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Rapid Communication

Biosecurity monitoring of Harmful Algal Bloom (HAB) species in Western Australian waters: first confirmed record of *Alexandrium catenella* (Dinophyceae)

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Abstract

The Australian National System for the Prevention and Management of Introduced Marine Pest Incursions has identified seven Harmful Algal Bloom (HAB) toxic dinoflagellate species as target species of concern. *Alexandrium minutum* Halim, 1960, and *Alexandrium cf. tamarense* (Lebour) Balech, 1995, are currently known to occur in south-western estuaries and coastal waters but with no documented impact on the seafood industry or human health. Monitoring of these species is challenging, time-consuming, expensive, and often relies on traditional morphotaxonomy. This reports the first confirmed detection of another HAB species, *Alexandrium catenella* (Whedon and Kofoid) Balech, 1995, in Western Australia (WA), using both microscopic and molecular methods. The confirmed detection of *A. catenella* (*A. pacificum* Group IV genotype) in WA hopefully will motivate discussion about better monitoring and control of toxic HAB species.

Key words: *Alexandrium catenella*, dinoflagellate, real-time PCR, HABs, biosecurity, Western Australia

Introduction

Harmful Algal Bloom (HAB) species can pose significant negative ecological and socio-economic impacts, produce toxins and can occur in concentrations high enough to have adverse effects on fish and human health (Hallegraeff 1993). Human illnesses resulting from the consumption of HAB species toxins include cyanobacterial toxin poisoning, ciguatera poisoning, amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), diarrheic shellfish poisoning (DSP), and paralytic shellfish poisoning (PSP). PSP is associated with the consumption of a potent toxin, saxitoxin, and is the most concerning of the HAB-related illnesses because extreme cases can result in muscular paralysis and death (Hallegraeff 1993). Reports of HAB have become increasingly frequent in recent decades, mostly as a result of increasing human impact on the marine environment, aquaculture development, and increased awareness of the problem (Anderson 1989; Hallegraeff 1993; Lilly et al. 2007; Anderson et al. 2012).

In Australia, seven HAB toxic dinoflagellate species have been listed as target biosecurity species under the National System for the Prevention and Management of Introduced Marine Pest Incursions (the National System) (NIMPGC 2010). This system aims to prevent new marine pest introductions, coordinate and support a response when a marine pest does arrive in a region, and minimize the spread and effects of those marine pests already established in Australia (DAFF 2010). Two of the seven listed HAB species include *Alexandrium monilatum* Balech, 1995, and *Dinophysis norvegica* Claparede and Lachmann, 1859, which have been associated with fish kills (May et al. 2010) and DSP (Freudenthal and Jijina 1988), respectively. These two species have, to the authors' knowledge, never been reported from Australian waters. A third species, *Pfiesteria piscicida* Steidinger and Burkholder, 1996, has previously been associated

with human illness and fish kills (Glasgow et al. 1995; Burkholder et al. 2001); however, this has more recently been attributed to misidentification of the dinoflagellate *Karlodinium veneficum* (Bergholtz et al. 2005; Zhang et al. 2008). The remaining four species, *Gymnodinium catenatum* Graham, 1943, *Alexandrium minutum* Halim, 1960, *Alexandrium tamarense* (Lebour) Balech, 1995, and *Alexandrium catenella* (Whedon and Kofoid) Balech, 1985, are causative agents of PSP and their presence in Australia has been reported on several occasions (Hallegraeff et al. 1988; Cannon 1990; Cohen et al. 2001; Bolch et al. 2002).

On a global scale, *Alexandrium tamarense* and *A. catenella* are amongst the most frequently occurring and noxious HAB species (Anderson et al. 2011) and have been reported in Australia along the coast of New South Wales (NSW), Victoria (Vic), South Australia (SA), and Tasmania (Tas) (Hallegraeff 1992; Ajani et al. 2001; Cohen et al. 2001; Bolch and Salas 2007). While *A. tamarense* is represented by both toxic and non-toxic strains, *A. catenella* is always reported as being toxic (Anderson et al. 2012). The first Australian medical record of PSP in NSW in 1935 is thought to have been a result of consumption of shellfish contaminated with *A. catenella* (Le Messurier 1935; Hallegraeff 1992), whose earliest identification in Australia dates to 1954 (Hallegraeff et al. 1991; Farrell et al. 2013). In Western Australia (WA), *A. minutum* is known to have been present in the Swan River since 1983 and is widespread in the south western estuaries and coastal waters (Hosja and Deeley 1994). Toxin-producing cultures have been established since 1996 from Bunbury waters (de Salas et al. 2001). In May 2000, a cyst survey of Fremantle's (Cockburn Sound) marine waters detected low concentrations of *A. tamarense*-like cysts in 12 out of 43 sites; however, these cysts failed to germinate – a requirement for conclusive identification (Huisman et al. 2008; JL Smith, School of Plant Sciences, University of Tasmania, unpubl. data). Without germination or molecular sequencing, *A. tamarense* and *A. catenella* cysts cannot be discriminated. There exists only a single report of viable *A. catenella* cysts entering WA waters; detected and cultured from Japanese cargo vessels at Port Hedland (Hallegraeff and Bolch 1992). To date no established populations have been detected by Department of Fisheries biosecurity monitoring. According to Hallegraeff (pers. comm. 2007, cited in Huisman et al. 2008), *A. catenella* has never been collected directly from WA waters.

Taxonomy of the *Alexandrium* '*tamarense* species-complex' (*A. tamarense*, *A. catenella* and *A. fundyense*) has long been a subject of debate (Scholin et al. 1994; Lilly et al. 2007; Anderson et al. 2012; John et al. 2014). Morphological identification of the three species (morphospecies) does not often correlate with the five identified genetic lineages within the '*tamarense* species-complex' (Scholin et al. 1994; Lilly et al. 2007). These five genetic lineages or 'ribotypes' were first identified by Scholin et al. (1994) and given geographic designations that reflected the origin of worldwide identified isolates: North American, Western European, Temperate Asian, Tasmanian, and Tropical Asian. However, for at least two of these genetic groups, the geographic names did not match the origin of the isolates. This led to further recommendations of genetic lineages within the '*tamarense* species-complex' being simply referenced as groups I, II, III, IV and V (Lilly et al. 2007). In the latest revision of the '*tamarense* species-complex' these five groups were assigned the following species names: Group I *A. fundyense*, Group II *A. mediterraneum*, Group III *A. tamarense,* Group IV *A. pacificum*, Group V *A. australiense* (see John et al. 2014 for more recent classification).

The classification of species within the *Alexandrium* '*tamarense* species-complex' based on genetic (rDNA) lineages highlights the need to integrate morphology with molecular identification methods such as real-time PCR and DNA barcoding. In this study, we report the first confirmed occurrence of *A. catenella* (*A. pacificum* Group IV) in open WA waters, using morphological and molecular identification methods.

Methods

Sampling

The Marine Biosecurity Research Group (MBR), part of the WA Department of Fisheries, undertakes regular biosecurity monitoring at high risk locations across the state. The annual monitoring of high risk locations (ports) includes plankton-net sampling to detect Harmful Algae (HA), which includes seven dinoflagellate species and three potentially harmful diatom species: *Alexandrium catenella* (HA), *Alexandrium minutum* (HA), *Alexandrium monilatum* (HA), *Alexandrium tamarense* (HA), *Chaetoceros concavicornis* Mangin, 1917, *Chaetoceros convolutus* Castracane, 1886, *Dinophysis norvegica* (HA), *Gymnodinium catenatum* (HA), *Pfiesteria piscicida* (HA) and *Pseudo-nitzschia seriata* Peragallo, 1899. During 2011 to 2013,

Table 1. Monitoring surveys for target HAB species. Location (port), year, number of plankton tows, and total volume of water sampled.

Location	Year	Total number of plankton tows	Total volume sampled (m^3)
Dampier	2011	15	424.1
	2013	11	311.0
Port Hedland	2011	7	197.9
	2013	8	226.1
Fremantle	2011	36	1017.8
	2013	36	1017.8
Geraldton	2012	12	339.2
Garden Island	2012	14	395.8

phytoplankton samples were collected with 30 cm diameter plankton nets (20 µm mesh) towed for 150 m (Table 1). Samples were preserved in 70% ethanol and later screened for target pest species.

Morphological identification

For the initial taxonomic sorting and identification (by JMH), samples were mounted on slides and phytoplankton cells examined under light microscopy for diagnostic morphological characters. Suspected HA cells were identified based on descriptions provided by Steidinger and Tangen (1996), Faust and Gulledge (2002) and Hallegraeff et al. (2010). Cells suspected of belonging to the toxic genus *Alexandrium* were examined in detail for the presence of a ventral pore on the first apical plate (1'), and a prominent apical pore complex. One sample collected from Henderson (Fremantle Port) in 2011 contained several cells suspected to be *A. catenella*. A subsample was sent to Gustaaf Hallegraeff, University of Tasmania, for scanning electron microscopy (SEM) and final identification of the specimens.

Molecular identification

The remainder of the sample (10 ml out of 100 ml sampled) containing suspected *A. catenella* (Fremantle Port) in 2011 was processed for molecular identification. The sample was stirred for homogenisation and two 0.5 ml subsamples (A and B) were collected with a Pasteur pipette and transferred to Eppendorf tubes. The two subsamples were then centrifuged at 4000 rpm for two minutes, and the supernatant carefully removed with a micropipette to avoid resuspension of the pellet. DNA was extracted from the pellet using a Fisher Biotec Favorgen FavorPrep Tissue Genomic DNA Extraction Mini Kit, following the manufacturer's instructions. Incubation was performed overnight (≈16 h) and samples eluted in a final volume of 100 µl. DNA extracts were stored at -20 ºC.

Water samples have a complex composition of plankton and particulate matter, some of which are known to inhibit PCR, potentially producing erroneous results. This problem can be detected and often overcome by diluting the DNA extracts as this also dilutes any inhibitors in the extract. For each of the DNA extracts of subsamples A and B, dilutions of 1:10 and 1:100 were made using PCR-grade water (Fisher Biotec) to check for PCR inhibition. To exclude the influence of PCR inhibitors and guarantee accurate and reproducible results, real-time PCR Critical Threshold (Ct) values for 10-fold dilutions should differ by around 3.3 cycles (100% efficiency) and the correlation (R^2) between Cts and dilution factor should be close to 1 (Applied Biosystems 2011; Pfaffl 2004).

Real-time PCR was performed on all DNA extracts and dilutions using the primers and probes as described in Hosoi-Tanabe and Sako (2005). The probes were used with slight modifications that consisted of the use of a TaqMan®-MGB quencher and FAM and VIC fluorophores to screen, respectively, for the presence of North American (Group I) and Temperate Asian (Group IV) ribotypes of the *Alexandrium* '*tamarensis* species-complex'. All reactions were conducted in a final volume of 10 µl containing 1 µl of DNA template, 1x TaqMan® Fast Advanced master mix (Applied Biosystems), 450 nM of each primer, and 200 nM of each TaqMan® probe (Applied Biosystems). All assays were performed in duplicate on an ABI Step One Plus™ real-time PCR system using a cycling profile of 50 ºC for 2 min (UNG incubation) and 95ºC for 20 s (DNA polymerase activation) followed by 45 cycles of 95ºC for 1 s (denaturation) and 60ºC for 20 s (annealing/ extension).

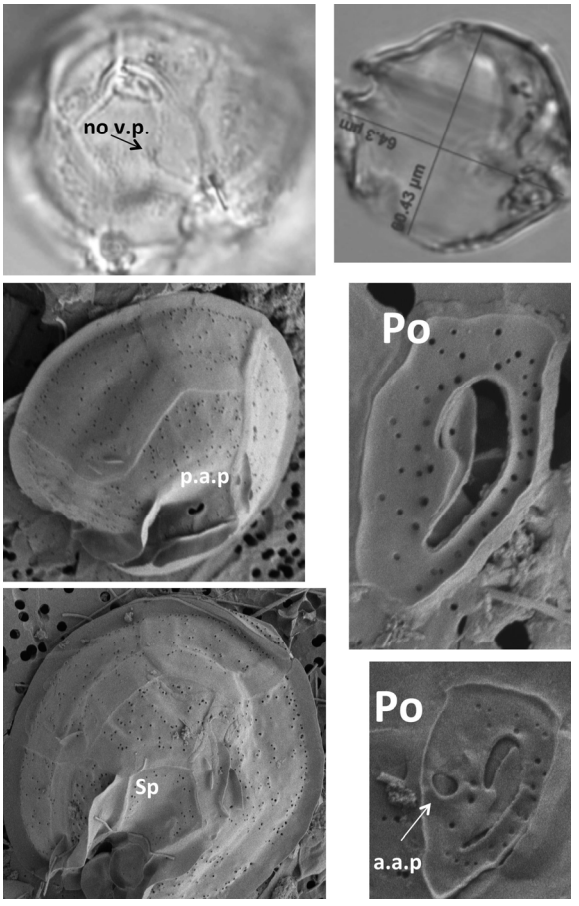

Figure 1. Light and Scanning Electronic Microscope micrographs of *Alexandrium catenella*. Figure showing morphological diagnostic features including cell measurements (above right), absence of ventral pore (no v.p., above left), presence of posterior apical pore (p.a.p., centre left) near the right margin of the wide posterior sulcal plate, presence of anterior apical pore (a.a.p., bottom right), triangular pore plates (Po, centre and bottom right) and anterior sulcal plate (Sp, bottom left) (images G. Hallegraeff).

Results

Morphological identification

In 2011, a single sample collected from Henderson (Fremantle Port) contained cells matching the description of *Alexandrium catenella*. We observed round armoured phytoplankton cells; slightly wider than long and anterior-posteriorly compressed. A ventral pore appeared absent in these cells, which fitted the description of the target HAB species, *A. catenella*. Other characteristics, such as the ability to form chains of cells, were not evident in the preserved sample under light microscopy.

The SEM analysis provided confirmation of diagnostic *A. catenella* characters, including: the absence of a ventral pore; sparsely-porulated thecal plates; the presence of anterior and posterior attachment pores (indicating the ability to form chains); the presence of a broad and triangular apical pore complex that widens dorsally; an apical pore plate with a characteristic fish hook foramen; a first apical plate in contact with the pore plate; and the wide posterial sulcal plate housing the posterior apical pore near the right margin (Figure 1). The plate formula for the armoured dinoflagellate was Po, 4', 6'', 6c, 8s, 5''', 2'''' with epitheca and hypotheca nearly equal in height. The median cingulum was lipped, deeply concave, and displaced in a descending fashion one times its width. The sulcus was observed to be deeply impressed and widened posteriorly.

The abundance of these cells was approximately 2 cells ml^{-1} in a concentrated 10 ml of plankton sample, which corresponds to about 0.02 cells per m^3 in the water column in Fremantle Port at the time of sampling.

In the 2013 survey, *A. catenella* was recorded for a second time in open waters of Fremantle Port at the Gages Roads sub-location (FRE03 13GAGEPT07). However, this sample was only morphologically identified as not enough material was available for filtration and subsequent real-time PCR analysis.

Molecular identification

The real-time PCR analyses were positive for the Temperate Asian (Group IV) strain for subsample A and its dilutions. Duplicate assays yielded comparable results (Critical Threshold Ct values) for subsample A (assay 1: Ct 35.09 and assay 2: Ct 35.51) and its 1:10 dilution (assay 1: Ct 38.15 and assay 2: Ct 38.45). For subsample A 1:100 dilution, assay 1 yielded a Ct of 41.79 while assay 2 resulted in a negative detection (Figure 2a). No detections were obtained for subsample B or its dilutions for either the North American (Group I) or the Temperate Asian (Group IV) strain. No detections were obtained from negative controls. The standard curve obtained for the detections of the Temperate Asian (Group IV) ribotype from the plankton sample showed a reaction efficiency of 98.5% (-3.25 slope) and R^2= 0.99 (Figure 2b).

Discussion

This study reports the first confirmed detection of *Alexandrium catenella* (*A. pacificum* Group IV genotype) in WA open waters and used a combination of morphological and molecular diagnoses.

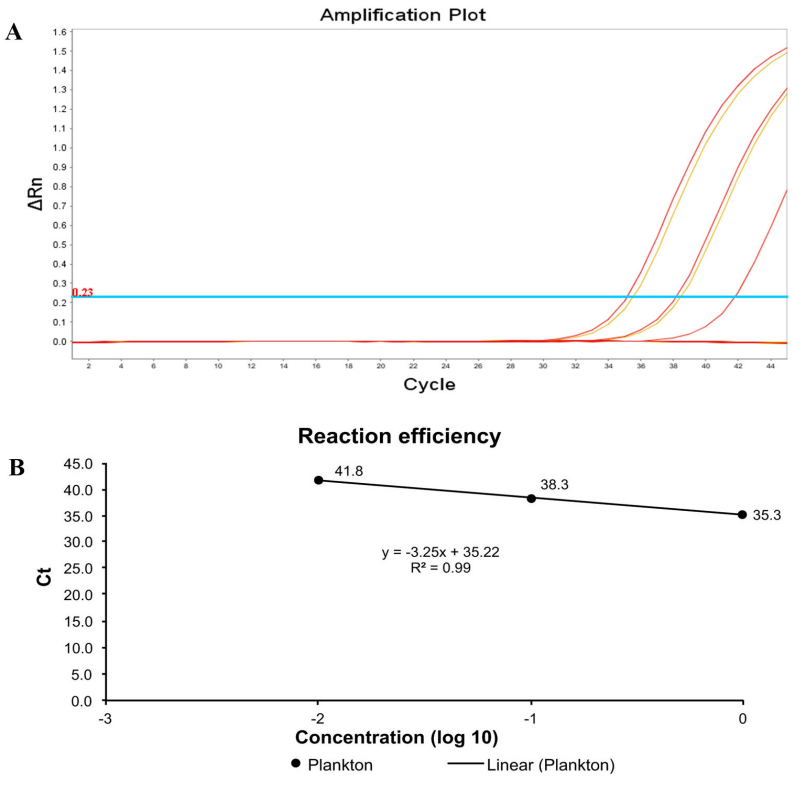

Figure 2. Real-time PCR amplification plot (A) and reaction efficiency (B). A. Amplification plot showing reaction Threshold (0.23) and Critical Threshold (Ct) values for amplifications of the *"tamarense species-complex*" temperate Asian ribotype. B. Reaction efficiency of the Temperate Asian specific Taqman®-MGB probe calculated using average values for DNA extracted from subsample A (Ct 35.3, SD±0.3) and its 1:10 dilution (Ct 38.3, SD±0.2), and value obtained for 1:100 dilution in assay 1 (Ct 41.8).

A. catenella is a pest species targeted by the National System (NIMPGC 2010) and is a causative agent of PSP. This detection has potential implications for state biosecurity and as a health-related threat to the shellfish aquaculture industry in the Cockburn Sound area.

Sampling and monitoring

Biosecurity surveillance employs a range of methodologies designed to target different life stages or environments that a potential pest may inhabit. This suite of sampling methods along with repeated sampling times adds confidence to the marine pest surveillance program. However, some of the methods used, such as plankton tows, are time consuming because sorting of samples can also be onerous and expensive. Also, most organisms are very difficult to identify at early larval life stages when diagnostic morphological characters can be undeveloped or absent.

The majority of target-pest species listed by the National System possess a 'final' stage that will settle and become more easily identifiable. One of the major exceptions to this are the seven listed HAB species and the three listed species of diatoms, which are mostly planktonic or, like *A. catenella*, have alternate resistant micro-cyst stages that can persist in the sediment for extended periods (Hallegraeff et al. 1998). The current approach to sampling for cysts of HAB species has been to collect and analyze numerous plankton tows and sediment cores. No *A. catenella* cysts were previously detected from sediments, and no cells from plankton samples were collected during monitoring surveys. However, given the low abundance and the localized and periodic nature of sediment and plankton sampling and analysis, the possibility that *A. catenella* cysts could have been present previously cannot be ruled out. Detection of toxin-producing cultures established from *A. catenella* cysts from sediments of cargo vessels at Port Hedland (Hallegraeff and Bolch 1992) and of *A. tamarense*-like cysts from Fremantle port in 2000 (JL Smith, School of Plant Sciences, University of Tasmania, unpubl. data) highlights the potential weakness in current bio-security monitoring and analysis for these species.

From a biosecurity point of view, prevention remains the most important requirement in the

fight against the introduction of HAB pest species. Studies have shown the seasonality of vessels arriving in Australia and testing positive for toxic dinoflagellate cysts closely reflects the seasonality of overseas plankton blooms (Hallegraeff 1998). An international warning network for algal blooms in ports has been previously suggested as an effective way to minimize pre-border risks (Hallegraeff 1998; Anderson et al. 2012). Finally, one should note that vessels travelling between Australian jurisdictions are not currently subject to Ballast Water Exchange (BWE) regulations (such as those of the IMO 2004). Preventing introductions resulting from the ballast water of these vessels could be achieved through targeted local monitoring and alert systems for HAB in the ports. Particular attention should be devoted to times of the year when environmental conditions are likely to favour the growth of HAB species, as most ballast water is likely to intake cells from these blooms and only to a lesser extent, cysts from re-suspended sediments (Hallegraeff 1998; Anderson et al. 2012).

Taxonomic and molecular identification

In the armoured phytoplankton cells found in Fremantle waters, the presence of diagnostic characters such as the absence of ventral pore, ability to form chains, and shape and position of attachment pores, supported the first confirmed detection of *Alexandrium catenella* in WA. Identification of morphospecies in the '*tamarense* species-complex' can only be achieved through morphological identification (Scholin et al. 1994; Anderson et al. 2012). The accurate species-level identification of phytoplankton is challenging, time consuming, and expensive. Taxonomic expertise for this group of species relies on a relatively small number of taxonomists worldwide. Taxonomic species-level identification, although essential, is not a practical method for a first-line of detection because of the time and costs involved.

Molecular identification of toxic HAB dinoflagellate species has become popular in recent years as it offers a cost-effective, faster, alternative that is better suited for routine identification (Galluzzi et al. 2004; Kamikawa et al. 2008; Anderson et al. 2012; Jedlicki et al. 2012). The high throughput potential of molecular techniques allows a large number of samples to be screened simultaneously, further reducing time and costs, and the sensitivity of probe-based methods like real-time PCR makes it possible to identify cells and cysts from complex water and sediment samples (Dyhrman et al. 2006; Erdner et al. 2010; Garneau et al. 2011). In taxonomically difficult and genetically complex HAB species, molecular markers have proved useful in identifying phylogenetic relationships, biogeography and strains, and their relation to important and distinct toxicology profiles (Scholin et al. 1994; MacKenzie et al. 2004; Hosoi-Tanabe and Sako 2005; Anderson et al. 2012; John et al. 2014).

The real-time PCR method developed by Hosoi-Tanabe and Sako (2005) proved to be a useful and rapid technique to detect cells of the Temperate Asian (Group IV) ribotype of the *Alexandrium* '*tamarense* species-complex'. We failed to pick individual cells of *A. catenella* for molecular diagnosis but we are confident in the analysis as HABs are often monospecific (Anderson et al. 2012) and the sample was screened by two experts who did not identify other species of the '*tamarense* species-complex'. We attribute the negative real-time PCR detection from subsample B to the low concentration of cells in the original sample as this could have led to no cells being collected in the subsample. Hosoi-Tanabe and Sako (2005) attributed a Ct~37 to the detection of a single *A. catenella* cell, leading us to think that in the concentrated subsample A, we were likely to have around 2–4 cells ml^{-1} (as reported from morphological identification screenings), corresponding to a detection of 0.02–0.04 cells per m^3 in the field. One should note however that the capacity to detect very low concentrations of these cells in the field will always be dependent, not only on the real-time PCR method sensitivity but, ultimately, on a comprehensive sampling approach of the affected area.

The efficiency in detecting 10-fold dilutions of DNA extracted from subsample A suggested little or no interference from inhibitors in the amplification, and we think the failure in detecting the 1:100 dilution of this subsample was likely due to the initial small amount of DNA in the subsample or pipetting error. The assigning of the detected *A. catenella* cells to the Temperate Asian (Group IV) ribotype allowed us to infer the primary region of origin of this introduced species but not the immediate source of introduction. *A. catenella* is thought to have been present in Australia since 1935 (Le Messurier 1935; Hallegraeff 1992); therefore, its introduction could have been from either an Asian or an already-established eastern Australian population. Molecular markers, such as microsatellites, able to analyse *A. catenella* populations at a finer

Table 2. HAB species targeted in Australia (NIMPGC 2010). Species name, confirmed detection in Australian states, most relevant associated effects, molecular methods available for detection, molecular methods sensitivity and respective references.

Family	Species	Detection in Australia	Effects	Molecular detection methods and sensitivity	Reference
Dinophyceae	*Alexandrium catenella*	Vic, NSW, SA, WA	PSP	PCR, qPCR assay able to detect single cells	Scholin et al. 1994; Hosoi-Tanabe and Sako 2005
	Alexandrium tamarense	Vic, Tas, SA	PSP	PCR, qPCR assay able to detect single cells	Scholin et al. 1994; Hosoi-Tanabe and Sako 2005
	Alexandrium minutum	Vic, NSW, Tas, SA, WA	PSP	PCR, qPCR assay able to amplify ≥ 0.2 equivalent of algal lysate	Scholin et al. 1994; Galluzi et al. 2004
	Alexandrium monilatum		Fish kills	PCR from culture cells, sensitivity not tested	Rogers et al. 2006
	Dinophysis norvegica		DSP	PCR from single cells	Hart et al. 2007
Gymnodiniaceae	*Gymnodinium catenatum*	Tas, Vic, SA	PSP	PCR able to detect ≥5 cyst from environmental samples	Patil et al. 2005
Pfiesteriaceae	*Pfiesteria piscicida*	Tas	Fish kills Human illness	PCR, qPCR assay can detect one zoospore in 1 ml of water, 10 resting cysts of sediment, or 10 fg in 1 g of heterologous DNA	Bowers et al. 2000; Saito et al. 2002

population genetic scale are needed to clarify this issue (Masseret et al. 2009).

Taxonomic identification of HAB species is labour-intensive, often requires additonal analysis (e.g. use of SEM), and only small number of cells can be analysed. In addition, processing of samples for SEM poses limitations when the method is applied to estuarine water samples (Bowers et al. 2000). There are currently a range of molecular methods available for the identification of HAB species, including the seven listed species in the National System (Table 2). Additional molecular methods are available that detect any species with saxitoxin-producing genes (Murray et al. 2011; Stuken et al. 2013). We think employing molecular methods as a first line of screening could save significant time and cost. In this study, outsourcing of taxonomic identification of phytoplankton species represented an added cost of ~ AU$ 100–200 per sample, in alternative to in-house real-time PCR analysis (~ AU$ 10–20 per sample). Molecular detection would also be fast enough to allow for a return to the sampling site within a few hours or days; in time to collect live cells for characterisation of toxic profiles. It would also limit the requirement for expert taxonomic screening and identification of HAB species, greatly reducing costs and time.

Conclusions

The detection of toxic HAB species is a growing problem worldwide and represents a serious threat to seafood security and human health. Monitoring of these species is challenging, time-consuming, and expensive and given the apparent absence of high-biomass, health threatening HABs, no comprehensive monitoring has been performed or routinely put in place to date in WA. Bio-security monitoring for HAB species at high-risk ports in WA is currently being performed; however, due to resource limitations, and the lack of feasible and efficient eradication methods, this surveillance program for HAB is being reviewed. Local routine monitoring of HAB species would unarguably be more practical and cost efficient using molecular methods. The use of molecular methods as a first line of screening for toxin producing HAB species would further speed up the identification and reporting to relevant authorities. The confirmed detection of *A. catenella* (*A. pacificum* Group IV) in WA using molecular tools will hopefully motivate discussion about improved surveillance for these toxic exotic HAB species.

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References

Ajani P, Hallegraeff G, Pritchard T (2001) Historic overview of algal blooms in marine and estuarine waters of New South Wales, Australia. *Proceedings of the Linnean Society N.S.W.* 123: 1–22

Anderson D (1989) Toxic algal blooms and red tides: a global perspective. In: Okalch T, Anderson D, Nemoto T (eds), Red tides. Biology, environmental science and toxicology. Elsevier, New York, pp 11–16

Anderson DM, Alpermann TJ, Cembella AD, Collos Y, Masseret E, Montresor M (2011) The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* 14: 10–35, http://dx.doi.org/10.1016/j.hal.2011.10.012

Anderson DM, Cembella AD, Hallegraeff GM (2012) Progress in Understanding Harmful Algal Blooms: Paradigm Shifts and New Technologies for Research, Monitoring, and Management. *Annual Review of Marine Science* 4: 143–176, http://dx.doi.org/10.1146/annurev-marine-120308-081121

Applied Biosystems (2011) Real-time PCR: Understanding Ct. Life Technologies Application Note, USA, 6 pp

Bergholtz T, Daugbjerg N, Moestrup O, Fernandez-Tejedor M (2005) On the identity of *Karlodinium veneficum* and description of *Karlodinium armiger* sp. Nov. (Dinophyceae), based on light and electron microscopy, nuclear-encoded LsU rDNA, and pigment composition. *Phycological Society of America* 42: 170–193

Bolch CJS, Blackburn SI, Hallegraeff GM, Vaillancourt RE (2002) Genetic variation among strains of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Journal of Phycology* 35: 356–367, http://dx.doi.org/10.1046/j.1529-8817.1999.3520356.x

Bolch CJS, de Salas MF (2007) A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates *Gymnodinium catenatum* and the *Alexandrium "tamarensis* complex" to Australasia. *Harmful Algae* 6: 465–485, http://dx.doi.org/10.1016/j.hal.2006.12.008

Bowers HA, Tengs T, Glasgow HB, Burkholder JM, Rublee PA, Oldach DW (2000) Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. *Applied and Environmental Microbiology* 66: 4641–4648, http://dx.doi.org/10.1128/AEM.66.11.4641-4648.2000

Burkholder JM, Glasgow HB, Deamer-Melia NJ (2001) Overview and present status of the toxic *Pfiesteria* complex. *Phycologia* 40:106–134, http://dx.doi.org/10.2216/i0031-8884-40-3-186.1

Cannon JA (1990) Development and dispersal of red tides in the Port River, South Australia. In: Graneli E et al. (eds), Toxic marine phytoplankton. Fourth international conference. Elsevier Science Publishing Co. Inc, pp 110–115

Cohen BF, McArthur MA, Parry GD (2001) Exotic marine pests in the Port of Melbourne, Victoria. In: Marine and Freshwater Resources Institute Report No. 25 Marine and Freshwater Resources Institute, 68 pp

DAFF (2010) Australian Marine pest monitoring guidelines. The National System for the Prevention and Management of Marine Pest Incursions. Department of Agriculture, Fisheries and Forestry, 57 pp

De Salas MF, van Emmerik M., Hallegraeff GM, Negri AP, Vaillancourt, RE, Bolch CJ (2001) Toxic Australian *Alexandrium* dinoflagellates: introduced or indigenous? In: Hallegraeff GM et al. (eds), Harmful Algal Blooms 2000, Proceedings of the Ninth International Conference on Harmful Algal Blooms, pp 214–217

Dyhrman ST, Erdner D, La Du J, Galac M, Anderson DM (2006) Molecular quantification of toxic *Alexandrium fundyense* in the Gulf of Maine using real-time PCR. *Harmful Algae* 5: 242–250, http://dx.doi.org/10.1016/j.hal.2005.07.005

Erdner DL, Percy L, Keafer B, Lewis J, Anderson DM (2010) A quantitative real-time PCR assay for the identification and enumeration of *Alexandrium* cysts in marine sediments. *Deep Sea Research* 57: 279–287, http://dx.doi.org/10.1016/j.dsr2.2009.09.006

Farrell H, Brett S, Ajani P, Murray S (2013) Distribution of the genus *Alexandrium* (Halim) and paralytic shellfish toxins along the coastline of New South Wales, Australia. *Marine Pollution Bulletin* 72: 133–145, http://dx.doi.org/10.1016/j.marpolbul.2013.04.009

Faust MA, Gulledge RA (2002) Identifying Harmful Marine Dinoflagellates. Contributions from the United States National Herbarium 42: 1–144, Smithsonian Institution, Washington, DC, ISSN 0097-1618

Freudenthal AR, Jijina JL (1988) Potential hazards of *Dinophysis* to consumers and shellfisheries. *Journal of Shellfish Research* 7: 695–701

Galluzzi L, Penna A, Bertozzini E, Vila M, Garce´s E, Magnani M (2004) Development of a Real-Time PCR Assay for Rapid Detection and Quantification of *Alexandrium minutum* (a Dinoflagellate). *Applied and Environmental Microbiology* 70: 1199–1206, http://dx.doi.org/10.1128/AEM.70.2.1199-1206.2004

Garneau M, Schnetzer A, Countway PD, Jones A C, Seubert EL, Caron D (2011) Examination of the Seasonal Dynamics of the Toxic Dinoflagellate *Alexandrium catenella* at Redondo Beach, California, by Quantitative PCR. *Applied and Environmental Microbiology* 7: 7669–7680, http://dx.doi.org/10.1128/AEM.06174-11

Glasgow HB Jr, Burkholder JM, Schmechel DE, Tester PA, Rublee PA (1995) Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. *Journal of Toxicology and Environmental Health* 46: 501–522, http://dx.doi.org/10.1080/15287399509532051

Hallegraeff GM, Steffensen DA, Wetherbee R (1988) Three estuarine Australian dinoflagellates that can produce paralytic shellfish toxins. *Journal of Plankton Research* 10: 533–541, http://dx.doi.org/10.1093/plankt/10.3.533

Hallegraeff GM, Bolch CJ, Blackburn S, Oshima Y (1991) Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters. *Botanica Marina* 34: 575–587, http://dx.doi.org/10.1515/botm.1991.34.6.575

Hallegraeff GM (1992) Harmful algal blooms in the Australian region. *Marine Pollution Bulletin* 25: 186–190, http://dx.doi.org/10.1016/0025-326X(92)90223-S

Hallegraeff GM, Bolch CJ (1992) Transport of diatom and dinoflagellate resting spores in ships' ballast water: Implications for plankton biogeography and aquaculture. *Journal of Plankton Research* 14: 1067–1084, http://dx.doi.org/10.1093/plankt/14.8.1067

Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia* 32: 79–99, http://dx.doi.org/10.2216/i0031-8884-32-2-79.1

Hallegraeff GM (1998) Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Marine Ecology Progress Series* 168: 297–309, http://dx.doi.org/10.3354/meps168297

Hallegraeff GM, Marshall JA, Valentine JP, Hardiman S (1998) Short cyst-dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella. Marine and Freshwater Research* 49: 415–420, http://dx.doi.org/10.1071/MF97264

Hallegraeff GM, Bolch CJS, Huisman JM, de Salas MF (2010) Planktonic dinoflagellates. In: Hallegraeff GM, Bolch CJ, Hill DRA, Jameson I, Le Roi JM, McMinn A, Murray S, de Salas M, Saunders K. Algae of Australia: Phytoplankton of Temperate Coastal Waters. ABRS, Canberra, CSIRO Publishing, Melbourne, Australia, pp 145–212

Hart MC, Green DH, Bresnan E, Bolch CJ (2007) Large subunit ribosomal RNA gene variation and sequence heterogeneity of *Dinophysis* (Dinophyceae) species from Scottish coastal waters. *Harmful Algae* 6: 271–287, http://dx.doi.org/10.1016/j.hal.2006.10.001

Hosja W, Deeley D (1994) Harmful phytoplankton surveillance in Western Australia. Waterways Commission Report, 43, 99 pp

Hosoi-Tanabe S, Sako Y (2005) Rapid detection of natural cells of *Alexandrium tamarense* and *A. catenella* (Dinophyceae) by fluorescence in situ hybridization. *Harmful Algae* 4: 319–328, http://dx.doi.org/10.1016/j.hal.2004.04.002

Huisman JM, Jones DS, Wells FE, Burton T (2008) Introduced marine biota in Western Australian waters. *Records of the Western Australian Museum* 25: 1–44

IMO (2004) International Convention for the Control and Management of Ships' Ballast Water and Sediments. International Maritime Organization, 38 pp

Jedlicki A, Fernandez G, Astorga M, Oyarzun P, Toro JE, Navarro JM, Martinez V (2012) Molecular detection and species identification of *Alexandrium* (Dinophyceae) causing harmful algal blooms along the Chilean coastline. *AoB Plants,* pls033, http://dx.doi.org/10.1093/aobpla/pls033

John U, Litaker RW, Montresor M, Murray S, Brosnahan ML, Anderson DM (2014) Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. *Protist* 165: 779–804, http://dx.doi.org/10.1016/j.protis.2014.10.001

Kamikawa R, Hosoi-Tanabe S, Yoshimatsu S, Oyama K, Masuda I, Sako Y (2008) Development of a novel molecular marker on the mitochondrial genome of a toxic dinoflagellate, *Alexandrium* spp, and its application in single-cell PCR. *Journal of Applied Phycology* 20: 153–159, http://dx.doi.org/10.1007/s10811-007-9200-9

Le Messurier DJ (1935) A survey of mussels on a portion of the Australian coast. *Medical Journal of Australia* 1: 490–492

Lilly EL, Halanych KM, Anderson DM (2007) Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). *Journal of Phycology* 43: 1329–1338, http://dx.doi.org/10.1111/j.1529-8817.2007.00420.x

MacKenzie L, de Salas M, Adamson J, Beuzenberg V (2004) The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. *Harmful Algae* 3: 71–92, http://dx.doi.org/10.1016/j.hal.2003.09.001

Masseret E, Grzebyk D, Nagai S, Genovesi B, Lasserre B, Laabir M, Collos Y, Vaquer A, Berrebi P (2009) Unexpected genetic diversity among and within populations of the toxic dinoflagellate *Alexandrium catenella* as revealed by nuclear microsatellite markers. *Applied and Environmental Microbiology* 75: 2037–2045, http://dx.doi.org/10.1128/AEM.01686-08

May SP, Burkholder JM, Shumway SE, Hégaret H, Wikfors GH, Frank D (2010) Effects of the toxic dinoflagellate *Alexandrium monilatum* on survival, grazing and behavioral response of three ecologically important bivalve molluscs. *Harmful Algae* 9: 281–293, http://dx.doi.org/10.1016/j.hal.2009.11.005

Murray SA, Wiese M, Stüken A, Brett S, Kellmann R, Hallegraeff G, Neilan BA (2011) sxtA-Based Quantitative Molecular Assay To Identify Saxitoxin-Producing Harmful Algal Blooms in Marine Waters. *Applied and Environmental Microbiology* 77: 7050–7057, http://dx.doi.org/10.1128/AEM.05308-11

NIMPCG (2010) Australian Marine Pest Monitoring Guidelines. Version 2. The National Introduced Marine Pest Coordination Group, Commonwealth of Australia, Canberra, 47 pp

Patil JG, Gunasekera RM, Deagle BE, Bax NJ, Blackburn SI (2005) Development and Evaluation of a PCR Based Assay for Detection of the Toxic Dinoflagellate, *Gymnodinium catenatum* (Graham) in Ballast Water and Environmental Samples. *Biological Invasions* 7: 983–994, http://dx.doi.org/10.1007/s10530-004-3119-8

Pfaffl MW (2004) Quantification strategies in real-time PCR. In: Bustin SA (ed), A-Z of Quantitative PCR. La Jolla: International University Line (IUL). Chapter 3, 25 pp

Rogers JE, Leblond JD, Moncreiff CA (2006) Phylogenetic relationship of *Alexandrium monilatum* (Dinophyceae) to other *Alexandrium* species based on 18S ribosomal RNA gene sequences. *Harmful Algae* 5: 275–280, http://dx.doi.org/10.1016/j.hal.2005.08.005

Saito K, Drgon T, Robledo JA, Krupatkina DN, Vasta GR (2002) Characterization of the rRNA locus of *Pfiesteria piscicida* and development of standard and quantitative PCR-based detection assays targeted to the nontranscribed spacer. *Applied and Environmental Microbiology* 68: 5394–5407, http://dx.doi.org/10.1128/AEM.68.11.5394-5407.2002

Scholin CA, Herzog M, Sogin M, Anderson DM (1994) Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). Part II. Sequence analysis of a fragment of the LSU rRNA gene. *Journal of Phycology* 30: 999–1011, http://dx.doi.org/10.1111/j.0022-3646.1994.00999.x

Steidinger KA, Tangen K (1996) Dinoflagellates. In: Tomas, CR (ed.), Identifying Marine Phytoplankton. Academic Press, San Diego, pp 387–584, http://dx.doi.org/10.1016/b978-012693015-3/50006-1

Stuken A, Dittami SM, Eikrem W, McNamee S, Campbell K, Jakobsen KS, Edvardsen B (2013) Novel hydrolysis-probe based qPCR assay to detect saxitoxin transcripts of dinoflagellates in environmental samples. *Harmful Algae* 28: 108–117, http://dx.doi.org/10.1016/j.hal.2013.06.003

Zhang H, Litaker W, Vandersea MW, Tester P, Lin S (2008) Geographic distribution of *Karlodinium veneficum* in the US east coast as detected by ITS-ferredoxin real-time PCR assay. *Journal of Plankton Research* 30: 905–922, http://dx.doi.org/10.1093/plankt/fbn047