bay. However *Alexandrium* were virtually absent from the shallow (1-2m) turbid waters of those estuaries, and also were absent from deeper (100m) offshore waters dominated instead by spring bloom diatoms.

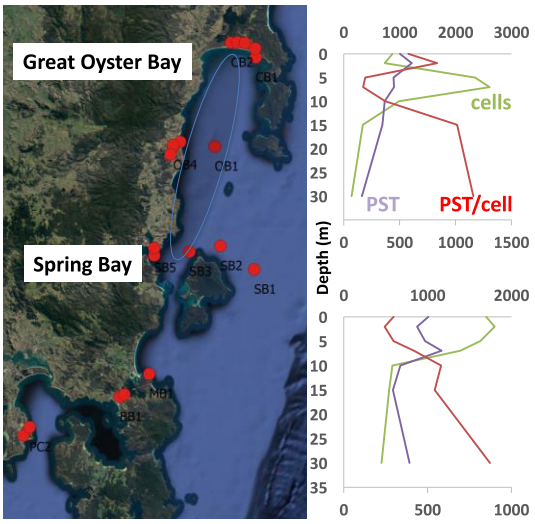

Fig.4. Left: *Alexandrium* bloom patch contained on the inner shelf of east coast Tasmania in August 2016, with no cells detected in offshore deeper waters. Right: Depth profiles of *Alexandrium* cell abundance (top scale), total PST toxins (ng/L) and pg PST eq per cell (bottom scale) in weakly stratified waters of Great Oyster Bay (top) and Spring Bay (bottom).

In 2016 the peak of the *Alexandrium* bloom coincided with a major high rainfall/ flood event that resulted in salinity stratified coastal waters (Fig. 2), while northward flow on the inner shelf was consistent with downwelling favorable conditions along the entire coast. In 2015 the situation was different however with anomalously cold water flowing out of Great Oyster Bay resulting in thermally stratified coastal waters. While both stratified and downwelling conditions are known to favor dinoflagellates over diatoms (Condie & Bormans 1997, Condie & Sherwood 2006), further research is in progress on how these processes control *Alexandrium* blooms off eastern Tasmania.

Alexandrium cyst surveys during August 2016 along the entire east coast of Tasmania found consistently low abundances of cysts (0.1-3 cysts per gram of sediment wet weight), but no dense cyst beds. Most sediments comprised coarse sands reflective of strong current regimes. Preliminary cyst culture experiments indicated a short dormancy period of 1-2 months (compare Hallegraeff *et al.* 1998 for New South Wales *Alexandrium* cysts) suggestive of rapid cycling between plankton and benthos. To protect tourism and human health, the area has now been sign-posted with permanent public PST warnings, which is a first for Australia.

Acknowledgements

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