**Title**

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

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**Highlights:**

- First extensive description of the genus Alexandrium for NSW, Australia.
- Genus is the main cause of microalgal related harvest closures in the region.
- Analysis of a 6.5 year phytoplankton monitoring data set for 31 estuaries.
- Findings propose target variables for site-specific studies.
- Results provide a baseline for development of predictive models for the region.

**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 8,649 fortnightly samples from 31 estuaries spread over 2,000 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 *Alexandrium 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, Alexandrium 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 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 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, 2011). 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 I) 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) (2011). 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, 2011).

A total of 8,649 (Table I), 500-1,000 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, 4,282 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. 0.5ml) of preserved water sample was placed on a slide and Calcofluor White was utilized 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, 2011) outlines "Phytoplankton Action Limits" (PAL) 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, 2011). The regulatory limit for PSP toxins is listed in the MBMP as being greater than or equal to 80 μg of saxitoxin equivalent/100g 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 *Alexandrium catenella* cultures established to date from the NSW region are of Group IV genotype (ie 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.

2.4.1 Environmental data

NSW tide tables (Fort Denison, Sydney 2005 - 2011) were used to determine tidal range (i.e., the difference in height between high tide and the subsequent low tide) for Brisbane Water, the Hawkesbury River and the Georges River. Along the south-eastern coastline of Australia, tides are semidiurnal with a pronounced difference in heights between consecutive high tides (Redden et al., 2009). Due to this, values of tidal range were averaged between successive tidal cycles, with the largest resulting value used as a daily estimate. The tidal ranges for the entrance to the Hawkesbury River/Brisbane Water and Botany Bay are similar to that of Sydney (NSW Office of Environment and Heritage, 2012). Given the orientation of Brisbane Water away from the open coast, the tidal range at the site would be expected to be lower than what is projected in the tide tables. For example, the tidal attenuation between Broken Bay and Ettalong (Fig. 1B) is ~15% (Manly Hydraulic Laboratory, 2004). Estimations of tidal range from the tide tables, however, provide an indication of what stage in the tidal regime that *Alexandrium* blooms occurred.

Automatic water level recording station data (NSW Public Work's Manly Hydraulics Laboratory (MHL)) were used to determine the maximum daily range in water level at sites near Brisbane Water (Ettalong, Fig. 1B) and the Hawkesbury River (Patonga, Fig. 1B and C). Data were recorded at 15 minute intervals, and following quality control processing of the data by MHL, the water level data output was accurate to ± 20 mm. Water level range was averaged between successive tidal cycles from which a daily maximum was obtained. At Brisbane Water (Fig. 1B), data were unavailable for the water level site at Ettalong between December 2006 and June 2009. However, missing water levels values were extrapolated from data collected at Patonga (Fig. 1C) to estimate the ranges in water level for Ettalong for the same period.

As part of the Shellfish Safety Program, sampling officers that collected water samples for microbiological indicators (e.g. faecal coliforms) also collected measurements of surface temperature and salinity. As part of the program, a range of instrument types were used and these varied between sampling sites. Temperature was recorded using either a thermometer or a digital temperature sensor (e.g Hanna Instruments Pty Ltd, USA). Salinity was measured using a refractometer (e.g. Issco Pty Ltd, Aus), or was calculated from the surface temperature and a surface density value derived from a hydrometer (e.g. S. Brannan & Sons Ltd, UK; Hanna Instruments Pty Ltd, USA.). Instrument calibrations were carried out annually. While this surface data were not sampled concurrently with phytoplankton samples, it provides an indication of surface water conditions for the estuaries between 2005 and 2011. For times when phytoplankton samples were collected, values of surface water temperatures were estimated from a 2-point linear regression on the dates closest to the sampling period.

2.4.2 Meteorological data

The Australian Bureau of Meteorology (BOM) records daily measurements of rainfall (mm) and global solar exposure (total solar radiation) ($MJm^{-2}$). Data between 2005 and 2011 were accessed from online archives (http://www.bom.gov.au/climate/data/) of weather stations near the Georges River (Sydney Airport AMO, Station ID: 66037) and the Hawkesbury River and Brisbane Water (Terrey Hills AWS Station ID: 66059) (Fig. 1). Photosynthetically active radiation (PAR) was estimated as 50% of the available solar radiation (Monteith, 1969) and expressed in $\mu E\ m^{-2}\ s^{-1}$. This data was considered as an indicator of seasonal light availability at the surface of the water column.

## 3. Results

3.1 Distribution and abundance of *Alexandrium* species

*Alexandrium* species were identified at all sites along the NSW coastline and in 1,674 (19.3%) of the 8,668 samples analysed (Table I). Seven taxa were identified during the period: *Alexandrium catenella, A. fraterculus, A. margalefi, A. minutum, A. ostenfeldii, A. pseudogonyaulax,* and *A. tamarense.* Of these species, *A. fraterculus*, *A. margalefi,* and *A. pseudogonyaulax* are not known PSP toxin producers (Anderson et al., 2012). Occasionally, cells of *Alexandrium* were found which were too difficult to identify to species level due to poor preservation of the theca, without the aid of more complex diagnostic tools. During analysis, these species were grouped as "unidentified *Alexandrium* species". Each estuary in Fig. 1 had observations of more than two species of *Alexandrium,* with the greatest species number of species (8 taxa) reported at two estuaries (Shoalhaven/Crookhaven River and Wagonga Inlet) (Table I). Based on the total *Alexandrium* species concentrations observed during the study period, *A. catenella* had the greatest relative abundance (45.5%) (Fig. 2), followed by *A. pseudogonyaulax* (22.83%). *Alexandrium fraterculus* had the lowest relative abundance (1.73%).

3.2 Temporal and spatial distribution of *Alexandrium* and PSP biotoxins

Examination of the seasonal distribution of the non-toxic and toxic species types showed that *Alexandrium* spp. were present during all months. The greatest cell abundances (up to 23,500 cells $L^{-1}$) from the combined cell concentrations of potentially toxic *Alexandrium* species were seen in the warmer months of spring and summer (months 1, 9-12), which also coincided with the majority of positive results from shellfish flesh tests for PSP toxins (Fig. 3A). *Gymnodinium catenatum,* which is the only other species present in the region known to produce PSP toxins, did not exceed PAL limits during the reported PSP events. Peaks in cell abundance (up to 3,900 cells $L^{-1}$) of

potentially toxic species of *Alexandrium* were also seen during autumn (months 2-5). Lower cell concentrations (<1000 cells $L^{-1}$) were observed during the Austral winter (months 6-8). Examination of individual species distributions showed the greatest seasonality in *A. tamarense* (Fig. 3B) and *A. catenella* (Fig 3C), both of which followed the pattern described above. In contrast, maximum cell concentrations of *A. minutum* were observed to occur during late summer/autumn (months 2-4) (Fig 3F). *Alexandrium ostenfieldi* and unidentified *Alexandrium* spp. did exhibit a seasonal pattern but with lower cell concentrations observed in seasonal peaks (<800 cells $L^{-1}$).

Between July 2005 and December 2011, *Alexandrium* related events accounted for 38 of the 72 harvest area closures in NSW (Table II). For the same period, analysis of shellfish flesh gave 63 positive results for PSP toxicity. *Alexandrium tamarense* was identified as the causative agent for PSP closures along the north coast. In the central and south coast regions of the state *A. catenella*, *A. minutum*, and *A. ostenfieldi* were linked to positive PSP events. In some cases the causative species were not confirmed, as water samples were not taken during extant closure periods (refer Table II). With the exception of 2008, where there were no closures or toxic events caused by *Alexandrium* spp., the number of harvest zone closures and positive samples increased annually from 2005 to 2010. During 2010, reports of PSP toxicity were notably higher than in previous years. The greatest number of closures (12 zones with a combined number of closures days of 312) and positive biotoxin samples (29) occurred.

There was no discernable pattern between the number of species present and latitudinal distribution (Table I). Although seasonality was apparent for individual species (refer Fig. 3), annual blooms did not occur at all sites. From the temporal and spatial distribution of the species (Fig. 4), it was apparent that the northern region of the study area (north of 32°S) had the fewest observations of *Alexandrium* spp. The majority of *Alexandrium* spp., classified as being either potentially toxic or non-toxic, were present south of this delineation.

*Alexandrium catenella* was the primary causative agent of PSP toxicity during the study period (Table II). The species had a distribution between Richmond and Wonboyn (Fig. 4A) and was observed to frequently bloom, resulting in harvest zone closures between 33°S - 35°S at Brisbane Water (max. concn. 1,200 cells $L^{-1}$), Hawkesbury River (max. concn. 2,500 cells $L^{-1}$) and Georges River (max. concn. 22,000 cells $L^{-1}$). On one occasion during 2005, cells exceeded the PAL limit of 200 cells $L^{-1}$ in Wallis Lake. Three proliferations of cells (2007, 2008 and 2009) were recorded in samples derived from the Shoalhaven/Crookhaven Rivers. In addition during 2010, notable cell

concentrations (> 500 cells $L^{-1}$) were observed at Nelson Lagoon. However, no toxicity was reported for these events (Table II). In contrast, during November 2010 at Wagonga Inlet, a bloom of 10,000 cells $L^{-1}$ resulted in up to 50 days of harvest zone closures (Table II).

Cells of *A. tamarense* were also observed between Richmond and Wonboyn (Fig. 4A), however, concentrations rarely exceeded 200 cells $L^{-1}$. *Alexandrium tamarense* was linked to HAB events at the Bellinger and Hastings Rivers and at Wallis Lake (Table II). The greatest proliferation of this species (11,000 cells $L^{-1}$) occurred at Wallis Lake during October 2010, although toxin analysis results were negative. The presence of cells at the Hastings River was linked to a positive PSP biotoxin result in November 2010 (Table II).

Unidentified species of *Alexandrium* (Fig. 4B), *A. ostenfeldii* (Fig. 4B) and *A. minutum* (Fig. 4C) were distributed all along the NSW coastline, however blooms of the species were infrequent and only reported south of 33°S (Fig. 4). *Alexandrium minutum* was the causative species of two toxic events resulting in harvest closures, in the Hawkesbury River during 2007 (March) and at Port Stephens in 2009 (December) (Table II). *Alexandrium ostenfeldii* was the only species (150 cells $L^{-1}$) identified during a positive PSP event in Twofold Bay in 2007 (Table II), however, no culture was established from this event, and the toxicity of this species in NSW waters needs to be further verified.

*Alexandrium fraterculus* was the species with the lowest relative abundance (Fig. 2), and was only observed south of 34°50'S (refer Table I and Fig. 4D). At Wagonga Inlet this species exceeded 200 cells $L^{-1}$ during late summer/early autumn in 2007, 2009 and 2011.

*A. margalefi* was observed all along the NSW coastline between Tweed Heads and Wonboyn (Fig. 4E). On several occasions, the recorded cell concentration exceeded 200 cells $L^{-1}$, usually in October or November. These observations were made across five different estuaries (Hunter River, Brisbane Water, Tuross Lake, Wagonga Inlet and Wonboyn River).

*Alexandrium pseudogonyaulax* was also observed between Tweed Heads and Wonboyn with the greatest abundance of species recorded south of 33°S (Fig. 4F).

3.3 Blooms of *A. catenella* in the Brisbane Water, Hawkesbury River and Georges River areas.

**3.3.1 Brisbane Water**

*Alexandrium catenella* has been recorded in Brisbane Water since 2006, with the greatest cell concentrations (up to 1,200 cells $L^{-1}$) observed at Station 46 (Fig. 1B). Cells of *A. catenella* were observed annually between June and December with maximum cell concentrations occurring between September and October (Fig. 5A,i). Monthly averages of daily global solar exposure showed blooms occurring after a threshold value of ca. 15 $MJm^{-2}$ was reached. This was equivalent to a surface PAR value of approximately 400 $\mu$ E $m^{-2}$ $s^{-1}$. The actual irradiance profile of the water column at the time of sampling is unknown. However, following the Lambert Beer Law for the exponential decrease of irradiance with depth:

$$I_z = I_0.e^{-k_e.z}$$

(where $I_z$ = irradiance at a depth (z, m) below the water surface; $I_0$ = surface irradiance and $k_e$ = extinction coefficient ($m^{-1}$, 0.1 – 0.9 $m^{-1}$ from Parlsow et al., 1999) values of PAR were expected to range between 4 – 400 $\mu$ E $m^{-2}$ $s^{-1}$ within a 5m water column, depending on localised water column conditions.

In this region, measurements of surface salinity ranged between 25.6 - 35.5 ppt. For each seasonal bloom, linear regression analysis demonstrated a significant relationship ($R^2$ = 0.56, p = 0.02, n=9) between increasing cell concentrations (>25 cells $L^{-1}$ to bloom maxima) and surface temperature. Generally, blooms coincided with water temperatures greater than 16°C (Fig. 5A,ii). Although peaks in cell abundance were seen in Brisbane Water in 2006 and 2011, surface water temperatures were ~15°C or less, and cell concentrations of bloom proportions did not develop (Fig. 5 A,i).

The distribution of daily tidal range for Fort Denson (Sydney) tides in Fig. 5 clearly shows the monthly spring and neap cycles (see Fig. 6). Averaged tidal range values up to 1.7 m occurred during March and September, while lower tidal ranges (up to 1.3 m) were observed during June and December. Brisbane Water was found to be a "wave dominated estuary" with greater freshwater influences than both the Hawkesbury and Georges Rivers, which were found to be tide dominated (Roy et al., 2001). At Brisbane Water, recorded water level range (max = 1.12; min = 0.41) was, as expected, lower than the predicted tidal range (Fig. 6A,i-iv). When cell maxima of *A. catenella* from Station 46 at Brisbane Water were compared to the daily water level range estimated at Ettalong (Fig. 1B), peaks in cell abundance appeared to be associated with periods of reduced water level range (Fig. 6A,i-iv). For blooms that occurred between 2007 and 2010 in Brisbane Water, with the exception of 2010, cell maxima occurred in months that had the lowest average water level range during the seasonal bloom period. Further examination of water level ranges during these bloom years, showed values of water level range between 0.64 m and 0.96 m associated with the

early stages of the bloom (Fig. 6A,i-iv). It is worth noting that while cell concentrations of *A. catenella* did not exceed 500 cells $L^{-1}$ in the nearby Patonga Creek (Fig. 1B), peaks in cell concentrations were observed annually and biotoxin test results were positive for PSP during 2009 and 2010 (Table II). Although cell concentrations were lower, a similar pattern of cell maxima occurring at low water level range was observed in the data from Patonga (data not shown).

Differences in bloom intensities were observed from year to year and appeared to be associated with rainfall events. Although daily rainfall values recorded near Brisbane Water during the study period were variable, two seasonal peaks (60 - 130 mm) were observed between February and April and June and September. Lower daily rainfall values (<50 mm) were reported between October and January. Total rainfall recorded in the two weeks prior to cell maxima >500 cells $L^{-1}$ of *A. catenella* between 2007 and 2011 (Table III), was less than 20mm. Notably, for the maximum observations (1,200 cells $L^{-1}$) at Brisbane Water in 2009, less than 6mm of rain were recorded in the 14 days before the bloom.

**3.3.2 Hawkesbury River**

Blooms of *A. catenella* were observed to occur predominately between September and November in the Hawkesbury River (Fig. 5B,i), although the blooms were not concurrent with those observed at Brisbane Water. In the Hawkesbury River, the greatest cell concentrations were seen at Station 34 (Fig. 1C). At this location, recorded values of surface salinity ranged between 21.9 ppt and 35.4 ppt. The linear relationship between bloom size and temperature from the Hawkesbury samples was not significant ($R^2 = 0.31$, $p = 0.25$, $n=6$) (Fig. 5B,ii). This may have been as a result of fewer samples with >25 cells $L^{-1}$ preceding bloom events (see Fig 5B,i), and no temperature data being available for the 2011 bloom season. Nonetheless, blooms appeared to coincide with surface water temperatures greater than 16°C (Fig. 5B,ii) and monthly averages of daily global solar exposure greater than 15 $MJm^{-2}$ (equiv. 400 $\mu$ E $m^{-2}$ $s^{-1}$ PAR).

Data from the Patonga water level recorder (Fig. 1B) revealed ranges in water level from 0.48m - 1.7m. Given the greater tidal influence within the Hawkesbury River (Roy et al., 2001), the distribution of water level range closely resembled that of the tidal range (Fig. 6B,i-iv). Seasonal blooms were observed to occur where values of water level range were < 1.05 m, either during neap tides or during the period of low water during the onset of, or immediately after, a neap tide (Fig. 6,B,i-iv).

Rainfall data for the Hawkesbury River was derived from the same weather station (Terrey Hills AWS Station ID: 66059) as that used for Brisbane Water. Examination of daily rainfall levels between 2007 and 2011 revealed total values less than 25mm in the two weeks preceding blooms of *A. catenella* (Table III). The most intense bloom (2,500 cells $L^{-1}$ 2009, Fig. 5B,ii) followed a period of 14 days where total rainfall was less than 6 mm.

### 3.3.3 Georges River

*Alexandrium catenella* was recorded during all months (except April) in the Georges River. While smaller blooms (up to 750 cells $L^{-1}$) were recorded between May and September, the greatest cell concentrations (up to 22,000 cells $L^{-1}$) were seen between the months of October and January (Fig. 5C). There was a significant relationship ($R^2 = 0.72$, $p < 0.001$, $n=12$) between temperature and cell concentrations leading up to seasonal *Alexandrium* proliferations in the Georges River (Fig. 5C,ii). Blooms were observed in 2005, 2006, 2007, 2010 and 2011, with associated surface water temperatures greater than 20°C and monthly averages of daily global solar exposure of more than 15 $MJm^{-2}$ (equiv. 400 $\mu$ E $m^{-2}$ $s^{-1}$ PAR). The observed surface salinity range during the study period was 22 - 35.8ppt.

No automated water level data were available for the Georges River. Based solely on tidal range data (Fig. 6C) the larger blooms observed during 2005 (8,500 cells $L^{-1}$) (Fig 6C,i) and 2010 (22,000 cells $L^{-1}$) (Fig 6C,iii) followed the lower tidal ranges (<1.4m), occurring after the Spring equinox, at the end of September/start of October. In 2006 (Fig 6C,ii), spring tidal ranges were >1.4m until the start of November and this additional dilution may have resulted in the delay in the bloom until Jan 2007 (3,900 cells $L^{-1}$). Similarly, the higher tidal ranges (up to 1.6m) observed during November 2011 (Fig. 6C,iv) may have caused sufficient dilution to cause the collapse of a bloom of 8,000 cells $L^{-1}$.

Rainfall levels were also variable near the Georges River. Daily values exceeding 45 mm were reported between February and October, with maximum values observed in March (106 mm) and July (91 mm). Values less than 45 mm were recorded between October and January. While blooms recorded for Georges River in 2006, 2007 and 2011 occurred after periods of low rainfall (<25mm in the preceding 14 days), a bloom in 2010 of 22,000 cells $L^{-1}$ was observed after a much larger rainfall event (>100 mm in the preceding 14 days) (Table III).

## 4. Discussion and Conclusion

Prior to 1970, reports of toxic *Alexandrium* blooms were only documented from temperate zones in the Northern Hemisphere (Europe, Japan and North America) (Hallegraeff, 1993). Since then, incidences of *Alexandrium* related PSP toxicity have been documented more frequently and with greater intensity (Glibert et al., 2005). Increased reports of blooms of harmful species across the globe have been linked to factors such as enhanced sampling and monitoring capabilities, intensified coastal development, transport of species to other countries via ship's ballast water and human induced climate change (Hallegraeff, 2003). Regardless of cause, these escalations of harmful blooms threaten both public health and regional economies. Species of the genus *Alexandrium* have now been recorded worldwide and throughout the southern hemisphere (Taylor et al., 2003). Globally, cell densities during blooms of $10^3 – 10^6$ cells $L^{-1}$ *Alexandrium* blooms have been reported (Wyatt and Jenkinson, 1997 e.g. *A. minutum* in the Mediterranean (Villa et al., 2005; Van Lenning et al., 2007) and *A. fundyense* in the Gulf of Maine (Anderson et al., 2005)). Cell concentrations of up to 25,000 cells $L^{-1}$ of *Alexandrium* spp. were observed during this study and the high potency of toxic members of the genus (Todd, 2001; Hallegraeff, 2003) reinforces the requirement of low target cell concentrations for monitoring purposes (NSW Food Authority MBMP, 2011). It is crucial for the shellfish industry to understand the underlying dynamics of HAB events, particularly blooms of *Alexandrium*, which account for the majority of algal related harvest zone closures in NSW.

Between 2005 and 2011, known toxin producing species of *Alexandrium* were observed at all sampling sites, within major oyster producing estuaries, along the NSW coastline. In 1999, the CSIRO documented that Newcastle Port (32° 55' S) was the northern most limit of *A. catenella* in NSW (CSIRO, 1999). During the current survey period (2005-2011), reports of *A. catenella* were made over 480 km further north, with an upper limit at the Richmond River (28° 53' S). Blooms of *A. minutum*, *A. tamarense* and *A. ostenfieldii* were rarer than those of *A. catenella*. Newcastle Port was also the northern limit within the state in previously published literature for these species (CSIRO, 1997; Pollard and Pethebridge, 2002a,b, Bolch and de Salas, 2007, Hallegraeff et al., 2010; Murray et al., 2011). However, the lower number of historical observations are likely to be a reflection of the reduced cell numbers observed in the northern part of the study area during this study. Based on the species distributions along the NSW coastline, the greatest abundance of both potentially toxic and non-toxic species of *Alexandrium* were observed at the mid range to southern latitudes of the study area (32° - 37°S). This was also true for the occurrence of positive PSP events in the region. There is no significant correlation between estuary classification (i.e., bays, tide dominated, wave dominated, intermittent estuaries and freshwater bodies) and latitude along the

NSW coastline (Roy et al., 2001), and the apparent greater species diversity of in the southern latitudes of the state is considered to be linked to a range of environmental factors within each individual estuary (Ajani et al., 2012). In the case of *Alexandrium*, the presence (or absence) of a source cyst bed is also a dominant factor accounting for presence and abundance, as recurrent annual blooms of *Alexandrium* have been linked to seasonal germination of a sedimentary cyst stage (Wyatt and Jenkinson, 1997; Anderson, 1998). Sediment surveys undertaken in NSW estuaries have shown the presence of *Alexandrium* cysts along the coastline, reaching as far north as Newcastle Port (Hallegraeff et al., 1998, CSIRO, 1999). It is to be expected, based on the seasonality observed for each of the *Alexandrium* species observed during this study that regional blooms are derived from a cyst seed bed source rather than being advected into the region's estuaries.

At the three sites chosen for further investigation, annual blooms of *A. catenella* coincided with a seasonal window of increasing solar radiation and subsequent increases in surface water temperature. Even with a limited temperature data set, the link between bloom size and surface temperature was evident in this study. Larger blooms (>800 cells $L^{-1}$) observed in Brisbane Water in 2007 and 2009 occurred between 16°C and 20°C. At lower temperatures cell concentrations did not exceed PAL levels. Similarly, bloom cell maxima in the Hawkesbury River were observed at surface temperatures greater than 16°C. In the Georges River the greatest cell concentrations reported were associated with temperatures greater than 20°C. This is consistent with findings for *A. catenella* cell growth in laboratory experiments (Ni, Rathaille et al., 2009; Laabir et al., 2011) and field observations (Ni Rathaille and Raine, 2006), which shows that temperatures greater than 20 degrees and underwater irradiance in excess of 100 $\mu$ E $m^{-2}$ $s^{-1}$ tends to support maximum growth, with photoinhibition being observed at irradiance levels greater than 250 $\mu$ E $m^{-2}$ $s^{-1}$.

Depending on regional circulation patterns, reduced freshwater input can increase population residence times and cell concentrations (Anderson, 1997). During this study, periods of low rainfall were observed to generally precede maximum bloom concentrations of *A. catenella*. Deviating from this trend, the largest bloom (22,000 cells $L^{-1}$) of *A. catenella* reported at the Georges River occurred after a period of intense rainfall. This suggests that dispersive influences may have less of an impact, if cell concentrations can reach sufficient levels in order to maintain bloom concentrations. In small bays and estuaries (e.g., Cork Harbour, Ireland and the Penzé Estuary, France) links between reduced tidal mixing and enhanced *A. minutum* and *A. tamarense* cell abundance have been established (Maguer et al., 2004; Chambouvet et al., 2008; Ni Rathaille et al., 2009). It was hypothesised for this study that the reduced water level ranges, coinciding with low

rainfall, during the optimal period of temperature and irradiance in Brisbane Water provided a suitably retentive physical environment for blooms of *A. catenella* to proliferate. Similarly, in the Hawkesbury and Georges Rivers, blooms of the species could be linked to periods of low tidal exchange, during the appropriate seasonal window.

Although our current knowledge of specific water column conditions is limited, this study has demonstrated associations between annual cell maxima of *A. catenella* and water temperature, solar radiation, rainfall and estuarine flushing at three locations along the NSW coastline, where harvest closures are frequent. The presence of *Alexandrium* along the NSW seaboard presents a serious threat to the region's aquaculture industry, with potentially toxic species occurring at all of the extant harvest areas. The risk is further accentuated by the increase in intensity and frequency of PSP events since 2005. The majority of aquaculture zones are exposed to *Alexandrium* blooms on an annual basis and while this vulnerability has been realized, the dynamics of these events in south-eastern Australia are not yet understood. Intensive site-specific studies are imperative to determine detailed correlations between estuaries and HAB events at the resolution required for the development of predictive models.

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## Tables

Table 1: Summary of NSW estuaries where phytoplankton samples were collected on a fortnightly basis between 2005 and 2011. The number of samples collected at each estuary, along with observations of *Alexandrium* spp., are noted. Estuary Locations are shown in Fig. 1.

| Estuary Name | Lat. (S) | Sites identification number for phytoplankton collection | No. of samples analysed for harmful (toxic and related) phytoplankton | Observations of *Alexandrium* spp. | |
|---|---|---|---|---|---|
| Tweed River | 28° 10' | 12,13 | 92, 91 | 4 | *A. margalefi*, *A. minutum*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Richmond River | 28° 53' | 10,11 | 72, 11 | 4 | *A. catenella*, *A. ostenfeldii*, unidentified *Alexandrium* species, *A. tamarense* |
| Clarence River | 29° 25' | 11 | 55 | 2 | *A. catenella*, unidentified *Alexandrium* species |
| Wooli Wooli River | 29° 53' | 7 | 41 | 6 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Bellinger/ Kalang River | 30° 30' | 17,18 | 38, 51 | 5 | *A. catenella*, *A. minutum*, *A. ostenfeldii*, *Alexandrium* sp., *A. tamarense. tamarense* |
| Nambucca River | 30° 39' | 13,14 | 107, 110 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense*. *tamarense* |
| Macleay River | 30° 52' | 18,19,20 | 100, 57, 103 | 5 | *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Hastings River | 31° 25' | 26,27,28 | 135, 135, 124 | 6 | *A. catenella*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Camden Haven River | 31° 38' | 21,22,23 | 150, 101, 89 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Manning River | 31° 53' | 39,40,41 | 74, 71, 21 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Wallis Lake | 32° 13' | 1,2,3 | 139, 139, 139 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| Port Stephens | 32° 42' | 25,26,27, 33,52,75,76,89, 90,100,117 | 155,155,153,156, 155,154,153,154, 156,137,155 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Hunter River | 32° 55' | 13, 14 | 54,18 | 2 | *A. margalefi*, *A. pseudogonyaulax* |
| Brisbane Water | 33° 31' | 46,47,48,49,54 | 157,17,152,155,135 | 7 | *A. catenella/fundyense*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Patonga Creek | 33° 32' | 7 | 144 | 6 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Hawkesbury River | 33° 34' | 31,32,33, 34,44,50 | 142,155,12, 129,15,1 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Georges River | 34° 01' | 7 | 156 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| Shoalhaven/ Crookhaven River | 34° 53' | 35,36,37,38 | 149,143,148,127 | 8 | *A. catenella*, *A. fraterculus*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Jervis Bay | 35° 06' | 9 | 22 | 3 | *A. margalefi*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Conjola Lake | 35° 16' | 6 | 14 | 5 | *A. catenella/fundyense*, *A. margalefi*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Clyde River | 35° 42' | 1,2,3 | 146,142,145 | 6 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| Tuross Lake | 36° 04' | 1,2 | 138,59 | 6 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Wagonga Inlet | 36° 13' | 1,2 | 168,169 | 8 | *A. catenella*, *A. fraterculus*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Bermagui River | 36° 26' | 9 | 113 | 6 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Wapengo Lagoon | 36° 38' | 1,2 | 146,147 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| Nelson Lagoon | 36° 41' | 1,3 | 4,141 | 5 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. pseudogonyaulax*, unidentified *Alexandrium* species |
| Merimbula Lake | 36° 54' | 1,2 | 164,163 | 7 | *A. catenella/fundyense*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| Pambula Lake | 36° 57' | 16,17 | 15,136 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species *A. tamarense* |
| Twofold Bay | 37° 05' | 1,2,3,4 | 125,18,99,108 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species, *A. tamarense* |
| Wonboyn River | 37° 17' | 1,2 | 165,165 | 7 | *A. catenella*, *A. margalefi*, *A. minutum*, *A. ostenfeldii*, *A. pseudogonyaulax*, unidentified *Alexandrium* species*A. tamarense* |
| **TOTAL** | | **79** | **8649** | | |

Table 2: Summary of the number of algal related harvest zone closures, positive PSP toxin tests encountered and causative species (if determined) for each year between 2005-2011 at each of the oyster producing estuaries monitored by the NSW Shellfish Safety Program.

| Estuary Name | No. of harvest zone closures due to algal blooms (suspected or confirmed) July 2005 – Dec 2011 | No. of harvest zone closures due to *Alexandrium* spp. (suspected or confirmed) July 2005 – Dec 2011 | No. of days of closure per zone due to *Alexandrium* spp. (suspected or confirmed) July 2005 – Dec 2011[1] | | | | | | | Total Positive PSP Biotoxin | PSP Toxins encountered (Positive toxin test on shellfish flesh) | | | | | | | Causative species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | |
| Tweed River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Richmond River | 2 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Clarence River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Wooli Wooli River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Bellinger River | 1 | 1 | - | 8 | - | - | - | - | - | 0 | - | - | - | - | - | - | - | *A. tamarense* |
| Nambucca River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Macleay River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Hastings River | 0 | 0 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | 1 | - | *A. tamarense*[3] |
| Camden Haven River | 1 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Manning River | 4 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Wallis Lake | 3 | 3 | - | - | - | - | - | 3(7) | - | 0 | - | - | - | - | - | - | - | *A. tamarense* |
| Port Stephens | 4 | 2 | - | - | - | - | 2(7) | - | - | 5 | - | - | - | - | 5 | - | - | *A. minutum* |
| Hunter River | 0 | 0 | - | - | - | - | - | - | - | 1 | - | - | 1 | - | - | - | - | undetermined[2] |
| Brisbane Water | 6 | 4 | - | - | - | - | 50,14 | 2(74) | - | 18 | - | - | - | - | 5 | 13 | - | *A. catenella* |
| Patonga Creek | 4 | 2 | - | - | - | - | 29 | 2 | - | 8 | - | - | - | - | 4 | 4 | - | *A. catenella* |
| Hawkesbury River | 21 | 14 | - | - | 4(5),6,16 | - | 3(12), 42 | 2(16) | 28,47 | 9 | - | - | 6 | - | - | 1 | 2 | *2007: A.minutum*, 2010-11*: A. catenella* |
| Georges River | 7 | 6 | 14 | 11 | - | - | - | 30 | 3,51,66 | 8 | 1 | 1 | - | - | - | 3 | 3 | *A. catenella* |
| Shoalhaven/ Crookhaven River | 2 | 1 | - | 10 | - | - | - | - | - | 1 | - | 1 | - | - | - | - | - | undetermined[2] |
| Jervis Bay | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Conjola Lake | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Clyde River | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Tuross Lake | 1 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Wagonga Inlet | 5 | 3 | - | - | - | - | - | 50,16,14 | - | 7 | - | - | - | - | - | 7 | - | *A. catenella* |
| Bermagui River | 2 | 0 | - | - | - | - | - | - | - | 1 | - | 1 | - | - | - | - | - | undetermined[2] |
| Wapengo Lagoon | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Nelson Lagoon | 2 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Merimbula Lake | 3 | 1 | - | - | 7 | - | - | - | - | 1 | - | - | 1 | - | - | - | - | undetermined[2,4] |
| Pambula Lake | 0 | 0 | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | |
| Twofold Bay | 3 | 1 | - | - | 13 | - | - | - | - | 2 | - | - | 2 | - | - | - | - | *A. ostenfeldii* |
| Wonboyn River | 1 | 0 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 | undetermined[2,5] |
| | | | | | | | | | | | | | | | | | | |
| Number of zone closures | | | 1 | 3 | 8 | 0 | 9 | 12 | 5 | | | | | | | | | |
| Total number of closure days | | | 14 | 29 | 62 | 0 | 185 | 313 | 195 | | | | | | | | | |
| **TOTAL** | **72** | **38** | | | | | | | | **63** | **1** | **3** | **10** | **0** | **14** | **29** | **6** | |

1. Number of days of closure for individual zones are indicated in brackets.
2. Not apparent in cell counts.
3. Harvest area closed due to rainfall exceeding the trigger level in the harvest area management plan.
4. Within period of cessation of harvest.
5. Within period of harvest closure due to algal bloom (not *Alexandrium* spp.).

Table 3: Levels of rainfall (mm) for the fourteen days preceding blooms (>200 cells $L^{-1}$) of *A. catenella* reported at Brisbane Water, Patonga Creek, Hawkesbury River and Georges River between 2005 and 2011. Rainfall data were sourced from weather stations operated by the Australian Bureau of Meteorology at Sydney Airport AMO (Station ID: 66037) and Terrey Hills AWS (Station ID: 66059).

| | **Estuary Class*** | **Date of observation** | ***A. catenella* (max cells $L^{-1}$ observed)** | **Total Rainfall (mm) (14 days preceding cell maximum)** |
|---|---|---|---|---|
| **Brisbane Water** | III | 3-Oct-07 | 830 | 4.6 |
| | | 15-Sep-08 | 250 | 94 |
| | | 20-Sep-09 | 1,200 | 5.4 |
| | | 28-Jul-10 | 500 | 17.4 |
| | | | | |
| **Hawkesbury River** | II | 3-Sep-07 | 480 | 21 |
| | | 1-Jan-09 | 1,900 | 14 |
| | | 28-Sep-09 | 2,500 | 5.8 |
| | | 2-Sep-10 | 650 | 0.8 |
| | | 15-Nov-11 | 680 | 24.2 |
| | | | | |
| **Georges River** | II | 24-Nov-05 | 8,500 | 11 |
| | | 23-Jan-07 | 3,900 | 0 |
| | | 10-Sep-07 | 400 | 37.4 |
| | | 6-Oct-09 | 480 | 14.2 |
| | | 17-Nov-10 | 22,000 | 108.8 |
| | | 24-Oct-11 | 8,000 | 22.8 |
| | | | | |

*I=oceanic embayments; II=tide dominated estuary, III= wave dominated estuary, IV=intermittently closed estuary, V=freshwater/brackish (Roy et al., 2001)

**Figure Legends**

Fig. 1: (A) Location of oyster producing estuaries along the coastline of New South Wales, Australia. The location of two BOM weather stations (Sydney Airport AMO, Station ID: 66037 and Terrey Hills AWS Station ID: 66059) referred to in the text are shown. Map of Brisbane Water and Patonga Creek (B), Hawkesbury River (C) and Georges River (D). Phytoplankton sampling stations are indicated by numbers as assigned by the NSW FA. Also indicated are the locations of automated water level sampling devices and the positions of stations, closest to those where phytoplankton samples were collected, where measurements of surface salinity and temperature were recorded during the study period (scale bar (B-D) = 2km).

Fig. 2: Composition of *Alexandrium* spp. in NSW Coastal waters. Relative percentage abundance was calculated based on total observations of *Alexandrium* from all NSW Food Authority phytoplankton monitoring data samples collected between July 2005 and December 2011.

Fig. 3: Seasonal distribution of potentially toxic *Alexandrium* spp. and positive PSP toxin results from analysis of shellfish flesh (A). Cell concentration data (black dots) were derived from observations of all potentially toxic *Alexandrium* species (*A. catenella*, *A minutum*, *A. ostenfeldii*, unidentified *Alexandrium* species and *A. tamarense*) from all NSW Food Authority phytoplankton monitoring data samples, collected between July 2005 and December 2011. Positive PSP results (bar graphs) were based on analysis by either HPLC analysis or a positive or negative Jellet test result. (Note: Austral winter = months 6-8.). Seasonal distributions for the same period were shown for each species individually: *A. tamarense* (B); *A. catenella* (C); unidentified *Alexandrium* species (D); *A. ostenfeldii* (E) and *A minutum* (F).

Fig. 4: Distribution and abundance of species of *Alexandrium* spp. (A: *A. tamarense* and *A. catenella*; B: unidentified *Alexandrium* species and *A. ostenfeldii*; C: *A minutum*; D: *A. fraterculus*; E: *A. margalefi*; F: *A. pseudogonaulax*) along the NSW Coastline (July 2005 – December 2011). Data were derived from all NSW Food Authority phytoplankton monitoring data samples, collected between July 2005 and December 2011. Locations where samples were collected but no *Alexandrium* was observed are denoted by a +.

Fig. 5: Distribution of *A. catenella* (cells $L^{-1}$) and surface water temperature (°C) for Brisbane Waters (A,i), the Hawkesbury River (B,i) and the Georges River (C,i). Distribution of *A.*

*catenella* (> 25 cells $L^{-1}$ to bloom maxima) and surface temperature (°C) for Brisbane Waters (A,iii), the Hawkesbury River (B,iii) and the Georges River (C,iii). Cell concentrations of *A. catenella* at each location are based on all NSW Food Authority phytoplankton monitoring data samples collected at each station between July 2005 and December 2011. Surface water measurements were taken in the vicinity of the phytoplankton sampling stations as part of the NSW Food Authority Shellfish Safety Program (refer Fig. 1B-C). Temperature data for part Fig. 5 B were estimated from Fig. 5 A for each location).

Fig. 6: (i) Distribution of *A. catenella* (cells $L^{-1}$), tidal range (m) and water level range (m) for Brisbane Water Station 46 (A), Hawkesbury River Station 34 (B) and the Georges River Station 7 (C) between July 2005 and December 2011. Sampling station locations for phytoplankton and the position of automated water level recorders are provided in Fig. 1. (Note: No water level recorder data was available for the Georges River) Tidal range data were derived from NSW tide tables (Fort Denison, Sydney 2005 - 2011).

**Figures**
**Fig 1.**

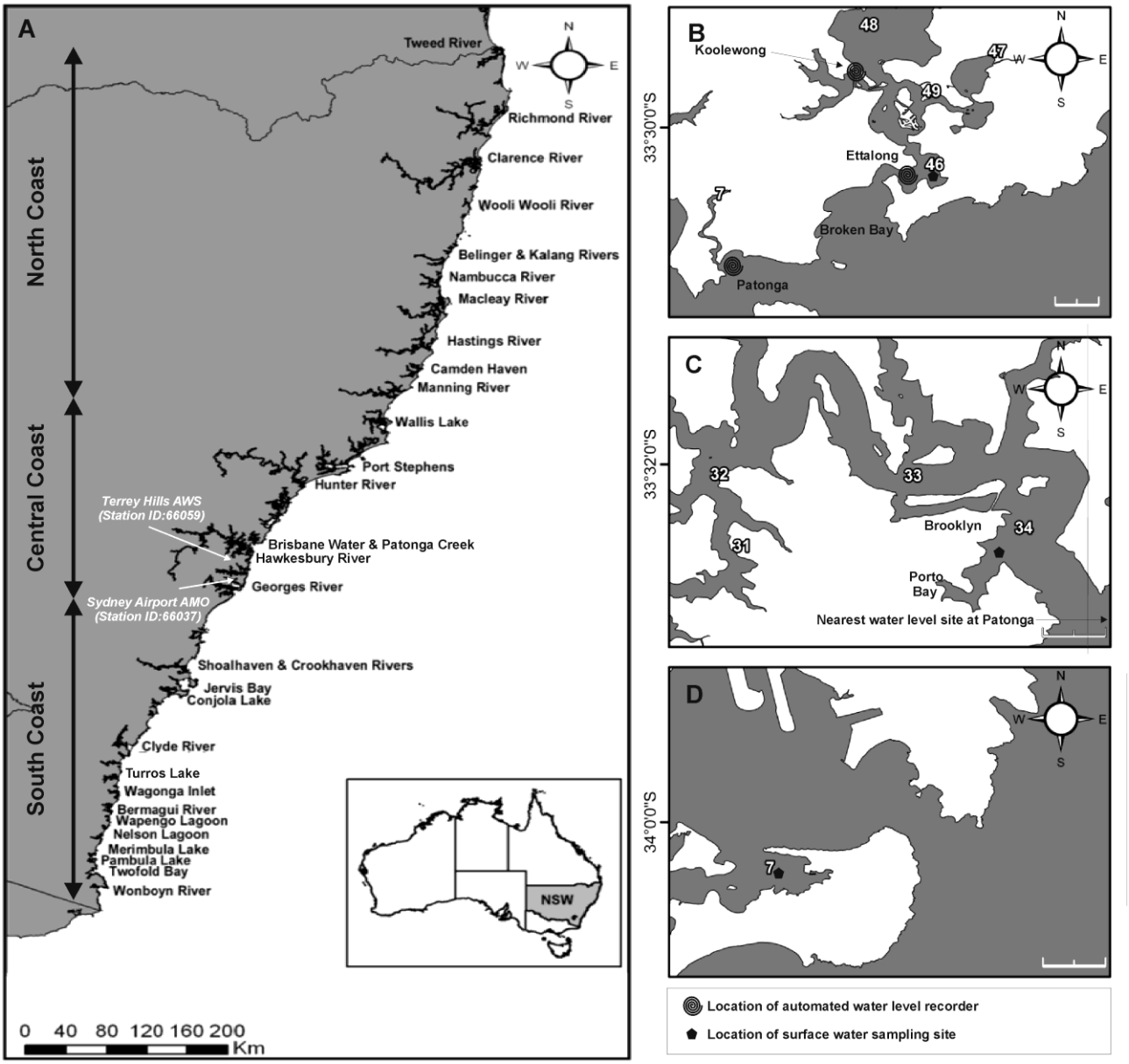


**Fig. 2**

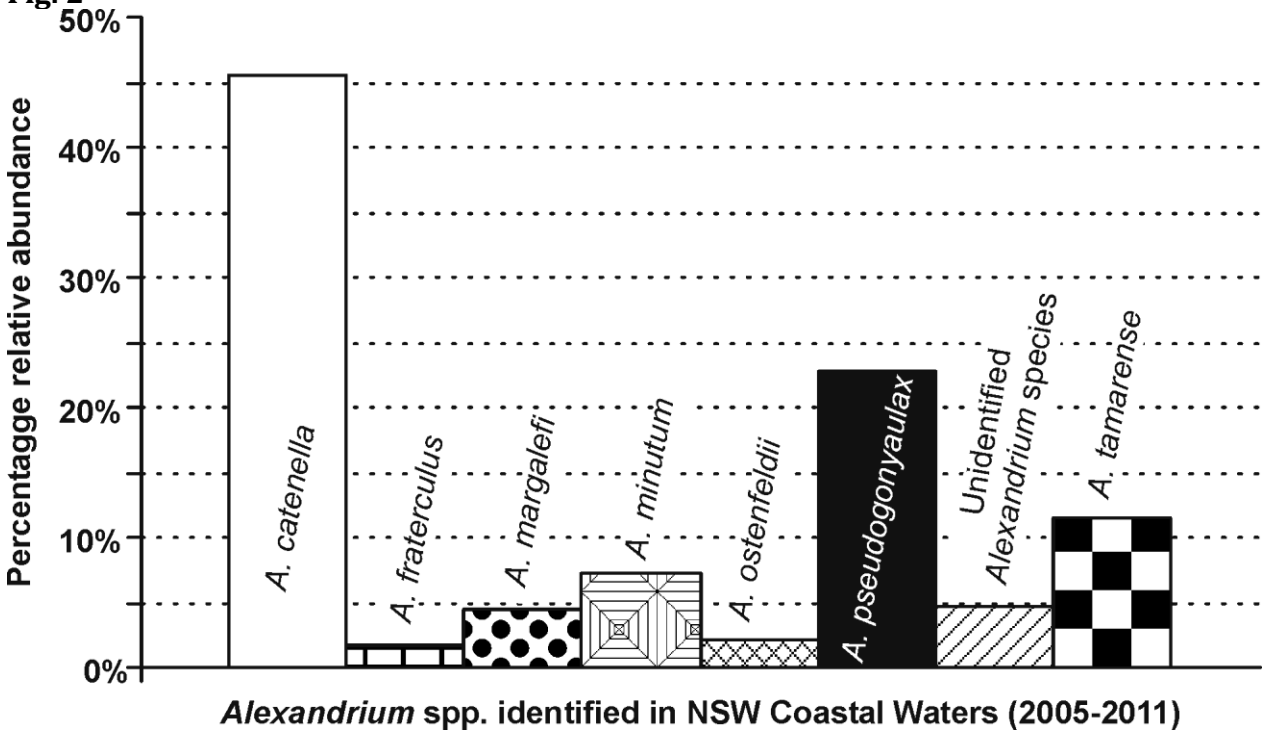

50%
40%
30%
20%
10%
0%
Percentagge relative abundance
A. catenella
A. fraterculus
A. margalefi
A. minutum
A. ostenfeldii
A. pseudogonyaulax
Unidentified Alexandrium species
A. tamarense
Alexandrium spp. identified in NSW Coastal Waters (2005-2011)

**Fig. 3**

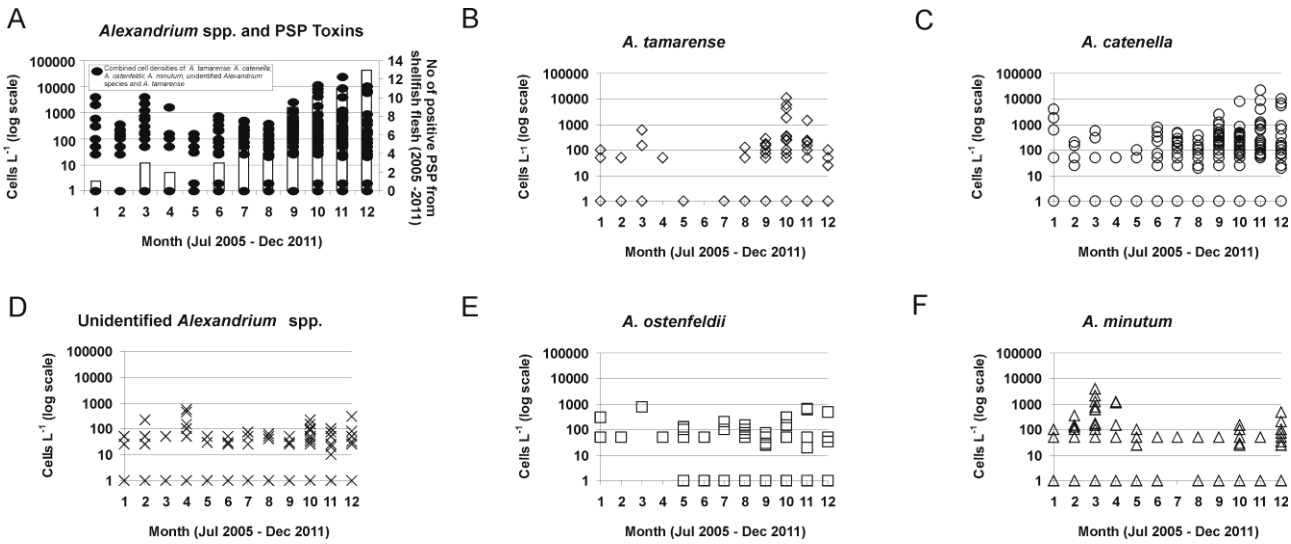

A
Alexandrium spp. and PSP Toxins
Cells L-1 (log scale)
No of positive PSP from shellfish flesh (2005 -2011)
Month (Jul 2005 - Dec 2011)
B
A. tamarense
C
A. catenella
D
Unidentified Alexandrium spp.
E
A. ostenfeldii
F
A. minutum

**Fig. 4**

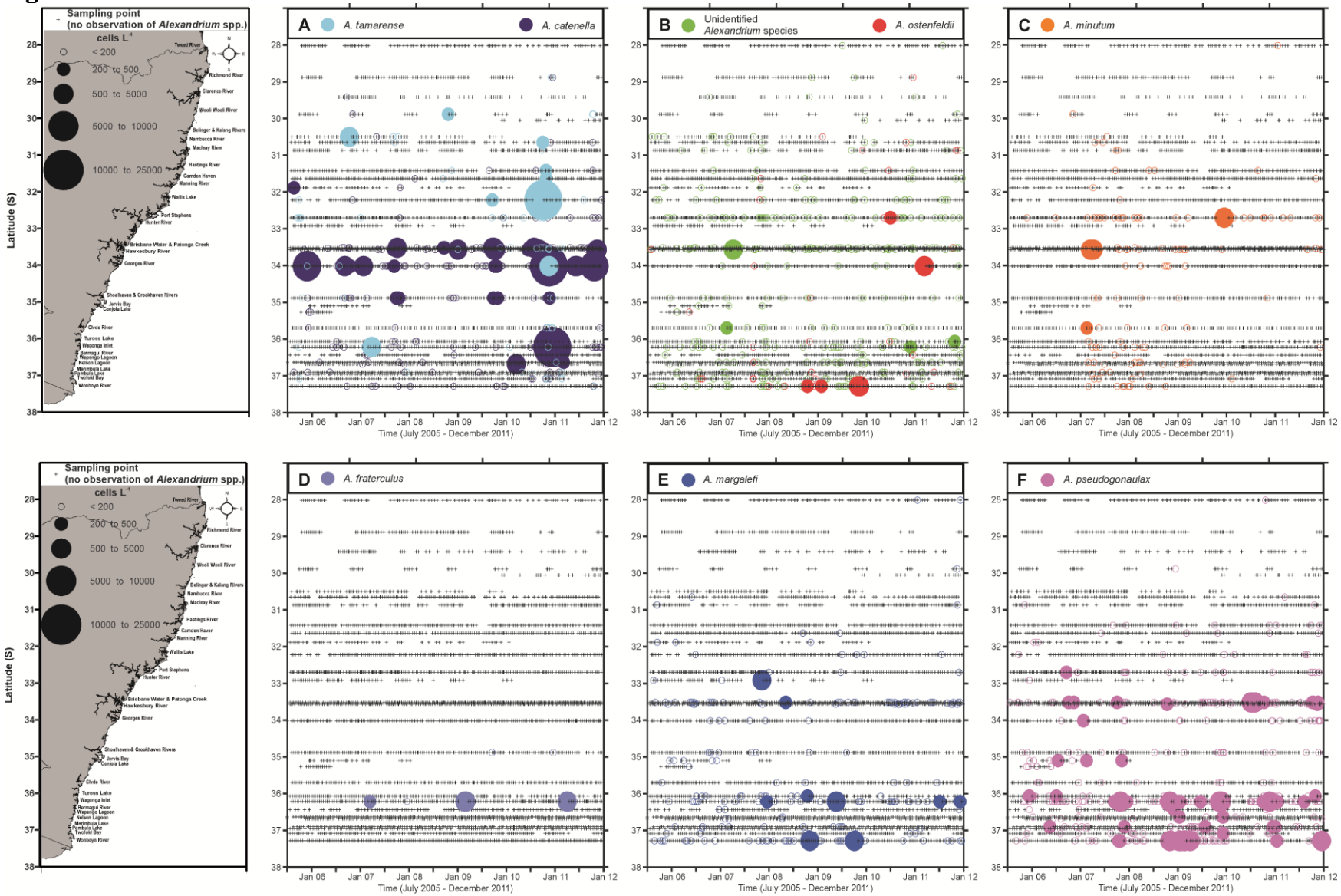

Sampling point
(no observation of Alexandrium spp.)
cells L-1
< 200
200 to 500
500 to 5000
5000 to 10000
10000 to 25000
Latitude (S)
A A. tamarense
A. catenella
B Unidentified Alexandrium species
A. ostenfeldii
C A. minutum
D A. fraterculus
E A. margalefi
F A. pseudogonaulax
Jan 06
Jan 07
Jan 08
Jan 09
Jan 10
Jan 11
Jan 12
Time (July 2005 - December 2011)

**Fig. 5**

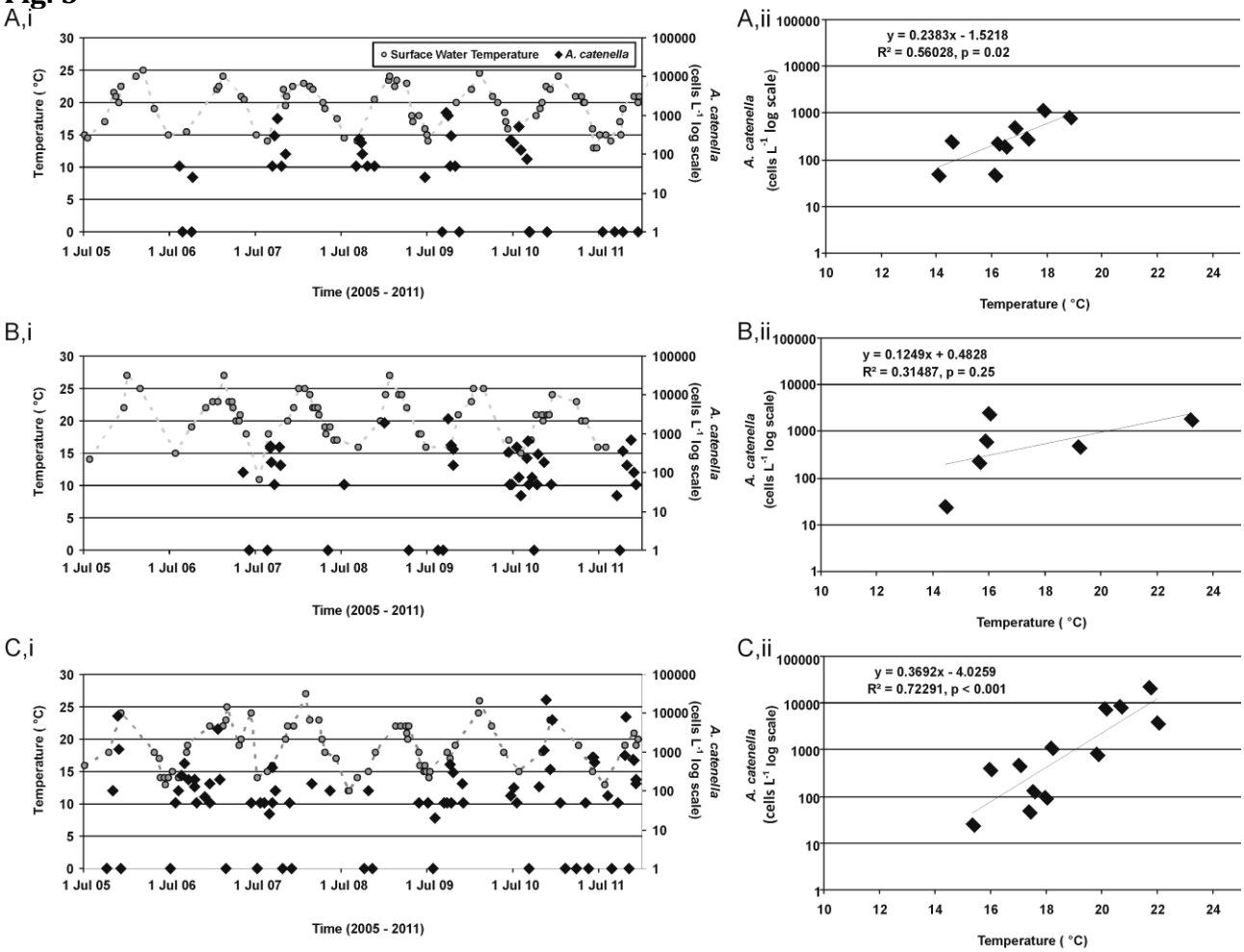
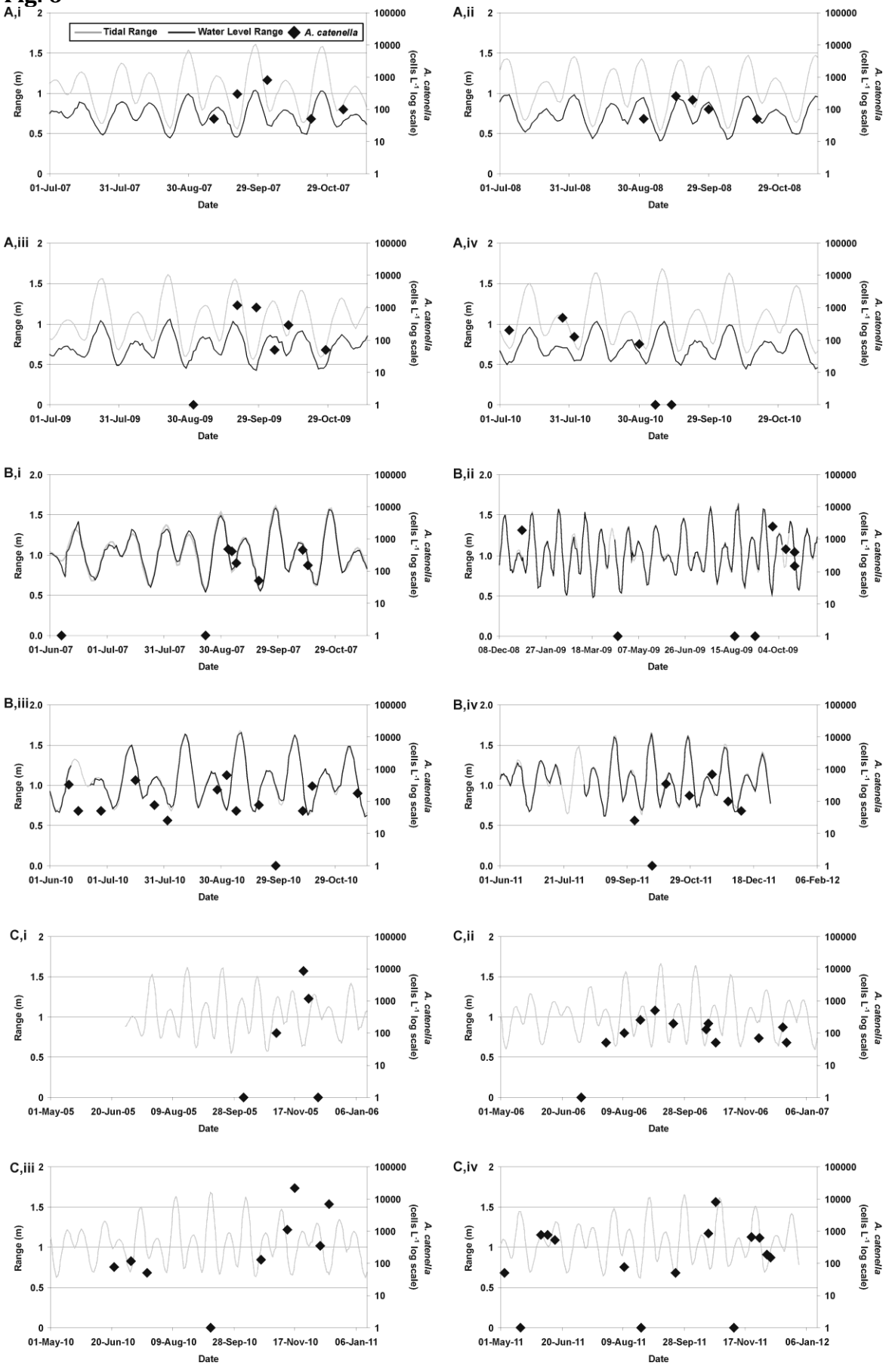

A,i
Surface Water Temperature
A. catenella
Temperature ( °C)
A. catenella (cells L-1 log scale)
1 Jul 05
1 Jul 06
1 Jul 07
1 Jul 08
1 Jul 09
1 Jul 10
1 Jul 11
Time (2005 - 2011)
A,ii
y = 0.2383x - 1.5218
R² = 0.56028, p = 0.02
A. catenella (cells L-1 log scale)
Temperature ( °C)
B,i
Temperature ( °C)
A. catenella (cells L-1 log scale)
Time (2005 - 2011)
B,ii
y = 0.1249x + 0.4828
R² = 0.31487, p = 0.25
A. catenella (cells L-1 log scale)
Temperature ( °C)
C,i
Temperature ( °C)
A. catenella (cells L-1 log scale)
Time (2005 - 2011)
C,ii
y = 0.3692x - 4.0259
R² = 0.72291, p < 0.001
A. catenella (cells L-1 log scale)
Temperature ( °C)

**Fig. 6**


A,i
Tidal Range
Water Level Range
A. catenella
Range (m)
A. catenella (cells L-1 log scale)
01-Jul-07
31-Jul-07
30-Aug-07
29-Sep-07
29-Oct-07
Date
A,ii
01-Jul-08
31-Jul-08
30-Aug-08
29-Sep-08
29-Oct-08
A,iii
01-Jul-09
31-Jul-09
30-Aug-09
29-Sep-09
29-Oct-09
A,iv
01-Jul-10
31-Jul-10
30-Aug-10
29-Sep-10
29-Oct-10
B,i
01-Jun-07
01-Jul-07
31-Jul-07
30-Aug-07
29-Sep-07
29-Oct-07
B,ii
08-Dec-08
27-Jan-09
18-Mar-09
07-May-09
26-Jun-09
15-Aug-09
04-Oct-09
B,iii
01-Jun-10
01-Jul-10
31-Jul-10
30-Aug-10
29-Sep-10
29-Oct-10
B,iv
01-Jun-11
21-Jul-11
09-Sep-11
29-Oct-11
18-Dec-11
06-Feb-12
C,i
01-May-05
20-Jun-05
09-Aug-05
28-Sep-05
17-Nov-05
06-Jan-06
C,ii
01-May-06
20-Jun-06
09-Aug-06
28-Sep-06
17-Nov-06
06-Jan-07
C,iii
01-May-10
20-Jun-10
09-Aug-10
28-Sep-10
17-Nov-10
06-Jan-11
C,iv
01-May-11
20-Jun-11
09-Aug-11
28-Sep-11
17-Nov-11
06-Jan-12

**Supplementary material**

Details of taxonomic features used to distinguish potentially toxic Alexandrium spp. in water samples collected in NSW estuaries.

***Supplementary information.***

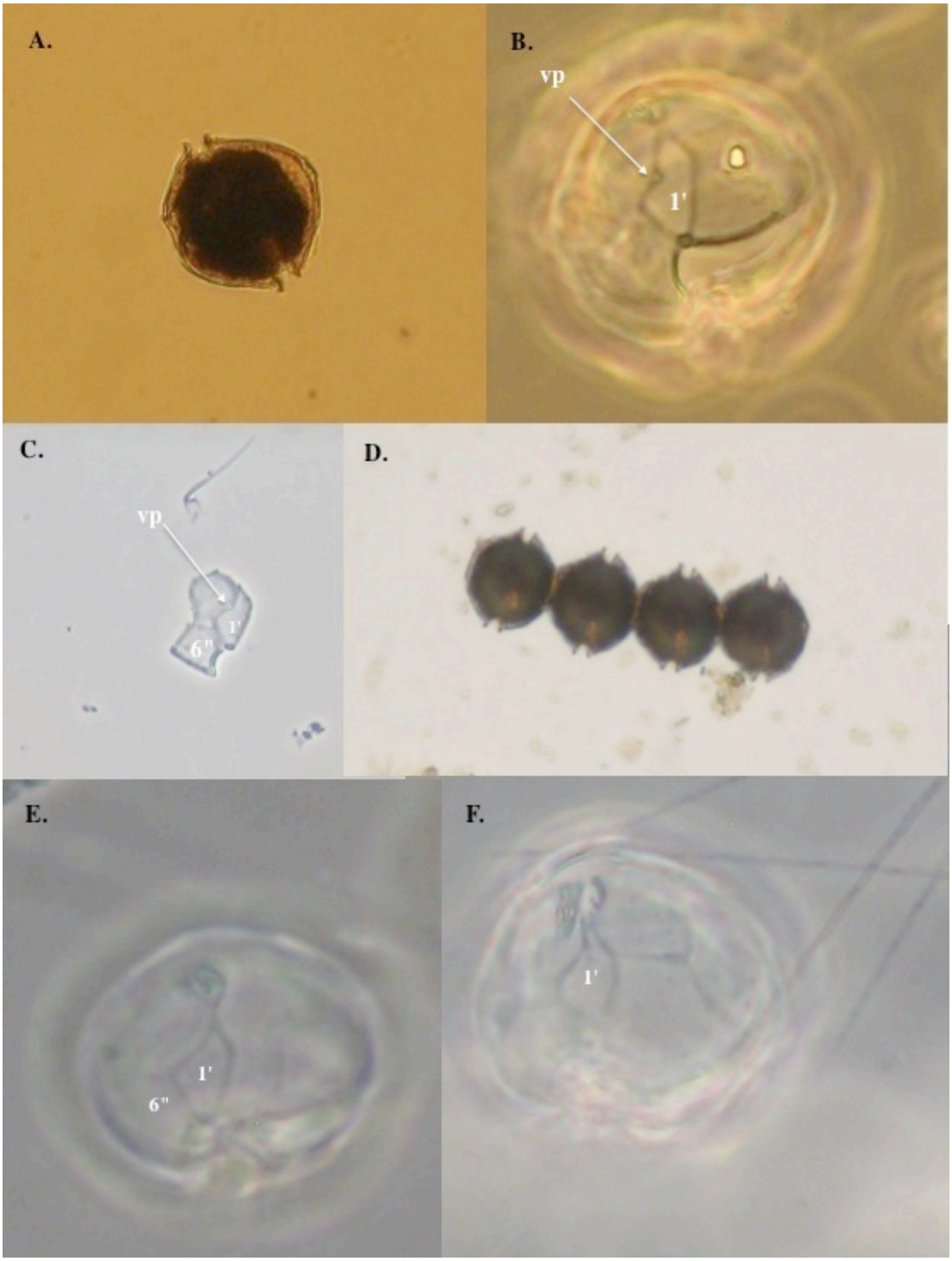



Figure S1: A, B, C: *A. tamarense* (LM); D, E, F: *A. catenella* (LM). (Refer to Table S1 for sampling locations). Note: vp = ventral pore, 1'=first apical plate, 6''= sixth precingular plate.

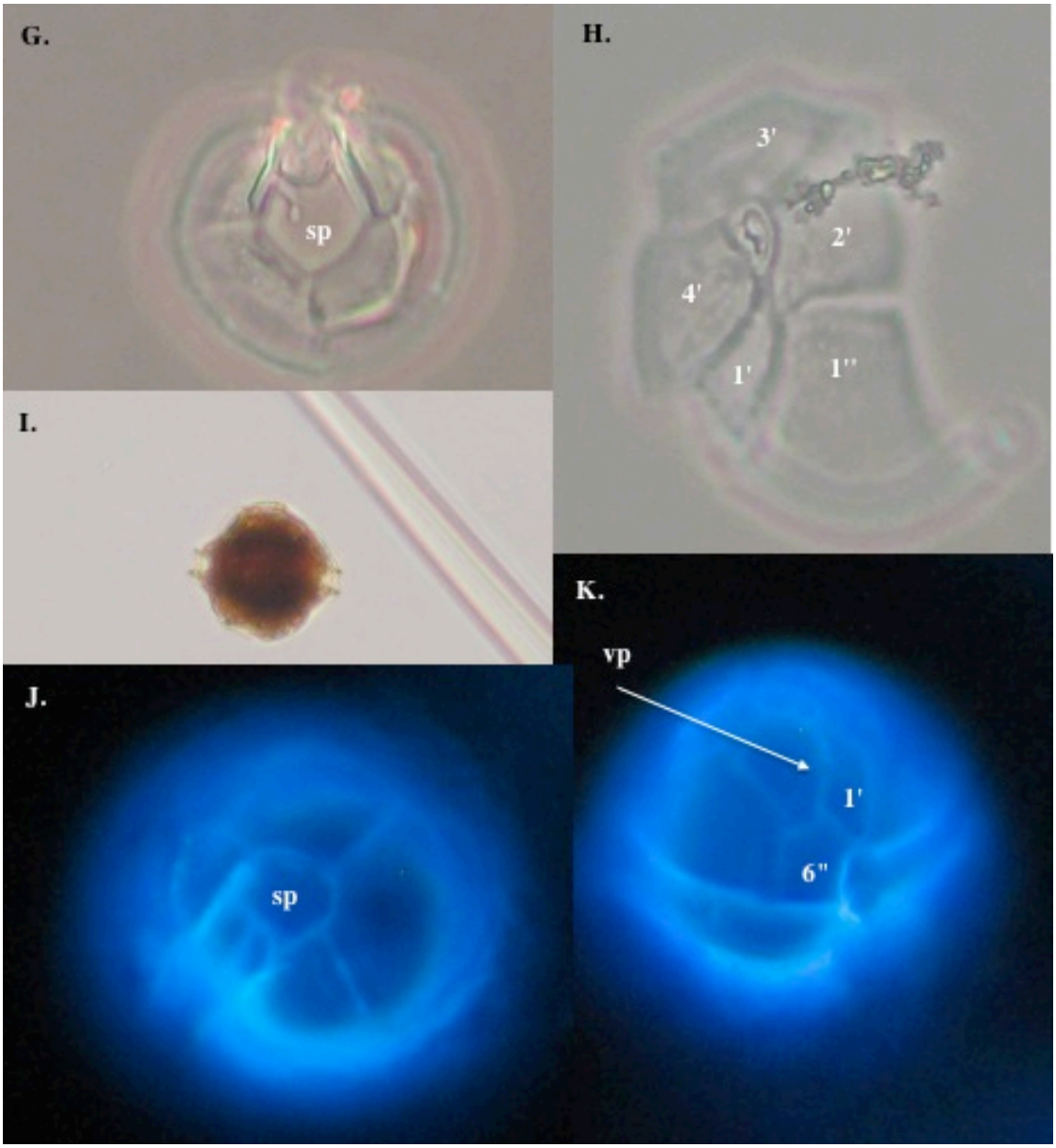


Figure S1 cont'd: G, H: *A. catenella* (LM); I, *A. minutum* (LM); J, K: *A. minutum* (Calcofluor fluorescence). (Refer to Table S1 for sampling locations). Note: vp = ventral pore, 1'=first apical plate, 6''= sixth precingular plate, sp = sulcal plate.

Identification of *Alexandrium* spp. were based on cell size, shape and thecal plate pattern. Images of *A. minutum*, *A. tamarense* and *A. catenella* from the shellfish monitoring program water samples collected in NSW estuaries are provided (see Table S1). Species were distinguished from the shape, and presence/absence of a ventral pore (vp) on the first apical plate (1'), the shape of the sixth precingular plate (6") and the shape and presence/absence of a pore on the sulcal plate (sp).

In summary:

- *Alexandrium tamarense* (Figure S1, A-C) was distinguished by the shape of the sulcal plate and the sixth precingular plate (6"), which is wider in *A. tamarense* than in *A. minutum*.
- *Alexandrium catenella* (Figure S1, D-H) is a chain forming species, although chains may not always remain intact in preserved samples. Cells are

dorsoventrally flattened without a ventral pore on 1'. Another pore is present on the sulcal plate.

- *Alexandrium minutum* (Figure S1, H-K) is identifiable as a small cell. Its size generally less than <27 μm. *Alexandrium catenella* and *A. tamarense* are larger (>27 μm). In *A. minutum* the 6" is narrow and the sulcal plate has a greater width than length.

Table S1: List of NSW estuaries from which images of *Alexandrium* spp. in Figure S1 were derived.

| Figure S1 | Species | Estuary | Date Sampled |
|---|---|---|---|
| A | *A. tamarense* | Wallis Lake | 21/09/09 |
| B | *A. tamarense* | Wallis Lake | 21/09/09 |
| C | *A. tamarense* | Port Stephens | 16/09/05 |
| D | *A. catenella* | Hawkesbury River | 02/10/09 |
| E | *A. catenella* | Hawkesbury River | 23/11/11 |
| F | *A. catenella* | Georges River | 7/12/11 |
| G | *A. catenella* | Georges River | 19/11/10 |
| H | *A. catenella* | Georges River | 3/12/10 |
| I | *A. minutum* | Port Stephens | 20/12/09 |
| J | *A. minutum* | Hastings River | 28/04/12 |
| K | *A. minutum* | Hastings River | 28/04/12 |