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Bloom drivers of the potentially harmful dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller in a south eastern temperate Australian estuary

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Abstract

Harmful algal blooms are an increasing concern in the estuarine reaches of the Hawkesbury-Nepean River, one of the largest coastal rivers systems in south eastern Australia. In the austral spring of 2016, an unprecedented bloom of the harmful mixotrophic dinoflagellate *Prorocentrum minimum* occurred in Berowra Creek (maximum cell abundance 1.9E+06 cells L^{-1}, 89% of the total phytoplankton community), a major tributary of this river system. In response to this bloom, our study utilizes an estuary-wide, thirteen-year time series of phytoplankton abundance and environmental data to examine the spatial and temporal patterns of this harmful algae and its potential bloom drivers in this system. ~~We found that~~ *P. minimum* cell densities and environmental parameters varied over large spatial scales, with sites located in the main channel of the estuary significantly differing from those in the more urbanized tributary of Berowra Creek. Generalised additive modelling outputs suggested that blooms of *P. minimum* are complex, but generally corresponded to a spatial gradient of eutrophication and salinity, whereby *P. minimum* growth and concomitant high chlorophyll-a concentrations were enhanced at sites that were generally less saline and more eutrophic than others. Furthermore, temporal patterns suggested that blooms occurred abruptly and lasted up to three weeks, most often during the austral autumn to spring.~~, most likely following a significant increase in available prey.~~ While significant correlations were observed between rainfall and nutrients at all other sites, suggesting a pathway for nutrient availability, the association between rainfall and nutrient delivery was generally not observed in Berowra Creek (a 15-meter deep site) suggesting that a continual supply of nutrients, coupled with unique bathymetry and water residence time at this site, are the most likely contributing factors to phytoplankton growth. This study presents the most comprehensive examination of *P. minimum* in any southern hemisphere estuary to date and highlights the importance of

continued monitoring of HABs and the important role that anthropogenic inputs have in driving blooms of *P. minimum* in this oyster-growing river/estuary system.

1.1 Introduction

Certain species of microalgae can form harmful algal blooms (HABs) which may have both ecosystem and human health consequences (Anderson et al. 2012). High biomass microalgal blooms can cause oxygen depletion in the water column, reduce light availability for aquatic organisms including macroalgae or seagrass, and/or alter food webs (Diaz and Rosenberg 2008 and references therein). Other monospecific microalgal blooms can produce toxic compounds that can bioaccumulate within the aquatic ecosystem, affecting both marine species and eventually human health via the consumption of seafood (Hallegraeff et al. 2003).

Several species of the dinoflagellate genus *Prorocentrum* produce toxins that can cause harmful impacts, while some species belonging to this genus can reach sufficiently high cell densities to have negative ecological consequences (Glibert et al 2001). At least nine species of *Prorocentrum* are known to produce toxins (including okadaic acid, dinophysistoxins, borbotoxin and other compounds yet to be characterised), with *Prorocentrum lima* being the most consistently toxic species known to date (Hoppenrath et al. 2014). ~~Moreover, a~~At least six planktonic species are known to form high-biomass blooms, with the globally distributed *Prorocentrum minimum* (Pavillard) J. Schiller 1933 considered to be the most problematic (Heil et al 2005, Glibert et al. 2008, 2012).

Prorocentrum minimum (syn. *P. cordatum* (Ostenfeld) Dodge 1975) is a relatively small, mixotrophic microalga (14-22 µm long and 10-15 µm wide), with a growth rate from 0.70 to >4 divisions day^{-1} (Heil et al 2005, Glibert et al. 2012). Certain strains of this species produce a water-soluble neurotoxin, which has not yet been chemically characterized. In high doses this toxin has been found to cause death in mice (Grzebyk et al. 1997). When experimentally~~?~~ fed ~~cells of~~ *P. minimum*, detrimental effects have been reported for scallops, oysters and clams (Glibert et al. 2007). Poor larval development, tissue pathologies, systemic immune responses or no effect at all, were among the variable results from these molluscan shellfish feeding experiments (Denardou-Queneherve et al. 1999, Wikfors 2005). Strain specific toxicity (Heil et al. 2005) or transient toxin expression (Wikfors 2005) are both possible explanations for this response variability. *P. minimum* has been linked to fish, shellfish and zoobenthos mortalities, and shellfish toxicity with associated, yet unconfirmed, human impacts from a variety of coastal environments ~~as well as being associated with human poisonings in several countries~~ ~~throughout the world~~ (Japan, France, Norway, Netherlands and USA) (Heil et al. 2005 and references therein). Furthermore, the detection of tetrodotoxin (TTX) in the Mediterranean mussel (*Mytilus galloprovincialis*) has been linked to *P. minimum* (Vlamis et al. 2015). Additional work by Rodriguez et al (2017) suggested that symbiotic bacteria of *P. minimum* could be associated with TTX production. Whilst toxin production has been unequivocally confirmed from certain benthic species of *Prorocentrum* (see above)~~okadaic acid and its analogues, *Dinophysis* toxins, borbotoxins, prorocentrolides, and other unidentified toxins, Hoppenrath et al. 2014 and references therein)~~, there is no scientific consensus on the toxicity and human health effects associated with *P. minimum* thus far.

~~The factors leading to b~~Blooms of *P. minimum* have been extensively investigated and are multifarious. *P minimum* demonstrates high physiological flexibility under varying environmental conditions such as light, temperature and salinity (Fan and Glibert 2005, Heil et al. 2005, Glibert et al. 2008, 2012, Li et al. 2015). Laboratory studies have confirmed that *P. minimum* is ecologically plastic in response to changing salinity, allowing *P. minimum* to successfully invade and proliferate in unstable, brackish water environments (Olenina et al. 2016, Skarlato et al. 2017). High biomass blooms of *P. minimum* in eutrophic coastal environments occur during periods of high irradiance, when conditions are relatively warm, salinity is low to moderate and there is low turbulence (Carreto et al. 2018 and references therein). The link between *P. minimum* growth and nutrient availability is complex. Laboratory-based experiments show that the growth of *P. minimum* can be stimulated by inorganic nutrient levels just below the Redfield N:P ratio in culture, while in field studies *P. minimum* has been observed to bloom at high N:P ratios following rainfall and runoff events, or in regions of high dissolved inorganic nitrogen (DIN) (e.g. nitrate) and phosphorus (DIP) exports which are strongly linked to anthropogenic sources (Heil et al. 2005, Glibert et al. 2008, 2012, Sahraoui et al. 2013, Ou et al. 2014). Significant heterogeneity in the rate of nutrient uptake and the extent to which urea input suppresses nitrate uptake at the single-cell level has also been observed, suggesting that heterogeneous populations of *P. minimum* can proliferate under varying environmental conditions (Matantseva et al. 2016). ~~The p~~Population dynamics of *P. minimum* are further controlled by species-species (Telesh 2016) and predator-prey relationships (Glibert et al. 2012). *P. minimum*, an active swimmer (Smayda 2002), can acquire nutrients by feeding on cyanobacteria, cryptophytes, haptophytes, diatoms and dinoflagellates (Stoeker et al 1997). In turn, *P. minimum* transfers nutrients to higher trophic levels such as mixotrophic and heterotrophic dinoflagellates and ciliates (Wikfors 2005).

P. minimum blooms are thought to be increasing in frequency while concurrently expanding their global range in a direct relationship with global increases in eutrophication of coastal and estuarine waterways (Glibert et al. 2008). Long-term microalgal community assessments in European and North American waters including the Baltic Sea, the Black Sea, Chesapeake Bay and the Neuse River Estuary (US) all suggest that *P. minimum* has increased in abundance in direct response to increasing nutrient loading (Glibert 2012 and references therein). To this end, *P. minimum* has been identified as an invasive alien species in the Baltic Sea, its spread leading to recognizable environmental effects on the native microalgal community, the pelagic habitat and the overall ecosystem functioning of this region (Pertola et al. 2005, Olenina et al. 2010). ~~However, t~~There are few reports on the abundances of *P. minimum* from estuarine and coastal waters in the southern hemisphere, and it is therefore not known whether this species is changing its distribution in these regions (Heil et al. 2015).

In 2016, an intense and unprecedented monospecific bloom of *P. minimum* (max 1.90E+07 cells L^{-1}) was observed in Berowra Creek, a tributary of the larger Hawkesbury-Nepean river system (HNRS) which is one of the largest coastal rivers systems in south eastern Australia (Fig. 1). Significant alteration of the natural river flow, intensive urban and industrial development and an increasing demand for water supply have increased the threat of eutrophication in the HNRS. This particular bloom occurred, however, following a decade of significant reduction in point source nitrogen from two wastewater treatment plants (a consequence of enhanced effluent treatment) and a concomitant decline in salinity (increased rainfall) (Larsson et al. 2017). Here, using a long-~~-~~term microalgal and physico-chemical dataset, our aim was to examine the spatial and temporal patterns of *P. minimum* in the Hawkesbury River estuary and to identify the key drivers of *P. minimum* blooms. A greater understanding the complex mechanisms of microalgal blooms in this highly modified estuary will be invaluable for future management purposes.

2. 1 Materials and Methods

2.1.1 Sampling Sites

Seven sampling sites of varying depths (3-15m), were established in the Hawkesbury River for long-term water quality and algal bloom assessment (HSC 2017) (Fig. 1). Five sites are located in the main estuary channel with the most downstream being 15 km from the estuary mouth (site 150) and the most upstream being 75 km from the mouth (site 153). Two sites (sites 60 and 61) are located within Berowra Creek, an important tributary of the Hawkesbury estuary, located ~24km from the ocean. This tributary's headwaters are highly urbanized including residential, commercial and industrial occupancy, whilst all other sites are largely surrounded by protected areas (HSC 2017).

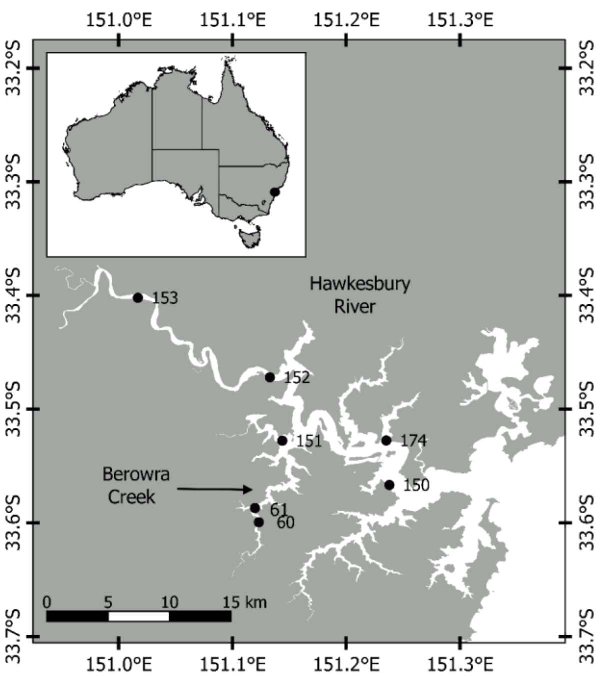

Figure 1. Sampling locations for Hawkesbury River phytoplankton and water quality sampling (2003-2016). Sites 60 and 61 are located within Berowra Creek; all other sampling locations (150, 174, 151, 152 and 153) are located within the main channel of the estuary.

2.1.2 Phytoplankton collection and enumeration

Water samples (500 ml) were collected from a depth of 0.5 m from each site at approximately 3 to 4-week intervals over a sampling period ranging from 3 to 13 years depending on the site (Table 1). Samples were preserved with Lugol's iodine solution for later identification and enumeration of phytoplankton. In the laboratory, samples were concentrated by gravity-assisted membrane filtration and phytoplankton cell counts were undertaken in a Sedgewick Rafter counting chamber. Cell enumeration and detailed examination of cells were carried out using Zeiss Axiolab or Standard microscopes equipped with phase contrast. Cells were identified to the closest taxon using light microscopy (maximum magnification ×1,000). Cell counts were undertaken to determine the abundance of each phytoplankton taxa, including *P. minimum* and total phytoplankton cell (> 5 μm) numbers. *P. minimum c*ells were counted to a minimum detection threshold of 500 cells L^{-1}.

2.1.3 Environmental Variables

In situ measurements of temperature (°C), salinity (ppt), turbidity (NTU), dissolved oxygen (mg L^{-1}) and pH from a depth of 0.5 - 1 m depth were made at the time of microalgal sampling using a YEOKAL™ 615 Water Quality Analyser (NSW, Australia). The instrument was calibrated at the commencement of each sampling day in accordance with manufacturer's specifications and a quality control check done at the end of the day (i.e. to ensure there was no drift in the measurements).

Site Number	Location (lat, long)	Depth (m)	Sampling Date Range	Daily Rainfall Data – BOM Station Name and No.
60	-33.599, 151.123	6	6/05/2003-14/12/2016	Goodwyn Rd 067052 & Ledora Farm 067052
61	-33.587, 151.120	15	7/04/2003-20/12/2016	Goodwyn Rd 067052 & Ledora Farm 067052
150	-33.567, 151.238	10	22/06/2010-20/12/2016	Palm Beach 066128
151	-33.528, 151.144	4	16/11/2010-20/12/2016	Canoelands 067023
152	-33.472, 151.133	7	16/11/2010-20/12/2016	Lower Mangrove 061216
153	-33.402, 151.017	8	22/06/2010-20/12/2016	Gunderman 067040
174	-33.528, 151.236	3	23/10/2013-20/12/2016	Palm Beach 066128

Table 1. List of sampling sites, site codes, GPS location, depth, sampling date range and nearest rainfall gauge sites (Australian Government Bureau of Meteorology (BOM), http://www.bom.gov.au) accessed 23/8/2017) used in model analyses. Those sites listed above the dotted line are those sites located within Berowra Creek; those sites below the dotted line are those sites located within the main channel of the Hawkesbury River estuary.

At the same time as the microalgal samples and *in situ* environmental data were collected, water samples for chemical data were collected using a pole sampler with attached prewashed 200 ml bottle from 0.5 - 1 m depth. Samples were analysed for the following nutrients: oxidised nitrogen (nitrite NO_2^- and nitrate NO_3^-), ammonium nitrogen (NH_4^+), total nitrogen (TN), soluble reactive phosphorus (SRP) and total phosphorus (TP) (mg L^{-1}). Two further one L samples were collected for chlorophyll-a (chl-a, μg L^{-1}) and suspended solids determination (mg L^{-1}). Once collected, all samples were transported to a NATA (National Association of Testing Authorities) accredited laboratory for nutrient analyses as per the methods and detection limits as listed in Supplementary Table 1.

To test the effect of rainfall on the abundance of *P. minimum* at each of the sampling sites, rainfall data ~~was~~ were obtained from the closest Bureau of Meteorology weather station to each site (Table 1). For sites 60 and 61, rainfall data were obtained from two weather stations and averaged across both stations for each day measured (mm day^{-1}) (Fig. 1). Rainfall data linked to each station was then averaged over the 7 days prior to the phytoplankton-sampling day to incorporate a measure of exposure to this variable at each sampling location (MHL 1998).

To assess the effects of nutrient ratios on *P. minimum* abundance, we also included the Redfield ratio as a predictor variable (Redfield, 1934). This stoichiometric ratio is an average of the elemental composition in plankton and was calculated as the atomic ratio between total nitrogen and total phosphorus as per the following equation:

$$\text{Redfield ratio} = \frac{[\text{Total N}]/14}{[\text{Total P}]/31}$$

Beginning in 2004, a real-time telemetry water quality probe was deployed at sited 61 (only). At this site, a thermistor chain was deployed which collects temperature (°C) data every 15 mins from the surface (30 cm) and every 100 mm to the bottom (1530 cm). Despite a reduced temporal coverage, this data provided an additional opportunity to assess the effects of thermal stratification (TS), defined as the temperature difference between 0.3 m and 15 m measured at midday, on *P. minimum* blooms at this location. All water quality data, including thermistor data, are publicly available at http://www.mhlfit.net/users/HornsbyShireCouncil-HistoricalBeroCR8

2.1.4 Data Treatment and Analyses

As there were a large number of environmental variables, initial exploratory analyses of the relationships between these were carried out. Scatterplot matrices and correlation analyses were undertaken and the information from these use in the model building process to ensure that models remained stable. This same methodology was employed for a similar previous study (Ajani et al. 2016).

To model the relationship between the abundance of *P. minimum* and the environmental variables, generalised additive models were used (Hastie and Tibshriani 1990, Wood 2000). By employing generalised additive models, we can treat the *P. minimum* abundance as count data, rather than using a log transformation to make the count continuous, and as such can handle zero counts. Furthermore, as our initial analyses indicated that several of the environmental variables e.g. time of year had a nonlinear relationship with abundance, these models allow us to include smoother functions to incorporate this relationship into the model. These models were fitted in version 3.3.3 of the R statistical package (Team R Core 2013), using the GAM (Generalised Additive Model) function in version 1.8-17 of the 'mgcv' package (Wood 2006).

Some of the environmental variables contained missing values for some observations. The number of missing values for each variable is given in Table 2 but mainly corresponded to levels of SRP between 2012 and 2014. When modelling, if one of the variables had a missing value for one of the observations, the entire observation was not used in model fitting. As a result of this data limitation, two models were developed for each sampling site: one using all variables collected over the sampling period for each site and one without SRP. For site 61, four models were developed: one using all variables; one without SRP; one using all variables including thermal stratification (data collected from the temperature probe); and finally, one using all variables including thermal stratification but not SRP. This resulted in a total of sixteen models across the sampling sites. When comparing different models within each site (using the Akaike Information Criterion (AIC), Akaike 1973), the number of observations in the models that were compared remained constant, however, due to the "patchy" nature of some variables they were not comparable across models.

For each site, the relationships between the environmental variables and *P. minimum* were visually examined. Where the relationship appeared to be nonlinear, spline based smoothings were utilised with the fitting algorithm attempting to minimise the order of the spline. Subsequently, if the fit suggested that a linear relation was sufficient, the model was further simplified and the spline fit replaced by a linear fit.

3.1 Results

3.1.2 P. minimum *Abundance*

Water samples were collected for microalgal enumeration from site 60 and 61 from 2003 to 2016, from sites 150, 151, 152 and 153 from 2010 to 2016, and from site 174 from 2013 to 2016 (Table 1). *P. minimum* reached a maximum of 96.5 % of the total microalgal abundance (cells > 5 µm) at site 60 on 12/7/2006 (maximum cell concentration 1.60E+07 cells L^{-1}), 98% at site 61 on 4/7/2003 (maximum 1.88E+07 cells L^{-1}), 10.6% at site 151 on 19/10/2016 (maximum 1.6E+05 cells L^{-1}), and <5% across all sampling dates for the other sampling sites. The maximum *P. minimum* concentration across all sites was reported at site 61, 1.88E+07 cells L^{-1} (1.96E+05, SE ± 7.93E+04) on 10/1/2012, whilst highest mean concentration across all sampling times was at site 60, 2.15E+05 cells L^{-1} (SE ± 1.12E+052), and lowest mean abundance reported at site 152, 4.41E+02 cells L^{-1} (SE ± 1.17E+02) (Supplementary Table 2).

Cell concentrations of *P. minimum* were variable across weeks/seasons with highest cell densities generally observed in the austral autumn (weeks 9-17) and spring (weeks 34-47) (Fig. 2A-G). When analysed across years, *P. minimum* appeared to be highly variable with no clear pattern emerging at sites 60, 151, 152 and 174, and a possible yearly increase towards the present at sites 61, 150 and 153 (Fig. 3A-G).

3.1.2 Environmental Variables

Over all sampling periods, average temperature, dissolved oxygen (DO), pH, ammonium (NH_4), total phosphorus (TP) and soluble reactive phosphorus (SRP) were similar across sites (see Supplementary Table 2). Mean turbidity was notably higher at sites 151, 152 and 174 (12.21, 12.52 and 11.23 NTU, respectively) than sites 60, 61 and 150 (2.57, 2.74 and 6.33 NTU, respectively), whereas the highest mean turbidity and suspended solids (SS) were at site 153 (19.62 NTU and 15.22 mg L^{-1} respectively) (Supplementary Table 2). Mean concentrations of oxidised nitrogen (NO_x) and total nitrogen (TN) were highest at the most upstream sites 152 (0.12 and 0.41 mg L-1, respectively) and 153 (0.17 and 0.52 mg L^{-1}, respectively) compared to all other sites. Mean values of salinity were highly variable across sites ranging from the most upstream and freshest site 153 (5.59 ppt) to the most saline and downstream site 150 (30.74 ppt). Mean chlorophyll concentrations were notably higher at sites 61 (10.92 µg L^{-1}) and 153 (12.10 µg L^{-1}) compared to other sites, whilst total phytoplankton cells were significantly higher at sites 61 (1.09E+07) and 60 (3.99E+0.6) compared to all other sites which ranged between 7.20E+05 (site 150) and 1.21E+06 at site 152. Redfield ratios varied from 30.81 at site 150 to 42.07 at site 152. Average daily rainfall was highest at sites 60 and 61 (5.60 and 4.76 mm day^{-1} respectively) compared to all other sites at ~1.5 - 3 mm day^{-1} (Supplementary Table 2).

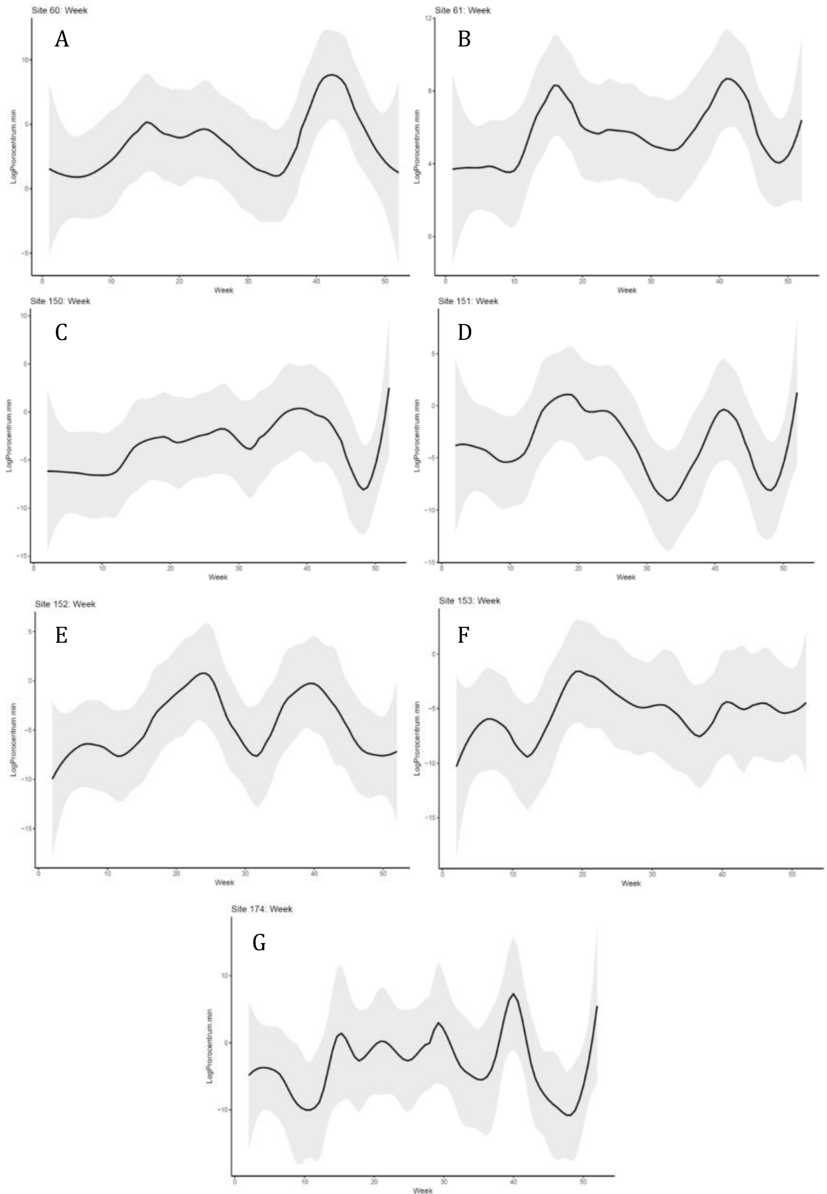

Figure 2A-G. Seven-day moving average (black line) and standard error (shaded area) of *P. minimum* cell abundance across each week in a year for each site across the sampling period; note different y-axis scales between sites.

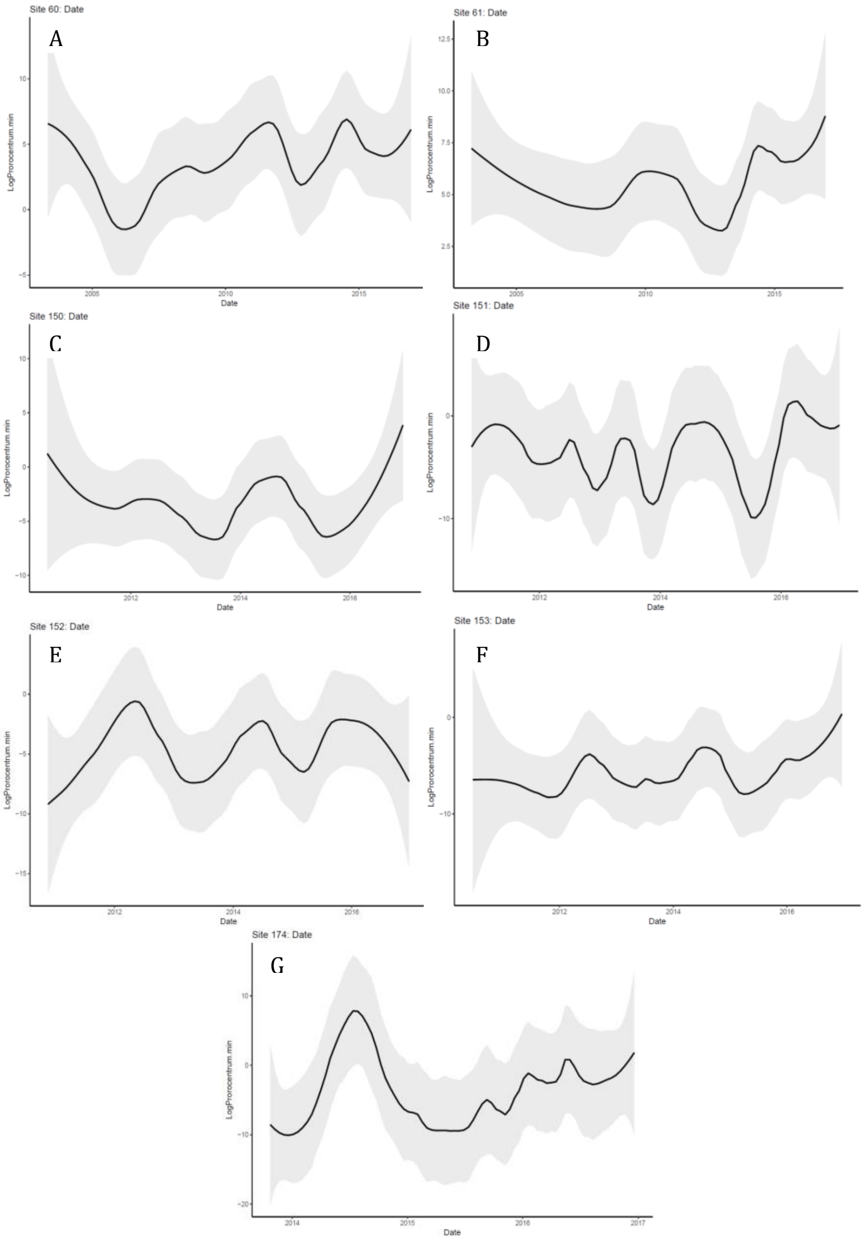

Figure 3A-G. Seven-day moving average (black line) and standard error (shaded area) of *P. minimum* cell abundance across years for each site across the sampling period; note different y-axis scales between sites.

Correlation coefficients were calculated among every pair of environmental variables and suggested strong positive relationships (r > 0.7) for most sites between NO_x, NH_4 and TN (Supplementary Table 3). Salinity showed a strong negative relationship to TN at sites 60, 150 and 174 (r < -0.7); with NO_x at site 150 and 174; and with the Redfield Ratio at site 174. Temperature and DO were negatively correlated at sites 151, 152, 153 and 174. Additionally, pH and TP showed strong negative correlations; and both turbidity and NH_4 were positively correlated to SRP at site 151. At sites 151, 152 and 153 turbidity and TP were positively correlated; while SRP and pH were negatively correlated at sites 152 and 153. At site 153 SRP was positively correlated to turbidity while negatively correlated to pH; and rainfall was positively correlated to SS and all nutrients.

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All correlations described were then considered when fitting the models. Where both correlated variables were included in the model, both variables were removed to see the impact on the overall model.

3.1.3 Case Study:* P. minimum *bloom Sept-Oct 2016

An unusual and immense water discolouration, appearing green, consisting of a dense bloom of *P. minimum*, occurred at site 61 within Berowra Creek during the austral spring, 2016 (Fig. 4). ~~reported to be associated with the P. minimum *bloom*~~Two significant rainfall events preceded the bloom: 32 mm on 07/09/2016 when *P. minimum* reached a cell density of 9.40E+04 cells L^{-1} (1% of the overall phytoplankton community); and a second rainfall event of 16 mm (18/09/2016), when *P. minimum* cell densities increased 6-fold to 5.6E+05 cells L^{-1} (14% of the total phytoplankton counts, 20/09/2016). A visible water discoloration (max pH 8.23) appeared a week later with cell concentrations reported as 1.4E+06 cells L^{-1} (28/09/2016, 67% of the total phytoplankton). This water discolouration persisted for an additional 25 days with an almost monospecific

Figure 4. Visible slick of *Prorocentrum minimum* bloom in Berowra Creek, Hawkesbury River estuary, south eastern Australia on 15/Oct/2016). Image credit: Robert Atlee.

bloom of *P. minimum* and reaching a maximum cell concentration of 1.9E+06 cells L^{-1} (89% of the total phytoplankton community). Water temperatures over the course of the bloom increased gradually from 17℃ to 22℃, whilst salinity levels ranged from 14.6 to 23.4 ppt. Nutrient concentrations, prior and during the bloom, were within the long-term average concentrations for this site, however it was observed that both total and dissolved nitrogen concentrations were elevated prior to the bloom (0.7 - 0.98 mg L^{-1} TN). In contrast, phosphorus (all forms) were higher during the second and highest peak of the bloom (max 0.037 mg L^{-1} TP). Chlorophyll-a concentrations were 12 µg L^{-1} and 17.7 µg L^{-1} during the first and second peaks of the bloom respectively. In total, the *P. minimum* bloom in Berowra Creek lasted ~ 2 months. One month prior (10/08/2016) to the immense *P. minimum* bloom, a succession of other phytoplankton preceded the *P. minimum* bloom. This began with high densities of the chain-forming diatom *Chaetoceros spp.* (2.3E+06 cells L^{-1}), followed by a bloom of small flagellates which was dominated by the cryptophytes *Plagioselmis prolonga*; (1.8E+06 cells L^{-1}) and *Hemiselmis sp.* (1.9E+06 cells L^{-1}); the prymnesiophyte *Chrysochromulina spp.* (2.6E+06 cells L^{-1}); the prasinophyte (*Pyramimonas spp.*; 1.5E+06 cells L^{-1}); and the pedinellophyte *Apedinella spinifera* (1.3E+06 cells L^{-1}) (Supplementary Fig. 1). *Prorocentrum minimum* cell concentrations increased abruptly approximately as the other phytoplankton groups declined (Fig. 5). No negative effects on humans or benthic fauna were reported to be associated with the *P. minimum* bloom.

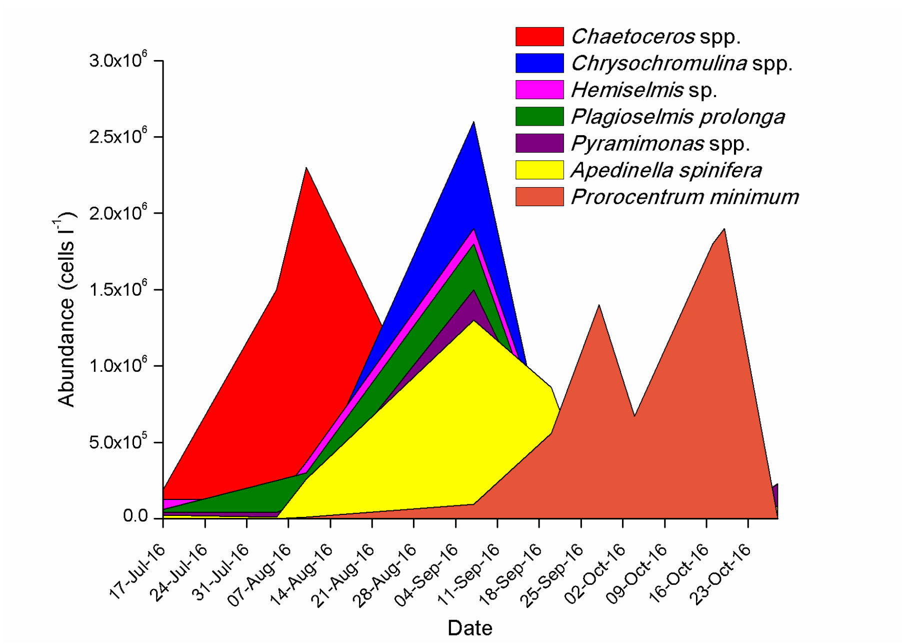

Figure 5. Phytoplankton succession patterns prior to the *P. minimum* bloom of 2016.

3.1.4 Modelling **P. minimum** *blooms in the Hawkesbury River*

Throughout the model selection process, several variables were removed as they were not significant in any of the models. These variables included electrical conductivity and TN, although the latter contributed to the Redfield ratio, which was significant in some models. There were no common variables which remained significant in all models at all sites, although when models were restricted to being calibrated on data points where measurements of (SRP) were available, this variable was a significant predictor at every site.

Model reduction (determined by the continued lowering of the AIC) was done iteratively with between seven and 15 iterations for each. The reduced model results showing the effect of each significant environmental variable on *P. minimum* abundance for each site are detailed below. Twelve out of a possible 16 models were deemed satisfactory, each explaining between 20% and 70% of the deviance. The models for site 153 were deemed of little value. Although we had monitoring data for this site between 2011 and 2016, there were large gaps in complete coverage (for example, no information from 2012) and, in the remaining data points, only once did the abundance of *P. minimum* rise above 1000 cells L-1. Similarly, for site 152 and site 174, the models which did not include SRP were of no value. This observation was confirmed by the high explanatory power of this variable when included in the other model for those sites. The effect of each significant environmental variable by site in each model analysis is presented in Figure 6 (with supporting data in Supplementary Tables 4-5 and Supplementary Figure 1).

To summarise the modelling results:

1. *P. minimum* abundance at site 60, when SRP was not included in the model, was significantly linked to an increase in chl-a ($p < 0.001$) and a marginal decrease in NOx

($p < 0.1$) and turbidity ($p < 0.05$). When SRP was included in the model, *P. minimum* abundance was linked to a significant decrease in turbidity ($p < 0.001$), a significant increase in DO ($p < 0.001$), an increase in chl-a ($p < 0.\ 01$), a decrease in NOx ($p < 0.01$) and a marginal decrease in salinity ($p < 0.05$).

2. For site 61 four models were run. The first which included all variables except SRP showed *P. minimum* abundance to be significantly linked to an increase in chl-a ($p < 0.001$), temperature and DO ($p < 0.01$). When SRP was included in the model increasing chl-a, decreasing temperature and decreasing SRP were all linked to increasing *P. minimum* abundance ($p < 0.001$), whilst decreasing salinity and week of year ($p < 0.01$) although this later variable was difficult to interpret and was likely due to collinearities with other variables. When thermal stratification, but not SRP, was included in the model increasing chl-a and decreasing TN were significantly linked to *P. minimum* abundance ($p < 0.001$), as well as decreasing salinity ($p < 0.01$). When all variables including thermal stratification and SRP were included, highly significant effect were seen for increasing chl-a, week of year (cell densities were lowest between weeks 25-35), decreasing water temperature and SRP ($p < 0.001$).
3. At site 150, when all variables except SRP were included in the model, the Redfield ratio significantly decreased as *P. minimum* abundance increased ($p < 0.001$), while a marginal influence from increasing dissolved oxygen and pH was observed (both $p < 0.05$). When SRP was included in the model decreasing dissolved oxygen was highly significant ($p < 0.001$), while SRP (decreasing) and the Redfield ratio (increasing) were also significant ($p < 0.01$).
4. For both models at site 151 (with and without SRP), *P. minimum* abundance was significantly linked to both decreasing Redfield ratios and decreasing rainfall both ($p < 0.01$ when SRP was included and $p < 0.001$ when it was not included).

5. Only one model (all variables including SRP) was informative at site 152. *P. minimum* rose in abundance at this site with an increasing Redfield ratio and a decreasing SRP concentration (both $p < 0.001$) and dissolved oxygen concentration ($p < 0.01$).
6. Only one model was informative at site 174 (all variables including SRP) with *P. minimum* significantly linked to a decreasing Redfield ratio ($p < 0.001$) and decreasing rainfall, chl-a and SRP concentrations ($p < 0.01$).

4.1 Discussion

4.1.1 Spatial Trends and Temporal trends in P. minimum

In response to an unprecedented visual bloom in the Hawkesbury River in the austral spring of 2016, our study examines the spatial and temporal patterns of the harmful dinoflagellate *P. minimum* in this estuary, and the relationship between its abundance and various water quality parameters. Whilst the Hawkesbury River has a long history of algal blooms (Ajani et al. 2001), the 2016 bloom was the first water discoloration in many years in Berowra Creek (Rubio pers. comm.). Using a long-term dataset which tracks phytoplankton and physio-chemical parameters over decadal timescales, we found that *P. minimum* varied in its density and the factors driving this density over large spatial scales, with sites located in the main channel of the Hawkesbury River estuary significantly differing from those in the more urbanized tributary of Berowra Creek. These results suggested that blooms of *P. minimum* may favour a spatial gradient of eutrophication and salinity, whereby *P. minimum* and phytoplankton growth (as measured by chl- a) may be enhanced at sites that are generally less saline and more eutrophic than others in this river system. The notable exceptions to this interpretation were the upstream sites (152 and 153), which had similarly high nutrient concentrations to those in Berowra Creek but returned very low salinity levels as a result of

Figure 6. Modelling results for *Prorocentrum minimum* in the Hawkesbury River. Only significant variables are shown for each successful model. From left to right: chlorophyll-a [Chl-a](µg L^{-1}); ~~Turbitidy~~Turbidity [Turb](NTU); Redfield Ratio [RR](see methods); Temperature [Temp](°C), dissolved oxygen [DO](mg L^{-1}), oxidised nitrogen [NOx](mg L^{-1}), total nitrogen [TN](mg L^{-1}), Soluble Reactive Phosphorus [SRP] (mg L^{-1}), rainfall (average 7 days) [R7], salinity [sal](ppt), week of year sampled [week] and pH. Thermal stratification (TS) was only available for site 61. Size of arrow/sun indicates variable is highly significant (large arrow, large sun) <0.001, significant (medium arrow, medium sun) <0.01, or marginally significant (small arrow) <0.05; and direction of arrow indicates variable is significantly increasing (arrow up) or decreasing (arrow down) when *P. minimum* abundance is increasing in the model.

their geographical location (long-term average of 15 and 8ppt respectively) coupled with low *P. minimum* abundance, suggesting that this dinoflagellate may have reached its upstream threshold at these sites.

Temporal patterns in *P. minimum* abundance in the Hawkesbury estuary suggest that blooms occur abruptly and last up to three weeks, most often during the austral autumn to spring. They also appear to be increasing in frequency towards the present (at least at certain sampling locations) in this river system. As has been proposed in other parts of the world (Heil et al. 2005 and references therein), the use of *P. minimum* as an indicator of increasing eutrophication in SE Australia however, remains difficult. This is because there is very little historical data for *P. minimum* available, with its notable absence from the early works in SE Australia by Dakin and Colefax (1933, 1940) and Revelante and Gilmartin (1978). It was however, reported in the waters offshore from Sydney as early as 1978 (Hallegraeff and Reid 1986) with maximum density observed in October (Ajani et al. 2001a). In March 1995 and March 2000, *P. minimum* blooms were reported in Berowra Creek and Sydney Harbour respectively (Ajani et al. 2001b). A bloom of *P. minimum* was implicated in a mass mortality (15-100%) of Sydney rock oysters (*Saccostrea glomerata*) in Wonboyn Lake SE Australia (37°S) as early as 2002 (Ogburn et al. 2005). Despite the pristine nature of the Wonboyn catchment, limited tidal flushing combined with other climatic (major rain event/significant reduction in salinity), hydrological (fresh water influx from run-off) and biological (high loading of nutrients and dissolved organic matter) factors appeared to have allowed the proliferation of *P. minimum* and the concomitant mortality of oysters at this time (Ogburn et al. 2005). From around 2000 onwards, *P. minimum* has been observed as a common component of the microalgal community in other Australian coastal embayments (Hallegraeff et al. 2010), with anecdotal evidence suggesting that it is increasing in abundance in Sydney Harbour and various other urbanized estuaries of SE Australia (Ajani unpublished data).

Similar spatial and temporal patterns in *P. minimum* blooms have been observed in other estuaries around the world (Fan et al. 2003, Sahraoui et al. 2013, Li et al. 2015, Telesh et al. 2016, Olenina et al. 2016, Carreto 2018). Two well studied marine systems for this particular HAB species are Chesapeake Bay (USA) and the Baltic Sea. Over the past two decades, blooms of *P. minimum* have been observed to significantly increase in Chesapeake Bay, with blooms initiating in late spring in response to low salinities and chl-a concentration between 5 and 20 µg chl-a L^{-1} (Li et al. 2015). Blooms generally followed a spatial gradient of eutrophic conditions, with the majority occurring in the upper part of the bay and within its tributaries. Similarly, *P. minimum* has increased in abundance and occurrence in the brackish waters of the Baltic Sea since its invasion over three decades ago (Telesh et al. 2016). Using climate change driven projections in salinity (decrease) and nutrient loading (increase) into the Baltic Sea, modelling suggests that *P. minimum* may have a long-~~-~~term competitive advantage, leading to more extensive and prolonged blooms of this harmful dinoflagellate in this enclosed ~~mediterranean~~ European sea (Olenina et al. 2016).

***4.1.2 Effect of nutrients on* P. minimum**

The high physiological flexibility of *P. minimum* has been well established in laboratory studies and during field investigations, and under varying light, temperature and salinity conditions (refer to review by Heil et al. 2005 and references therein). In Australia, there are few published reports of blooms of *P. minimum*. Interestingly, and in a similar circumstance as the Hawkesbury River, mixed dinoflagellate blooms (including *P. micans*) have been recorded in the Port River, South Australia proximate to a sewage outfall (Cannon 1990).

These blooms, however, appear to be driven largely by water column stability despite high nutrient availability. While upgrades to the treatment plant(s) in Berowra Creek of the Hawkesbury River in 2003 resulted in a reduced nutrient load entering the system, N concentrations in the estuary remain relatively high (Larsson et al. 2017). With the apparent increase in *P. minimum* blooms in the Hawkesbury River and elsewhere, understanding nutrient forms, variability and availability is critical and highlights the value of long-~~-~~term water quality datasets. ~~On-going monitoring should be continued and if resources permit, other variables (e.g. other forms of N, species diversity) could be included in the program to augment the dataset.~~

The modelling component of this study reflects the ability of *P. minimum* to outcompete other algal species in nutrient-rich systems. Nutrient uptake by *P. minimum* is complex and the species can utilise a range of N types depending on their availability and ratios, combined with environmental variables such as temperature and light attenuation (Glibert et al. 2012 and references therein). Optimal (faster) growth rates for *P. minimum* occur at low N:P ratios (~12; Li et al., 2011, Glibert et al., 2012). In both regions of the estuary, high cell densities of *P. minimum* were associated with a decline in SRP. Glibert et al. (2012) proposed that a "flush" of organic or inorganic forms of N or P can stimulate a bloom. Other than direct land-based input sources, P could derive from existing sediment deposits (and be released during a decline in stratification or low DO). Accoroni et al. (2015) proposed a similar explanation for annual *Ostreopsis* cf. *ovata* blooms in the Northern Adriatic Sea with subsequent bloom maintenance a result of either (or a combination of mixotrophy, allelopathy or metabolic dissipatory strategies, particularly during non-optimal N:P ratios. On the other hand, while nitrogen loading to Berowra Creek has significantly declined over the past few decades, total nitrogen, ammonia and oxidised nitrogen concentrations remain well above levels recommended for estuaries in South East Australia (Larsson et al. 2017). This suggests that Berowra Creek remains P-limited, and that a significant decline in SRP as seen in the present study may be a direct result from phytoplankton uptake during bloom development.

The model outputs in the current study also demonstrated the natural hydrographic~~?~~ divide between the main channel of the Hawkesbury River estuary and Berowra Creek. A distinguishing feature was the strong correlation between *P. minimum* and the Redfield Ratio in the main channel of the Hawkesbury River estuary. *P. minimum* was not consistently correlated with an increase or decrease in the Redfield Ratio, and this further highlights the flexibility of this species. Lee et al. (2015) reported similar circumstances for *P. minimum* blooms which occurred under a wide range of DIN:DIP ratios (ranging from Redfield up to 300). The link between nutrients and HABs such as those produced by *P. minimum* therefore, is not straightforward, and common metrics of eutrophication such as total N and total P do not always reflect this complexity. Nutrient proportions and forms, as well as the distinct eco-physiological characteristics of taxa, are now important considerations in understanding HAB responses (Glibert et al. 2017).

The almost monospecific presence of *P. minimum* was apparent in the correlation between increasing chl-a and *P. minimum* cell concentration~~,~~ in ~~the~~ Berowra Creek~~ portion of the estuary~~. Mixotrophy represents another competitive advantage for *P. minimum*, and a likely explanation for sustained bloom events (Glibert et al. 2012). *P. minimum* utilises other microplankton as a source of vital nutrients when dissolved inorganic nutrients are limited (Stoecker et al. 1997, Stoecker 1998), being particularly effective at gaining P from ingested prey (Johnson 2014). ~~While t~~This mixotrophic response is not a direct result of limited light attenuation (Stoecker et al. 1997). ~~, a~~As other phytoplankton become prey for *P. minimum* combined with *P. minimum's* ability to adapt to low light conditions (Coats and Harding 1988, Fan and Glibert 2005), *P. minimum* can outcompete predominately phototrophic species.

4.1.3 The 2016* P. minimum *bloom event

The *P. minimum* bloom within Berowra Creek during the austral spring of 2016 reached a maximum cell density of 1.9E+06 cells L^{-1} and was the main component of the water column phytoplankton (89%). Whilst higher cell densities have been reported at this site (1.88E+0.07 cells L^{-1} during the summer of 2012), these cell densities are within the range of those reported from other estuaries around the world. For example, maximum cell densities of 7.00E+07 cells L^{-1} were reported from Golden Horn Estuary, Turkey (Tas and Okus 2011); 2.28E+07 cells L^{-1} from Masan Bay, Korea (Jeong et al. 2013); ~3.50E+0.6 cells L^{-1} from the Baltic Sea (Telesh et al. 2016); and >5.00E+0.5 cells L^{-1} from Chesapeake Bay (Li et al, 2015).

While significant correlations were observed between rainfall and nutrients at all other sites in this estuary system (and thus suggesting a pathway for nutrient availability), the association between rainfall and nutrient delivery was generally not observed at site 61 (Supplementary Table 3). We hypothesize instead, that a continual supply of nutrients from two upstream sewage treatment facilities, coupled with the unique bathymetry and residence time of this site (Larsson et al. 2017), are the most likely contributing factors to favourable bloom conditions at this site. Indeed, the harmful dinoflagellates *Dinophysis acuminata* and *Dinophysis caudata* have also been observed to bloom more frequently and more intensely at this site compared to other sites sampled within this estuary (maximum cell concentrations of 4.50E+03 cells L-1 and 1.20E+04 cells L-1 respectively, Ajani et al. 2016).

Short-term phytoplankton species successions were observed prior to the *P. minimum* bloom in September 2016 at site 61, beginning with chain-forming diatoms and followed by various small flagellates (Supplementary Fig. 2). These short-term blooms themselves, or the collapse of these blooms, may have provided food particles into the water column allowing *P. minimum* to flourish using a mixotrophic mode of nutrition (see above). In support of this hypothesis, Stoecker et al. (1997) discusses *P. minimum's* ability to utilise a tube feeding mechanism which ingests prey organelles and moves them into intracellular food vacuoles. These food vacuoles, observed by Stoecker et al. both in the field and under laboratory conditions, indicate that *P. minimum* can supplement its carbon and trace element nutrition with active feeding and thus dominate when nutrients become depleted. In addition to providing an abundance of food particles, these short-term phytoplankton species successions also increased pH above the usual levels (pH 8.23). *Prorocentrum minimum* has a high pH tolerance (Hansen 2002) and these elevated levels, may have provided a competitive advantage for this species, over other mixotrophic dinoflagellates.

Analysis of samples for presence of toxins (phytoplankton culture or shellfish tissue) or potential toxic effects (i.e. shellfish histology) did not occur during this bloom event or as part of this study. Within the Hawkesbury River system, DSTs have not been detected since the establishment (2004) of the routine shellfish quality assurance program in the estuary (NSW Food Authority, 2017 and NSW Food Authority, unpublished data). *P. minimum* blooms during the study period were not linked to any negative effects in humans or marine fauna. The apparent increase in frequency and intensity of *P. minimum* blooms~~, in the Hawkesbury River (this study) and elsewhere~~ globally (e.g. Glibert et al. 2001, 2008, 2012, Heil et al. 2005, Tango et al. 2005, Olenina et al. 2016, Skarlato et al. 2017), is a potential threat to coastal ecosystems. While the link between human illnesses and *P. minimum* blooms is inconclusive

(Heil et al., 2005), the potential impact of dense *P. minimum* blooms to aquaculture and coastal resources is of concern. DST-production in some species of *Prorocentrum* has been linked to several variables, including low N or P concentrations (Chun-Hung Lee et al. 2016). It follows that the acquisition of nutrients via mixotrophy by *P. minimum* under nutrient deplete conditions has revealed a possible link between bacteriovory and toxic-like effects on shellfish physiology (Wikfors and Fernandez 2013).

Further work into blooms in this estuary should include a comprehensive investigation into whole water column characteristics including diurnal dissolved oxygen measurements, other forms of nutrients, species successions, the effect of mixotrophy and pH on *P. minimum* abundance, and the presence of DSTs. Moreover, changes climate predictions for Australia include warmer temperatures and more intense rainfall events (www.csiro.au/state-of-the-climate) which, coupled with laboratory studies by Peperzak (2003) which demonstrated higher growth rates for *P. minimum* under warmer more stratified conditions, might indicate that *P. minimum* will continue to be successful under future climate change scenarios. To examine this more broadly, future work should consider multiple stressors against this background of global change (Glibert et al. 2017).

Coastal environments are rich in natural and cultural resources and provide a suite of economic, social, tourist and recreational activities. These demands, however, pose unique challenges for sustainable management, especially during algal bloom events. Some harmful algal blooms have low cell concentrations and low visual impact but may still pose ecosystem and human health risks due to the potent toxins they produce. Others, such as the *P. minimum* bloom reported here, are highly visible blooms, posing threats to ecosystem services and recreational activities alike. ~~For example, as *P. minimum* blooms increase in biomass, and subsequently limit light attenuation, they can also damage submerged aquatic vegetation (Gallegos and Bergstrom 2005), which can have negative consequences for higher trophic levels. Either way, i~~Intensive monitoring of harmful microalgae informs the early detection of blooms, supports appropriate management efforts, and with increasing temporal scales, can provide a valuable forecasting capability which can protect and promote ecosystem health, tourism and food security.

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Supplementary Table 1. Variable, method used for analyses and detection limits for each analyte measured.

Analyte	Reference Method	Detection Limit
Chlorophyll-a	APHA 10200 H	<0.2 µg L-1
Total Nitrogen	APHA 4500 NO3 I	<0.05 mg L-1
Soluble Reactive Phosphorus	APHA 4500 P G	<0.002 mg L-1
Total Phosphorus	APHA 4500 P H	<0.002 mg L-1
Oxidised Nitrogen Low Level	APHA 4500 NO3 I	<0.01 mg L-1
Ammonia NH_3-N Low Level	APHA 4500 NH3 H	<0.01 mg L-1
Suspended solids	APHA 2540 D	<1 mg L-1

Supplementary Table 2. Summary statistics for all cell concentrations and environmental variables in the generalised linear modelling for all sites over the sampling period 2003 to 2016.

Attached Excel file

Supplementary Table 3. Correlation matrices showing correlation coefficients for every pair of predictor variables employed in the generalised additive models over the specific sampling period for each site. Values in the upper dataset are correlation values (-1 to 1) with those shaded green being those that suggested moderate to strong relationships ($r > 0.5$ or $r < -0.5$) between variables while the lower dataset is the p-value for each correlation with highlighted cells showing a significant value of $<2.5\times10^{-4}$, which is 0.05 corrected for approximately 20 paired comparisons.

Attached Excel file

Supplementary Table 4. Model results for *P. minimum* at all sites for the total sampling period 2003 to 2016 inclusive.

```
Site 60 (All, -SRP, -TS)
Family: Negative Binomial(0.113)
Link function: log
Formula:
Prorocentrum.min ~ s(Turbidity, k = 3) + Rainfall7 + OxidisedNitrogen +
    ChlorophyllA + SuspendedSolids

Parametric coefficients:
                 Estimate Std. Error z value Pr(>|z|)        Cont
(Intercept)       9.31331    0.49047  18.989  < 2e-16 ***
Rainfall7         0.04021    0.04041   0.995   0.3197        -1.9%
OxidisedNitrogen -4.69508    2.47009  -1.901   0.0573 .      5.2%
ChlorophyllA      0.11422    0.02776   4.115 3.87e-05 ***    24.6%
SuspendedSolids   0.02397    0.03034   0.790   0.4294        -11.4%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
               edf Ref.df Chi.sq p-value      Cont
s(Turbidity) 1.735   1.93  5.337  0.0421 *    -0.1%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  -2.02e+03   Deviance explained = 42.7%
-REML = 1204.8  Scale est. = 1         n = 145
> AIC(TTT)
[1] 2408.23

Site 60 (ALL, -TS)
Family: Negative Binomial(0.128)
Link function: log
Formula:
Prorocentrum.min ~ s(Turbidity, k = 3) + Salinity + s(Dissolvedoxygen,
    k = 3) + AmmoniumNitrogen + OxidisedNitrogen + ChlorophyllA +
    SolubleReactivePhosphorus + SuspendedSolids

Parametric coefficients:
                          Estimate Std. Error z value Pr(>|z|)       Cont
(Intercept)                9.26110    2.48375   3.729 0.000192 ***
Salinity                  -0.13485    0.07064  -1.909 0.056263 .     9.4%
AmmoniumNitrogen           2.79320   15.11216   0.185 0.853361       -0.1%
OxidisedNitrogen          -9.46363    4.49058  -2.107 0.035080 *     1.3%
ChlorophyllA               0.06693    0.03079   2.174 0.029739 *     10.9%
SolubleReactivePhosphorus 37.86484   49.63885   0.763 0.445579       0.8%
SuspendedSolids            0.04798    0.03057   1.570 0.116497       1.2%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                     edf Ref.df Chi.sq p-value       Cont
s(Turbidity)       1.905  1.991 11.025 0.00593 **    10.0%
s(Dissolvedoxygen) 1.000  1.000  8.509 0.00354 **    -3.3%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.279   Deviance explained = 36.9%
```

```
-REML = 779.99  Scale est. = 1         n = 94
> AIC(TTT)
[1] 1586.288
```

Site 61 (All, -SRP, -TS)

```
Family: Negative Binomial(0.158)
Link function: log

Formula:
Prorocentrum.min ~ s(Week, k = 6) + Salinity + Temperature +
    Dissolvedoxygen + ChlorophyllA

Parametric coefficients:
                Estimate Std. Error z value Pr(>|z|)        Cont
(Intercept)     10.85835    1.78779   6.074 1.25e-09 ***
Salinity        -0.02630    0.02997  -0.878 0.380175        0.9%
Temperature     -0.11351    0.04550  -2.495 0.012603 *      2.8%
Dissolvedoxygen  0.35495    0.15100   2.351 0.018741 *      -0.4%
ChlorophyllA     0.06410    0.01916   3.346 0.000819 ***    6.0%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value   Cont
s(Week) 1.004  1.007  0.675   0.414   1.2%

R-sq.(adj) =  -0.673   Deviance explained = 21.3%
-REML = 2086.2  Scale est. = 1         n = 203
> AIC(TTT)
[1] 4164.065
```

Site 61 (All, -TS)

```
Family: Negative Binomial(0.15)
Link function: log

Formula:
Prorocentrum.min ~ s(Week, k = 6) + Salinity + Temperature +
    SolubleReactivePhosphorus + ChlorophyllA

Parametric coefficients:
                            Estimate Std. Error z value Pr(>|z|)     Cont
(Intercept)                 17.00273    1.15505  14.720  < 2e-16 ***
Salinity                    -0.07170    0.03370  -2.128   0.0334 *   7.5%
Temperature                 -0.21286    0.04771  -4.462 8.13e-06 *** 3.9%
SolubleReactivePhosphorus -125.36065   30.77667  -4.073 4.64e-05 *** 12.4%
ChlorophyllA                 0.09264    0.01995   4.643 3.43e-06 *** 19.6%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq p-value   Cont
s(Week) 1.005  1.009  4.597  0.0328 * 9.4%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =   -333   Deviance explained = 38.9%
-REML = 1556.6  Scale est. = 1         n = 155
> AIC(TTT)
```

```
[1] 3117.794
```

Site 61 (All, -SRP)

```
Family: Negative Binomial(0.179)
Link function: log

Formula:
Prorocentrum.min ~ s(Week, k = 6) + TotalNitrogen + Salinity +
    Temperature + ChlorophyllA

Parametric coefficients:
              Estimate Std. Error z value Pr(>|z|)          Cont
(Intercept)   13.71557    3.05233   4.493 7.01e-06 ***
TotalNitrogen -5.90186    1.55995  -3.783 0.000155 ***       21.0%
Salinity      -0.11328    0.04563  -2.482 0.013048 *         22.4%
Temperature    0.03231    0.14143   0.228 0.819284           -1.7%
ChlorophyllA   0.12626    0.01885   6.699 2.10e-11 ***       31.2%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
         edf Ref.df Chi.sq p-value    Cont
s(Week) 4.07  4.666  5.947   0.143    -6.1%

R-sq.(adj) =  -1.94e+03   Deviance explained = 51.4%
-REML = 1562.6  Scale est. = 1         n = 151
> AIC(TTT)
[1] 3119.907
```

Site 61 (All)

```
Family: Negative Binomial(0.167)
Link function: log

Formula:
Prorocentrum.min ~ s(Week, k = 6) + Redfield + Salinity + Temperature +
    SolubleReactivePhosphorus + ChlorophyllA

Parametric coefficients:
                            Estimate Std. Error z value Pr(>|z|)     Cont
(Intercept)                 24.39820    4.53815   5.376 7.61e-08 ***
Redfield                    -0.02577    0.01427  -1.805   0.0710 .  1.1%
Salinity                    -0.04602    0.04413  -1.043   0.2971    1.9%
Temperature                 -0.59181    0.20934  -2.827   0.0047 ** -11.1%
SolubleReactivePhosphorus -128.76254   54.94000  -2.344   0.0191 *  8.9%
ChlorophyllA                 0.09630    0.02318   4.155 3.26e-05 *** 23.3%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
          edf Ref.df Chi.sq  p-value         Cont
s(Week) 4.548  4.917  34.39 2.21e-05 ***      -6.2%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =   -523   Deviance explained =   53%
-REML = 1041.4  Scale est. = 1         n = 104
> AIC(TTT)
```

```
[1] 2079.194
```

Site 150 (All, -SRP, -TS)

```
Family: Negative Binomial(0.046)
Link function: log

Formula:
Prorocentrum.min ~ s(pH, k = 3) + Redfield + ChlorophyllA + Dissolvedoxyge
n

Parametric coefficients:
                Estimate Std. Error z value Pr(>|z|)        Cont
(Intercept)      0.42075    8.78761   0.048   0.9618
Redfield        -0.29103    0.07246  -4.016 5.91e-05 ***     25.8%
ChlorophyllA    -0.61260    0.73407  -0.835   0.4040         12.2%
Dissolvedoxygen  2.33078    1.26554   1.842   0.0655 .       14.9%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
      edf Ref.df Chi.sq p-value       Cont
s(pH)   1      1  2.955  0.0857 .     21.75%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =   -415   Deviance explained = 26.4%
-REML = 180.29  Scale est. = 1         n = 46
> AIC(TTT)
[1] 376.0651
```

Site 150 (All, -TS)

```
Family: Negative Binomial(0.041)
Link function: log

Formula:
Prorocentrum.min ~ Redfield + ChlorophyllA + Dissolvedoxygen +
    SolubleReactivePhosphorus

Parametric coefficients:
                            Estimate Std. Error z value Pr(>|z|)     Cont
(Intercept)                4.387e+01  1.734e+01   2.530  0.01140 *
Redfield                   2.144e-01  9.016e-02   2.378  0.01741 *   15.2%
ChlorophyllA               1.921e+00  1.012e+00   1.898  0.05764 .   43.9%
Dissolvedoxygen           -5.919e+00  2.188e+00  -2.705  0.00682 **  39.0%
SolubleReactivePhosphorus -1.270e+03  5.863e+02  -2.166  0.03030 *   51.24
%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


R-sq.(adj) =  -1.86e+07   Deviance explained = 55.2%
-REML = 89.261  Scale est. = 1         n = 25
> AIC(TTT)
[1] 213.3259
```

Site 151 (All, -SRP, -TS)

```
Family: Negative Binomial(0.041)
Link function: log

Formula:
Prorocentrum.min ~ Redfield + ChlorophyllA + Dissolvedoxygen +
    Rainfall7

Parametric coefficients:
                Estimate Std. Error z value Pr(>|z|)         Cont
(Intercept)     10.00441    6.86885   1.456 0.145258
Redfield        -0.27241    0.07926  -3.437 0.000588 ***     22.32%
ChlorophyllA     0.08390    0.30439   0.276 0.782828         -0.5%
Dissolvedoxygen  1.29661    1.01029   1.283 0.199352         -1.1%
Rainfall7       -1.16739    0.39698  -2.941 0.003275 **      12.9%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


R-sq.(adj) =  -12.3   Deviance explained = 30.5%
-REML =  173.8  Scale est. = 1         n = 44
> AIC(TTT)
[1] 360.0156
```

Site 151 (All, -TS)

```
Family: Negative Binomial(0.036)
Link function: log

Formula:
Prorocentrum.min ~ Redfield + Dissolvedoxygen + Rainfall7

Parametric coefficients:
                Estimate Std. Error z value Pr(>|z|)         Cont
(Intercept)      21.3622     9.1264   2.341  0.01925 *
Redfield         -0.3883     0.1353  -2.869  0.00411 **      38.87%
Dissolvedoxygen   0.5870     1.6241   0.361  0.71779         5.8%
Rainfall7        -2.4614     1.0117  -2.433  0.01497 *       31.7%
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


R-sq.(adj) =  -11.3   Deviance explained = 47.2%
-REML = 86.304  Scale est. = 1         n = 24
> AIC(TTT)
[1] 187.6089
```

Site 152 (All, -TS)

```
Family: Negative Binomial(0.053)
Link function: log

Formula:
Prorocentrum.min ~ Redfield + Dissolvedoxygen + SolubleReactivePhosphorus

Parametric coefficients:
                            Estimate Std. Error z value Pr(>|z|)     Cont
(Intercept)                  17.4849     7.8424   2.230 0.025779 *
Redfield                      0.5229     0.1403   3.726 0.000195 *** 25.1%
Dissolvedoxygen              -2.4876     1.1915  -2.088 0.036812 *    1.1%
SolubleReactivePhosphorus -2900.9780   661.5429  -4.385 1.16e-05 ***  30.8
%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


R-sq.(adj) =  -4.32e+07   Deviance explained = 66.6%
-REML =  77.59  Scale est. = 1         n = 23
[1] 184.9074
> summary(TTT)
```

Site 174 (All, -TS)

```
Family: Negative Binomial(0.027)
Link function: log

Formula:
Prorocentrum.min ~ pH + Redfield + Rainfall7 + ChlorophyllA +
    SolubleReactivePhosphorus

Parametric coefficients:
                            Estimate Std. Error z value Pr(>|z|)     Cont
(Intercept)                 223.9954   161.6187   1.386   0.1658
pH                          -19.2277    19.8729  -0.968   0.3333     -0.1%

Redfield                     -0.6420     0.2401  -2.674   0.0075 **  -0.3%
Rainfall7                    -1.8817     0.8521  -2.208   0.0272 *   9.2%
ChlorophyllA                 -9.4640     3.9185  -2.415   0.0157 *   37.5%
SolubleReactivePhosphorus -2610.9364  1080.4583  -2.417   0.0157 *   67.48
%
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


R-sq.(adj) =  -1.44e+06   Deviance explained = 69.6%
-REML = 53.885  Scale est. = 1         n = 24
> AIC(TTT)
[1] 154.8333
```

Supplementary Table 5. *Prorocentrum minimum* model summary for each site sampled in the Hawkesbury River estuary. Those models indicated by grey lines are those which were not informative and therefore not included.

Site	Variables included in model	No. of observations in model	Significant variables	Deviance Explained
60	All (-SRP, -TS)	145	chl-a***, NOx*	42.7
	All (-TS)	94	turb***, DO***, chl-a**, NOx**, sal*	36.9
61	All (-SRP, -TS)	203	chl-a***,temp**, DO**	21.3
	All (-TS)	155	chl-a***, temp***, SRP***, sal**, week**	38.9
	All (-SRP)	151	chl-a***, TN***, sal**	51.4
	All	104	chl-a***, temp***, week***, SRP**	53.0
150	All (-SRP, -TS)	46	RR***, DO*, pH*	26.4
	All (-TS)	25	DO***, SRP**, RR**	55.2
151	All (-SRP, -TS)	44	RR***, R7***	30.5
	All (-TS)	24	RR***, R7**	47.2
152	All (-SRP, -TS)	42	-	0.01
	All (-TS)	23	RR***, SRP***, DO**	66.6
153	All (-SRP, -TS)	43	-	14.6
	All (-TS)	24	-	42.4
174	All (-SRP, -TS)	44	-	0.89
	All (-TS)	24	RR***, R7**, chl-a**, SRP**	69.9

All = total cells (cells L-1), temperature [temp](°C), turbidity [turb](NTU), dissolved oxygen [DO](mg L-1), pH, salinity [sal](ppt), suspended solids (mg L-1), ammonium-nitrogen [NH3](mg L-1), oxidised nitrogen [NOx](mg L-1), total nitrogen [TN](mg L-1), total phosphorus [TP](mg L-1), chlorophyll-A [chl-a](μg L-1), rainfall (average 7 days) [R7], Redfield ratio [RR]; week

SRP= Soluble Reactive Phosphorus [SRP] (mg L-1)

TS=thermal stratification (only available at site 61)

Significance codes: <0.001 '***', 0.01 '**', 0.05 '*'

Supplementary Figure 1

Attached Word document