

## Scientific Bases for Establishing Chlorophyll-*a* Thresholds for San Francisco Bay

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## 1 **Abstract**

2 San Francisco Bay (SFB) receives high nutrient loads from agricultural runoff, storm water,  
3 and treated wastewater effluent from 37 Publically Owned Treatment Works (POTWs), although  
4 to date the estuary appears resistant to classic symptoms of eutrophication. Recent trends of  
5 increasing chlorophyll-*a* (*chl-a*), harmful algal blooms (HAB), and dissolved oxygen  
6 concentrations (DO) suggest this resistance may be weakening. These findings motivated  
7 development of water-quality criteria (WQC) for SFB protective from adverse effects of nutrient  
8 over-enrichment. WQC consisting of thresholds of phytoplankton biomass as *chl-a* are based on  
9 strong relationships between nutrients, *chl-a*, and water-quality impairments in several estuaries.  
10 Although plankton ecology is well chronicled for SFB, data from several decades of monitoring  
11 have not been used heretofore to support WQC. Here, we analyze long-term data on *chl-a* (1993-  
12 2014), phytoplankton species composition (1993-2014), algal toxins (2012-2014), and DO  
13 (1993-2014) to derive: (1) quantitative relationships of HAB abundances, toxin levels, and DO to  
14 *chl-a*; and (2) *chl-a* thresholds and related uncertainties corresponding to “protected” and “at  
15 risk” categories based on WQC for DO and HAB alerts. Although *chl-a* is lower and DO higher  
16 in SFB than comparable estuaries experiencing nutrient over-enrichment, we report trends of  
17 increasing *chl-a*, declining DO, ubiquitous presence of HAB species, and toxin concentrations  
18 exceeding alert levels in ~35% of samples over the last 20 years. Quantile regressions of *chl-a*  
19 with HAB abundance and DO were significant, indicating SFB is poised for increased risk of  
20 impairments by HAB and low DO with increasing phytoplankton biomass. Coordinated  
21 statistical analyses showed *chl-a* thresholds associated with HAB and DO impairments  
22 converged on comparable values. We identified monthly mean *chl-a* < 13 mg m<sup>-3</sup> as an inflection  
23 point, below which probabilities for exceeding alert levels for HAB abundances and HAB toxins

24 were reduced. This HAB-based *chl-a* threshold was similar to a *chl-a* threshold of 13 - 16 mg m<sup>-3</sup>  
25 for meeting the WQC for DO of 7 mg L<sup>-1</sup>. At the high-end of risk, *chl-a* thresholds from 25 - 40  
26 mg m<sup>-3</sup> corresponded to a 0.5 probability of exceeding alert levels for HAB abundance, and with  
27 consistent excursions of DO in lower South Bay (LSB) and South Bay (SB) below the WQC of  
28 5.0 mg L<sup>-1</sup> for DO. We suggest that if available nutrients in SFB were assimilated into  
29 phytoplankton biomass, mean *chl-a* in all sub-embayments of SFB could reach “high risk”  
30 thresholds. These findings justify the establishment of *chl-a* thresholds to support nutrient  
31 management of SFB, given uncertainty about the future trajectory of water quality in this  
32 important estuarine ecosystem.

### 33 **Introduction**

34 Nutrient over-enrichment of the world's estuaries has led to multiple ecosystem impairments  
35 that express cultural eutrophication (Nixon, 1995; Paerl 1997; Cloern 2001; Diaz and Rosenberg,  
36 2008; Bricker et al. 2008). Identifying specific water-quality goals for nutrients has proven  
37 difficult, however, because ecological responses to nutrients are complex. San Francisco Bay  
38 (SFB) is a well-documented example of a nutrient-enriched estuary that exhibits this complexity  
39 (Cloern and Jassby, 2012). Extensive long-term data suggest that, to date, SFB has been resistant  
40 to classic symptoms of nutrient over-enrichment such as high phytoplankton biomass, harmful  
41 algal blooms (HAB), and low dissolved oxygen (DO). A number of factors have precluded  
42 widespread development of these symptoms in SFB, including high turbidity and concomitant  
43 light-limitation of primary productivity, intense tidal mixing that reduces biomass accumulation  
44 and DO depletion, and grazing by large populations of filter-feeding clams that regulates  
45 phytoplankton biomass in some areas of the bay (cf. Cloern and Jassby, 2012; Cloern et al.,  
46 2007; Kimmerer and Thompson, 2014).

47 Recent evidence suggests resistance to nutrient over-enrichment may be weakening in SFB,  
48 such as: (1) a three-fold increase of chlorophyll-*a* (*chl-a*) in South Bay (SB) during summer-fall  
49 since 1999 (Cloern et al., 2007); (2) regular occurrences of HAB species (Lehman et al., 2005;  
50 Cloern et al., 2005; Cloern and Dufford, 2005); and (3) diurnal depressions of DO to hypoxic  
51 conditions with  $DO < 2.8 \text{ mg L}^{-1}$  in restored salt ponds (Thebault et al., 2008; Topping et al.,  
52 2009). These observations call for a water-quality framework to inform management actions,  
53 consisting of thresholds for key properties that would be "protective" from adverse effects of  
54 nutrient over-enrichment. Phytoplankton biomass as *chl-a* is an integrative indicator of nutrient  
55 loadings with established links to water-quality impairments, commonly used to assess

56 eutrophication and support regulatory goals (Bricker et al., 2003; Zaldivar et al., 2008; Harding  
57 et al., 2014). Quantitative thresholds leading to management endpoints can be based on  
58 deviations from “reference” conditions when data prior to degradation are available (Andersen et  
59 al. 2010, 2015), or on ecosystem impairments such as low DO, HAB, or water clarity (e.g.,  
60 Harding et al., 2014). We lack *chl-a* records for SFB prior to human disturbance, limiting the use  
61 of reference conditions, but long-term data on *chl-a* support quantitative analyses of relationships  
62 between *chl-a* and potential impairments.

63 Two pathways of nutrient over-enrichment that culminate in adverse effects on humans,  
64 marine mammals, and other aquatic life include: (1) low DO associated with excess organic  
65 matter; and (2) increased HAB occurrences (Rosenberg et al., 1991; Diaz and Rosenberg, 1995;  
66 Kirkpatrick et al., 2004; Glibert et al., 2005; Baustein and Rabalais, 2009). Recognizing that  
67 factors other than nutrients affect low DO and HABs, causal links are established for nutrient  
68 loadings, *chl-a*, hypoxia, and HABs (Tett et al., 2007; Heisler et al., 2008; Anderson et al.,  
69 2012). Such links have been used to support water quality criteria (WQC) for Chesapeake Bay,  
70 relating risk of impairments to increased *chl-a* (e.g., Harding et al., 2014). As in many estuaries,  
71 *chl-a* has increased significantly over the past 15-20 years in SFB, amounting to a three-fold  
72 increase from the mid-1990s to mid-2000s in South Bay (SB) and Lower South Bay (LSB)  
73 (Cloern et al., 2007). Of particular concern are regular occurrences of fall blooms of  
74 phytoplankton in SB and LSB since the late 1990s, areas that rarely experienced such outbreaks  
75 in the past (Cloern and Jassby, 2012), and significant increases of *chl-a* in other sub-embayments  
76 during the same period. Despite these upward trends of *chl-a* and reports of HAB occurrences  
77 (see Cloern et al., 1994), routine monitoring for algal toxins has not been conducted (Cloern and  
78 Dufford, 2005). Moreover, long-term data on *chl-a*, phytoplankton species composition, and DO

79 to quantify risk of low DO or HAB occurrences with increasing *chl-a* have yet to be assembled  
80 for various sub-embayments of SFB.

81 Here, we present relationships between DO, HAB, and *chl-a* in SFB to derive quantitative  
82 thresholds based on water-quality impairments. We then apply these thresholds as endpoints to  
83 support assessments of status and trends of water quality required by both scientists and  
84 managers (Sutula et al., 2015). Our goals were to: (1) determine relationships DO, HAB  
85 occurrences, and algal toxins to *chl-a*; and (2) quantify *chl-a* thresholds and associated  
86 uncertainties using statistical approaches that identify “protected” and “at risk” categories in the  
87 context of WQC for DO and HAB alerts.

## 88 **Materials and Methods**

### 89 *Study Area*

90 SFB is the largest estuary in California, consisting of several major sub-embayments  
91 (Nichols et al., 1986). The estuary receives nutrient loads from 37 publicly owned wastewater  
92 treatment works (POTW) serving the area’s population of 7.2 million (Fig. 1). Most POTW  
93 perform only secondary treatment without additional nitrogen (N) or phosphorus (P) removal.  
94 Freshwater flow into SFB comes from two major sources, the Sacramento and San Joaquin  
95 Rivers, large rivers that drain 40% of California’s landscape. Intense agriculture in the heavily  
96 farmed Central Valley combined with urban sources such as Sacramento ~100 km upstream of  
97 Suisun Bay (SUB) contribute to high nutrient loads entering the northern estuary from the  
98 Sacramento/San Joaquin Delta. Storm-water runoff from densely populated urban areas  
99 surrounding SFB also contributes significant nutrients.

## 100 *Conceptual Approach*

101 Long-term data from the SFB Research Program (1993-2014) of the US Geological Survey  
102 (USGS) and concurrent measurements of algal toxins (2012-2014) supported analyses of trends  
103 for DO and HAB. Relationships of DO and HAB to *chl-a* were used to identify *chl-a* thresholds  
104 that correspond to risks of low DO or HAB alerts. Increased *chl-a* does not uniformly correspond  
105 to increased HAB occurrences, particularly for a single phytoplankton species or toxin, and both  
106 high-biomass and high-toxicity events are well described (Anderson et al., 2012). For the former,  
107 significant relationships between HAB and *chl-a* have been used to support WQC (Shutler et al.,  
108 2012; Schaeffer et al., 2012; Harding et al., 2014). Predominance of a particular taxonomic  
109 group, i.e., diatoms, expressed as cell counts or fraction of bio-volume is often accompanied by  
110 increased *chl-a*. Conditions that support increased *chl-a*, however, are known to increase  
111 abundance of the entire phytoplankton community, not just HAB species (Barber and Hiscock,  
112 2006). For our analyses, we assumed increased *chl-a* reflected increased abundance of all  
113 phytoplankton, including potentially toxic HAB based on previous studies (Bricker et al., 2008;  
114 Glibert et al., 2005).

115 Decomposition of excess phytoplankton biomass supports DO consumption, leading to  
116 hypoxia ( $\text{DO} < 2.8 \text{ mg L}^{-1}$ ) in stratified conditions. Spatial and temporal displacement of high  
117 *chl-a* and DO depletion commonly occurs in estuaries (Rabalais et al., 2014), reflecting strong  
118 seasonality of production and consumption (e.g., Wheeler et al., 2003). Empirical relationships  
119 between DO and *chl-a* exhibit time lags, with analyses requiring consideration of relevant time  
120 and space scales for individual ecosystems. Accordingly, we aggregated DO and *chl-a* data for a  
121 range of time scales for the six sub-embayments to evaluate the strength of these relationships.

122 *Data Sources*

123 USGS SFB Research Program. Our analyses drew on time-series data collected on regular  
124 cruises by the USGS along a 145-km transect from 1993 – 2014. These observations provided a  
125 complete record of *chl-a*, DO, conductivity, temperature, turbidity, and photosynthetically  
126 available radiation (PAR) (<http://sfbay.wr.usgs.gov/access/wqdata/query/index.html>). Vertical  
127 profiles were conducted with a Seabird Electronics SBE9+ CTD and rosette sampler equipped  
128 with a Turner Designs C3 fluorometer, Li-Cor LI 192 transmissometer, and Seabird SBE 43 DO  
129 electrode. Concurrent grab samples were collected for identification and enumeration of  
130 phytoplankton species. Discrete measurements of DO and *chl-a* were used to calibrate  
131 instruments and correct for turbidity.

132 Data were aggregated by sub-embayment (Fig. 1) as geomorphology and nutrient loadings  
133 affect ecological responses to nutrient inputs in SFB (Jassby et al., 1997). Sub-embayments  
134 consist of: (1) Lower South Bay (LSB), the area south of Dumbarton Bridge; (2) South Bay  
135 (SB), from Dumbarton Bridge to San Bruno Shoal; (3) Central Bay (CB), from San Bruno Shoal  
136 to Angel Island; (4) North Central Bay (NCB), from Angel Island to Pt. San Pablo; (5) San Pablo  
137 Bay (SPB), from Pt. San Pablo to Martinez; and (6) Suisun Bay (SUB), east of Martinez. USGS  
138 stations corresponding to these sub-embayments are 34-36, 24-32, 23-20, 18-16, 15-10, and 4-8,  
139 respectively. For some analyses, data from several sub-embayments were combined based on the  
140 statistical similarities to obtain bay-wide metrics, and to increase sample sizes for uncommon,  
141 but potentially deleterious HAB species.

142 HAB Species and Toxins. HAB species identified by the USGS were used for this analysis  
143 (Table 1). Seasonal and inter-annual patterns were identified for the three most common HAB  
144 species in SFB, *Pseudo-nitzschia sp.*, *Alexandrium sp.*, *Dinophysis sp.*, for several dinoflagellate



145 species, including *Heterosigma akashiwo*, *Karenia mikimotoi*, *Karlodinium veneficum*, and for  
146 cyanobacteria including the genera *Microcystis*, *Oscillatoria*, *Planktothrix*, *Anabaenopsis*, and  
147 *Anabaena*. Some rare species with low frequencies-of-occurrence were excluded from the  
148 analyses. SFB does not currently have established guidance for potentially deleterious HABs, so  
149 we used alert levels from the literature, monitoring programs, and analyses of available data.  
150 These included:  $10^6$  cells  $L^{-1}$  for cyanobacteria (WHO 2003), presence/absence for *Alexandrium*  
151 (<http://www.scotland.gov.uk/Publications/2011/03/16182005/37> ),  $10^2 - 10^3$  cells  $L^{-1}$  for  
152 *Dinophysis spp.* (<http://www.scotland.gov.uk/Publications/2011/03/16182005/37>; Vlamis et al.,  
153 2014), and  $10^5 - 5 \times 10^5$  cells  $L^{-1}$  for *Pseudo-nitzschia*. The dinoflagellates *H. akashiwo*, *K.*  
154 *mikimotoi*, and *K. veneficum* lack guidance on alert levels, so we used  $5 \times 10^5$  cells  $L^{-1}$  based on  
155 expert opinion. No defined alert levels exist for toxin concentrations estimated using Solid Phase  
156 Adsorption Toxin-Tracking (SPATT - MacKenzie et al., 2004), thus alert levels were defined as  
157  $1 \text{ ng g}^{-1}$  for microcystins (MCY), and  $75 \text{ ng g}^{-1}$  for domoic acid (DA) based on laboratory  
158 calibrations and studies at the Santa Cruz Municipal Wharf and Pinto Lake, California (Lane et  
159 al., 2010; Kudela, 2011; Gobble and Kudela, 2014).

160 We deployed SPATT samplers in the flow-through system of the *R/V Polaris* (~1 m intake)  
161 from October 2011 to November 2014 to assess the presence of DA and MCY. Individual  
162 SPATT deployments encompassed South SFB (stations 36-18, representing LSB+SB+CB), and  
163 typically stations 36-24 for full-bay cruises (representing LSB+SB), NCB (stations 21-16), SPB  
164 (stations 15-9), and SUB and the Delta (stations 8-657). Data were binned by sub-embayment,  
165 with SB and South-CB defined as stations 36-24 and 36-18, respectively. SPATT were operated  
166 as described previously (Lane et al., 2010; Kudela, 2011; Gobble and Kudela, 2014) for MCY,  
167 and reported as the total of LR, RR, YR, and LA congeners and domoic acid (DA). SPATT toxin

168 concentrations were reported in units of ng toxin g<sup>-1</sup> resin, and represented a weighted-average  
169 for the length of deployment (sub-embayment).

### 170 *Statistical Analyses*

171 Three statistical approaches were used: (1) seasonal Mann-Kendall Test to quantify temporal  
172 trends of DO, HAB and *chl-a*; (2) ordinary least squares regression (OLS), robust (e.g., Least  
173 Absolute Deviation - LAD), and quantile regression to determine relationships of DO, HAB, and  
174 *chl-a*; and (3) quantile regression and conditional probability analyses (CPA) to derive  
175 quantitative thresholds for *chl-a* based on a failure to achieve DO benchmarks, or an increased  
176 risk of reaching HAB abundances to trigger HAB alerts. Quantile regression was used to  
177 determine the 10<sup>th</sup> and 50<sup>th</sup> (median) quantiles of DO or HAB cell densities conditional on *chl-a*.  
178 Quantile regression is statistically analogous to rank-based correlation; it is based on ordering the  
179 observations, is robust to extreme values, and does not require assumptions about distributions of  
180 residuals (Cade and Noon, 2003). LAD regression is similar to quantile regression when the  
181 median quantile is used, although LAD uses ranks. CPA was used to analyze risk of DO below a  
182 WQC, or HAB abundance above a set alert level based on  $chl-a \geq$  a specified concentration (R  
183 package *CProb*; Hollister et al., 2008). The baseline probability is the overall probability of  
184 exceedance among all observations, without regard to *chl-a* (i.e.,  $chl-a >$  minimum value).  
185 Inflection points in the relationship are interpreted as *chl-a* above which probability of an  
186 adverse DO or HAB event increases at a faster rate relative to increases of *chl-a*. A probability of  
187 0.5 is nominally defined as a benchmark of “elevated risk” because above this level, an adverse  
188 event is more likely to occur than not.

189 Statistical Analyses of HAB – *Chl-a*. HAB cell densities and toxins were analyzed in the  
190 context of seasonal and inter-annual patterns of *chl-a*. Near-surface samples ( $\leq 2$  m) collected

191 from April - November were used for these analyses. “Calculated *chl-a*” consisting of  
192 fluorescence calibrated by discrete samples was also used for these analyses. All cell counts for  
193 known HAB species were used, regardless of depth or location, to increase sample sizes. More  
194 than 95% of HAB were from near-surface samples. USGS enumerates phytoplankton for  
195 samples with *chl-a*  $\geq 5 \text{ mg m}^{-3}$ , introducing a possible sampling bias by neglecting HAB at low  
196 *chl-a*. Another potential bias in the data is a lack of records for *Microcystis* spp., suggesting these  
197 cells were not identified by microscopy although they are regularly observed in northern SFB.

198 OLS and LAD regressions of HAB cell counts and SPATT toxin concentrations on *chl-a*  
199 were based on log-transformed data to improve normality. For toxin analysis, log<sub>10</sub>-transformed  
200 SPATT were compared to mean or maximum *chl-a* from corresponding SFB sub-embayments.  
201 Cell counts and *chl-a* were transformed by natural logarithm. HAB alert levels (see above) were  
202 used to derive probabilities that HAB or toxins would reach problematic levels with increased  
203 *chl-a*. Selection of alert level influenced the probability derived from CPA (see below).

204 Quantile regression and CPA were used to identify *chl-a* thresholds based on the risk of  
205 exceedances of HAB cell densities or toxin alert levels. First, CPA was conducted on HAB cell  
206 densities and SPATT data aggregated for all sub-embayments. A “HAB event of concern” was  
207 classified as a site with at least one HAB species exceeding cell-density alert levels. Second,  
208 quantile and OLS regressions were used to quantify relationships between cell densities of  
209 *Alexandrium*, *Dinophysis*, *Heterosigma*, *Karlodinium*, and *Pseudo-nitzschia* and *chl-a*.  
210 Corresponding analyses were performed for SPATT data aggregated among years and sub-  
211 embayments. Additional analyses were conducted by sub-embayment, but the results were  
212 similar (albeit with reduced statistical power) and were omitted for brevity. Analyses were also  
213 performed with and without *Alexandrium*, potentially biasing the analysis because this HAB has

214 a low alert level. Concern that patterns may be strongly influenced by exchange with the open  
215 coast led to CPA on the complete time-series stratified by sub-embayment. Finally, data were  
216 divided into pre- and post-2002 to identify potential decadal differences.

217 Statistical Analyses of DO - *Chl-a*. Relationships between DO and *chl-a* were derived using  
218 the USGS data (1993-2014). Mean *chl-a* from depths  $\leq 2$  m was calculated for each station for  
219 periods-of-interest identified in previous analyses as showing *chl-a* changes (Cloern et al., 2007).  
220 These periods include: 1) spring bloom (February-May), 2) summer baseline (June-September),  
221 and 3) combination of these two periods (February-September). Mean February-September *chl-a*  
222 proved integrative of changing phytoplankton productivity in SFB and was chosen as the time  
223 period to derive thresholds of risk of low DO.

224 The evaluation period for DO was based on periods of non-compliance using existing WQC  
225 for DO in SFB: 1) an instantaneous WQC  $> 7$  mg L<sup>-1</sup> upstream and  $> 5$  mg L<sup>-1</sup> downstream of  
226 the Carquinez Bridge, not to fall below these values more than 10% of the time (SFRWQCB,  
227 2011); and 2)  $> 80\%$  saturation in running three-month medians in any sub-embayment of SFB.  
228 Medians for percent saturation and concentration (mg L<sup>-1</sup>) of DO were computed from vertical  
229 profiles at each station. The number of stations below the WQC was tabulated by sub-  
230 embayment over the 20-year period. The three-month intervals with the most DO exceedances  
231 were used for further statistical analyses as these periods are sensitive to low DO and would  
232 correspond to protective thresholds for *chl-a*.

233 Quantile regression was used to investigate relationships between DO and *chl-a* by sub-  
234 embayment for several time lags. DO percent saturation was preferred to DO concentration as it  
235 removed variability associated with effects of temperature and salinity on solubility. Median  
236 (i.e.,  $\tau = 0.5$ ) quantiles of percent DO saturation were used to test the significance and slope of the

237 relationship between DO and *chl-a* for three periods of integration. Sub-embayments for which  
238 a there was a positive slope between median percent DO saturation and *chl-a* were omitted from  
239 further analyses.

240 For sub-embayments with significant ( $p < 0.05$ ) negative relationships between DO and  
241 *chl-a*, thresholds of increased risk of falling below DO benchmarks were quantified using two  
242 approaches. First, quantile regression using  $\tau = 0.1$  was used to predict the mean and 95%  
243 confidence intervals of *chl-a* at which a gradient of DO percent saturation of 80%, 72%, 57%,  
244 and 46% would be attained 90% of the time. The remaining 10% non-attainment corresponds to  
245 California State Water Resource Control Board guidance for listing of impaired waters  
246 (SWRCB, 2005). These percent DO saturation are equivalent to DO concentrations of 7.0, 6.3,  
247 5.0 and 4.0 mg L<sup>-1</sup> at mean summer temperature 15° C and salinity 24. Benchmark  
248 concentrations of 6.3 and 5.0 mg L<sup>-1</sup> are the lowest DO concentrations to which salmonid and  
249 non-salmonid fish, respectively, can be exposed indefinitely without resulting in > 5% impact to  
250 estuarine populations (Bailey et al., 2014). 7.0 and 5.0 mg L<sup>-1</sup> benchmarks are the established  
251 WQC for DO for SFB sub-embayments. In addition, *chl-a* at which DO percent saturation was  
252 expected to meet the median three-month percent saturation WQC of 80% for SFB was  
253 estimated as the 50<sup>th</sup> quantile regression line. Finally, CPA was used to identify change points in  
254 the probability of DO falling below established WQC for DO with increasing *chl-a*.

## 255 **Results**

### 256 *HAB Cell Densities and Algal Toxins*

257 HAB species were detected in ~50% of samples and exceeded alert levels in ~35% of  
258 samples. Of samples exceeding alert levels, 53% were associated with *Alexandrium*, 11% with  
259 *Dinophysis*, and 7% with *Pseudo-nitzschia* (Fig. 2). Few toxic HAB events have been reported

260 for SFB, but SPATT data confirm common occurrences of toxins DA and MCY (Fig. 3). Of 158  
261 SPATT samplers we deployed, 72% showed detectable MCY and 97% showed detectable DA.  
262 Mean concentrations were 0.75 ng g<sup>-1</sup> for MCY and 57 ng g<sup>-1</sup> for DA, with ranges of detectable  
263 toxin for SPATT from 0.01 - 25.5 ng g<sup>-1</sup> for MCY and from 1.69 - 1650 ng g<sup>-1</sup> for DA (Fig. 3a-  
264 b).

#### 265 *DO, HAB Cell Densities, Algal Toxins, and Chl-a*

266 Significant decreases of DO and increases of *chl-a* occurred in all sub-embayments from  
267 1993 – 2013 ( $p < 0.05$ ) based on a seasonal Mann-Kendall test (Fig. 4). Particularly notable were  
268 increases of summer baseline *chl-a* throughout SFB, with the largest increases in central and  
269 southern sub-embayments (Fig. 4). Sen slopes ranged from -0.9 to -1 percent saturation yr<sup>-1</sup> for  
270 DO and from 0.041 to 0.096 mg m<sup>-3</sup> yr<sup>-1</sup> for *chl-a*. Cell counts were aggregated for all sub-  
271 embayments to increase sample sizes, except for *Pseudo-nitzschia* ( $n = 166$ ) and *Alexandrium* ( $n$   
272  $= 261$ ). HAB organisms *Alexandrium*, several cyanobacteria, *Dinophysis*, *Heterosigma*,  
273 *Karlodinium*, and *Pseudo-nitzschia* showed no significant increases based on Kendall's Tau tests  
274 ( $p > 0.1$ ), while *Karenia* showed a significant, positive trend ( $p < 0.05$ ). Cell counts for *Pseudo-*  
275 *nitzschia* or *Alexandrium* analyzed by sub-embayment showed no significant trends (ANCOVA,  
276  $p > 0.05$ ).

277 The 10<sup>th</sup> percentile of summer DO ranged from 5.7 – 7.8 mg L<sup>-1</sup> on south to north transects  
278 (Supplemental Material, Table S1 and Fig. S1). DO was  $> 5$  mg L<sup>-1</sup> from 97.1 to 100% of the  
279 time along these transects, with DO  $> 7$  mg L<sup>-1</sup> in SUB 100% of the time. For most sub-  
280 embayments, evaluation periods that most frequently fell below the WQC for DO consisting of a  
281 three-month running median of 80% saturation were May-July and June-August (Supplemental  
282 Material, Table S2), and these periods were used in quantile regressions.

283 *Relationships of HAB and Chl-a*

284 Relationships of HAB abundance and SPATT toxins to *chl-a* showed considerable scatter.  
285 Cell counts of *Alexandrium*, *Dinophysis*, *Karlodinium*, and *Pseudo-nitzschia* increased with  
286 increasing *chl-a* and slopes of LAD regressions were significant ( $p < 0.05$ ). Slopes and  
287 corresponding [ $R^2$ ] for these HAB were 0.48 [0.25], 0.56 [0.33], 1.4 [0.44], and 0.43 [0.45]  
288 respectively. Cyanobacteria, *Heterosigma*, and *Karenia* showed no significant trends. SPATT  
289 data analyzed by sub-embayment showed a significant increase of MCY and DA with increasing  
290 mean *chl-a*, and a significant increase of DA with maximum *chl-a*.

291 *Relationships of DO and Chl-a*

292 Median DO in May-July and June-August showed similar patterns with consistently negative  
293 slopes for SUB, SPB, SB, and LSB, regardless of the evaluation period for *chl-a* (Table 1; Fig.  
294 5). Slopes were generally steepest and most significant for mean February-September *chl-a*.  
295 Unlike other sub-embayments, the DO - *chl-a* relationship for SB was relatively insensitive to  
296 the evaluation period for *chl-a*, with significant relationships for most combinations. DO - *chl-a*  
297 relationships were significant in LSB for several evaluation periods. The June-September mean  
298 was only negative and significant in SPB, SB, and LSB, while the February-May mean was only  
299 negative and significant when correlated with SB and LSB (Table 1). In contrast, slopes were  
300 often positive for CB and NB. Quantile regressions of SUB and SPB, while significant,  
301 contained relatively few observations at high *chl-a* (Fig. 5). In addition, NB and CB had  
302 insufficient exceedances of WQC for DO to run CPA. For this reason, all sub-embayments  
303 except SB and LSB were omitted from further analyses to derive DO-related *chl-a* thresholds.

304 *Thresholds Based on Chl-a*

305 HAB Relationships to Chl-a. The baseline probability of HAB occurrences for the full range  
306 of *chl-a* was 0.35 - 0.40 (Fig. 6a). Interpretation of this baseline probability is that 35-40% of all  
307 samples from 1993-2014 exceeded HAB alert levels based on abundance (cells L<sup>-1</sup>). A mean  
308 probability of 0.5 to exceed HAB alert levels corresponded to *chl-a* > 37.5 mg m<sup>-3</sup> with an upper  
309 95% confidence interval of 13.5 mg m<sup>-3</sup>. An inflection point for probability corresponding to  
310 increased risk occurred at *chl-a* > 25 mg m<sup>-3</sup>. We repeated this analysis after removing  
311 *Alexandrium* to determine if this species with low alert level affected CPA outputs (Fig. 6b). The  
312 relationship between HAB abundance and *chl-a* was weaker at higher *chl-a*, but  
313 presence/absence of *Alexandrium* did not affect the baseline probability. Setting an *Alexandrium*  
314 alert level other than “present” had little effect on CPA outputs as mean abundance was ~8,000  
315 cells L<sup>-1</sup> (range: 100-290,000 cells L<sup>-1</sup>), and an alert level of > 1,000 cells L<sup>-1</sup> gave similar  
316 patterns.

317 The *chl-a* thresholds derived using CPA were consistent with relationships of HAB species  
318 to *chl-a* using quantile regressions or LAD, with a 0.50 probability of HAB corresponding to a  
319 broad range of *chl-a* from 3.5 - 40 mg m<sup>-3</sup>. Low-biomass, highly toxic genera such as  
320 *Alexandrium* and *Dinophysis* occupied the low end of the *chl-a* range, while high-biomass genera  
321 such as *Heterosigma* and *Pseudo-nitzschia* occurred at the other high end. CPA for individual  
322 sub-embayments were affected by sample size with limited observations at high *chl-a*, but  
323 comparable thresholds were derived using spatially aggregated data. Exceptions included NCB  
324 and CB that showed flat relationships with *chl-a* (e.g., Fig. 7). Other sub-embayments showed  
325 increased probabilities of HAB occurrences with increasing *chl-a*, exceeding 0.80 at highest  
326 *chl-a* in SPB and SB. More than 90% of *chl-a* observations in NB and CB were < 13 mg m<sup>-3</sup> for



327 the 20-year record, and only in SB and LSB were *chl-a* commonly  $> 13 \text{ mg m}^{-3}$  (18% and 26%,  
328 respectively; Supplemental Materials, Fig. S2).

329 OLS regressions of SPATT toxin on *chl-a* were not statistically significant, but CPA on  
330 toxins and *chl-a* gave similar inflection points as we derived for HAB organisms. The baseline  
331 probability for DA began at  $\sim 0.35$  (i.e., across all *chl-a* levels) and increased to  $\sim 0.6$  for  
332 observations with *chl-a*  $> 13 \text{ mg m}^{-3}$  (Fig. 8a). MCY showed a similar pattern, with a baseline  
333 probability of  $\sim 0.3$  (Fig. 8b). Very few SPATT observations exceeded *chl-a* thresholds for HAB  
334 alert levels ( $> 13 \text{ mg m}^{-3}$ ), but an increased probability of exceeding toxin thresholds at *chl-a*  $> 10$   
335  $\text{mg m}^{-3}$  was consistent with the probability of exceeding alert levels for HAB abundance based  
336 on CPA (Fig. 6a-b).

337 Thresholds Relating DO to *Chl-a*. Quantile regression of mean *chl-a* from February-  
338 September and DO from May-July in SB and LSB showed consistently significant ( $p < 0.1$ ),  
339 negative slopes for  $\tau = 0.1$  and  $0.5$  using all three *chl-a* evaluation periods (Table 1). Slopes were  
340 slightly steeper and more significant for May-July than for June-August. Based on quantile  
341 regressions for SB using DO from May-July, a mean *chl-a* from February - September of  $14 \text{ mg}$   
342  $\text{m}^{-3}$  was associated with a low frequency of DO falling below the WQC for DO, while the  
343 likelihood was higher at *chl-a* of  $17 \text{ mg m}^{-3}$  (Table 2). Comparison of predicted *chl-a* values for a  
344 gradient of DO is instructive. At *chl-a* of  $14 \text{ mg m}^{-3}$ , 90% of DO observations were predicted to  
345 exceed  $7 \text{ mg L}^{-1}$ , while at *chl-a* of  $42 \text{ mg m}^{-3}$ , 90% of DO observations were predicted to exceed  
346  $5.0 \text{ mg L}^{-1}$  (Table 2). For context, the February-September *chl-a* measured at SB sites was below  
347  $14 \text{ mg m}^{-3}$  95% of the time over the 20-year record (supplemental materials, Fig. S2).

348 DO in LSB was predicted to fall below DO benchmarks at lower *chl-a* than in SB, although  
349 confidence intervals were larger. At a mean *chl-a* from February-September of  $16 \text{ mg m}^{-3}$ , there

350 was an elevated risk of falling below WQC for DO based on the three-month median for percent  
351 saturation (Table 2). A 10% probability of exceeding the WQC for DO was associated with *chl-a*  
352 of  $4 \text{ mg m}^{-3}$ , with a negative lower 95<sup>th</sup> CI. This suggests advection of DO-depleted water into  
353 the study area such that even at extremely low values of *chl-a*, the probability of falling below  
354 the WQC for DO is high. Similarly, the CPA showed a baseline probability of 0.2 for falling  
355 below the WQC for DO (Fig. 9). This baseline was moderately high considering a mean  
356 probability of 0.5 based on the WQC for DO to *chl-a*  $> 14 \text{ mg m}^{-3}$  with an upper 95% confidence  
357 interval of  $> 10 \text{ mg m}^{-3}$ . We interpret this result to mean *chl-a* at or above these thresholds entails  
358 increased risk of DO below the WQC for DO of 80% saturation with increased *chl-a* (Fig. 9).  
359 Applying the CPA and comparing results to DO and *chl-a* distributions in SFB, we observed that  
360 90% of DO values would exceed  $6.3 \text{ mg L}^{-1}$  and  $5.0 \text{ mg L}^{-1}$ , respectively, at *chl-a* of  $15 \text{ mg m}^{-3}$   
361 and  $36 \text{ mg m}^{-3}$  (Table 2). Long-term data for 20 years showed 95% of *chl-a* measured in LSB  
362 was  $< 25 \text{ mg m}^{-3}$  (Supplemental Materials, Fig. S2), and hypoxia associated with high *chl-a*  
363 remains uncommon in the open channel habitat of LSB.

## 364 **Discussion**

### 365 *Current Status and Potential for Eutrophication in SFB*

366 Humans have enriched the world's bays and estuaries with nitrogen and phosphorus, but the  
367 responses to enrichment vary widely across ecosystems (Cloern, 2001). Nutrient supply sets the  
368 potential for environmental degradation through excess production of algal biomass, but the  
369 realization of that potential – i.e., the efficiency with which exogenous nutrients are converted  
370 into biomass – depends on factors that regulate phytoplankton population growth, including light  
371 availability, toxins, grazing, pathogens, and transport processes. Nutrient concentrations in SFB  
372 exceed those that have led to degradation of water quality in other estuaries, but its

373 phytoplankton biomass (mean *chl-a* concentration) is lower and DO concentrations higher than  
374 for other enriched estuaries such Chesapeake Bay, Neuse Estuary, Seine Bay, and the  
375 Westerschelde (Bricker et al., 2007; Cloern and Jassby 2008, Fig. 1). However, estuaries are  
376 highly dynamic ecosystems that exhibit complex responses to human disturbances, climate  
377 variability, and climate change (Cloern et al., 2015, Harding et al., 2015). Changes in SFB during  
378 the past two decades include significant increases of *chl-a*, ubiquitous presence of HAB species  
379 known to be toxic in other nutrient-enriched estuaries, and significant decreasing trends of DO.

380 HAB cell densities exceeded alert levels in ~35% of samples from SFB, indicating the  
381 potential for adverse effects on ecosystem health. HAB species are expected to occur at some  
382 baseline level, based on the cosmopolitan distributions of many species (Lundholm and  
383 Moestrop, 2006). However, the probability of a HAB event is high, once seeded, due to nutrient  
384 over-enrichment that characterizes SFB. The high baseline of occurrence documented in this  
385 study reflects strong connectivity with at least two documented sources of HAB seed  
386 populations. The first is the coastal ocean adjacent to SFB, a source of toxic phytoplankton  
387 species that lead to closures of shellfish harvesting half the year because of potential exceedances  
388 of alert levels based on HAB abundance (Lewitus et al., 2012). The second source is the South  
389 Bay salt ponds where the presence of dinoflagellates, *Alexandrium* spp. and *Karenia mikimotoi*,  
390 the raphidophyte, *Chattonella marina*, and the cyanophytes, *Anabaenopsis* spp. and *Anabaena*  
391 spp. has been confirmed (Thébault et al., 2008). Samples from SB contained other HAB species  
392 that were rare in SFB prior to the opening of the Salt Ponds, including *Karlodinium veneficum*,  
393 *Chattonella marina*, and *Heterosigma akashiwo*, while abundances of *K. mikimotoi* and *K.*  
394 *veneficum* in LSB and SB increased after breaching of the Salt Ponds. Distributions of these

395 species show a spatial pattern reveal expansion into the rest of the SFB, suggesting that they pose  
396 an emerging threat.

397 While presence of HAB species above a defined alert level indicates a potential threat, the  
398 presence of toxins elevates that threat considerably as it demonstrates that environmental  
399 conditions within SFB or connected habitats are conducive to toxin production. SFB is not  
400 routinely monitored for algal toxins and no acute wildlife mortalities or human illnesses have  
401 been directly attributed to HAB from 1993-2014. MCY in SFB, however, has been linked to  
402 negative impacts on aquatic food webs (Lehman et al., 2010), and there is increasing evidence  
403 that chronic, sub-lethal exposure to DA constitutes a significant impairment (Goldstein et al.,  
404 2008; Montie et al., 2012). *Pseudo-nitzschia* exceeded alert levels in only 11% of samples, and  
405 cyanobacteria cells were not recorded, nonetheless, 72% and 96% of SPATT showed measurable  
406 quantities of the toxins MCY and DA, respectively. SPATT detects low concentrations of toxins  
407 compared to traditional methods (Lane et al., 2010; Kudela, 2011), and removal of SPATT data  
408 with the lowest toxin levels still left ~35% of samples with toxin levels of concern. These  
409 findings suggest that dissolved toxins are widely distributed in SFB. SPATT were not analyzed  
410 for other toxins that may occur in SFB, and the threat of HAB toxins remains requires further  
411 study.

412 Significant relationships of HAB abundance to *chl-a* were detected in SFB, while low DO  
413 and high *chl-a* were rarely observed and relationships differed by sub-embayment. In Northern  
414 SFB, DO was high and relatively low *chl-a* accompany depressed primary production with  
415 several possible causes, including inhibition and grazing (Dugdale et al., 2007; Cloern et al.,  
416 2014). The lack of consistent, significant relationships between DO and *chl-a* in SUB, SPB and  
417 CB sub-embayments suggests that physical processes, such as strong tidal mixing and a lack of

418 persistent stratification, partially alleviate the development of low DO, despite high  
419 phytoplankton biomass (Smith and Hollibaugh, 2006). These modulating factors appear  
420 important in both CB and SUB, sub-embayments that are adjacent to the coastal ocean and the  
421 Sacramento/San Joaquin Delta, respectively. In contrast to CB and SUB sub-embayments, DO  
422 was lowest and *chl-a* was highest in LSB, a lagoonal sub-embayment with a long residence time  
423 that is near productive intertidal habitats that experience hypoxia, such as the restored salt ponds  
424 in SB (Thebault et al., 2008) and tidal sloughs (Senn et al., 2014).

425 SFB is responsive to both climate forcing and climate change (Cloern et al., 2015), and these  
426 factors can lead to shifts in the efficiency of nutrient assimilation into phytoplankton biomass, as  
427 reported for the Baltic Sea (Riemann et al., 2015). The high ambient nutrient concentrations that  
428 characterize SFB suggest a potential for accumulation of phytoplankton biomass sufficient to  
429 impair water quality. To evaluate this potential, we computed median concentrations of dissolved  
430 inorganic nitrogen (DIN) and *chl-a* for four sub-embayments (Table 3). We then estimated  
431 potential *chl-a* as the sum of measured *chl-a* plus the amount of *chl-a* that would be produced if  
432 all remaining DIN was assimilated into phytoplankton biomass, assuming a conversion factor of  
433 1 g *chl-a* per mol N (Eppley et al., 1971). We found median *chl-a* in all sub-embayments of SFB  
434 would increase an order of magnitude if this potential was realized. Given uncertainty about the  
435 future trajectory of water quality in SFB, a potential for increased phytoplankton biomass  
436 justifies establishment of *chl-a* thresholds to support nutrient management directed at reducing  
437 risk of impairments.

438

439 *Chl-a as the Basis to Assess Water-Quality Impairments*

440 *Chl-a* is an integrative measure of water quality that has been used to assess eutrophication in  
441 estuaries around the world (Bricker et al., 2003; Zaldivar et al., 2008). Our analyses have related  
442 specific water-quality impairments in SFB to *chl-a*, consistent with published work that applies  
443 *chl-a* as a pivotal indicator of nutrient over-enrichment. We present several key findings that  
444 support this approach. First, we documented significant relationships between HAB abundance,  
445 DO and *chl-a* using quantile regressions. Our results are consistent with a conceptual model of  
446 increased risk for HAB abundance, toxins, and low DO at increased phytoplankton biomass  
447 (Cloern, 2001). Second, several statistical approaches yield consistent ranges for *chl-a* threshold  
448 based on HAB and DO. An inflection point at mean monthly *chl-a* < 13 mg m<sup>-3</sup> was a threshold  
449 below which the probability of potentially deleterious conditions quantified by HAB abundance  
450 and SPATT-derived toxins decreased. This *chl-a* threshold was similar to mean seasonal *chl-a* of  
451 13 - 16 mg m<sup>-3</sup> associated with attainment of the WQC for DO in SFB, based on the three-month  
452 median percent saturation of 7 mg L<sup>-1</sup>. At the opposite end of the risk continuum, inflection  
453 points of heightened risk of HAB cell density (*chl-a* from 25 - 40 mg m<sup>-3</sup>) corresponded well to  
454 mean seasonal *chl-a* thresholds of 35-40 mg m<sup>-3</sup> required for LSB and SB to fall more  
455 consistently below the 5.0 mg L<sup>-1</sup> DO WQC. Third, *chl-a* thresholds we derived for SFB were in  
456 agreement with published water-quality criteria using a variety of assessment methods. Several  
457 examples are consistent with *chl-a* thresholds that we derived for SFB based on relationships  
458 with HAB and DO. Harding et al. (2014) reported that mean summer *chl-a* from 7.2 – 11 mg m<sup>-3</sup>  
459 precluded low DO in the deep waters of Chesapeake Bay, and that mean annual *chl-a* of 15 mg  
460 m<sup>-3</sup> was associated with decreased risk of *Microcystis* spp. toxins. Bricker et al. (2003)  
461 designated > 20 mg m<sup>-3</sup> as a threshold of “high” risk for eutrophication, a value agreed upon by  
462 expert judgment. Similarly, *chl-a* thresholds of 10, 20 and 50 mg m<sup>-3</sup> are used to define

463 categories of low, high, and very high risk of eutrophication in the Phytoplankton Biological  
464 Quality Element for the European Union (EU) Water Framework Directive (WFD) proposed in  
465 the United Kingdom (Devlin et al., 2011).

466

#### 467 “Risk Assessment” and Uncertainty in *Chl-a* Thresholds

468 Environmental management and regulation are firmly grounded in a paradigm of “risk  
469 assessment” (US Environmental Protection Agency, 1998). For this reason, risk represents a  
470 useful context to express *chl-a* thresholds and uncertainties with respect to WQC for SFB. Here,  
471 we used CPA and quantile regression to derive *chl-a* thresholds corresponding to low and high  
472 risk of exceeding HAB alert levels. A similar approach is commonly used to derive WQC for  
473 freshwater ecosystems, but few applications exist for the marine environment (Paul et al., 2005).  
474 Both CPA and quantile regression provide quantitative measures of uncertainty, a key element to  
475 support environmental decision-making (National Research Council, 2009).

476 CPA and quantile regression provided estimates of statistical uncertainty for *chl-a* thresholds  
477 based on HAB and DO, but other sources of uncertainty should be considered when applying  
478 these thresholds to nutrient management. First, the ecological significance of HAB species in  
479 SFB is not well known. Data needs include bio-accumulation of particulate and dissolved toxins  
480 in the biota, and acute and chronic impairments of ecosystem health. Such efforts should be  
481 coupled to an improved understanding of relationships between HAB toxins and *chl-a* specific to  
482 each sub-embayment. Second, spatial and temporal dynamics of low summer DO and seasonal  
483 maxima of *chl-a* that support DO consumption require additional study. Conceptually, it is  
484 possible that the mechanism behind this relationship is that high primary production on seasonal  
485 to annual time scales is expected to promote increased abundance of detritus, which, during the

486 summer, leads to an increased probability of net ecosystem heterotrophy (Caffrey, 2003). Large  
487 spring blooms and subsequent fall blooms that were prominent features of the annual  
488 phytoplankton cycle in 2000 (Cloern et al., 2007) have not occurred in the past five years; in  
489 contrast, the summertime baseline that has seen the largest magnitude increase from 1993 to  
490 2014 (Fig. 4). In-depth investigations into phytoplankton contribution to the SFB carbon budget  
491 and its relative influence on the coupling of pelagic and benthic metabolism are needed to better  
492 understand the relationships behind these empirical relationships between DO and *chl-a* in SB  
493 and LSB (e.g., Murrell et al., 2013).

494 Finally, there is a need to review the relevance and adequacy of scientific data supporting  
495 WQC for DO in SFB, specifically in LSB. Over the last 20 years, LSB has met  $5.7 \text{ mg L}^{-1}$ , the  
496 benchmark proposed by Best et al. (2007) that corresponds to the highest ecological condition  
497 category in EU estuaries. However, it has frequently not met the WQC based on three-month  
498 median percent DO saturation of  $> 80\%$ , a value that at mean summer salinities and temperatures  
499 is equivalent to  $7 \text{ mg L}^{-1}$ . The question is whether  $7 \text{ mg L}^{-1}$  is a reasonable expectation for DO in  
500 LSB, given that this sub-embayment is strongly influenced by highly productive, intertidal  
501 habitats (Thebault et al., 2008; Shellenbarger et al., 2008).

502 Such investigations should be nested within an improved monitoring program, as the  
503 complexity of these patterns remind us that SFB is in a continuing state of change, one that is  
504 likely to continue over the next century (Cloern et al., 2011). Although it is attractive to consider  
505 relationships of impairments such as HAB abundance and low DO to *chl-a* as constant, we  
506 recognize that *chl-a* thresholds are responsive to changes in fundamental drivers of  
507 phytoplankton dynamics, such as oceanic exchange, top-down grazing, and light limitation.  
508 Changes in the relationships of impairments to *chl-a* will almost certainly respond to climate



509 variability and climate change, as reported for this and other ecosystems (Cloern et al., 2014;  
510 Riemann et al., 2015).

## 511 **Summary**

512 This study demonstrated that, while DO is higher and *chl-a* lower in SFB than in other estuaries  
513 subject to nutrient over-enrichment, this important ecosystem is poised to express symptoms of  
514 cultural eutrophication. We found that evidence of ubiquitous HAB abundance, HAB toxins,  
515 declining DO, and increasing *chl-a*, supporting generalized conceptual models that describe  
516 increased risk of HAB cell densities and toxin concentrations and declining DO with increasing  
517 phytoplankton biomass. The majority of SFB subembayments are currently below *chl-a* < 13 mg  
518 m<sup>-3</sup>, representative of baseline probabilities of HAB occurrence and attainment of SFB's 3-  
519 month median percent saturation DO WQC. However, SFB has sufficient dissolved inorganic  
520 nutrients to reach *chl-a* levels defined by "high risk" thresholds in the range of 25-40 mg m<sup>-3</sup>  
521 *chl-a*, suggesting a potential for increased biomass accumulation that could lead to cultural  
522 eutrophication. Given the uncertainty in SFB's trajectory amidst global change, it is this potential  
523 for high biomass production that motivates establishment of *chl-a* water quality goals to support  
524 nutrient management of SFB, and underlines the need for continued monitoring of SFB to  
525 understand how these fundamental relationships may change in the future.

526

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716 **Figure Legends**

717 Fig. 1. SFB showing distribution of habitat types and locations of sub-embayments Suisun Bay  
718 (SUB), San Pablo Bay (SBP), North Central Bay (NCB), Central Bay (CB), South Bay (SB), and  
719 Lower South Bay (LSB), defined by Jassby et al. (1997), relative to the locations of major cities  
720 in region.

721 Fig. 2. Time series of major HAB in SFB from 1993-2014. Symbols indicate cell densities (cells  
722  $\text{mL}^{-1}$ ) by cruise. The station with the highest cell density is indicated for cruises with HAB  
723 enumerated at multiple locations. Inset values give cell densities at stations  $> 200$  cells  $\text{mL}^{-1}$ .

724 Fig. 3. Concentration of (a) DA ( $\text{ng g}^{-1}$ ) and (b) MCY (LR, RR, YR, and LA in  $\text{ng g}^{-1}$ ) from  
725 SPATT deployed in the R/V *Polaris* surface mapping system for regions representing the  
726 following sub-embayments: SUB+ Delta station, SPB, NCB, and SB+CB during full Bay  
727 cruises, and LSB+SB during South SFB only cruises sub-embayments. Circles indicate DA (top)  
728 or MCY concentrations (bottom); for DA  $> 400$   $\text{ng g}^{-1}$  and MCY  $> 10$   $\text{ng g}^{-1}$ , and numeric values  
729 indicate the concentrations.

730 Fig. 4. Monthly geomean and 95% CI of *chl-a* over the periods from 1993-1999 and 2000-2014,  
731 by subembayment, from north to south, (a) SUB, (b) SPB, (c) NCB, (d) CB, (e) SB, and (f) LSB.  
732 Comparison of *chl-a* before and after 1999 is important temporal benchmark as Cloern et al.  
733 (2007) identified a *chl-a* step change coincident with the shifting of the NE Pacific to its cool  
734 phase.

735 Fig. 5. Comparison of quantile regressions relating May-July DO percent saturation to *chl-a* in  
736 selected sub-embayments from north to south: (a) SUB, (b) SPB, (c) SB and (d) LSB. Lines for  
737 the 10<sup>th</sup> ( $\tau=0.1$ , red) and median quantiles ( $\tau=0.5$ , blue) are shown for the quantile regressions.  
738 Results of regression analyses are given in Table 1.

739 Fig. 6. Probability of HAB cell densities higher than alert levels as specified value of *chl-a* is  
740 exceeded for data in which (a) all HAB species are included and (b) excluding *Alexandrium*. The  
741 black line represents mean probability. Grey dashed lines are lower and upper 95% confidence  
742 intervals of bootstrap values (100 iterations).

743 Fig. 7. Mean probability of observing any HAB species at concentrations higher than defined  
744 alert levels if specified value of *chl-a* is exceeded, by sub-embayment for CB (open squares), SB  
745 (black triangle), and LSB (grey circle).

746 Fig. 8. Probabilities of DA (top panel) or MCY (bottom panel)  $> 75 \text{ ng g}^{-1}$  and  $1 \text{ ng g}^{-1}$ ,  
747 respectively, indicating risk when specified values of *chl-a* are exceeded. The black line  
748 represents mean probability. Dashed lines are lower and upper 95% confidence intervals from  
749 bootstrap (100 iterations).

750 Fig. 9. Probability of DO percent saturation  $< 80\%$  during the months of June-August in LSB as  
751 specified value of February – September mean *chl-a* is exceeded. The black line represents mean  
752 probability. Grey dashed lines are lower and upper 95% confidence intervals of bootstrap values  
753 (100 iterations).

#### 754 **Figure Legends (Supplemental)**

755 Fig. S1. Cumulative frequency distribution of minimum monthly DO by sub-embayment  
756 stations.

757 Fig. S2. Cumulative frequency distribution of (left panel) annual calendar mean and February-  
758 September *chl-a* for South and Lower South Bays as a proportion of site -years of 1993-2013 and  
759 (right panel) monthly *chl-a* by all sub-embayments as a proportion of site-years.

1 **Table 1. Slopes of quantile regressions at Tau= 0.1 and 0.5 by DO integrating period (May-July and June-August) and chlorophyll-a**  
2 **averaging period (February-May, June-September, and February-September mean chlorophyll-a). \* designates p< 0.1, \*\* designates**  
3 **p<0.05 and \*\*\* designates P< 0.01.**

Subembayment and DO  Integrating Period	Slope of Quantile Regressions and Significance Level					
	February-May Mean		June-September Mean		February-September Mean	
	0.1 Tau	0.5 Tau	0.1 Tau	0.5 Tau	0.1 Tau	0.5 Tau
<b>May-July</b>						
Lower South	0.06	-0.04	-0.22	-0.62**	-0.73**	-0.61**
South	-0.38***	-0.28***	-0.17	-0.58***	-0.78***	-0.73***
Central	-0.43	0.01	2.15***	0.74**	-0.73	0.15
North Central	-0.20	0.14	1.18	0.87	-0.84	0.85
San Pablo	-0.36	-0.44	-0.93	-0.58***	-0.77	-0.37
Suisun	-0.85	-0.57	-0.86	-0.45	-1.99***	-0.16
<b>June-August</b>						
Lower South	-0.14	-0.23***	0.62	0.39	-0.14	-0.20
South	-0.29***	-0.17***	0.16	-0.02	-0.60***	-0.39***
Central	-0.47	-0.10	0.74*	0.70**	0.27	0.27
North Central	-0.25	-0.13	0.98	0.60	0.39	0.39

San Pablo	-0.20	-0.11	-0.11	-0.36*	-0.33	-0.33
Suisun	0.02	0.05	-1.08	-0.82	0.49	-0.49

4

5 **Table 2. Comparison of mean and 95% CI (in parentheses) of predicted *chl-a* (mg m<sup>-3</sup>) from quantile**  
6 **regressions of February –September mean *chl-a* and May-July DO for specified DO benchmarks.**  
7 **80% saturation at a  $\tau= 0.5$  is equivalent to SFB’s percent saturation WQC. Predicted *chl-a* at  $\tau= 0.1$**   
8 **represent a 10% frequency of falling below a gradient of DO benchmarks from the literature (i.e.**  
9 **80%, 72%, 66% and 57% saturation, with corresponding to DO concentrations at mean summertime**  
10 **temperature of 15°C and salinity of 24 ppt in SB and LSB). All regressions were significant for  $p <$**   
11 **0.05 (Table 1).**

DO Percent saturation, with Equivalent DO Concentration	Predicted Mean <i>Chl-a</i> (95% CI)	
	LSB (N=48)	SB (N=161)
$\tau= 0.5$		
80% (~7 mg L <sup>-1</sup> )	15.6 (9.2 – 21.8)	17.3 (15.1 – 19.5)
$\tau = 0.1$		
80% (~ 7.0 mg L <sup>-1</sup> )	4.3 (-4.1 – 12.1)	14.3 (12.6 – 15.5)
72% (~6.3 mg L <sup>-1</sup> )	15.3 (5.3 – 29.3)	24.6 (21.9 – 24.7)
66% (~5.7 mg L <sup>-1</sup> )	23.5 (15.3 – 39.3)	32.3 (29.5 – 32.3)
57% (~ 5.0 mg L <sup>-1</sup> )	35.8 (30.3 – 54.3)	43.8 (40.5 – 45.9)
46% (~4.0 mg L <sup>-1</sup> )	50.9 (41.4 – 60.4)	57.9 (56.2 – 59.2)

12

13

14 **Table 3. Median values of dissolved inorganic nitrogen (DIN), measured *chl-a***  
15 **concentration, and potential *chl-a* concentration if all DIN was assimilated into additional**  
16 **phytoplankton biomass. Data from the USGS SFB water-quality measurement program**  
17 **for years 2000-2014.**

18

<b>Sub-embayment</b>	<b>DIN (<math>\mu\text{M}</math>)</b>	<b>Measured <i>Chl-a</i> (<math>\text{mg m}^{-3}</math>)</b>	<b>Potential <i>Chl-a</i> (<math>\text{mg m}^{-3}</math>)</b>
SUB	36.9	2.5	39.7
SPB	29.0	3.8	33.6
SB	31.4	5.5	39.2
LSB	57.5	7.5	67.0

19

20

21



## **SUPPLEMENTAL MATERIALS**

**Table A1. 10<sup>th</sup> Percentile of the vertical median and minimum summer (May-August) DO concentration over the period of 1993-2014 and percentage of time over that period that DO concentration was less than 5 mg L<sup>-1</sup>.**

<b>Sub-embayment</b>	<b>10th Percentile of Summer Vertical Median DO (mg L<sup>-1</sup>)</b>	<b>10th Percentile of DO Summer Vertical Minimum (mg L<sup>-1</sup>)</b>	<b>% of Time Summer DO &lt; 5 mg L<sup>-1</sup></b>
<b>Lower South Bay</b>	5.7	5.6	2.9
<b>South Bay</b>	5.9	5.8	0.5
<b>Central Bay</b>	6.5	6.5	0.2
<b>North Central Bay</b>	6.8	6.4	1.9
<b>San Pablo Bay</b>	7.1	7	0
<b>Suisun Bay</b>	7.8	7.7	0

**Table A4. Percent of site-events that fell below DO objectives of 3 month median < 80%**

**Saturation. Julian Day designates 3-month DO median aggregating period (e.g. Days 120-210 are May-July and 150-240 are June-August). N= Total number of site events.**

<b>Julian Day</b>	<b>Lower South</b>		<b>South</b>		<b>Central</b>		<b>No. Central</b>		<b>San Pablo</b>		<b>Suisun</b>	
	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
<b>30-120</b>	<b>63</b>	<b>0%</b>	<b>210</b>	<b>0%</b>	<b>82</b>	<b>0%</b>	<b>60</b>	<b>2%</b>	<b>126</b>	<b>1%</b>	<b>96</b>	<b>0%</b>
<b>60-150</b>	<b>63</b>	<b>0%</b>	<b>210</b>	<b>0%</b>	<b>82</b>	<b>0%</b>	<b>60</b>	<b>8%</b>	<b>126</b>	<b>4%</b>	<b>96</b>	<b>0%</b>
<b>90-180</b>	<b>63</b>	<b>6%</b>	<b>210</b>	<b>0%</b>	<b>82</b>	<b>2%</b>	<b>60</b>	<b>13%</b>	<b>126</b>	<b>3%</b>	<b>96</b>	<b>0%</b>
<b>120-210</b>	<b>61</b>	<b>18%</b>	<b>201</b>	<b>0%</b>	<b>80</b>	<b>5%</b>	<b>59</b>	<b>15%</b>	<b>126</b>	<b>1%</b>	<b>100</b>	<b>1%</b>
<b>150-240</b>	<b>54</b>	<b>28%</b>	<b>189</b>	<b>1%</b>	<b>77</b>	<b>0%</b>	<b>58</b>	<b>0%</b>	<b>126</b>	<b>0%</b>	<b>97</b>	<b>0%</b>
<b>180-270</b>	<b>53</b>	<b>13%</b>	<b>176</b>	<b>0%</b>	<b>73</b>	<b>0%</b>	<b>60</b>	<b>0%</b>	<b>126</b>	<b>0%</b>	<b>94</b>	<b>0%</b>
<b>210-300</b>	<b>50</b>	<b>6%</b>	<b>166</b>	<b>0%</b>	<b>68</b>	<b>0%</b>	<b>55</b>	<b>0%</b>	<b>117</b>	<b>0%</b>	<b>89</b>	<b>0%</b>

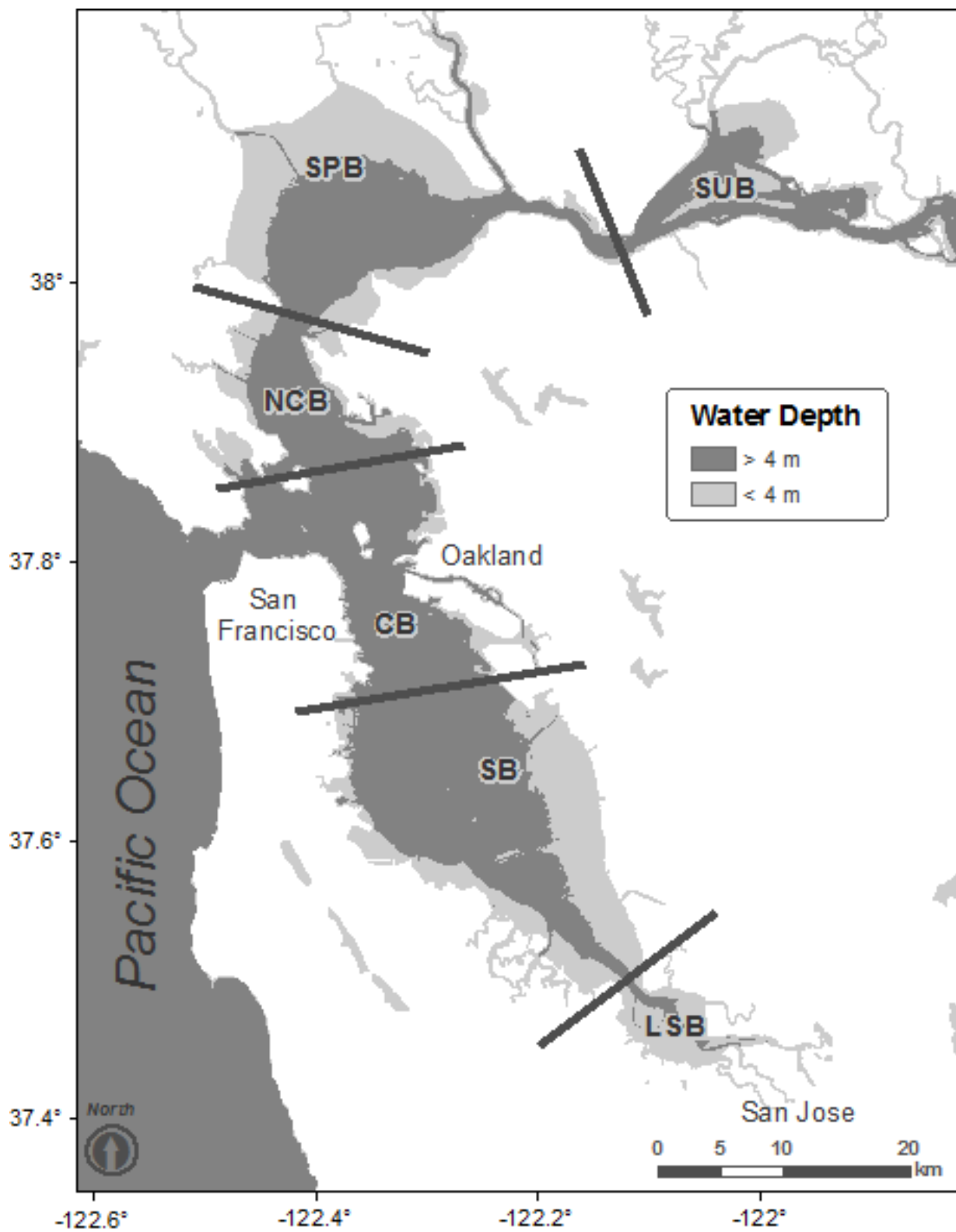


Fig. 1

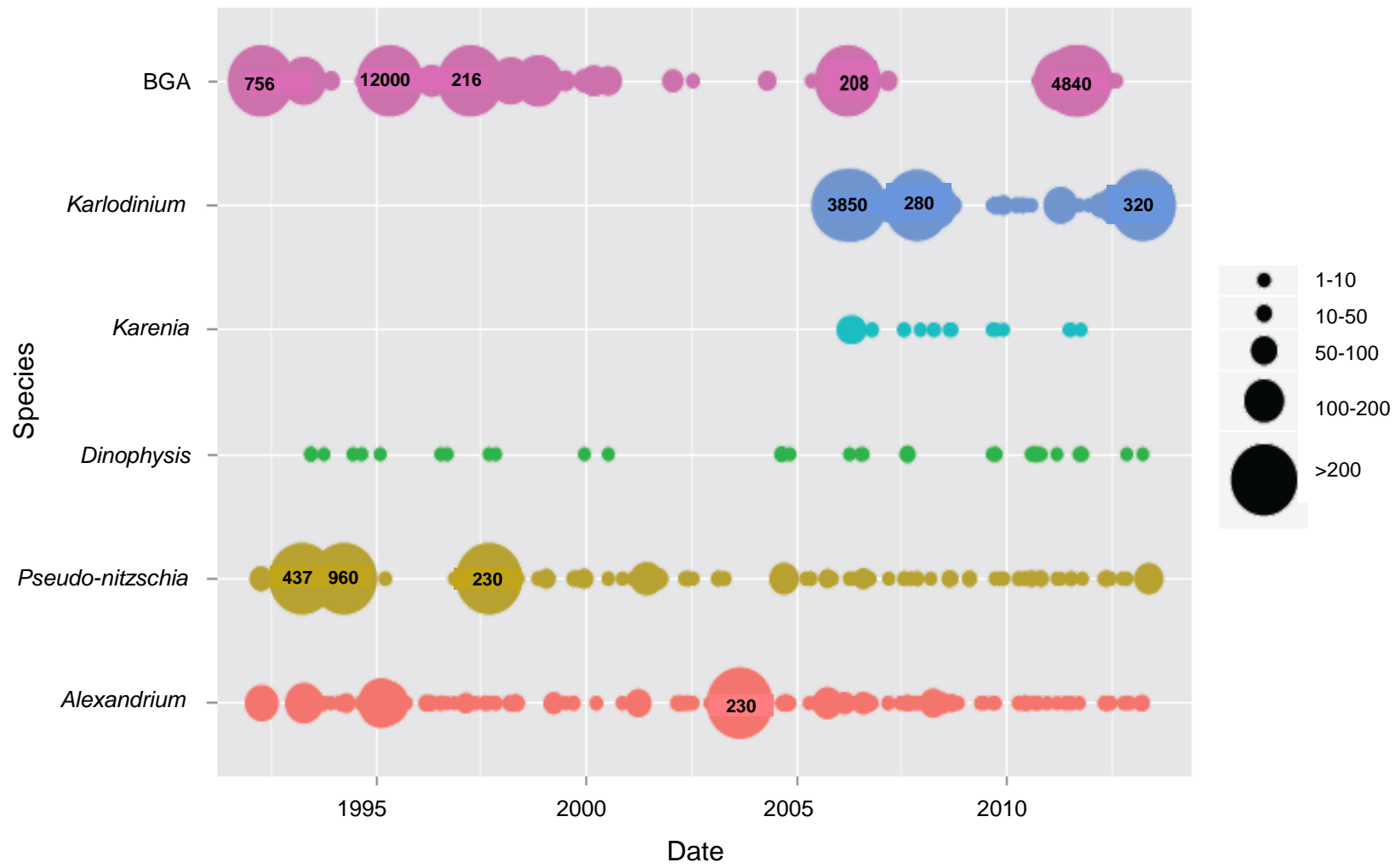


Fig. 2

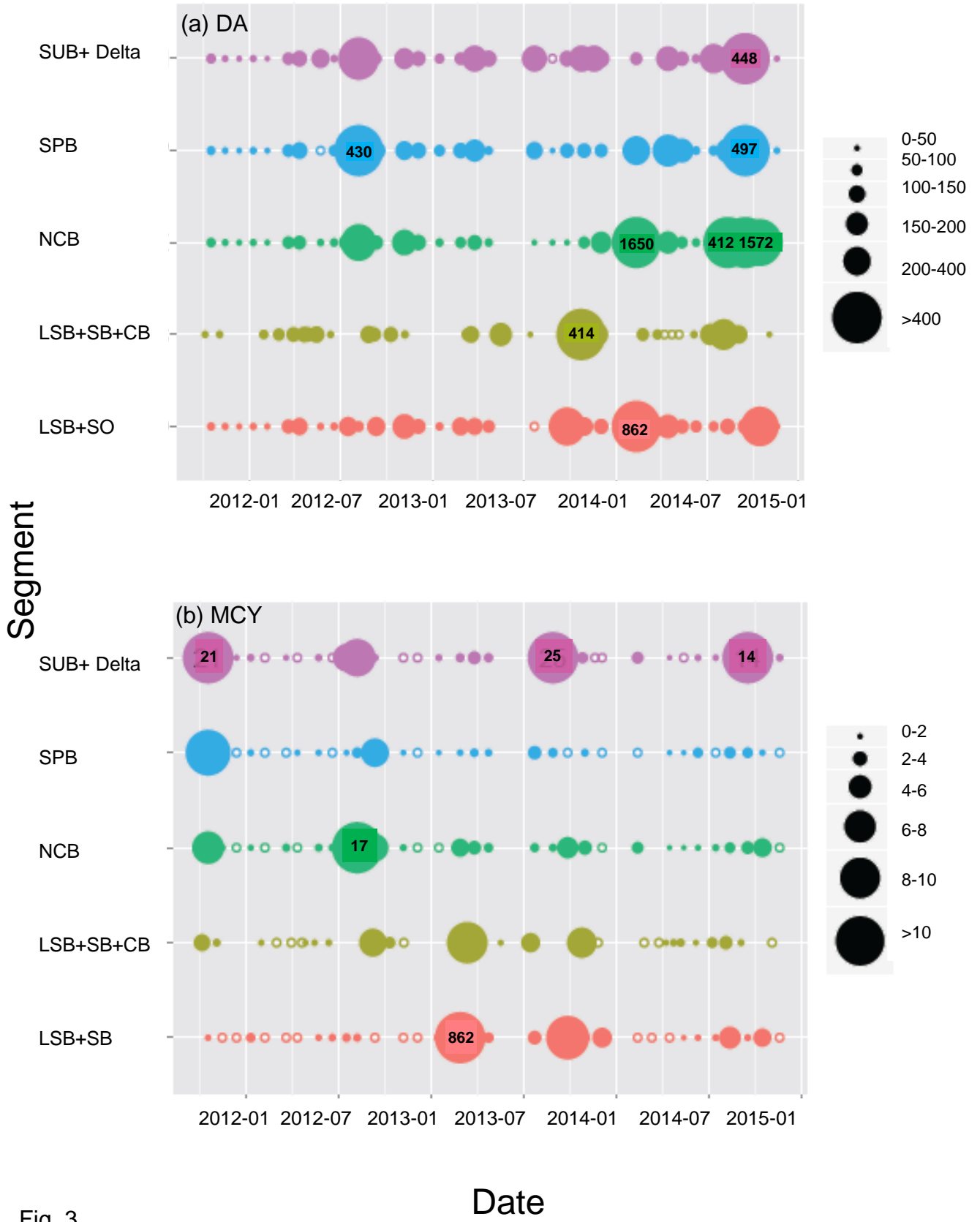


Fig. 3

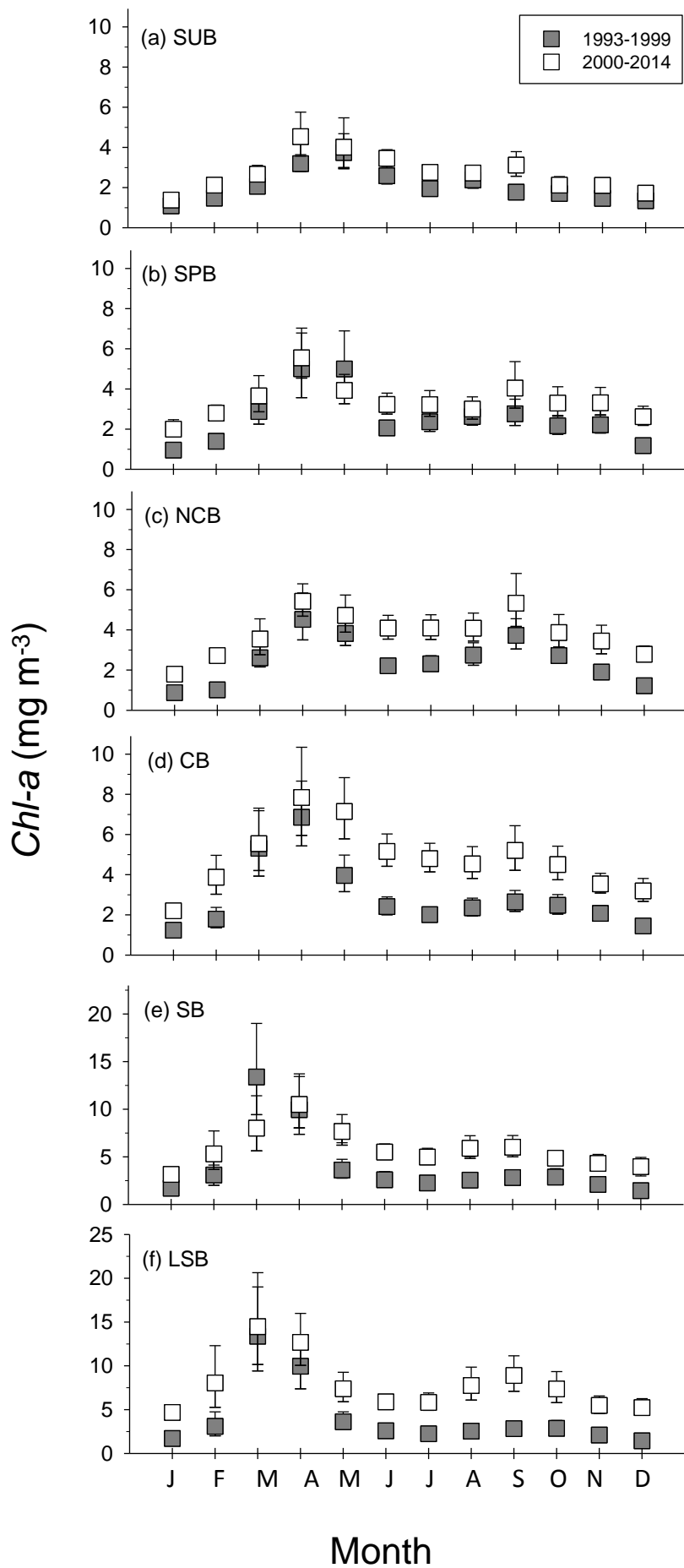


Fig. 4

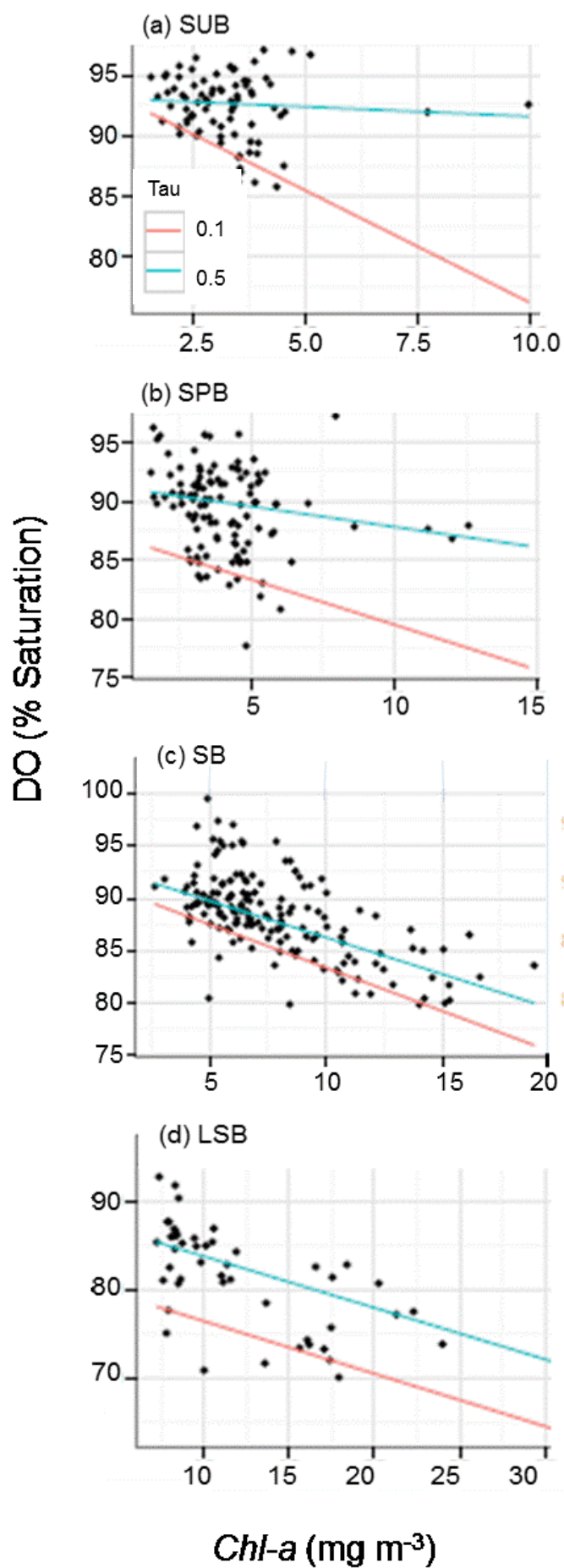


Fig. 5

*Chl-a* ( $\text{mg m}^{-3}$ )



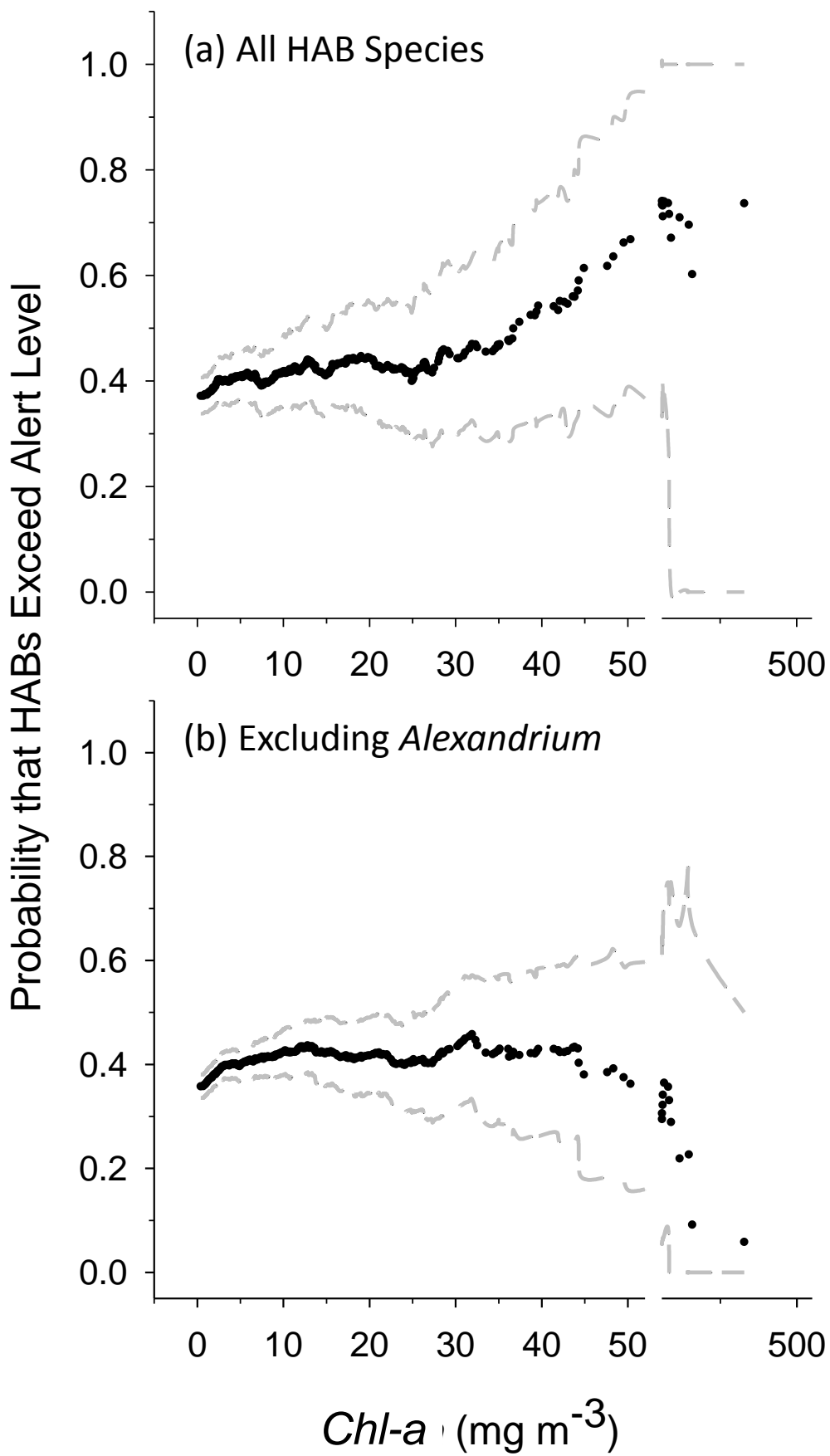


Fig. 6

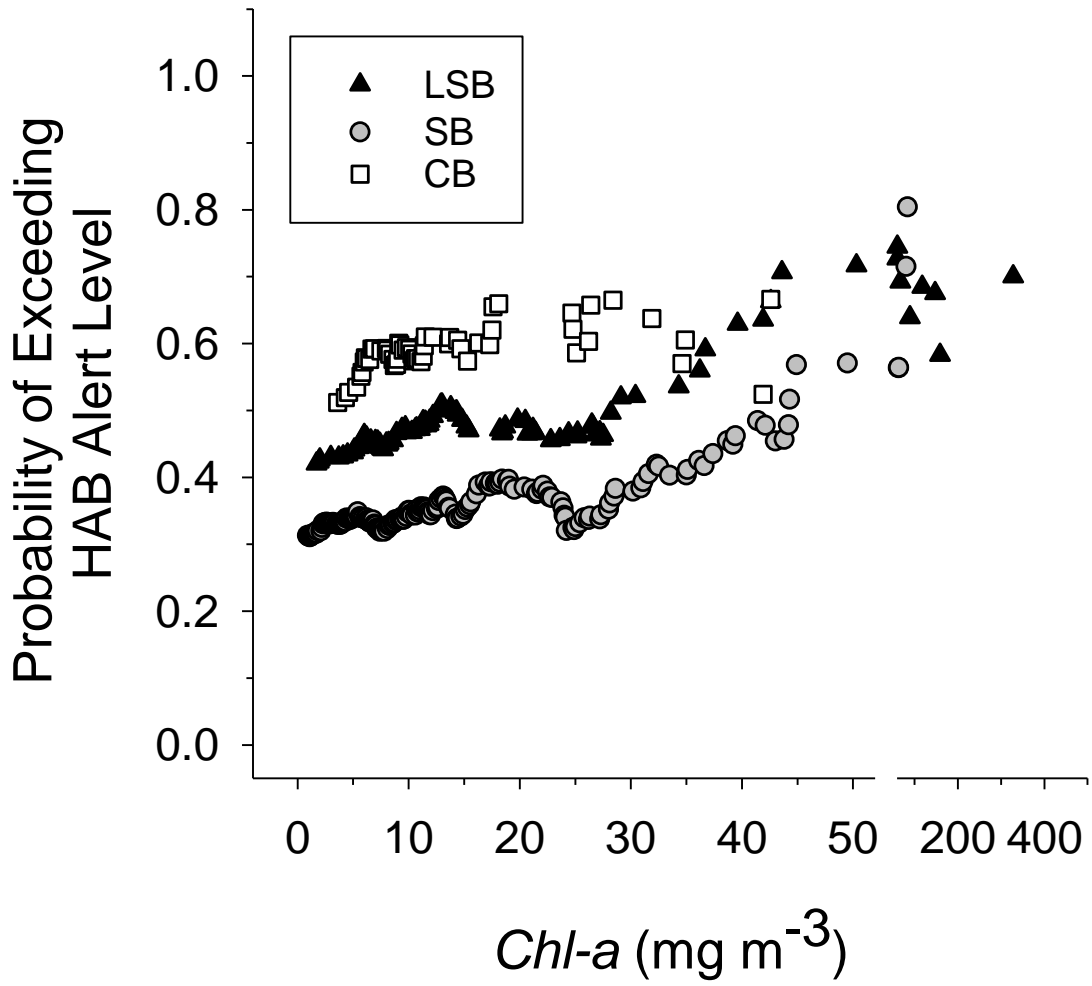


Fig. 7

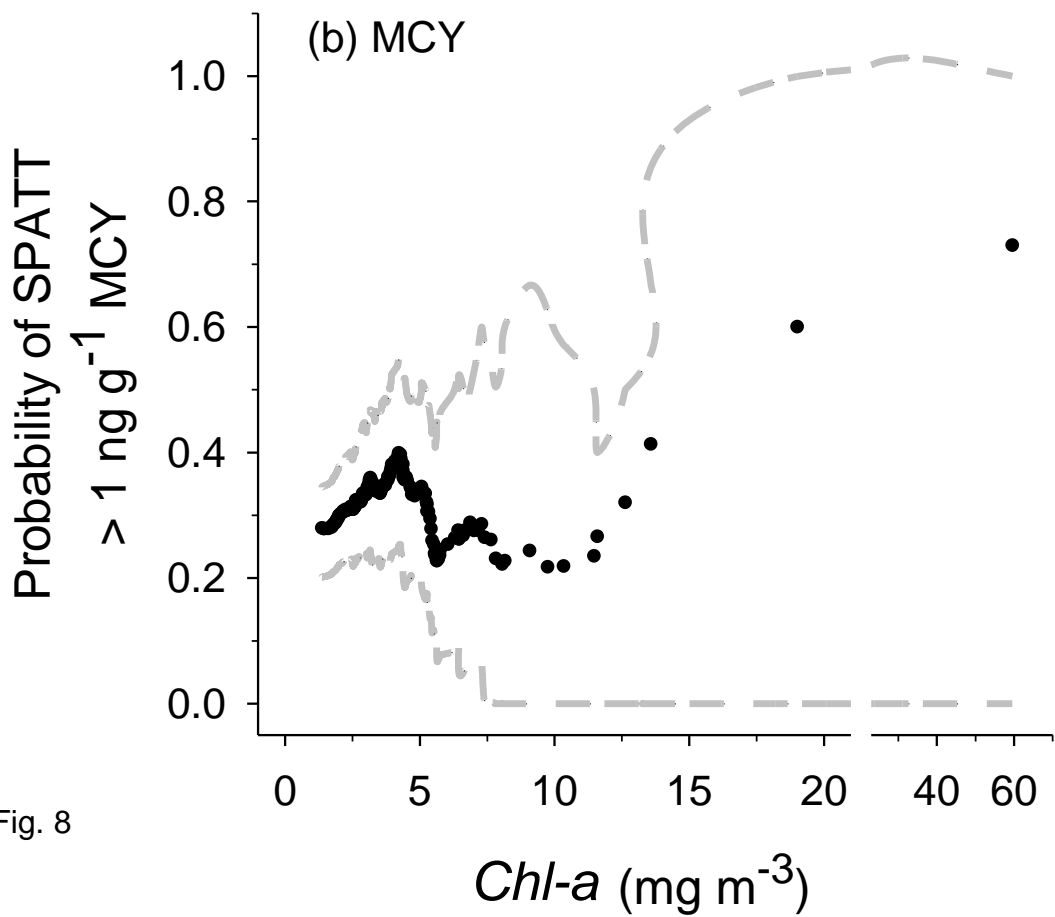
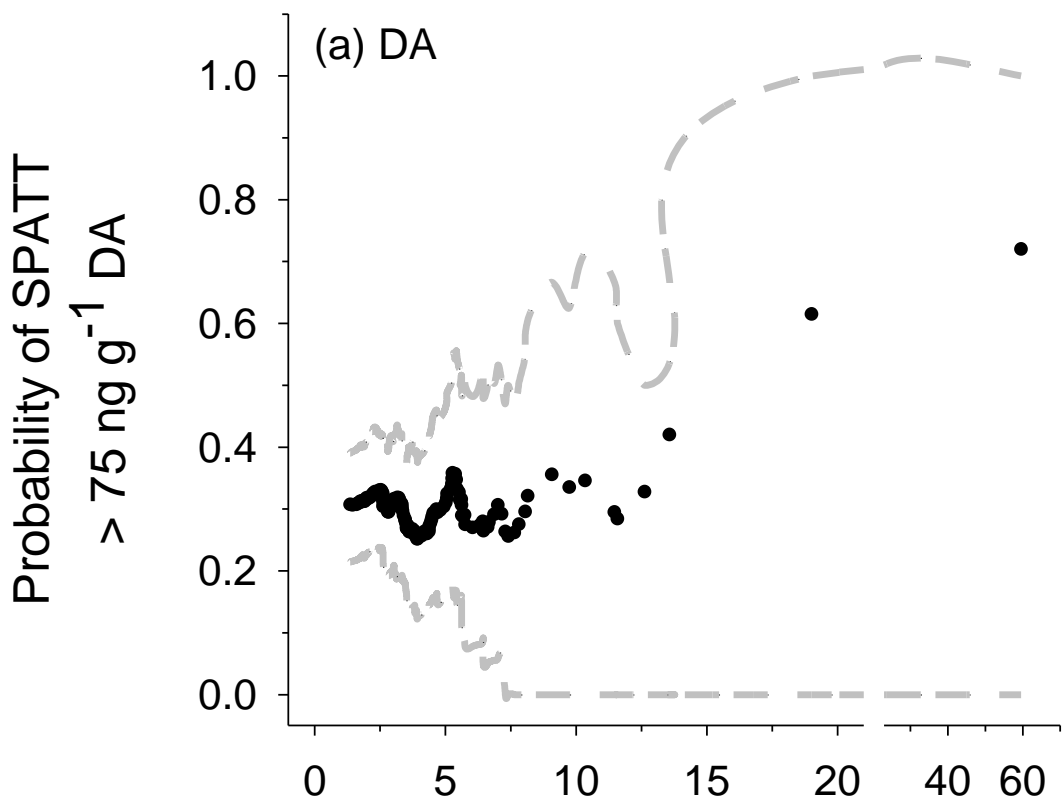


Fig. 8

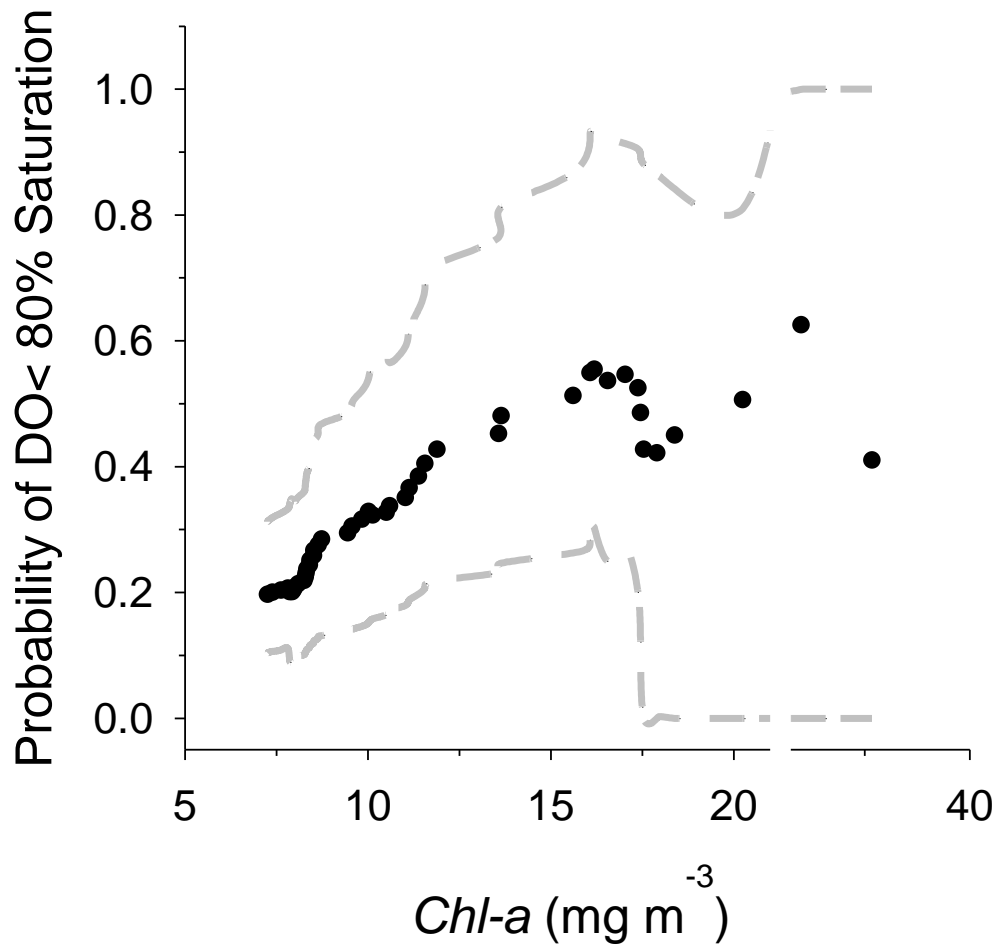


Fig 9