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Modelling bloom formation of the toxic dinoflagellates *Dinophysis acuminata* and *Dinophysis caudata* in a highly modified estuary, south eastern Australia

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Dinophysis acuminata (Spring)

D. acuminata abundance is significantly higher when dissolved oxygen increases (>7-8 mg/L)

D. acuminata abundance is significantly associated with a reduction in the Redfield Ratio TN:TP

D. acuminata abundance is significantly higher when rainfall is low (< 10mm day)

Estuary flow

O$_2$

TN   TP

Phytoplankton bloom

To the ocean

D. acuminata abundance is significantly linked to a water temperature of ~20 °C, a concomitant increase in stratification and a decrease in total phytoplankton abundance
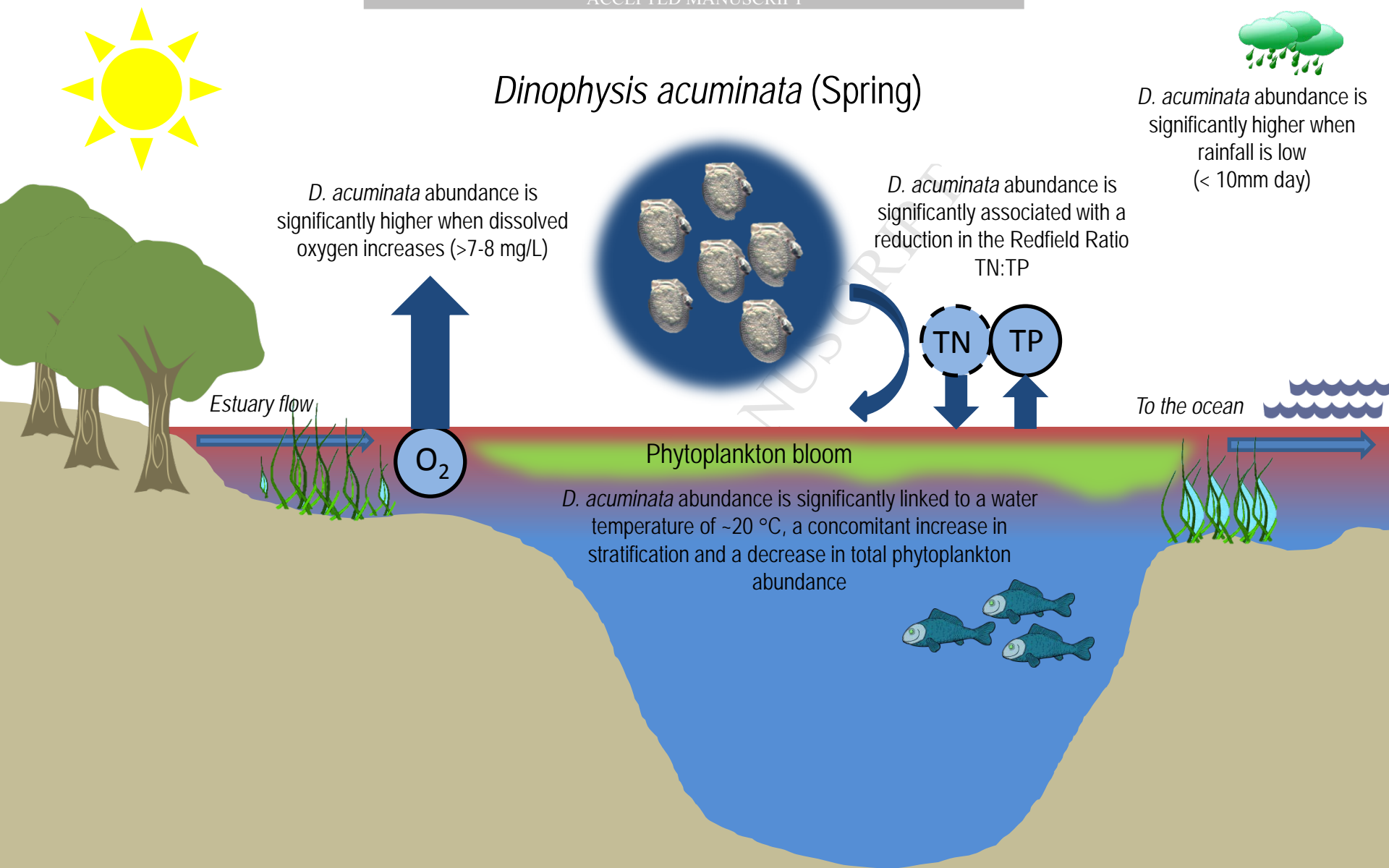
1 Modelling bloom formation of the toxic dinoflagellates *Dinophysis acuminata* and

2 *Dinophysis caudata* in a highly modified estuary, south eastern Australia

3 Penelope Ajani[1*], Michaela E. Larsson[1], Ana Rubio[2], Stephen Bush[3], Steve Brett[4] and Hazel

4 Farrell[5]

5 [1]*Plant Functional Biology and Climate Change Cluster (C3), University of Technology*

6 *Sydney, NSW 2007 Australia*

7 [2]*Natural Resources Branch, Hornsby Shire Council, Hornsby, NSW 2077 Australia*

8 [3]*School of Mathematical and Physical Sciences, University of Technology Sydney, NSW 2007*

9 *Australia*

10 [4]*Microalgal Services, Ormond VIC 3204 Australia*

11 [5]*New South Wales Food Authority, Newington NSW 2430 Australia*

12 [*]Corresponding Author: Email address: penelope.ajani@uts.edu.au [add telephone number at

13 poof stage]

14 **Abstract**

15 Dinoflagellates belonging to the toxigenic genus *Dinophysis* are increasing in abundance in

16 the Hawkesbury River, south-eastern Australia. This study investigates a twelve year time

17 series of abundance and physico-chemical data to model these blooms. Four species were

18 reported over the sampling campaign - *Dinophysis acuminata, Dinophysis caudata,*

19 *Dinophysis fortii* and *Dinophysis tripos*- with *D. acuminata* and *D. caudata* being most

20 abundant. Highest abundance of *D. acuminata* occurred in the austral spring (max.

21 abundance 4,500 cells l^{-1}), whilst highest *D. caudata* occurred in the summer to autumn (max.

22 12,000 cells l^{-1}). Generalised additive models revealed abundance of *D. acuminata* was

23 significantly linked to season, thermal stratification and nutrients, whilst *D. caudata* was

24 associated with nutrients, salinity and dissolved oxygen. The models' predictive capability

25 was up to 60% for *D. acuminata* and 53% for *D. caudata*. Altering sampling strategies during

26 blooms accompanied with *in situ* high resolution monitoring will further improve *Dinophysis*

27 bloom prediction capability.

28 **Key words**: harmful algal blooms, phytoplankton, toxins, okadaic acid, *Mesodinium rubrum*

29

30 **1.1 Introduction**

31 Certain species of phytoplankton can form harmful algal blooms (HABs) that may have both

32 ecosystem and human health consequences (Hallegraeff et al. 2003, Anderson et al. 2012,

33 Munday and Reeve 2013). HABs can produce potent phycotoxins that may accumulate in

34 marine animals (e.g. oysters, fish) and be transferred to higher trophic levels such as

35 mammals, seabirds and humans. One harmful dinoflagellate of global significance is the

36 genus *Dinophysis* (Dinophysaceae) Ehrenberg. Being cosmopolitan, this genus has over 100

37 species represented worldwide, ten of which (*Dinophysis acuminata* Claparède & Lachmann,

38 *Dinophysis acuta* Ehrenberg, *Dinophysis caudata* Saville-Kent, *Dinophysis fortii* Pavillard,

39 *Dinophysis infundibulum* J.Schiller, *Dinophysis miles* Cleve, *Dinophysis norvegica* Claparède

40 & Lachmann, *Dinophysis ovum* Schütt, *Dinophysis saccula* Stein and *Dinophysis tripos*

41 Gourret) have been unambiguously found to be toxic, producing diarrhetic toxins (okadaic

42 acid and dinophysistoxins) and pectenotoxins even at low cell densities ($< 10^3$ cells l^{-1})

43 (Reguera et al. 2012, Reguera et al. 2014, Simoes et al. 2015). Symptoms of vomiting and

44 diarrhoea in seafood consumers, have been reported from 'diarrhetic shellfish poisoning'

45 (DSP) in many parts of the world (Yasumoto et al. 1978, Lembeye et al. 2003, Taylor et al.

46 2013, Whyte et al. 2014) and for this reason, this genus is the focus of many harmful algal

47 monitoring programs throughout the world.

48

49 Despite its importance, many aspects of *Dinophysis* (life history, toxicity, genetic diversity,

50 and population heterogeneity) have remained undiscovered until very recently. This has been

51 due to an inability to successfully maintain cultures of these organisms in the laboratory

52 (Sampayo et al. 1993, Nishitani et al. 2003). Furthemore, due to their typically low cell

53 density in the water column, they have often escaped detection using standard quantitative

54 methods, making them even more elusive (Reguera et al. 2012). In 2006 however, Park et al.

55 (2006) successfully cultivated *Dinophysis* for the first time. Using a mixotrophic culture

56 approach, *Dinophysis* was successfully grown in the presence of its prey, the phototrophic

57 ciliate *Mesodinium rubrum* and chryptophyte *Teleaulax* spp. Since this breakthrough,

58 worldwide efforts to investigate this genus have increased rapidly, with new insights now

59 available into their toxicity, nutrition, population dynamics and polymorphic life cycle

60 (Reguera et al. 2012).

61

62 *Dinophysis* is widespread in Australian waters, with 36 species reported thus far (Hallegraeff

63 and Lucas 1988, Ajani et al. 2011, McCarthy 2013**).** Toxic representatives include *D.*

64 *acuminata* (Claparède & Lachmann)*, D. acuta* (Ehrenberg)*, D. caudata* (Saville-Kent), *D.*

65 *fortii* (Pavillard) and *D. hastata* (Stein). There have been three major DSP events in Australia

66 to date. In 1997 *D. acuminata* and *D. tripos* were implicated in the contamination of pipis

67 (*Plebidonax deltoides* Lamarck 1818) in New South Wales (NSW) (Quaine et al. 1997) in

68 which 102 people were affected, and 56 cases of gastroenteritis reported. In March 1998 a

69 second outbreak was reported in which 20 cases of DSP poisoning were reported from New

70 South Wales (Madigan et al. 2006 and references therein). In March 2000, a third outbreak

71 occurred in Queensland and was again linked to the consumption of pipis (Burgess and Shaw

72 2001). In this event only one individual was affected. In December 2003, a further *D.*

73 *acuminata* bloom was detected in the Eyre Peninsula, South Australia (SA) (Madigan et al.

74 2006). This was the first record of Diarrhetic Shellfish Toxins (DSTs) above the regulatory

75 limit for SA, however, with careful monitoring and harvest closures, no human illnesses were

3

76 reported. In 2013, a synthesis of harmful phytoplankton species in oyster growing estuaries of

77 NSW identified *Dinophysis* as one of three potentially high-risk genera for biotoxin events

78 (others being *Alexandrium* and *Pseudo-nitzschia*) (Ajani et al. 2013). The study found the

79 NSW Food Authority's regulatory "Phytoplankton Action Limit" (PAL) which triggers

80 shellfish flesh sampling (defined as 500 'total *Dinophysis*' cells l^{-1}, NSW MBMP 2015) was

81 exceeded in 136 samples across 31 estuaries over a 5 year period. It was concluded from this

82 meta-analysis that blooms of *Dinophysis* posed a potential threat to this AUD\$32M (farm

83 gate value) per annum industry (Trenaman et al. 2015). Since the commencement of routine

84 biotoxin monitoring from classified NSW shellfish aquaculture areas (predominately

85 *Saccostrea glomerata* with some *Crassostrea gigas, Ostrea angasi* and *Mytilus edulis*) in

86 2005, there have been 29 positive test results for the presence of DSTs recorded (<1%), with

87 no positive reports for the Hawkesbury River to date (NSW Food Authority, *unpublished*

88 *data*). Typically, higher concentrations of toxins associated with *Dinophysis* spp. have been

89 reported in wild harvest pipis (*Donax deltoides*) from the state (Farrell et al. 2015).

90

91 Regardless of the recent advances in toxic bloom research however, there remains a

92 significant gap in our knowledge regarding *Dinophysis* bloom initiation and development

93 (Reguera et al. 2012, 2014) and there has been no investigation into the dynamics of this

94 genus in any Australian embayment thus far. Berowra Creek (~33 °S, 151 °E), our study

95 location, is positioned within one of the southern arms of the Hawkesbury-Nepean River

96 which is one of the largest coastal rivers systems in south eastern (SE) Australia (Fig. 1). This

97 highly modified (Ozcoasts 2011) drowned river estuary (Roy et al., 2011) supports

98 commercial fishing, shellfish aquaculture and tourism (HSC 2015). Since the early 1990s,

99 Berowra Creek has been a 'hot spot' for algal blooms (Ajani et al. 2001, 2011 & 2013) with

100 variable residence times and nutrient loading implicated as possible bloom drivers (Coad

101 2012, Larsson et al. 2016). Whilst potentially harmful phytoplankton are often observed in

102 this estuary (Ajani et al. 2013), it has only been in more recent years that high cell densities

103 of *Dinophysis acuminata* have been reported as part of an ongoing water quality program.

104 This program was initiated in 2003 to investigate the effect of two sewage treatment upgrades

105 in the catchment on phytoplankton blooms. With this in mind, our study draws upon this

106 unique twelve-year time-series to model the biological, physical and chemical conditions

107 under which *Dinophysis* blooms occur in Berowra Creek, with the aim of identifying the

108 potential mechanism(s) for bloom development in south eastern Australia.

109

110 **2.1 Methods and Materials**

111 *2.1.1 Sampling Sites*

112 Water samples from two sites in Berowra Creek were collected at approximately 3-4 week

113 intervals over the period April 2003 to December 2014. The first site, hereafter known as site

114 60 (33.5995 °S, 151.1233 °E) had a water depth of approximately 6 m (Figure 1). The second

115 site (33.5870 °S, 151.1199 °E) hereafter known as site 61, was located approximately 1.5 km

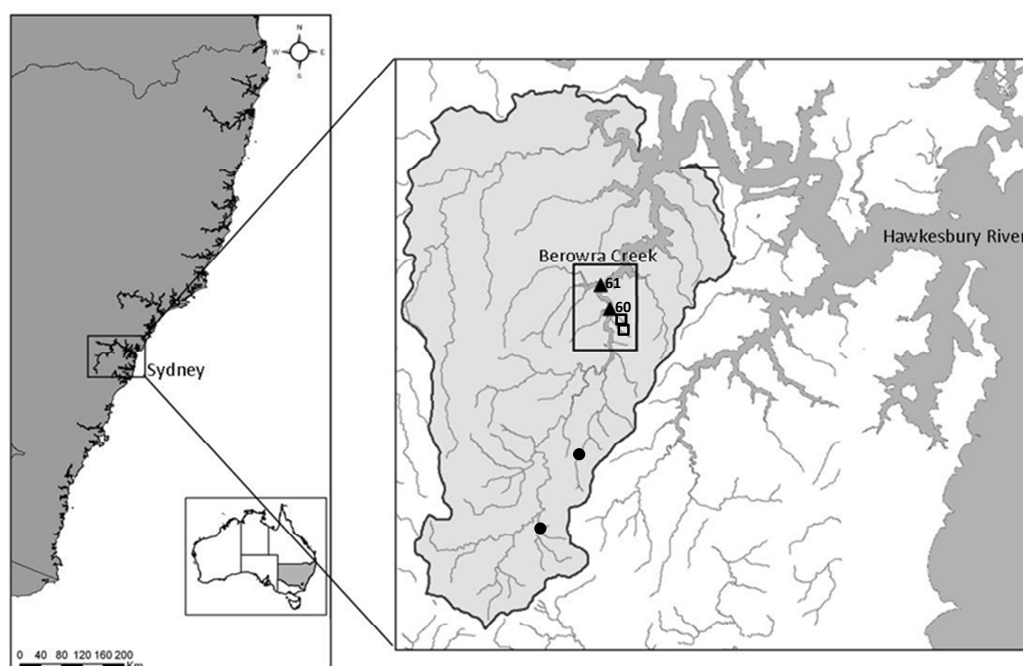116 downstream of site 60 and had a water depth of approximately 15 m (Figure 1).

117

129

130

131   Figure 1. Map of the New South Wales coastline (SE Australia) showing the Sydney region,

132   and the Hawkesbury River. The two sampling locations within Berowra Creek are shown as

133   sites 60 and 61 (dark triangles), the two sewage treatment plants (dark circles), and the two

134   rainfall stations (open squares).

135

136   *2.1.2 Species Enumeration*

137   At each of the two sampling sites, water samples (500 ml) were collected from a depth of 0.5

138   m and immediately preserved with Lugol's iodine solution for later identification and

139   enumeration of phytoplankton. In the laboratory, samples were concentrated by gravity-

140   assisted membrane filtration, and phytoplankton cell counts were undertaken in a Sedgewick

141   Rafter counting chamber. Cell enumeration and detailed examination of cells were carried out

142   using Zeiss Axiolab or Standard microscopes equipped with phase contrast. Cells were

143   identified to the closest taxon that could be accurately identified using light microscopy

144   (maximum magnification ×1,000). Cell counts were undertaken to determine the abundance

145   of individual *Dinophysis* species, the ciliate prey organism *Mesodinium rubrum* and total

146   phytoplankton cell (> 5 μm) numbers. Due to its small size (<10 um), the alternate prey of

147   *Dinophysis*, *Teleaulax* spp., was not enumerated in this study. *Dinophysis* cells were counted

148   to a minimum detection threshold of 50 cells l^{-1} while all other species were counted to a

149   minimum detection threshold of 500 cells l^{-1}.

150

151   *2.1.3 Environmental Variables*

152   *In situ* measurements of temperature (°C), salinity (psu), turbidity (ntu), dissolved oxygen

153   (mg l^{-1}) and pH from a depth of 0.5 m were made at the time of phytoplankton sampling

154   using a YEOKAL^{TM} 615 Water Quality Analyser (NSW, Australia). The instrument was

155 calibrated in accordance with manufacturer's specifications at the commencement of each

156 sampling day, and checked at the end of the day to identify and correct for calibration drift, if

157 required.

158

159 At the same time as the phytoplankton and *in situ* environmental data were collected, water

160 samples were collected using a pole sampler with attached prewashed 200 ml bottle from 0.5-

161 1 m depth and subsampled for nutrient analyses: oxidised nitrogen (nitrite NO_2^- and nitrate

162 NO_3^-), ammonium nitrogen (NH_4^+), total nitrogen (TN) and total phosphorus (TP) (mg l^{-1}).

163 Two further one litre samples were collected for chlorophyll-a (chl-a, µg l^{-1}) and suspended

164 solids determination (mg l^{-1}). Once collected, all samples were kept cool in the dark and

165 immediately transported to a NATA (National Association of Testing Authorities)-accredited

166 laboratory for analyses as per the methods and detection limits as listed in Supplementary

167 Table 1.

168

169 To test the effect of rainfall on the abundance of *Dinophysis* at the two sampling sites in

170 Berowra Creek, rainfall data was obtained from the two closest Bureau of Meteorology

171 weather stations at Berowra (Goodwyn Road, station no. 67052, 33.64 ° S, 151.14 ° E) and

172 Mount Kuring-Gai (Ledora Farm, station no. 66119, 33.64 ° S, 151.14 ° E). These stations

173 were located approximately 6 km upstream from sampling site 61 and 3 km from site 60 (Fig.

174 1). Rainfall data was averaged across both stations for each day measured (mm/day), and then

175 averaged over the 7 days prior to the phytoplankton sampling day to incorporate a measure of

176 exposure to this variable (MHL 1998).

177

178   To assess the effects of nutrient ratios on *Dinophysis* abundance, we also included the

179   Redfield ratio as a predictor variable (Redfield, 1934). This was calculated as the atomic ratio

180   between total nitrogen and total phosphorus as per the following equation:

181

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

183

184   Beginning in 2007 an automated testing probe was deployed at sited 61 (only). This probe

185   collected temperature (°C) data every 15 mins from the surface (30 mm) and every 100 mm

186   to the bottom (1530 mm). Despite a reduced temporal coverage, this data provided an

187   additional opportunity to assess the effects of thermal stratification, defined as the

188   temperature difference between 0 m and 15 m, on *Dinophysis* blooms at this location.

189

190   *2.1.4 Data Treatment and Analyses*

191   Given that there were a large number of environmental variables, analysis commenced with

192   an exploration of relationships between these variables. This was done through scatterplot

193   matrices and correlation analyses. Information from these analyses was used in the model

194   building process to ensure that models remained stable.

195

196   To model the relationship between the abundance of *Dinophysis* and the environmental

197   variables, generalised additive models were used (Hastie and Tibshriani 1990, Wood 2000).

198   There are two primary benefits of using generalised additive models. The first is that this type

199   of model treats the *Dinophysis* abundance as count data, rather than using a log

200   transformation to make the count continuous, and as such can handle zero counts. The second

201   is that several of the environmental variables e.g. time of year, had a nonlinear relationship

202   with abundance, so smoother functions were used to incorporate this relationship into the

203   model. These models were fitted in version 3.2.1 of the R statistical package (Team R Core

204   2013), using the GAM (Generalised Additive Model) function in version 1.8-7 of the 'mgcv'

205   package (Wood 2006).

206

207   To incorporate a measure of exposure experienced by the phytoplankton community, some of

208   the environmental variables were averaged over a period of time and then incorporated into

209   the model. Where there was a single measurement within these periods, the original value

210   was used. Where there were multiple measurements within this period, an average of up to 21

211   days prior to sampling was used for *M. rubrum* concentrations. This interval was based on

212   observed time-lags (2-21 days) between *M. rubrum* and *Dinophysis* blooms (Reguera et al.

213   2012, Velo-Suárez et al. 2014) and our maximum sampling interval. In addition, if more than

214   one sample was taken within a 7 day period, then the average of the past 7 days was used for

215   temperature, ammonium nitrogen, oxidised nitrogen, total phosphorus and total nitrogen to

216   incorporate a measure of exposure to these variables by the phytoplankton (Ajani et al. 2001).

217

218   In the first instance, four models were developed, one for each of the two commonly

219   observed species, *D. acuminata* and *D. caudata*, at each of the two sites, 60 and 61 using the

220   data collected from April 2003 to December 2014 (not including the probe temperature data).

221   Visual inspection of the relationships between environmental variables and *Dinophysis*

222   abundance indicated that month of year, temperature, dissolved oxygen, salinity, pH,

223   ammonium nitrogen, the Redfield ratio and rainfall may be nonlinear. Spline based smooths

224   were used to capture these nonlinear relationships, with the fitting algorithm attempting to

225   minimise the order of the spline. If the fit suggested that a linear relationship was sufficient

226   for all four models, the spline fit was replaced with a linear fit.

227

228 In the second instance, two additional models were developed, one for each of the two

229 commonly observed species, *D. acuminata* and *D. caudata* at site 61, using the

230 environmental variables and additional data collected from the temperature probe (thermal

231 stratification defined as the difference in temperature between the surface and bottom

232 temperatures). Visual inspection of the relationships between environmental variables and

233 *Dinophysis* abundance was again carried out, and where non-linear relationships were

234 observed, spline based smooths were used to capture these nonlinear relationships as

235 described above.

236

237 Some of the environmental variables contained missing values for some observations. The

238 number of missing values for each variable is given in Tables 1 and 2. When modelling, if

239 one of the variables had a missing value for one of the observations, the entire observation

240 was not used in model fitting. When comparing different models (using the Akaike

241 Information Criterion (AIC), Akaike 1973), the number of observations in the models that

242 were compared remained constant.

243

244 **3.1 Results**

245 *3.1.1 Species Abundance*

246 Over the twelve year sampling period (2003 - 2014), 142 water samples were collected for

247 phytoplankton enumeration from site 60, and 193 from site 61 (Table 1). *Dinophysis* species

248 identified included *D. acuminata, D. caudata, D. fortii* and *D. tripos* (Figs 2A, B). *D. fortii*

249 and *D. tripos* were observed on occasion in water samples; however both species were

250 observed in very low abundances (8% and 0% of total *Dinophysis* abundance at site 61

251 respectively and 0.2% and 0% at site 60). *D. acuminata* and *D. caudata* on the other hand,

252 made up 31% and 69% of total *Dinophysis* abundance at site 61 respectively, and 19% and

10

253 73% at site 60. Due to their low cell numbers *D. tripos* and *D forti* were not included in any

254 further statistical modelling.

255

256 *D. acuminata* concentrations were greatest at site 61 (123.63 ± 26.88 (mean ± standard error)

257 cells l^{-1} max. 4,500 cells l^{-1}, Table 1, Figs. 2A, B) when compared with site 60 (52.32 ± 10.71

258 cells l^{-1}, max. 1,000 cells l^{-1} Table 1, Figs. 2A, B). Similarly, *D. caudata* concentrations were

259 greatest at site 61 (486.09 ± 126.49 cells l^{-1}, max. 12,000 cells l^{1} Table 1, Figure 2A-B) when

260 compared with site 60 (116.34 ± 35.42 cells l^{-1}, max. 3,500 cells l^{-1} (Table 1, Figs. 2A, B).

261

262 Cell concentrations of *D. acuminata* and *D. caudata* varied across years, with *D. acuminata*

263 revealing highest cell densities in more recent years (Figs. 2A, B). When examined for

264 monthly or seasonal variation, the temporal distribution of *D. acuminata* was similar across

265 each site. This was also the case for *D. caudata*. Both species, however, showed a varying

266 temporal pattern within each site, with *D. caudata* elevated between weeks 0-20 and *D.*

267 *acuminata* between weeks 30-50, and both showing similar cell concentrations between

268 weeks 20-30 and 52 (Figs. 2C, D).

11

269   Table 1. Summary statistics for all cell concentrations and environmental variables used in the generalised linear modelling for site 60 and 61,

270   Berowra Creek, over the period 2003 to 2014.

|	Site 60							Site 61						
	min	max	median	mean	SE.mean	nbr.val*	nbr.na#	min	max	median	mean	SE.mean	nbr.val*	nbr.na#
D. acuminata (cells l⁻¹)	0.00E+00	1.00E+03	0.00E+00	5.23E+01	1.07E+01	142	0	0.00E+00	4.50E+03	0.00E+00	1.24E+02	2.69E+01	193	0
D. caudata (cells l⁻¹)	0.00E+00	3.50E+03	0.00E+00	1.16E+02	3.54E+01	142	0	0.00E+00	1.20E+04	0.00E+00	4.86E+02	1.26E+02	193	0
M. rubrum (cells l⁻¹)	0.00E+00	1.60E+05	5.00E-01	8.73E+03	1.83E+03	142	0	0.00E+00	8.00E+05	5.00E-01	1.66E+04	4.77E+03	193	0
Total Phytoplankton (cells l⁻¹)	1.69E+05	4.31E+07	1.86E+06	3.96E+06	5.08E+05	142	0	2.45E+04	1.51E+09	2.33E+06	1.26E+07	7.79E+06	193	0
Log Total Phytoplankton (cells l⁻¹)	5.23	7.63	6.27	6.31	0.04	142	0	4.39	9.18	6.37	6.38	0.04	193	0
Temperature (°C)	11.73	28.59	20.58	20.34	0.40	140	2	11.80	28.75	21.04	20.57	0.36	174	19
Turbidity (ntu)	0.00	21.90	1.29	2.13	0.27	140	2	0.00	24.00	1.30	2.25	0.25	174	19
Dissolved Oxygen (mg l⁻¹)	3.65	10.06	6.80	6.83	0.12	139	3	4.02	13.00	7.48	7.64	0.10	173	20
pH	6.60	7.97	7.49	7.44	0.02	140	2	6.75	8.28	7.67	7.62	0.02	174	19
Salinity (psu)	2.99	33.86	23.28	22.05	0.54	139	3	2.62	34.02	23.29	22.39	0.47	173	20
Suspended Solids (mg l⁻¹)	0.50	84.00	5.00	6.43	0.73	140	2	0.05	86.00	4.00	7.00	0.72	156	37
Chlorophyll-a (µg l⁻¹)	0.40	85.50	4.30	7.10	0.80	139	3	0.50	87.50	7.00	10.32	0.95	154	39
Average Rainfall (mm day⁻¹)	0.00	45.46	2.43	5.04	0.65	135	7	0.00	45.46	1.92	4.72	0.55	180	13
Average *M.rubrum* (cells l⁻¹)	0.00E+00	1.60E+05	5.00E-01	8.82E+03	1.82E+03	142	0	0.00E+00	4.25E+05	2.50E+03	1.47E+04	3.24E+03	193	0
Average Temperature (°C)	11.73	28.59	20.58	20.33	0.40	140	2	11.80	28.52	20.96	20.49	0.36	170	23
Average Ammonium Nitrogen (mg l⁻¹)	0.00	0.19	0.02	0.03	0.00	113	29	0.00	0.14	0.01	0.02	0.00	128	65
Average Total Nitrogen (mg l⁻¹)	0.15	1.52	0.38	0.42	0.02	113	29	0.14	1.21	0.37	0.40	0.02	128	65
Average Oxidised Nitrogen (mg l⁻¹)	0.00	0.61	0.06	0.10	0.01	139	3	0.00	0.09	0.03	0.03	0.00	155	38
Average Total Phosphorus (mg l⁻¹)	0.00	0.12	0.02	0.03	0.00	139	3	0.00	0.55	0.03	0.07	0.01	155	38
Redfield Ratio (Total N/Total P)	8.71	153.10	33.95	38.06	1.95	114	28	14.13	138.55	31.74	35.57	1.54	131	62

271   *nbr.val = total number of samples/dates analysed; #nbr.na = number of samples/dates missing for this variable

272

12

273 A.

274

B.

284 C.

D.

Figure 2A-B. Concentrations of *D. acuminata* (solid line) and *D. caudata* (dashed line) of the moving average of cell abundance at sites 60 and 61 across all years 2003 to 2014 in Berowra Creek; C-D. Weekly variation (moving average) in both *D. acuminata* and *D. caudata* cell concentrations at sites 60 and 61 across the entire sampling period. Shaded area in each figure

299   represents the standard error around each of the logged mean abundance values. Note:

300   approx. weeks 36-48 correspond to the austral spring.

301   A.   B.

310
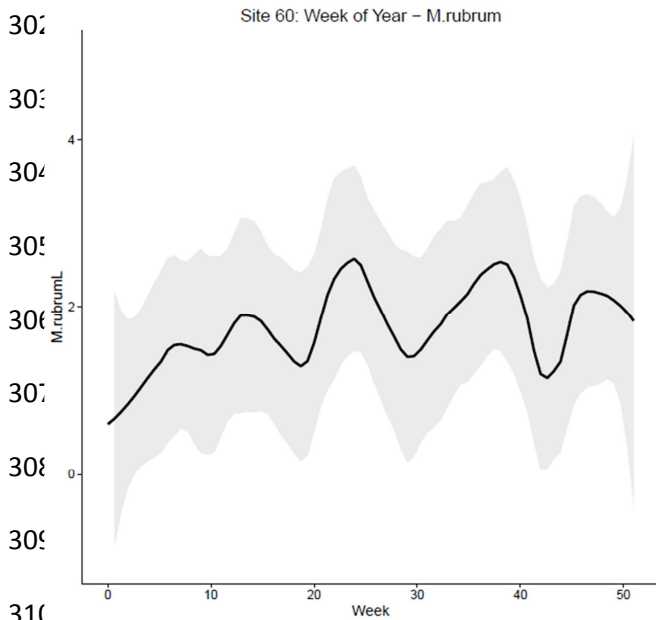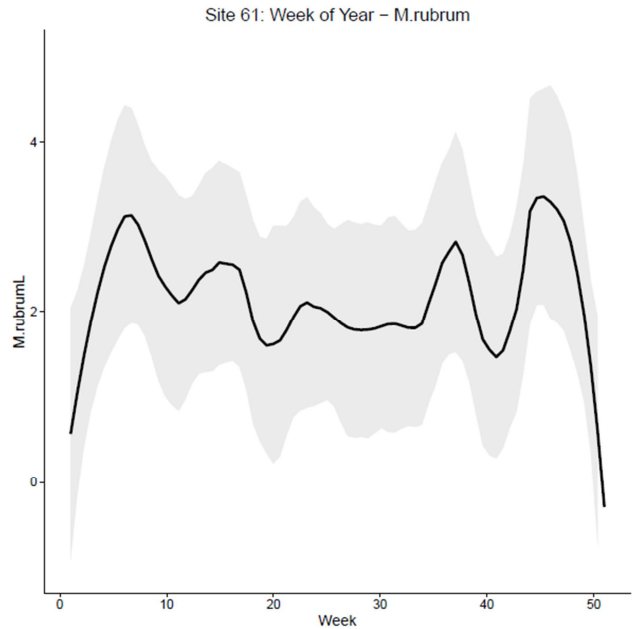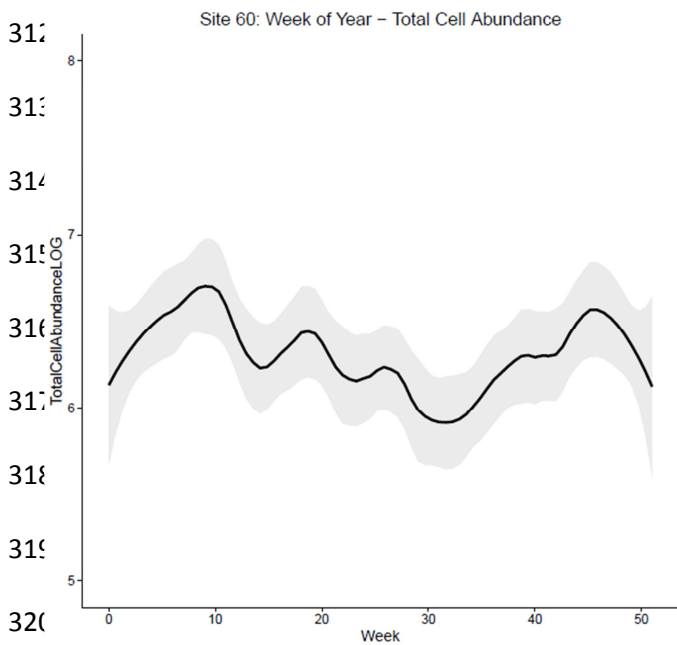
311   C.   D.
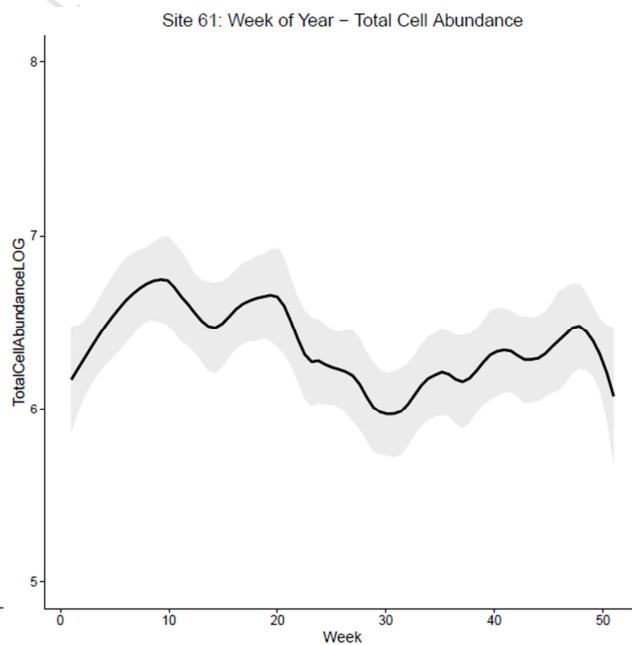
321   Figure 3A-D. Weekly variation in *Mesodinium rubrum* mean abundance at sites 60 (A) and

322   61 (B) and Total phytoplankton abundance at site 60 (C) and 61 (D) across the sampling

14

323 period 2003-2014 at Berowra Creek. Shaded area in each figure represents the standard error

324 around each of the logged mean cell concentrations.

325

326 The ciliate prey *Mesodinium rubrum* was present across all weeks (Figs. 3A, D), with

327 minimum abundance observed in winter/spring (0 cells l^{-1}) and maximum abundance in the

328 late summer and early autumn observed at both sites (800,000 cells l^{-1}, Table 1). Maximum

329 abundance was 4 times greater at site 61 compared to site 60 (800,000 cells l^{-1}, 160,000 cells

330 l^{-1} respectively) (Table 1). The weekly total phytoplankton abundance showed a similar

331 pattern across both sites, with some fluctuations between week 10-30 at site 60 (Figs. 3C, D).

332 The highest concentrations of total phytoplankton cell densities peaked at 1,500,000,000 cells

333 l^{-1} (10 April 2013) at site 61 (Table 1). On this sampling occasion, *D. caudata* reached 12,

334 000 cells l^{-1} and *D. acuminata* 400 cells l^{-1} (Table 1).

335

3.1.2 Environmental Variables

337 Over the twelve year sampling period (2003 - 2014) average temperature, turbidity, pH,

338 salinity, suspended solids, ammonium, total nitrogen and total phosphorus were comparable

339 between sites, dissolved oxygen and chlorophyll-a were notably lower at site 60 compared to

340 site 61 (6.83 and 7.64 mg l^{-1} for dissolved oxygen, and 7.10 and 10.32 µg l^{-1} for chlorophyll-

341 a, respectively), whilst averaged oxidised nitrogen was higher at site 60 compared to site 61

342 (0.10 and 0.03 mg l^{-1}, respectively) (Table 1).

343

344 The inclusion of thermal stratification at site 61 from 2007 to 2014 reduced the number of

345 observations to 123. The highest degree of stratification reported at this site was 5.56 °C with

346 an average of 0.11 °C difference recorded (Table 2). Whilst the summary statistics of this

347 reduced data set is presented in Table 2, it is considered a subset of the total dataset, and is

348 therefore not discussed in any further detail here.

349

350 Table 2. Summary statistics for all cell concentrations and environmental variables used in

351 the generalised linear modelling for site 61, Berowra Creek, over the sampling period 2007 to

352 2014.

	Site 61						
	min	max	median	mean	SE.mean	nbr.val*	nbr.na#
D. acuminata (cells l⁻¹)	0.00E+00	4.50E+03	0.00E+00	1.47E+02	4.08E+01	123	0
D. caudata (cells l⁻¹)	0.00E+00	1.20E+04	0.00E+00	3.53E+02	1.40E+02	123	0
M. rubrum (cells l⁻¹)	0.00E+00	8.00E+05	1.00E+03	2.13E+04	7.20E+03	123	0
Total Phytoplankton (cells l⁻¹)	1.27E+05	1.51E+09	2.37E+06	1.61E+07	1.22E+07	123	0
Log Total Phytoplankton (cells l⁻¹)	5.10	9.18	6.38	6.38	0.05	123	0
Temperature (°C)	11.97	28.22	20.98	20.62	0.43	118	5
Turbidity (ntu)	0.00	24.00	1.40	2.55	0.33	118	5
Dissolved Oxygen (mg l⁻¹)	4.02	13.00	7.44	7.55	0.13	117	6
pH	6.75	8.28	7.63	7.58	0.03	118	5
Salinity (psu)	2.62	29.57	21.58	20.70	0.58	117	6
Suspended Solids (mg l⁻¹)	0.50	27.00	3.50	5.09	0.50	98	25
Chlorophyll-a (μg l⁻¹)	0.50	87.50	8.65	10.96	1.29	98	25
Average Rainfall (mm day⁻¹)	0.00	45.46	1.42	4.93	0.79	110	13
Average *M.rubrum* (cells l⁻¹)	0.00E+00	4.25E+05	3.33E+03	1.88E+04	4.70E+03	123	0
Average Temperature (°C)	11.97	27.54	20.99	20.64	0.43	117	6
Average Ammonium Nitrogen (mg l⁻¹)	0.00	0.14	0.01	0.02	0.00	98	25
Average Total Nitrogen (mg l⁻¹)	0.17	1.21	0.37	0.40	0.02	98	25
Average Oxidised Nitrogen (mg l⁻¹)	0.01	0.09	0.03	0.03	0.00	98	25
Average Total Phosphorus (mg l⁻¹)	0.00	0.55	0.03	0.08	0.01	98	25
Redfield Ratio (Total N/Total P)	14.13	138.55	33.21	36.80	1.88	101	22
Degree of Stratification	-3.29	5.56	-0.03	0.11	0.13	121	2

353 *nbr.val = total number of samples/dates analysed; #nbr.na = number of samples/dates

354 missing for this variable

355

356 Correlation coefficients were computed among every pair of environmental variables and

357 suggested moderate to strong relationships ($r > 0.5$ or $r < -0.5$) between the average oxidised

358 nitrogen and average ammonium nitrogen at both sites 60 and 61 ($p < 0.0001$ in both cases),

359 as well as a significant relationship between rainfall and the average ammonium nitrogen at

360 both site 60 and site 61 ($r = 0.59$ and 0.62, respectively, $p < 0.0001$ in both cases)

361 (Supplementary Table 2). Salinity and the pH level were significantly correlated at site 60 (r

362 = 0.53, p < 0.0001) Inverse relationships (r < -0.6) were observed between salinity and

363 rainfall for both sites (site 60 r = -0.53, 61 r = -0.52, p < 0.0001 in both cases), salinity with

364 average oxidised nitrogen at both sites (site 60 r = -0.65, site 61 r = -0.69, p < 0.0001 in both

365 cases), and pH and average oxidised nitrogen at site 61 (r = -0.54, p < 0.0001).

366

367 Correlations coefficients were recalculated to include thermal stratification (Supplementary

368 Table 3). No significant correlations were observed between degree of stratification and any

369 other variable measured.

370

371 All correlations described were then taken into account when fitting the models. In particular,

372 where both of the correlated variables were included in the model, both variables were

373 removed to see the impact on the overall model.

374

375 Throughout the model selection process, several variables were removed as they were not

376 significant in any of the models. These variables included the average *M. rubrum* abundance,

377 turbidity, suspended solids and average water temperature. After seven iterations of model

378 reduction (determined by the continued lowering of the AIC) the reduced model indicated

379 that total phytoplankton abundance (*D. acuminata* p = 0.004, *D. caudata* p = 0.021), average

380 rainfall (p = 0.012, p = 0.04 respectively) and the Redfield ratio (p = 0.001, p = 0.029) were

381 related to the abundance of both species of *Dinophysis* at Site 61, but neither species at Site

382 60 (Supplementary Table 4). The concentration of chlorophyll-a (Site 60 p < 0.0001, Site 61

383 p = 0.002), level of salinity (p = 0.030, p < 0.001, respectively) and dissolved oxygen (p <

384 0.001, p = 0.000, respectively) were significantly associated with the abundance of *D.*

385 *caudata* at both sites, but dissolved oxygen was only related to *D. acuminata* abundance at

386 site 61 (p = 0.002). The time of year also had a significant effect on the abundance of *D.*

387 *caudata* at Site 61 (p < 0.001). At Site 60, the concentration of oxidised nitrogen was related

388 to *D. caudata* abundance (p = 0.044) (Supplementary Table 3).

389

390 To summarise the modelling results (without thermal stratification):

391   1. An increase in *D. acuminata* abundance at site 60 was marginally linked (p < 0.01) to

392   time of year (highest abundance in spring), high total phytoplankton and a decrease in

393   the Redfield ratio (number of observations = 104) (Supplementary Figure 1A-C).

394   2. An increase in *D. caudata* abundance at site 60 was significantly associated with an

395   increase in oxidised nitrogen, an increase in chlorophyll-a, a reduction in dissolved

396   oxygen (> 7 mg l^{-1}) and an increase in salinity (>18 ppt) (number of observations =

397   104) (Supplementary Figure 2A-D).

398   3. An increase in *D. acuminata* at site 61 was related to a decrease in total phytoplankton

399   abundance, a decrease in Redfield Ratio, an increase in dissolved oxygen (>7-8 mg l^{-}

400   ^{1}), a decrease in rainfall (<10 mm day) (number of observations = 107) (Figure 4A,

401   Supplementary Figure 3A-D).

402   4. An increase in *D. caudata* abundance at site 61 was significantly associated with the

403   time of year (summer to autumn), a decrease in phytoplankton abundance, a lower

404   Redfield Ratio, an increase in chlorophyll-a, a reduction in dissolved oxygen (~6-7

405   mg l^{-1}), a salinity of ~20 ppt and an increase in rainfall (~20 mm day^{-1}) (number of

406   observations = 107) (Figure 4B, Supplementary Figure 4A-G).

407

408 At both sites, the model for *D. caudata* abundance was substantially more predictive than the

409 model for *D. acuminata*. The models for *D. caudata* explained approximately 50% of

410 deviance at both sites, whilst the models for *D. acuminata* explained approximately 15-20%

411 of deviance at both sites.

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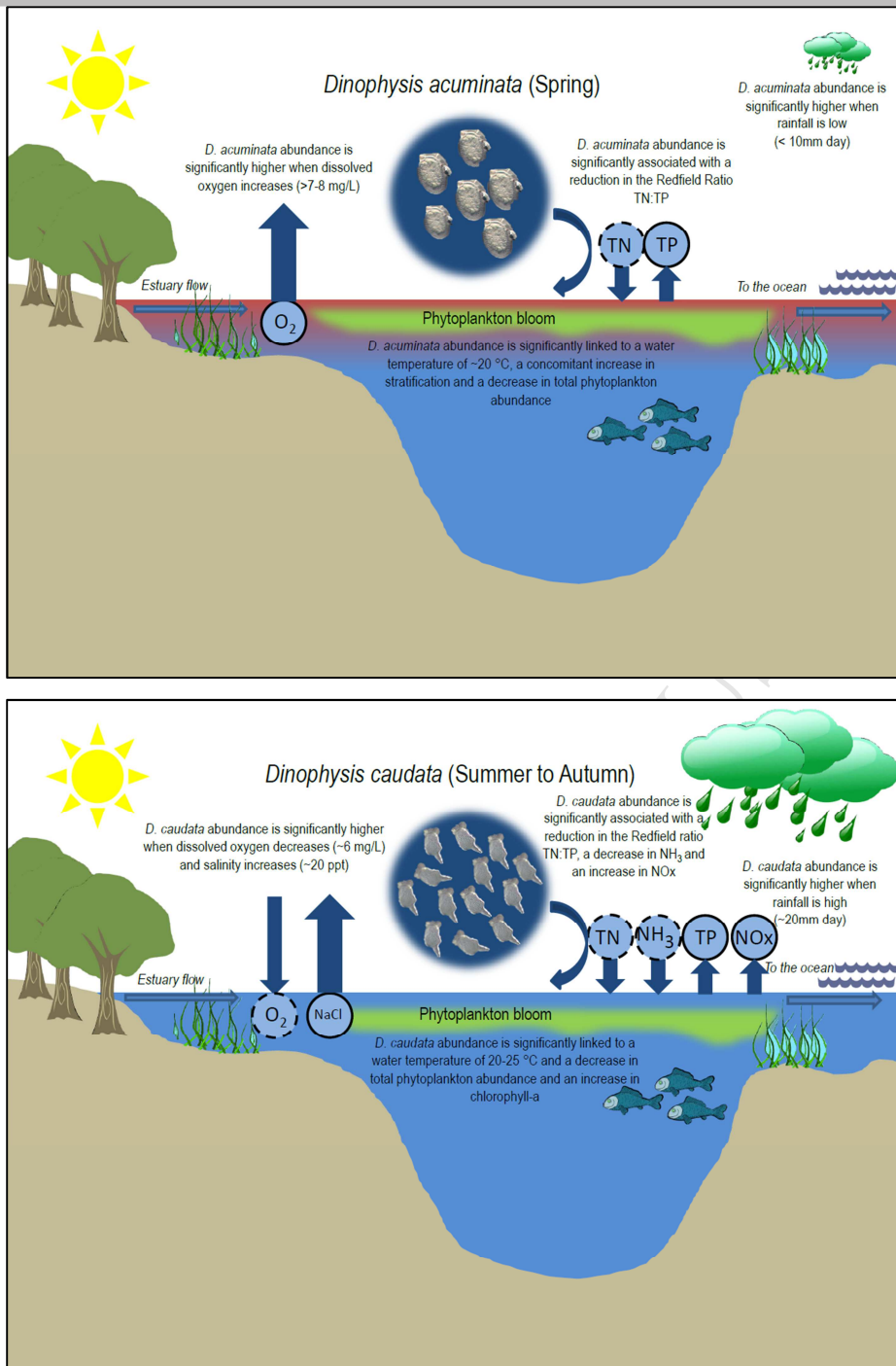418
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Figure 4A-B. Schematic diagram showing the modelled (with, shown in red, and without thermal stratification) mechanisms of *Dinophysis acuminata* (A) and *D. caudata* (B) bloom development at site 61 in Berowra Creek. Dotted lines (solid) around the environmental variables indicate decreasing (increasing) concentrations have a significant effect on *Dinophysis* cell concentrations. Seasons after species names indicate peak bloom periods.

19

3.1.3 Modelling Dinophysis Blooms with Thermal Stratification

After eight iterations of model reduction the reduced model indicated that the time of year (*D. acuminata* p=0.0018, *D. caudata* p = 0.0003), temperature (p = 0.000, p = 0.0002 respectively) and the Redfield ratio (p = 0.001, p = 0.0079) were related to the abundance of both species of *Dinophysis* at Site 61 (Supplementary Table 5). Additionally, the concentration of ammonium nitrogen and oxidised nitrogen were related to *D. caudata* abundance (p = 0.0.0193 and 0.0014) as well as salinity (p = 0.0001). Finally, *Dinophysis acuminata* abundance was significantly associated with degree of stratification at site 61 (p = 0.0009).

To summarise the modelling results (with thermal stratification):

5. An increase in *D. acuminata* at site 61 was significantly related to the time of year (highest abundance in spring), a temperature of ~20 °C, a decrease in the Redfield ratio, and an increase in degree of stratification (number of observations = 82) (Figure 4A, Supplementary Figure 5A-D).

6. An increase in *D. caudata* abundance at site 61 was significantly associated with the time of year (summer to autumn), a temperature of between 20 to 25 °C, a salinity of ~20 ppt and a lowering of the Redfield ratio. It was also associated with a decrease in ammonium nitrogen and an increase in oxidised nitrogen (number of observations = 82) (Figure 4B, Supplementary Figure 6A-F).

When thermal stratification was included in the analyses at site 61, the model substantially increased in its predictive capability for *D. acuminata* (19.4 % to 59.7%), whilst for *D. caudata* only a slight increase in model fit was observed (49.4% to 53.5%) (Supplementary Table 5).

4.1 Discussion

4.1.1 Temporal Variation in Dinophysis

Using a twelve-year time-series this study found that up to sixty per cent of the variability in *Dinophysis* cell concentrations in Berowra Creek could be predicted using the physico-chemical parameters measured. From a seasonal perspective, *Dinophysis caudata* and *D. acuminata* in Berowra Creek were intermittently high during the warmer austral spring to autumn periods. In a meta-analysis to assess the risk of harmful algal blooms in the oyster-growing estuaries of New South Wales, Ajani et al. (2013) identified a similar temporal pattern in the mean abundance of total *Dinophysis* spp., with a winter minimum observed across six other important oyster producing estuaries in NSW from 2005 to 2009. The data in the current study, however, also suggests distinct seasonal niches for *D. acuminata* and *D. caudata*, with mean abundance of *D. acuminata* highest in the austral spring and *D. caudata* highest in summer to autumn. Other studies have similarly reported disparate temporal windows for different *Dinophysis* species. Hallfors et al. (2011) observed *D. acuminata* to peak in the Baltic Sea during or after periods of high phytoplankton biomass in early and late summer (August), while *D. norvegica* was observed to be abundant during a shorter period, peaking one month after the first *D. acuminata* maximum. Additional studies around the globe revealed vastly different seasonal periods for *Dinophysis* bloom development, suggesting that temperature itself is not the most important factor driving bloom initiation and formation. In Greek coastal waters peak densities of *D. acuminata* have been shown to occur at low water temperatures, 10.5 to 14.8 °C (Koukaras & Nikolaidis 2004) whilst in the north western coast of the Netherlands, blooms of *D. acuminata* occurred in water temperatures in excess of 19°C (Peperzak et al. 1996).

487 In addition to temperature, hydrological forcing can induce the growth of a population and/or

488 the transport the bloom into a particular area. For example, wind-induced advection (Raine et

489 al. 2010, Batifoulier et al. 2013, Whyte et al. 2014), eddies and other retentive oceanic

490 structures (Xie et al. 2007, Farrell et al. 2014), current/wind-induced upwelling (Diaz et al.,

491 2013) or upwelling relaxation (Reguera et al. 1995, Velo-Suárez et al. 2008 & 2014), or a

492 combination of these features, can drive *Dinophysis* events. While the majority of these

493 features have been investigated in coastal oceans, Berowra Creek is located 24 km from the

494 ocean and unlikely to be influenced by coastal transport processes. Our data supports that

495 blooms of *Dinophysis* within Berowra are derived from an indigenous population which

496 responds to localised changes.

497

498 *4.1.2 Thermal Stratification*

499 Thermal stratification, however, was revealed as a highly significant hydrological forcing

500 variable in *Dinophysis* blooms at site 61 (deeper site) in this study, an association that has

501 been reported in many other studies. Koukaras & Nikolaidis (2004) observed that *D.* cf.

502 *acuminata* blooms occurred at the same time as water column stratification, with population

503 maxima in or just above the pycnocline. Likewise, highest abundances of *D. acuminata*, *D.*

504 *caudata* and *D. tripos* in Guaratuba Bay, Brazil, were associated with the upper halocline

505 layer in areas of the bay where water column stratification was more frequent (Junqueira de

506 Azevedo Tibirica et al. 2015). In the same way, Velo-Suárez and Gutiérrez-Estrada (2007)

507 reported the combined effects of wind relaxation, water column stratification and high

508 densities of the ciliate prey *M. rubrum* (see below) on *D. acuminata* cell densities in an

509 upwelling system embayment in north western Spain.

510

22

511 Stratification can occur in Berowra Creek in the warmer austral months when the temperature

512 difference (and density difference) between the upper water column and bottom waters is

513 greatest. This can be seen most noticeably at the deeper of the two sites (site 61) which also

514 has a greater water residence time (MHL 1998). The temperature and density gradient

515 interface set up during these times may allow for the accumulation of detrital material which

516 may provide nutrition for *Dinophysis* spp. Although our study did not characterise the

517 distribution of *Dinophysis* spp. throughout the water column at the time of this

518 stratification/interphase set up, it is hypothesised that incorporating sampling at depth

519 accompanied with *in situ* high resolution monitoring more recently employed in this system,

520 would assist to support the hypothesis of stratification as the predominant precursor to

521 blooms at this site.

522

523 *4.1.3 Nutrients*

524 Based on the model outputs at both sampling sites we also observed a significant correlation

525 between N:P ratios (decreasing) and greater *Dinophysis* spp. abundance. It is widely accepted

526 that the Redfield stoichiometry (which is based on the elemental composition of marine

527 organic matter across a range of oceans) can provide a crude value as to which nutrients are

528 limiting in a localized system (Redfield 1958). Although applicable in a broad sense to

529 Berowra Creek, there is great plasticity in biogeochemical dynamics and species and/or site

530 specific abilities to sequester and utilise nutrients, therefore allowing the proliferation of one

531 species over others under different nutrient regimes (Glibert et al. 2011, Davidson et al.

532 2014). Although there was no clear pattern as to whether a decreasing N or increasing P was

533 driving the nutrient shift in Berowra Creek (N and P were highly correlated and therefore

534 difficult to partition, Supplementary Figures 1-4), elevated concentrations of *D. caudata* were

535 significantly linked to elevated oxidised nitrogen (nitrite NO_2- and nitrate NO_3-, both which

536 are biologically available) at site 60, and elevated P was observed at times of very high *D.*

537 *caudata* abundance at site 61 suggesting that this species has both N and P requirements.

538

539 Dissolved inorganic nitrogen and phosphorus are the preferred (and most bioavailable) forms

540 of these nutrients for phytoplankton assimilation (Gabric and Bell 1993). A measure,

541 therefore, of ammonia, organic nitrogenous substances (e.g. urea) and dissolved inorganic

542 phosphorus would have allowed a more robust examination of nutrient availability by

543 *Dinophysis* spp. in Berowra Creek. Whilst other studies suggest *Dinophysis* growth can be

544 promoted by excessive nutrient loading, either directly (stimulating *Dinophysis* growth) or

545 indirectly (stimulating the growth of their prey), both field and laboratory studies have shown

546 that *Dinophysis* growth can be enhanced by both inorganic and organic nitrogen and

547 phosphorus (Singh et al. 2014, Hattenrath-Lehmann et al. 2015) and that nutrient loading

548 promotes the toxicity of *D. acuminata* populations (Hattenrath-Lehmann and Gobler 2015).

549 On the other hand, when *D. acuminata* was provided with dissolved nitrate and phosphate in

550 the presence of *M. rubrum*, these nutrient pools only contributed to the growth and biomass

551 of *M. rubrum*, and therefore only indirectly influenced *D. acuminata* growth (Tong et al.

552 2015). As mentioned above, the importance of nutrient stoichiometry i.e. varying nutrient

553 ratios on ecosystem productivity have been revealed as significant drivers of biogeochemical

554 dynamics and changing food webs in the San Francisco estuary now favouring the growth of

555 harmful algal species such as the dinoflagellate *Prorocentrum minimum* (Glibert et al. 2011).

556 Laboratory culture studies also reveal that *P. minimum* reaches maximal growth in inorganic

557 nutrient ratios just below Redfield proportions, yet in the field they bloom when nutrients are

558 at high N:P ratios. These results suggested that while a high growth rate allows these

559 microalgae to initiate blooms, they are maintained at less than maximal growth rates and at

560 non-optimal N:P ratios (Glibert et al. 2012).

561

4.1.4 Other environmental variables

563 Another environmental parameter that was significantly associated with high *Dinophysis* spp.

564 abundance in Berowra Creek in this study was dissolved oxygen (DO). Although the

565 association of *Dinophysis* with low oxygen may not be a direct causal relationship, low DO

566 can indicate excessive respiration in both the water column and benthos which is a symptom

567 of eutrophication, where DO is being consumed by chemical and biological reactions e.g. an

568 algal bloom, and/or stratification of the water body. At site 60, low DO and correspondingly

569 elevated *D. caudata* most likely relates to the shallow nature of this site (6 metres), where

570 solar penetration and chlorophyll-a may combine to reduce dissolved oxygen levels.

571 Similarly at site 61, an increase in *D. caudata* abundance was coupled with an increase in

572 chlorophyll-a and a decrease in DO, although this site is deeper. On the other hand, *D.*

573 *acuminata* at site 61 was observed to be associated with an increase in dissolved oxygen.

574 Being mixotrophic, *Dinophysis* may be in a heterotrophic mode at this time, feeding on other

575 phytoplankton and thus consuming more oxygen than they produce, whilst at the same time

576 reducing the oxygen produced from the autotrophic phytoplankters themselves.

577

578 At site 61, both species were also significantly linked to a decrease in total phytoplankton

579 abundance (when thermal stratification was removed from the analyses). A decrease in total

580 phytoplankton abundance, and a concomitant increase in chlorophyll-a, combined with

581 varying effects from rainfall such as the addition of essential micronutrients, may indicate

582 species-specific growth requirements in conjunction with a shift in species composition, or

583 succession, to larger species. It is also possible that *Dinophysis* spp. abundance may also be

584 indirectly affected by biological factors such as feeding and/or grazing. Although we found

585 no significant link between *M. rubrum* and *D. acuminata* or *D. caudata* abundance, our

586   sampling frequency (approximately three to four weeks) and lack of depth profile

587   measurements (current study only surface sampling) may have biased these results. In fact

588   Velo-Suárez et al. (2014) report an increase in *D. acuminata* in NW Spain coincided with an

589   upwelling-relaxation event and a short lived maximum of *M. rubrum*. A large percentage

590   (72%) of vacuolated *Dinophysis* one week later suggested that the bloom was triggered by the

591   heterotrophic feeding on co-occurring *Mesodinium*, but the low frequency with which

592   vacuolated cells occurred suggested that *D. acuminata* is prey limited most of the time and

593   does not require a constant supply of prey for long term survival. Another study in the Gulf of

594   Mexico found a direct, albeit positive time-lagged correlation between *Dinophysis ovum* and

595   *Mesodinium* spp., suggesting that the presence of *Mesodinium* may be a good indicator of

596   upcoming *D. ovum* blooms (Harred & Campbell 2014).

597

598   ***4.1.5 Future Work***

599   Whilst the relationship between *Dinophysis* cell numbers and DSTs is not a simple one, and

600   can vary considerably between geographic populations, *Dinophysis* spp. has shown high

601   toxicity even at low cell concentrations and a positive correlation between toxin production

602   and growth has been observed (*D. caudata*, Basti et al. 2015). Furthermore, diarrhetic

603   shellfish toxicity episodes throughout the world have identified bivalve molluscs (Suzuki et

604   al. 2001, Mafra et al. 2015a), crabs (Vale et al. 2002), octopus (Mafra et al 2015b) and fishes

605   (Mafra et al 2014) as potential vectors. With these factors in mind, and the likelihood of

606   aquaculture diversification in these waters, *Dinophysis* blooms may become an increasingly

607   important issue for Australia, and understanding their bloom dynamics and prediction may

608   become an imperative. Our models identified significant explanatory variables that explained

609   up to 60% of the observed variability in *Dinophysis* abundance in Berowra Creek, but other

610   sources of variability also need to be considered. Firstly, biological systems are intrinsically

26

611 variable; secondly, the model itself has only limited capability; and finally, the variables

612 measured may not be the most appropriate. More specifically, it may be the variables *not*

613 measured that may account for a significant portion of variability within the data (Zuur et. al.

614 2009). To refine these models, a more intimate knowledge of the seasonal and interannual

615 variability of *Dinophysis* spp., their lifecycle, including seeding mechanisms and vertical

616 migration, and their relationship to predators/prey is required. Furthermore the patterns we

617 observed relate to the surface layer of the water only (and not necessarily the whole water

618 column), and this may be important for any future shellfish grown on long-line systems.

619 Whilst general water quality monitoring programs can generate invaluable environmental

620 data, more focused, higher frequency (eg. biweekly during bloom events and/or at multiple

621 depths) sampling, coupled with *in situ* water quality monitoring as well as simple, short range

622 models will further increase the prediction of these harmful species. These, together with a

623 focus on key bloom triggers (nutrient ratios and physical processes) as we have identified in

624 this study, will assist in a more robust understanding of *Dinophysis* blooms in eastern

625 Australian.

626

633

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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}
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. Correlation matrix showing correlation coefficients for every pair of predictor variables employed in the generalised additive models employed over the sampling period 2003-2014 (thermal stratification not included). Values in yellow boxes are those that are significant at the 0.0001 level; values in orange highlighted by red text are those that suggested moderate to strong relationships (r > 0.5 or r< -0.5) between variables.

Site 60														
	Month	AvM.rubrum	TotalCellAbu	AvTemp	TURBIDITY	DO	pH	Salinity	SuspendedSo	AvAmmoniun	AvOxidisedN	Redfield	ChlorophyllA	Av Rainfall
Month	1.000													
AvM.rubrum	-0.107	1.000												
TotalCellAbundanceLOG	-0.106	0.187	1.000											
AvTemp	-0.327	0.133	0.319	1.000										
TURBIDITY	-0.103	0.050	0.209	0.228	1.000									
DO	0.435	-0.083	0.058	-0.496	0.066	1.000								
pH	0.256	-0.303	-0.083	-0.239	-0.374	0.495	1.000							
Salinity	0.162	-0.353	-0.315	-0.031	-0.485	0.007	0.580	1.000						
SuspendedSolids	0.100	0.081	-0.098	0.018	0.023	0.020	0.105	0.073	1.000					
AvAmmoniumNitrogen	-0.041	0.049	-0.054	-0.066	0.421	-0.148	-0.387	-0.454	0.015	1.000				
AvOxidisedNitrogen	-0.117	0.224	0.090	-0.242	0.366	-0.063	-0.411	-0.656	-0.064	0.717	1.000			
Redfield	0.094	-0.116	0.136	-0.392	-0.167	0.182	0.053	-0.158	-0.050	-0.058	0.171	1.000		
ChlorophyllA	-0.207	0.153	0.347	0.345	0.273	0.202	-0.009	-0.246	-0.002	-0.052	-0.020	-0.251	1.000	
Av Rainfall	-0.072	0.196	0.229	0.187	0.448	-0.001	-0.393	-0.606	-0.031	0.581	0.506	0.026	0.288	1.000
Site 61														
	Month	AvM.rubrum	TotalCellAbu	AvTemp	TURBIDITY	DO	pH	Salinity	SuspendedSo	AvAmmoniun	AvOxidisedN	Redfield	ChlorophyllA	Av Rainfall
Month	1.000													
AvM.rubrum	-0.109	1.000												
TotalCellAbundanceLOG	-0.187	0.206	1.000											
AvTemp	-0.342	0.051	0.361	1.000										
TURBIDITY	-0.086	0.051	0.143	0.309	1.000									
DO	0.270	-0.068	0.086	-0.352	0.049	1.000								
pH	0.172	-0.250	-0.039	-0.175	-0.401	0.598	1.000							
Salinity	0.107	-0.262	-0.240	0.010	-0.536	0.023	0.581	1.000						
SuspendedSolids	0.102	0.062	-0.011	-0.006	0.068	0.084	0.112	0.071	1.000					
AvAmmoniumNitrogen	0.073	0.117	-0.071	-0.065	0.442	-0.084	-0.365	-0.442	-0.031	1.000				
AvOxidisedNitrogen	-0.064	0.234	-0.032	-0.229	0.382	-0.106	-0.530	-0.703	-0.074	0.721	1.000			
Redfield	0.073	-0.003	0.057	-0.356	-0.111	0.115	-0.016	-0.283	-0.043	-0.015	0.213	1.000		
ChlorophyllA	-0.312	0.281	0.449	0.335	0.313	0.267	0.041	-0.162	0.076	-0.019	-0.080	-0.315	1.000	
Av Rainfall	-0.107	0.184	0.147	0.227	0.544	-0.074	-0.464	-0.653	-0.035	0.617	0.593	0.063	0.161	1.000

Supplementary Table 3. Correlation matrix showing correlation coefficients for every pair of predictor variables employed in the generalised additive models employed over the sampling period 2007-2014 (including thermal stratification). Values in yellow boxes are those that are significant at the 0.0001 level; values in orange highlighted by red text are those that suggested moderate to strong relationships ($r > 0.5$ or $r < -0.5$) between variables.

	Month	AvM.rubrum	TotalCellAbu	AvTemp	TURBIDITY	DO	pH	Salinity	SuspendedSo	AvAmmoniun	AvOxidisedN	Redfield	ChlorophyllA	Rainfall	Stratification
Month	1.000														
AvM.rubrum	-0.131	1.000													
TotalCellAbundanceLOG	-0.191	0.148	1.000												
AvTemp	-0.319	0.058	0.168	1.000											
TURBIDITY	0.005	0.093	0.161	0.275	1.000										
DO	0.163	-0.119	0.098	-0.385	0.092	1.000									
pH	0.106	-0.362	-0.134	-0.159	-0.336	0.606	1.000								
Salinity	0.119	-0.377	-0.320	0.096	-0.593	-0.105	0.522	1.000							
SuspendedSolids	-0.003	-0.037	0.026	0.248	0.366	0.068	-0.015	-0.188	1.000						
AvAmmoniumNitrogen	0.106	0.129	0.041	-0.027	0.529	-0.074	-0.438	-0.530	0.085	1.000					
AvOxidisedNitrogen	-0.072	0.373	0.122	-0.239	0.439	-0.042	-0.553	-0.753	0.031	0.693	1.000				
Redfield	-0.029	-0.009	0.175	-0.359	-0.118	0.086	0.033	-0.253	-0.105	-0.042	0.201	1.000			
ChlorophyllA	-0.331	0.112	0.461	0.347	0.343	0.336	0.065	-0.209	0.196	-0.036	-0.058	-0.312	1.000		
Rainfall	-0.108	0.232	0.204	0.253	0.579	-0.076	-0.490	-0.683	0.116	0.620	0.609	0.031	0.194	1.000	
Stratification	0.105	-0.113	-0.029	0.359	-0.031	0.114	0.280	0.378	0.016	-0.272	-0.371	-0.023	0.021	-0.136	1.000

Supplementary Table 4. Model results after seven iterations for *D. acuminata* and *D. caudata* at both sites 60 and 61, Berowra Creek from the sampling period 2003 to 2014 (not including thermal stratification). Highlighted p values indicate significance at 0.05 level.

Site 60: *D. acuminata*

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	Contribution
(Intercept)	-10.35	8.42	-1.23	0.219	
TotalCellAbundanceLOG	2.34	1.33	1.77	0.077	1.49%
AvOxidisedNitrogen	7.41	7.00	1.06	0.290	0.97%
Redfield	-0.06	0.04	-1.81	0.070	5.59%
ChlorophyllA	-0.01	0.07	-0.17	0.864	-0.01%

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(Week)	1.00	1.00	3.47	0.063	4.26%
s(DO)	1.00	1.00	1.88	0.170	1.41%
s(Salinity)	1.00	1.00	2.26	0.133	3.25%
s(Av Rainfall)	1.00	1.00	1.92	0.166	1.31%

R-sq.(adj) = -176 Deviance explained = 14.8%
-REML = 205.92 Scale est. = 1   n = 104

Site 60: *D. caudata*

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	Contribution
(Intercept)	-2.62	8.18	-0.32	0.749	
TotalCellAbundanceLOG	-0.12	1.24	-0.10	0.924	-0.05%
AvOxidisedNitrogen	12.80	6.37	2.01	0.044	5.28%
Redfield	-0.02	0.03	-0.62	0.536	7.29%
ChlorophyllA	0.27	0.08	3.51	0.000	7.04%

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(Week)	1.00	1.00	0.99	0.320	1.25%
s(DO)	1.88	1.98	17.80	0.000	-6.49%
s(Salinity)	1.81	1.96	7.35	0.030	15.95%
s(Av Rainfall)	1.84	1.97	1.27	0.483	-1.21%

R-sq.(adj) = -9.37 Deviance explained = 51.8%
-REML = 238.77 Scale est. = 1   n = 104

Site 61: *D. acuminata*

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	Contribution
(Intercept)	26.59	7.02	3.79	0.000	
TotalCellAbundanceLOG	-3.08	1.08	-2.85	0.004	1.25%
AvOxidisedNitrogen	12.79	6.74	1.90	0.058	2.43%
Redfield	-0.12	0.04	-3.29	0.001	13.10%
ChlorophyllA	-0.03	0.06	-0.42	0.673	-0.49%

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(Week)	1.00	1.00	1.62	0.203	-1.94%
s(DO)	1.00	1.00	9.51	0.002	-0.73%
s(Salinity)	1.00	1.00	0.29	0.594	0.15%
s(Av Rainfall)	1.00	1.00	6.32	0.012	5.79%

R-sq.(adj) = -25.6 Deviance explained = 19.4%
-REML = 292.39 Scale est. = 1   n = 107

Site 61: *D. caudata*

Parametric coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	Contribution
(Intercept)	16.11	5.36	3.01	0.003	
TotalCellAbundanceLOG	-1.95	0.85	-2.31	0.021	6.10%
AvOxidisedNitrogen	-3.42	5.82	-0.59	0.557	1.38%
Redfield	-0.05	0.02	-2.18	0.029	3.21%
ChlorophyllA	0.15	0.05	3.16	0.002	6.12%

Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(Week)	3.55	4.23	21.60	0.000	17.34%
s(DO)	1.31	1.52	16.24	0.000	5.79%
s(Salinity)	1.96	2.00	19.16	0.000	11.14%
s(Av Rainfall)	1.71	1.91	7.53	0.040	4.88%

R-sq.(adj) = -198 Deviance explained = 49.4%
-REML = 393.67 Scale est. = 1   n = 107

Supplementary Table 5. Model results, including thermal stratification, after eight iterations for *D. acuminata* and *D. caudata* at sites 61, Berowra Creek for the sampling period 2007 to 2014. Highlighted p values indicate significance at 0.05 level.

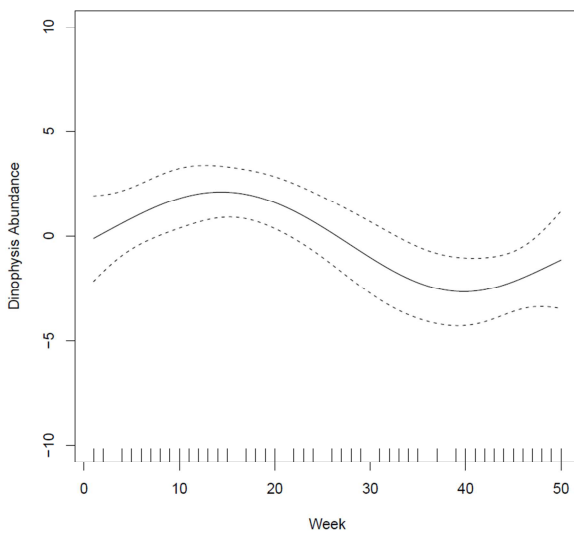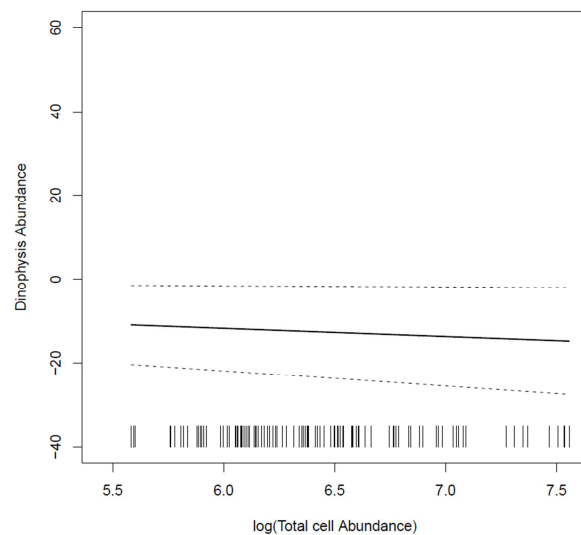| Site 61: *D. acuminata*							Site 61: *D. caudata*					
Parametric coefficients:							Parametric coefficients:					
	Estimate	Std. Error	z value	Pr(>|z|)	Contribution			Estimate	Std. Error	z value	Pr(>|z|	Contributio
(Intercept)	1.705	2.815	0.606	0.5448			(Intercept)	4.329	1.192	3.632	0.0003	
AvAmmoniumNitrogen	74.615	43.495	1.715	0.0863	-3.54%		AvAmmoniumNitrogen	-76.123	32.523	-2.341	0.0193	3.11%
AvOxidisedNitrogen	21.049	11.463	1.836	0.0663	9.44%		AvOxidisedNitrogen	29.328	9.193	3.190	0.0014	6.04%
Redfield	-0.136	0.041	-3.299	0.0010	9.32%		Redfield	-0.058	0.022	-2.656	0.0079	6.37%
Stratification	1.684	0.505	3.335	0.0009	13.27%		Stratification	-0.178	0.404	-0.439	0.6605	-0.57%
Approximate significance of smooth terms:							Approximate significance of smooth terms:					
	edf	Ref.df	Chi.sq	p-value				edf	Ref.df	Chi.sq	p-value	
s(Week)	1.000	1.000	9.749	0.0018	14.18%		s(Week)	1.001	1.002	13.204	0.0003	11.98%
s(AvTemp)	1.976	1.999	29.465	0.0000	25.30%		s(AvTemp)	1.900	1.990	16.646	0.0002	11.09%
s(Salinity)	1.000	1.000	3.779	0.0519	2.22%		s(Salinity)	1.925	1.994	18.210	0.0001	7.53%
s(Rainfall)	1.842	1.975	3.122	0.2423	2.55%		s(Rainfall)	1.774	1.949	5.653	0.0811	6.03%
R-sq.(adj) = -382 Deviance explained = 59.7%							R-sq.(adj) = -9.97 Deviance explained = 53.5%					
-REML = 214.23 Scale est. = 1 n = 82							-REML = 291.93 Scale est. = 1 n = 82					

43

Supplementary Figure 1A-C. Plots of the effect of each (marginally) significant environmental variable A. week; B. total phytoplankton abundance (log); C. Redfield ratio on the log *D. acuminata* counts at site 60. The expected *D. acuminata* count at each value of the variable is described by the solid line, with the dashed lines representing 95% confidence intervals of these expected counts. The vertical lines at the bottom of the chart indicate the distribution of the variable values amongst the site 60 observations.

A.
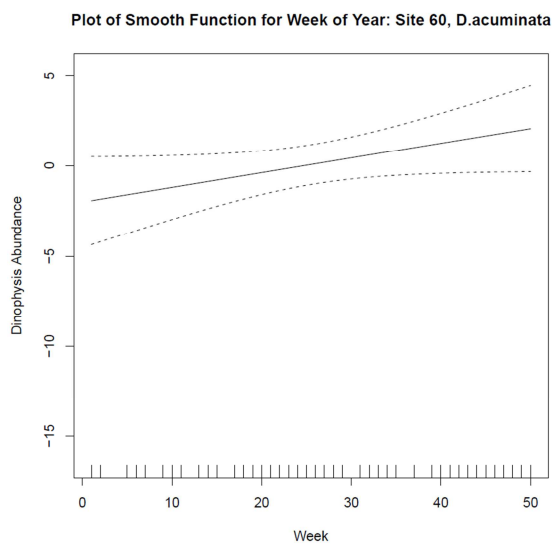
B.
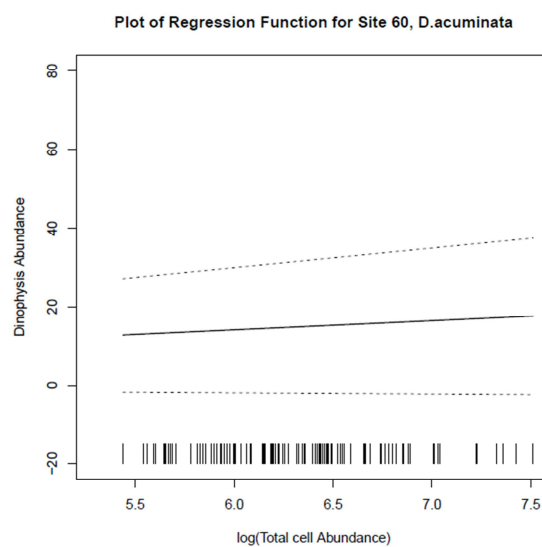
C.
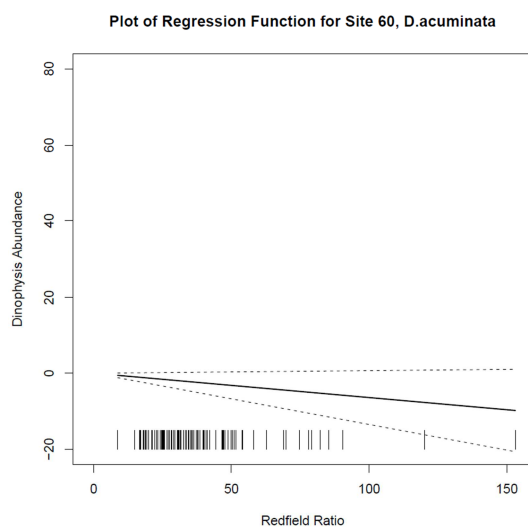
Supplementary Figure 2A-D. Plots of the effect of each significant environmental variable A. oxidised nitrogen; B. chlorophyll-a; C. dissolved oxygen; D. salinity on the log *D. caudata* counts at site 60. The expected *D. caudata* count at each value of the variable is described by the solid line, with the dashed lines representing 95% confidence intervals of these expected counts. The vertical lines at the bottom of the chart indicate the distribution of the variable values amongst the site 60 observations.

A.

B.

C.

D.
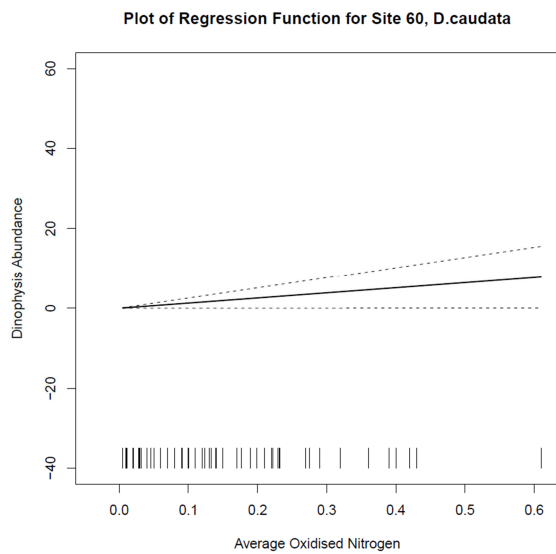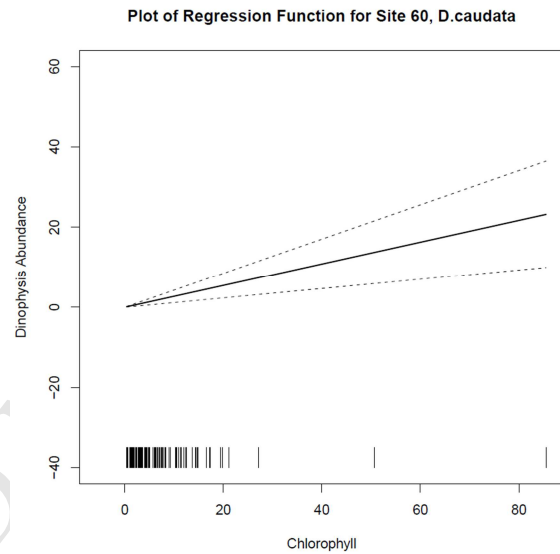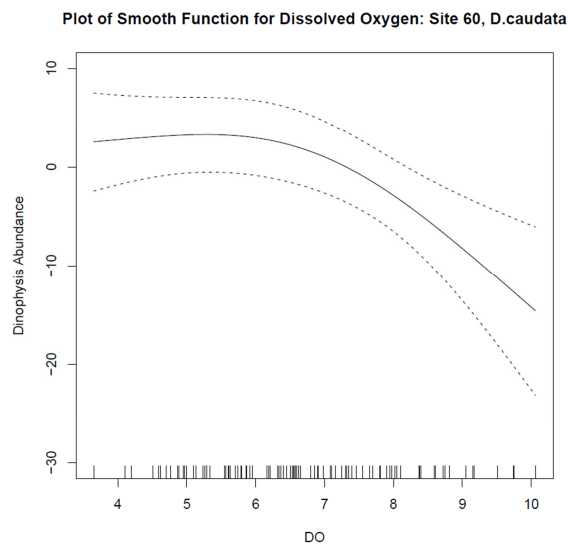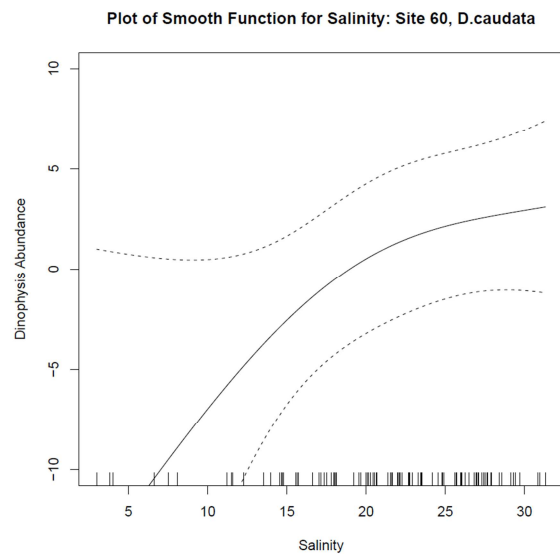
Supplementary Figure 3A-D. Plots of the effect of each significant environmental variable A. total phytoplankton abundance (log); B. Redfield Ratio; C. dissolved oxygen; D. rainfall on the log *D. acuminata* counts at site 61. The expected *D. acuminata* count at each value of the variable is described by the solid line, with the dashed lines representing 95% confidence intervals of these expected counts. The vertical lines at the bottom of the chart indicate the distribution of the variable values amongst the site 61 observations.
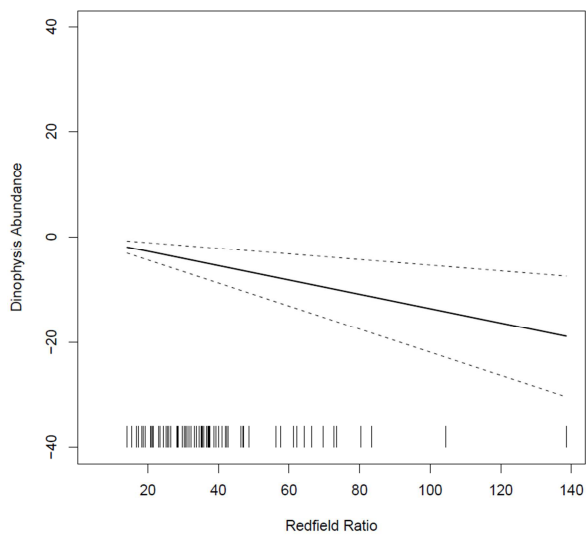
A.

B.

C.

D.
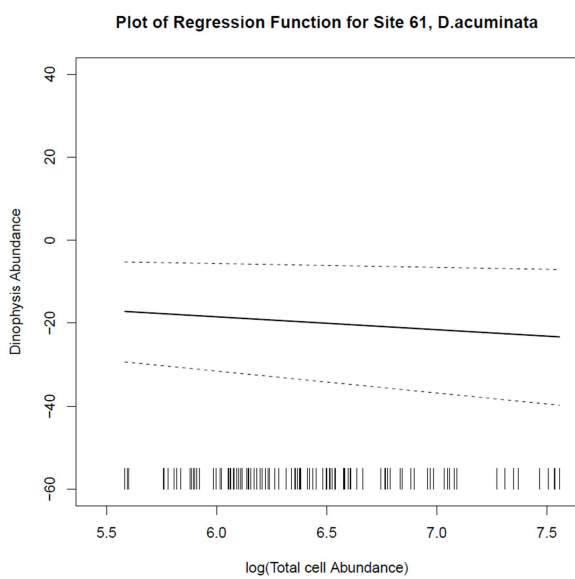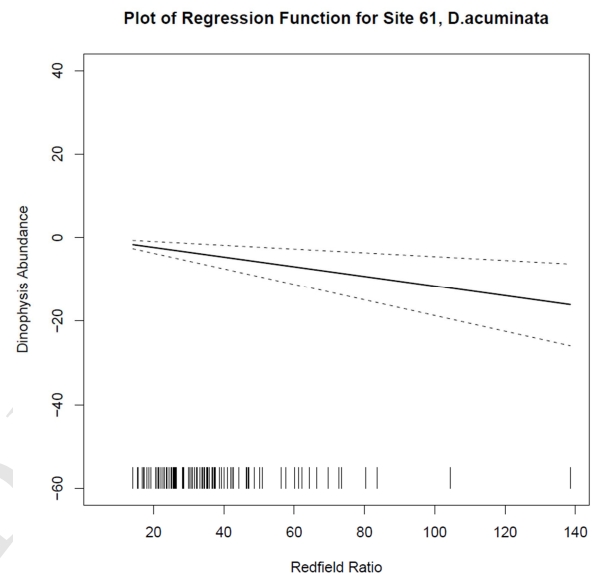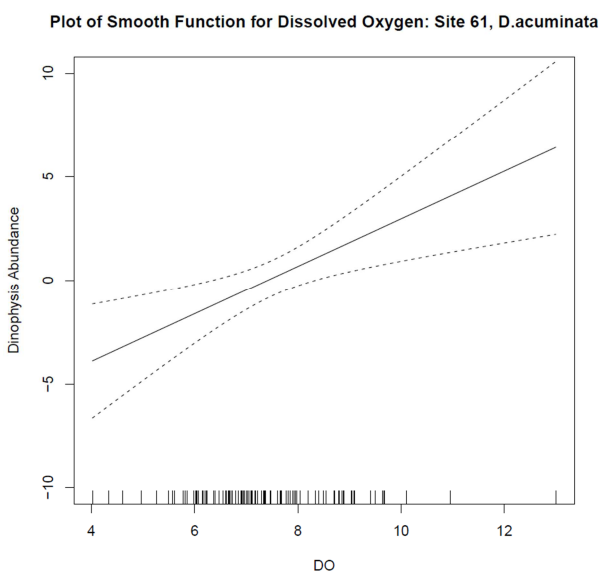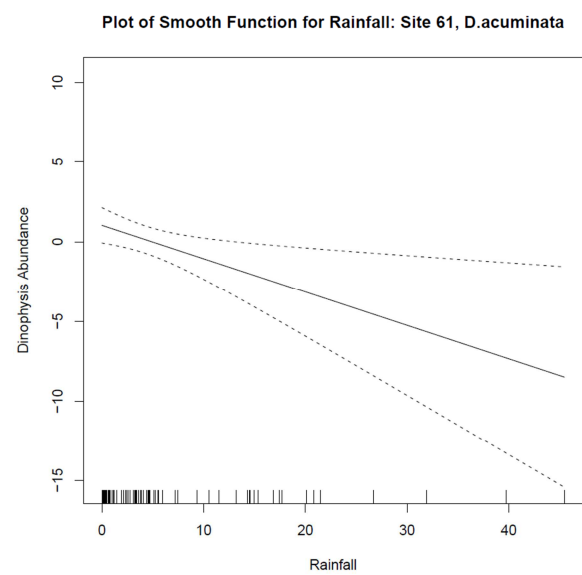
Supplementary Figure 4A-G. Plots of the effect of each significant environmental variable A. week; B. total phytoplankton abundance (log); C. Redfield Ratio; D. chlorophyll-a; E. dissolved oxygen; F. salinity; G. rainfall on the log *D. caudata* counts at site 61. The expected *D. caudata* count at each value of the variable is described by the solid line, with the dashed lines representing 95% confidence intervals of these expected counts. The vertical lines at the bottom of the chart indicate the distribution of the variable values amongst the site 61 observations.

A.

B.

C.

D.

E.

Plot of Smooth Function for Dissolved Oxygen: Site 61, D.caudata
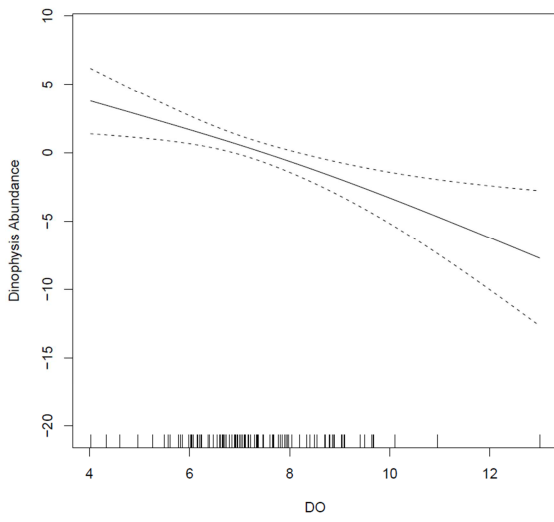
F.

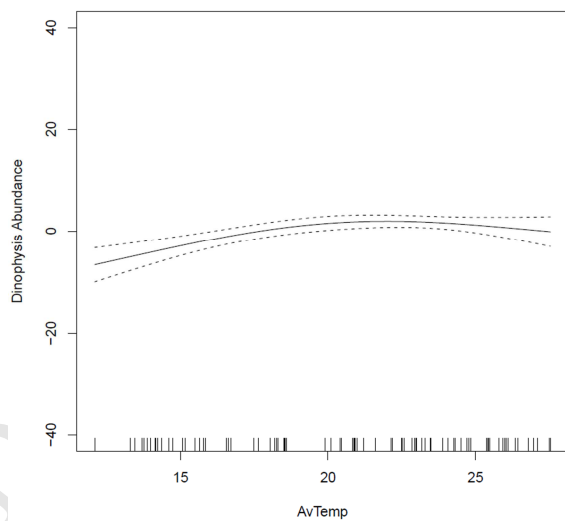Plot of Smooth Function for Salinity: Site 61, D.caudata
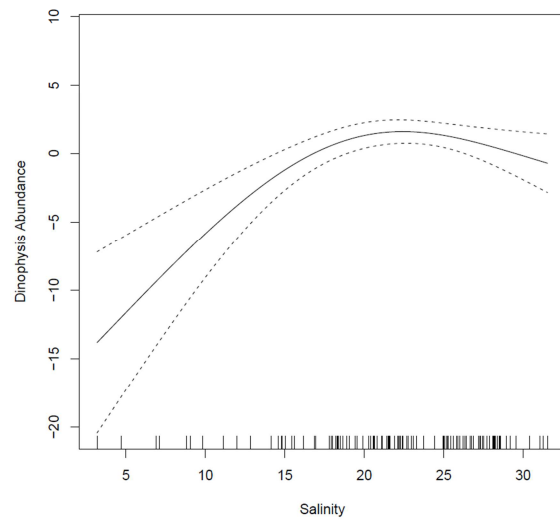
G.

Plot of Smooth Function for Rainfall: Site 61, D.caudata
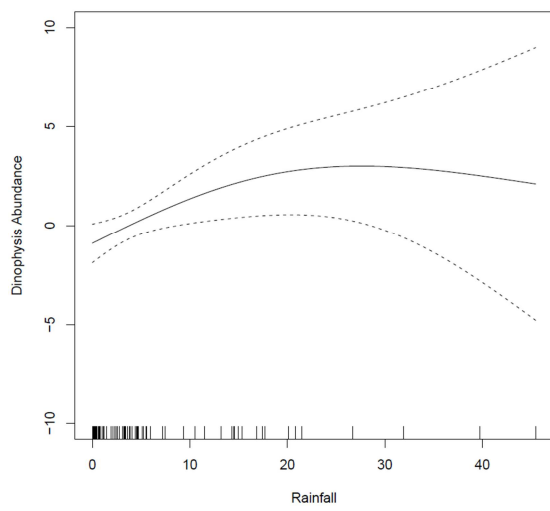
Supplementary Figure 5A-D. Plots of the effect of each significant environmental variable A. week; B. temperature; C. Redfield ratio; D. stratification. The expected *D. acuminata* count at each value of the variable is described by the solid line, with the dashed lines representing 95% confidence intervals of these expected counts. The vertical lines at the bottom of the chart indicate the distribution of the variable values amongst the site 61 observations.
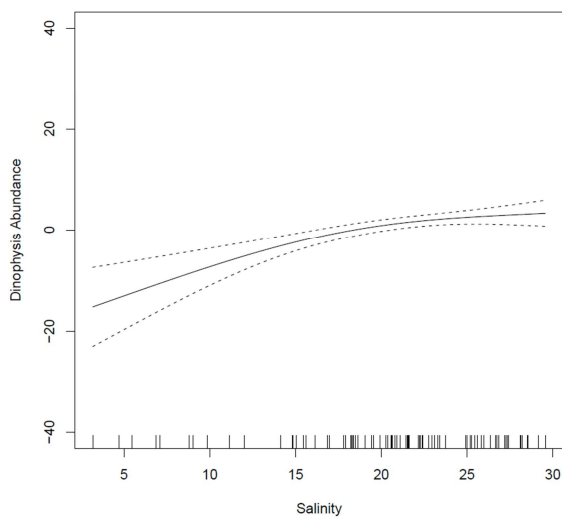
A.

Plot of Smooth Function for Week of Year: Site 61, D.acuminata
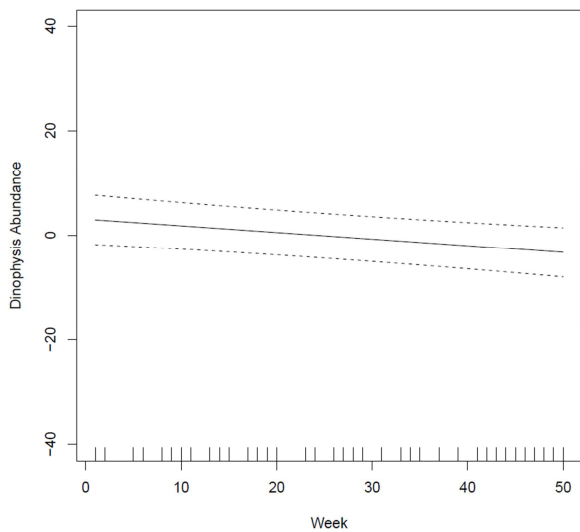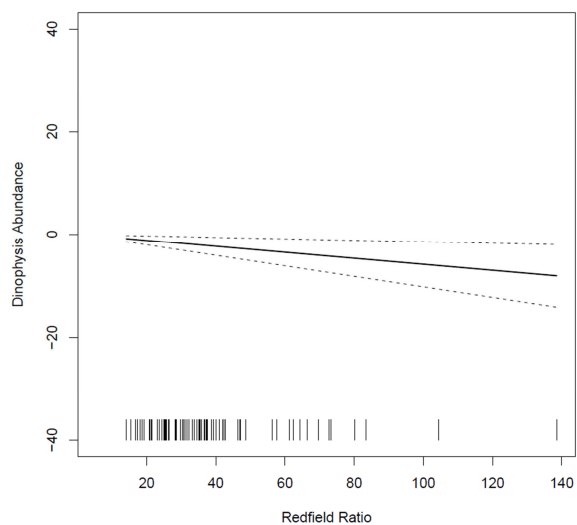
B.

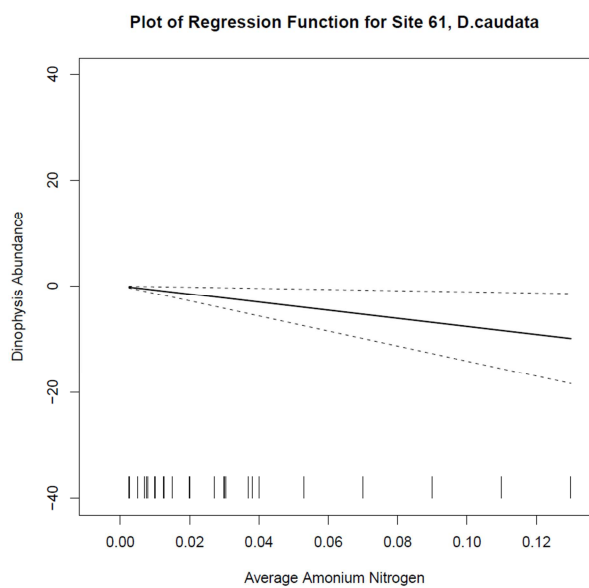Plot of Smooth Function for Average Temperature: Site 61, D.acuminata
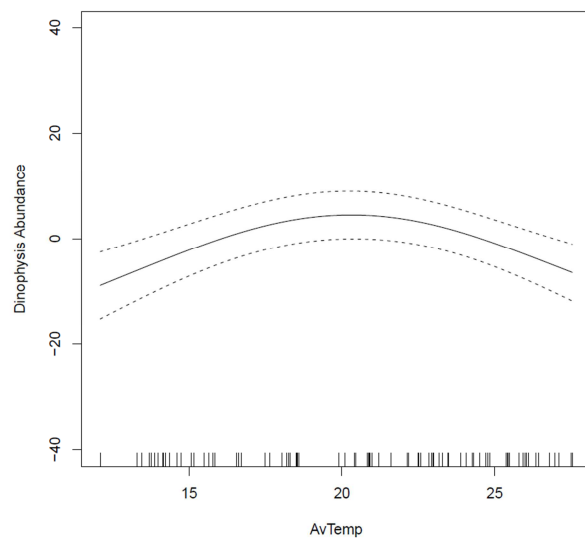
C.

Plot of Regression Function for Site 61, D.acuminata

D.

Plot of Regression Function for Site 61, D.acuminata

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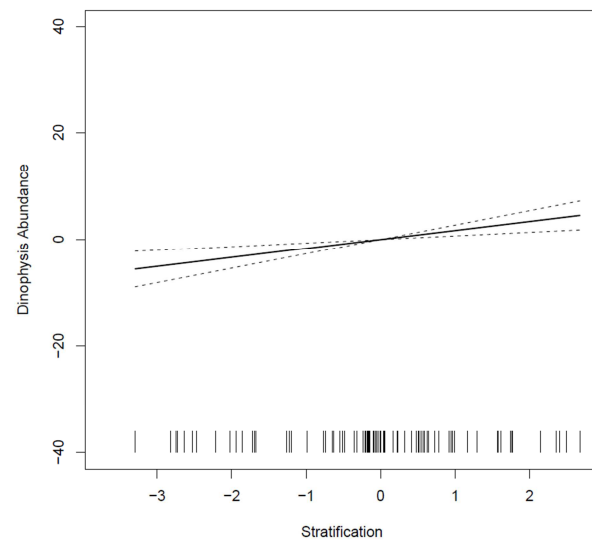
Supplementary Figure 6A-F. Plots of the effect of each significant environmental variable A.
week; B. temperature; C. salinity; D. Redfield ratio; E. ammonium nitrogen; and F. oxidised
nitrogen. The expected *D. caudata* count at each value of the variable is described by the
solid line, with the dashed lines representing 95% confidence intervals of these expected
counts. The vertical lines at the bottom of the chart indicate the distribution of the variable
values amongst the site 61 observations.

A.

B.

C.

D.

E.

Plot of Regression Function for Site 61, D.caudata

F.
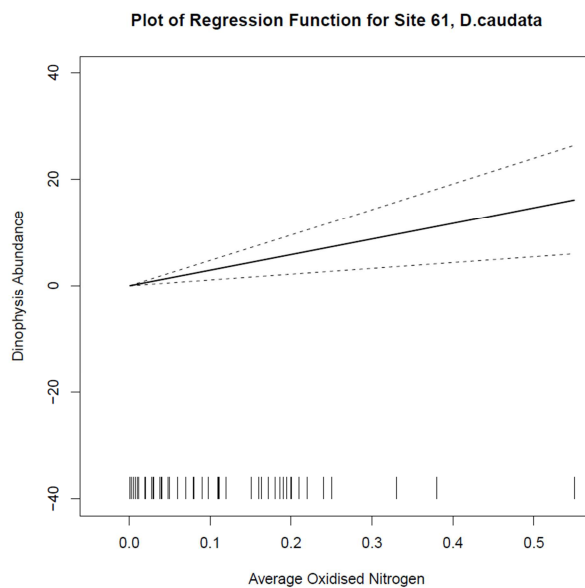
Plot of Regression Function for Site 61, D.caudata

E.

Plot of Regression Function for Site 61, D.caudata

F.

Plot of Regression Function for Site 61, D.caudata