Nutrient tipping points for estuarine phytoplankton communities

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ABSTRACT

Nutrient tipping points may be indicative of the point (i.e., nutrient concentration) at which there is a fundamental change in the way phytoplankton respond to further increases in nutrient loading. The ecological implication is that phytoplankton community processes and functions may shift from one state to another alternate state at tipping points. Nutrient concentration tipping points for the total phytoplankton community as well as individual phytoplankton groups in response to increases in dissolved inorganic nitrogen (DIN) concentrations were determined for two different estuarine systems, the low (2 – 4) salinity Winyah Bay and the high (22 – 36) salinity North Inlet Estuary. Phytoplankton were exposed to increasing N addition scenarios (0 – 100 µmol DIN l^{-1}) under two irradiance exposure conditions. Response trajectories for the total phytoplankton community, group diversity, and individual algal groups were determined after 72 h incubations in experimental bioassays. Phytoplankton from the low salinity estuary exhibited higher DIN tipping points (> double) than those of the high salinity estuary while irradiance (20% vs. 40% of ambient) did not affect tipping points. Our results suggest that ambient nutrient concentrations should not exceed nutrient tipping point concentrations to prevent possible shifts from one ecological state to another. Conservative estimates for maximum DIN concentrations for the high salinity estuary would be ca. 25 µmol l^{-1} and ca. 50 µmol l^{-1} for the low salinity estuary. These levels should be below the threshold for major alterations in phytoplankton community structure and function in these two estuarine systems.

INTRODUCTION

Fixed nitrogen (N) is usually the primary nutrient controlling or "limiting" estuarine and coastal phytoplankton primary production (Nixon 1995; Dame et al. 2000; Pinckney et al. 2001a). Anthropogenically-generated N compounds from agricultural, urban, and industrial expansion are key drivers of accelerating eutrophication by phytoplankton in N-sensitive waters (Nixon 1995; Vitousek et al. 1997; Bricker et al. 1999; Paerl et al. 2003; Bricker et al. 2008; Paerl et al. 2014). Development, agriculture, and a growing human population in the coastal zone has resulted in a general decline in water quality in nearby estuaries (Bricker et al. 1999). Concerns about this decline have led to the need to determine criteria to ensure acceptable water quality conditions for both biota and recreational uses (GASCET 2015). Consequently, state management agencies have been tasked with establishing nutrient criteria for impacted aquatic systems. A major component of numeric nutrient criteria modeling is the understanding of the quantitative relationship between nutrient loading and phytoplankton productivity responses, in terms of both total biomass and community composition (GASCET 2015).

An ecological tipping point or threshold can be defined as a bifurcation between alternate states that results in a system changing to a different state (Huggett 2005). These points are signals for a system rapidly changing from one condition to another due to a relatively small perturbation or minor trigger (Scheffer et al. 2012; Lenton 2013). Tipping points may be caused by a reduction in ecosystem resilience, resulting in regime shifts, loss of functional group diversity, and critical transitions (Holling 1973; Scheffer et al. 2001; Folke et al. 2004; Lenton 2013). Alterations in some control parameter frequently results in a qualitative change in steady state after passing the tipping point (Lenton 2013). Systems that exhibit multiple stable states or alternate states frequently have distinct tipping points (Scheffer et al. 2001; Folke 2004; Lenton 2013). In

addition, regime shifts in many ecosystems are preceded by a tipping point (de Young et al. 2008; Vanacker et al. 2015). A common consequence of these changes is that the system shifts from a desired to less desired state in its capacity to generate ecosystem services (Folke et al. 2004). In many cases, these regime shifts appear to be irreversible (Folke et al. 2004). In the current study, we define a tipping point as the nutrient concentration at which there is a significant change in the trajectory (i.e., the slope of the response) of phytoplankton biomass and community composition responses to increasing nutrient concentrations. The response (trajectory change) following a tipping point may be either a marked increase or decrease in the rate of change in diversity or algal group abundance with increasing nutrient concentration. Thus, tipping points identified in this study are indicative of the point (i.e., nutrient concentration) at which there is a fundamental change in the way that phytoplankton respond to further increases in nutrient concentrations. The ecological implication is that the phytoplankton community processes and functions have possibly shifted from one state to another alternate state.

Predicting tipping points in complex environmental systems using empirical data is difficult due to the large number of species and interactions, the stochastic nature of ecosystems and their drivers, and initial conditions (Scheffer et al. 2012; Lenton 2013; Moore 2018). However, phytoplankton communities are good systems to use for empirical determinations of tipping points due to their relatively rapid response times (days) and sensitivity to environmental alterations (Glibert et al. 2014; Vanacker et al. 2015). Knowledge of tipping points can be useful for establishing and prioritizing biodiversity conservation and natural resource management targets and actions (Huggett 2005).

86 Chlorophyll *a* (chl *a*), the primary photosynthetic pigment in microalgae, provides a surrogate
87 measure of phytoplankton biomass that is commonly used to indicate the designated endpoint for
88 aquatic community structure and function. Water quality regulatory criteria rely almost
89 exclusively on this relatively arbitrary bulk measure of phytoplankton biomass (i.e., chl *a*).
90 Typically, concentration thresholds for water quality management are set at >20 µg chl *a* l^{-1} (Van
91 Meerssche and Pinckney 2019). However, criteria based on the ecological characteristics of the
92 phytoplankton community offer a more valid, process-based approach for managing water
93 quality. In particular, the identification of nutrient tipping points for chl *a*, diversity, and
94 individual algal groups may better characterize phytoplankton responses to changes in limiting
95 nutrient concentrations.

96 Previous research in Winyah Bay and North Inlet Estuary (Fig. 1) indicates that the waters of the
97 bay and estuary are consistently N-limited with respect to phytoplankton growth (Ranhofer et al.
98 2009; Richardson et al. 2009; Gilde and Pinckney 2011; Allen et al. 2014; Kline and Pinckney
99 2014; Reed et al. 2016; Pinckney et al. 2017). Although turbid, with periodically high
100 concentrations of humics, phytoplankton growth is likely not light-limited due to the high
101 vertical mixing rates driven by tides and winds in the generally shallow waters (< 4 m) of the bay
102 (Yoder and Bishop 1985; Cloern 1987; Alpine and Cloern 1988; Mallin and Paerl 1992). Thus,
103 the concentration of fixed N in this estuary is most likely the major control of phytoplankton
104 concentrations and should be the primary target in the development of numeric nutrient criteria
105 for these systems (Van Meerssche and Pinckney 2019).

106 The present study uses a quantitative, empirical approach for predicting the magnitude of
107 phytoplankton group-specific (i.e., diatoms, cyanobacteria, dinoflagellates, chlorophytes, etc.)
108 responses to a range of nutrient loading conditions. In addition to the applied utility of this study

for water quality management, our results are novel in that we determine nutrient response tipping points for a natural phytoplankton assemblage in terms of total biomass (chl *a*) and group diversity as well as for individual algal groups within the assemblage. This approach allows the resolution of the responses of different phytoplankton groups within a mixed natural assemblage. We are unaware of any other published studies quantifying estuarine tipping points for different algal groups in natural estuarine phytoplankton assemblages.

Our primary objective was to determine nutrient concentration tipping points for the total phytoplankton community as well as individual phytoplankton groups in response increases in total N concentrations. Experimental bioassays of natural phytoplankton communities were exposed to increasing N concentration scenarios under both high and low light exposure conditions to quantify phytoplankton response trajectories. The goal of the study was to provide phytoplankton tipping points for consideration in nutrient management strategies for Winyah Bay and North Inlet Estuary and offer a "proof of concept" approach for determining ecologically based water quality criteria in similar estuarine systems.

MATERIALS AND METHODS

Study Location

North Inlet Estuary is a relatively undisturbed (by local anthropogenic factors) euhaline (25 – 40) bar-built system that receives limited freshwater input (ca. 1 – 5 $m^3 s^{-1}$) from a small protected watershed (3800 ha) (Gardner et al. 2006; Allen et al. 2014)(Fig. 1). Semidiurnal tides average 1.5 m and ca. 70% of the water volume in the estuary is exchanged with each tide, leading to a hydrodynamic turnover of ca. 15 h (Kjerfve 1986; Allen et al. 2014). The 33 km^2 estuary is

131 composed of an extensive tidal creek system bordered by the saltmarsh cordgrass *Sporobolus*
132 *alterniflorus (Spartina alterniflora)*. Sediments are primarily muddy sand and intertidal oyster
133 (*Crassostrea virginica*) reefs are common.

134 Winyah Bay is the third largest estuary on the east coast of the US based on the 4.7 million ha
135 forested and agricultural watershed area (Fig. 1). The Pee Dee, Black, and Sampit Rivers merge
136 with the Waccamaw River to form the bay. The estuary, which is ca. 30 km long and varies in
137 width from 1 to 7 km, has a surface area of 15,500 ha and a mean depth of 4.2 m. An 8.2 m deep
138 ship channel extends from the Port of Georgetown to the jetties at the mouth of the bay. Winyah
139 Bay is a Class B, partially-mixed drowned river valley estuary with a mean annual riverine input
140 of 450 m^3 s^{-1}. During storm and flood conditions, inputs may exceed 7,800 m^3 s^{-1}. The average
141 tidal range in the lower bay is 1.4 m and decreases to 1.2 m 18 km up the bay. Surface current
142 velocities often exceed 2 m s^{-1} and facilitate strong vertical and horizontal mixing of the water
143 column. Salinity varies from near 0 at the tidal freshwater river mouths to 36 at the bay entrance,
144 resulting in a salinity gradient that changes with tidal stage and meteorological conditions.
145 Winyah Bay is relatively impacted by anthropogenic activities (Bergquist et al. 2009; Allen et al.
146 2014). Extensive agriculture in its watershed accounts for total nitrogen concentrations that are,
147 on average, double that of North Inlet Estuary (Buzzelli et al. 2005; Table 1).

148 **Bioassays**

149 Ten nutrient addition bioassays were conducted in May, June, July, November in 2018 and
150 February and April of 2019 (Table 1). Phytoplankton-nutrient responses were measured using
151 bioassays of natural phytoplankton communities collected in North Inlet Estuary at Clambank
152 Landing (33.3339° N, 79.1930° W; CB site) and in Winyah Bay (33.3094° N, 79.2888° W; WB
153 site) (Fig. 1). Salinities at CB ranged from 22 - 36 compared to 2 – 4 at WB, depending on

154 sampling date (Table 1). Surface water (0.5 m depth) from both locations was dispensed into
155 250 ml clear polystyrene culture flasks (VWR, cat. # 10062–862) for the bioassays.

156 The fixed nitrogen (N) treatment for the bioassays consisted of a range of concentrations (1 –
157 100 µmol total N l^{-1} additions in increments of 5 to 10 µmol N l^{-1}) and were composed of an
158 equimolar mixture of nitrate (NO_3^-), ammonium (NH_4^+), and urea ($CO(NH_2)_2$) to simulate the
159 various types of N compounds likely available in the estuaries. Phosphate (as orthophosphate,
160 PO_4^{3-}) was added to all treatments in a ratio of 4N:1P to prevent P limitation or co-limitation.
161 Controls consisted of sample water without any addition of nutrients. Inorganic nutrients (N, P)
162 and total N were measured at the initiation the bioassays to calculate actual N exposure levels
163 (Table 1).

164 The flasks were submerged in temperature (ambient) controlled, flowing water tables and
165 incubated for 72 h at 40% (high light) or 20% (low light) of ambient solar irradiance (Table 1).
166 Light levels were achieved by shading the water tables with gray fiberglass neutral density
167 screen. The nutrient treatment levels and controls consisted of 5 replicates. Thus, each bioassay
168 consisted of 125 measurements of phytoplankton biomass and community composition over a
169 range of nutrient conditions. At the end of the incubations, samples were vacuum (-50 kPa)
170 filtered onto 2.5 cm GF/F glass microfiber filters and stored at -80˚C.

171 **Nutrient Analyses**

172 Nutrient concentrations were measured in the incubation water to determine ambient
173 concentrations and thereby quantify the exact N loading levels for the bioassays. Filtered (0.2
174 µm) samples were analyzed for total nitrogen (TN), total phosphorus (TP), orthophosphate
175 (PO_4^{3-}), nitrate + nitrite (NO^-_{2+3}), and ammonium (NH_4^+) using a Seal Analytical nutrient

AutoAnalyzer3. The TN and TP method was based on an alkaline potassium persulfate oxidation procedure (SM 4500-N C; Eaton et al. 2005) that converts all N in the sample to nitrate (NO_3^-) and all P to orthophosphate (PO_4^{3-}). SM 4500-N C was developed for the digestion of a whole or filtered water sample for the determination of N alone. However, its efficacy for the digestion of both total N and P is well-proven (Valderrama 1981, Gross & Boyd 1998). Concentrations of dissolved organic nitrogen (DON) and phosphorus (DOP) were calculated by difference between total and inorganic fractions, as determined by autoanalyzer. The basic analytical methods used for the determination of inorganic N were Standard Methods 4500-NH_3 G, 4500-NH_3 F, 4500-NO_3^- E, 4500-NO3$^-$ F (Eaton et al. 2005). The basic analytical methods used for the determination of inorganic P were Standard Methods 4500-P E and 4500-P F. Modifications of these methods included the recommendations of Loder (1978) for small sample volumes and Glibert et al. (1977) for samples in a saltwater matrix.

Phytoplankton Analyses

Phytoplankton photopigment concentrations were measured using HPLC (Roy et al. 2011). Filters were lyophilized for 24 h at -50 °C and extracted with 750 µL of 90% aqueous acetone solvent, followed by storage for 24 h at -20°C. Filtered (0.4 µm) extracts were injected (250 µL) into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing) and a polymeric (Vydac 201TP54, 0.46×25 cm, 5 µm packing) reverse-phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol : 20% 0.5 M ammonium acetate) and solvent B (80% methanol : 20% acetone) was used for the mobile phase (Pinckney et al. 2001b). Absorption spectra and chromatograms (440 ± 4 nm) were obtained at 2 second intervals using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure

199 standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma 10810) was
200 used as an internal standard. QA/QC procedures are outlined in Hooker et al. (2010).

201 The software ChemTax (v. 1.95) was used to determine the relative concentrations of major
202 phytoplankton groups based on measured photopigment concentrations (Pinckney et al. 2001b;
203 Higgins et al. 2011). This procedure partitions total chl *a* into the contribution of different algal
204 groups (e.g., diatoms, cyanobacteria, dinoflagellates, etc.). A two-step cluster analysis procedure
205 based on log-likelihood distance measures of photopigment variables was used to define
206 homogeneous groups for separate bins for ChemTax analyses (SPSS v. 26) (Higgins et al 2011).
207 Cluster analysis identified 3 separate clusters of 756 (60.5%), 253 (20.3%), and 241 (19.2%)
208 samples each. The five most important pigments for discriminating clusters were, in order from
209 highest to lowest, alloxanthin, chlorophyll c_2, fucoxanthin, 9' cis-neoxanthin, and violaxanthin.
210 Bioassay samples were examined by qualitative microscopy to confirm algal groups included in
211 the ChemTax analysis. The initial pigment ratio matrix used for this analysis was a combination
212 of matrices provided by Mackey et al. (1996), Lewitus et al. (2005), and Schlüter et al. (2000)
213 (Supplements 1 - 3). The initial ratio matrix randomization procedure outlined by Higgins et al.
214 (2011) was used to minimize errors in algal group biomass due to inaccurate pigment ratio seed
215 values. ChemTax provided estimates of the relative concentrations of major algal groups (e.g.,
216 chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, etc.) in units of μg chl *a* l^{-1}.

217 **Group Diversity Index**

218 Phytoplankton group diversity was calculated using the Shannon-Wiener diversity index (H')
219 (Krebs 1999; Clarke and Gorley 2001),

220 $$H' = -\sum p_i \ln_e (p_i)$$

where p_i is the proportion of the total group abundance (concentration of algal group in units of µg chl *a* l^{-1}) from the i^{th} group. Seven algal groups were used for diversity calculations (chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, euglenophytes, and prasinophytes) based on qualitative microscopic examinations of samples.

Tipping Point Analyses

Tipping points in phytoplankton vs. dissolved inorganic nitrogen (DIN; $NO_2^- + NO_3^- + NH_4^+$) concentration plots were determined using the *segmented* R package (Muggeo 2008; Vanacker et al. 2015; R Core Team 2019). This analysis uses broken-line linear regression models to determine relationships between the response variable (phytoplankton diversity, biomass, or group concentrations) and an explanatory variable (DIN concentration). Two or more lines are connected at unknown values referred to as breakpoints (e.g., tipping points). The result of the analysis is two or more slope parameters and breakpoint(s) where the linear regression relationship changes. The breakpoint (and standard error of the point) is estimated by assessing a relevant gap and a 'difference in slope' coefficient in the linear predictor (Vanacker et al. 2015). If there is no breakpoint, the difference in slope parameter is 0 and the result is a single linear regression equation. A complete discussion of this statistical method is detailed in Muggeo (2008).

RESULTS

Total Phytoplankton Biomass

Scatterplots of total phytoplankton biomass (chl *a*) vs. concentrations of DIN additions were analyzed to identify tipping points for the community. For the CB site, DIN tipping points

243 ranged from 8.9 to 64.8 µmol DIN l^{-1} (Table 2, Fig. 2). The medians for the high and low
244 irradiance exposures were 16.6 and 26.2, respectively (Table 3). Response trajectories were
245 generally rapidly increasing up to the tipping points, then showed a range of positive, negative,
246 or no change after the tipping point, depending on the bioassay date and exposure irradiance.

247 Similarly, tipping points ranged from 36.9 to 93.6 µmol DIN l^{-1} for the WB site, with high and
248 low irradiance medians of 63.3 and 39.7, respectively (Fig. 3, Table 3). Response vectors were
249 also variable between bioassay dates and irradiance levels. A comparison of tipping point
250 estimates for pooled high vs. low salinities and irradiances indicated that, although the tipping
251 points at the WB site appeared somewhat higher, they were not different (Kruskal-Wallis one-
252 way ANOVA, $X^2_{3df} = 7.06$, $p = 0.070$) (Fig.4). Likewise, the tipping points for the paired high
253 vs. low irradiance exposures were not different (Paired Sample Wilcoxon Signed Ranks Test, Z
254 = 0.237, p = 0.813). However, when the high and low irradiance treatments were pooled for
255 each site, the WB site had higher DIN tipping point values than the CB site (Mann-Whitney U
256 test, U = 12, Z = -2.49, p = 0.013). For the WB site, the median DIN tipping point was 49.4
257 µmol DIN l^{-1} compared to 20.3 for the CB site.

258 **Phytoplankton Community Composition**

259 The concentrations of photopigments were processed with ChemTax to determine the relative
260 concentrations of 7 algal groups (Supplements 1 - 3). The phytoplankton community
261 composition at the initiation (i.e., time 0) of the bioassays for the respective sampling dates and
262 locations are provided in Figs. 5 and 6. Diatoms were consistently the most abundant
263 community component for all bioassays. Other groups were present at low concentrations,
264 typically in the range of 0 to 2 µg chl *a* l^{-1}.

265 **Algal Group Diversity**

266 The Shannon group diversity index was calculated for experimental treatments in each of the
267 bioassays using the ChemTax-derived algal group concentrations (Table 2). Tipping points
268 could not be resolved for 2 of the 6 bioassays conducted for the CB site. For those that were
269 determined, the DIN tipping points ranged from 13.9 to 55.4 µmol DIN l^{-1}, with high and low
270 irradiance medians of 36.2 and 18.7, respectively (Fig. 7, Table 3). Following tipping points,
271 group diversity trajectories showed high variability in both magnitude and direction, depending
272 on bioassay date and irradiance level.

273 For the WB site, tipping points ranged from 25.2 to 91.8 µmol DIN l^{-1} with high and low
274 irradiance medians of 66.4 and 46.0, respectively (Fig. 8, Table 3). Group diversity changes after
275 tipping points were also variable depending on the bioassay date and irradiance level. DIN
276 tipping points for group diversity were not different between the two sites (Kruskal-Wallis one-
277 way ANOVA, $X^2_{3df} = 3.81$, $p = 0.283$) or when high light vs. low light paired samples were
278 compared (Paired Sample Wilcoxon Signed Ranks Test, $Z = 1.468$, $p = 0.156$) (Fig. 8).
279 However, there was the hint of a trend toward higher DIN tipping points for the WB site.

280 **Algal Groups**

281 *Chlorophytes* – DIN tipping points for chlorophytes ranged from 9.3 to 59.1 µmol DIN l^{-1} for the
282 CB site and from 66.4 to 93.5 µmol DIN l^{-1} at the WB site (Table 2, Fig. 9). The WB site had
283 higher DIN tipping points (Kruskal-Wallis one-way ANOVA, $X^2_{3df} = 12.77$, $p = 0.005$). Median
284 DIN tipping points for the pooled data for the CB site were 27.3 (high irradiance) and 38.8 (low
285 irradiance) (Table 3). For the WB site, medians were 72.8 and 72.4 for high and low irradiances,
286 respectively (Table 3). A paired comparison of high vs. low irradiance exposures for each

287 bioassay also indicated that there was no effect of irradiance on the DIN tipping points (Paired
288 Sample Wilcoxon Signed Ranks Test, Z = 1.468, p = 0.156).

289 *Cryptophytes* – Cryptophyte DIN tipping points ranged from 9.5 to 61.5 (medians = 36.8 and
290 32.4 for high and low irradiances, respectively) at the CB site (Tables 2, 3). At the WB site,
291 values ranged from 37.9 to 78.2 (medians = 75.0 and 47.9 for high and low irradiances,
292 respectively). DIN tipping points were not significantly different for the 2 sites and 2 irradiance
293 levels (Kruskal-Wallis one-way ANOVA, $X^2_{3df} = 6.22$, p = 0.102). However, graphical analysis
294 suggests that, like the chlorophytes, DIN tipping points tended to be higher at the WB site (Fig.
295 9). An analysis of the paired incubation irradiances indicated that the DIN tipping points were
296 higher in the high irradiance exposures (Paired Sample Wilcoxon Signed Ranks Test, Z = 2.141,
297 p = 0.032).

298 *Cyanobacteria* – Median tipping points for high and low irradiance exposures were 29.5 and
299 27.6 µmol DIN l^{-1} at the CB site and 57.5 and 40.9 at the WB site, respectively (Table 3).
300 Tipping points ranged from 7.3 to 83.3 µmol DIN l^{-1} for all bioassays (Table 2). Tipping points
301 at the WB site were marginally lower than those for the CB site (Kruskal-Wallis one-way
302 ANOVA, $X^2_{3df} = 7.81$, p = 0.050) and paired-comparisons of irradiance exposures indicated that
303 tipping points were lower in the low irradiance treatments (Paired Sample Wilcoxon Signed
304 Ranks Test, Z = 2.097, p = 0.036)(Fig. 9).

305 *Diatoms* – Diatoms were the most abundant group of phytoplankton in all of the bioassays, often
306 comprising > 75% of the total phytoplankton chl *a*. At the CB site, DIN tipping points ranged
307 from 8.6 to 58.5 µmol DIN l^{-1} compared to 36.9 to 85.8 at the WB site (Fig. 9, Table 2). Paired
308 comparisons of high vs. low irradiance tipping points were not different (Paired Sample
309 Wilcoxon Signed Ranks Test, Z = 1.262, p = 0.211). Pooled tipping points were also not

310 different (Kruskal-Wallis one-way ANOVA, $X^2_{3df} = 5.14$, $p = 0.162$), but also showed the trend
311 of higher tipping points for the WB site (Fig. 7). Median tipping point values for high and low
312 irradiance exposures for the CB site were 33.7 and 32.5 vs. 63.3 and 39.6 for the WB site,
313 respectively (Table 3).

314 *Dinoflagellates* – DIN tipping points ranged from 6.3 to 81.4 for all the bioassays (Fig. 9, Table
315 2) and there was no difference between the treatment levels (Kruskal-Wallis one-way ANOVA,
316 $X^2_{3df} = 2.02$, $p = 0.569$). Median tipping point values for the CB site and high and low irradiance
317 exposures were 39.1 and 40.4 μmol DIN l^{-1}, respectively compared to 54.2 and 49.1 at the WB
318 site (Table 3). Tipping points in paired high and low irradiance exposure samples were also not
319 different (Paired Sample Wilcoxon Signed Ranks Test, $Z = 0.490$, $p = 0.624$).

320 *Euglenophytes* – For euglenophytes, the DIN tipping points ranged from 5.8 to 53.4 μmol DIN l^{-1}
321 at the CB site. In comparison, the range for the WB site was 39.7 to 93.7 (Table 2). Tipping
322 points were different for the 4 treatments (Kruskal-Wallis one-way ANOVA, $X^2_{3df} = 10.54$, $p =$
323 0.015). *A posteriori* comparisons identified two homogeneous groups: 1) CB - high irradiance,
324 WB - high irradiance, and WB – low irradiance and 2) CB – high irradiance and CB – low
325 irradiance (Bonferroni, $p < 0.05$)(Fig. 9). Median values for DIN tipping points at the CB sites
326 were 42.5 (high irradiance) and 20.4 (low irradiance). At the WB site, medians were 66.6 and
327 54.5 for high and low irradiances, respectively (Table 3). Comparisons of paired high vs. low
328 irradiance exposures were not different (Paired Sample Wilcoxon Signed Ranks Test, $Z = 1.190$,
329 $p = 0.234$).

330 *Prasinophytes* – At the CB site, DIN tipping points ranged from 9.5 to 55.2 μmol DIN l^{-1}
331 compared to 34.2 to 93.7 at the WB site (Fig. 9, Table 2). The 4 treatment levels had different
332 DIN tipping points (Kruskal-Wallis one-way ANOVA, $X^2_{3df} = 8.97$, $p = 0.030$)(Fig. 7). Two

333 homogeneous groups were identified in the *a posterior* comparisons: 1) WB – high irradiance
334 and WB – low irradiance and 2) CB – high irradiance, CB – low irradiance, and WB – low
335 irradiance. DIN tipping point median values for the CB site were 19.6 and 26.6 for high and low
336 irradiances, respectively (Table 3). At the WB site, medians were 26.6 and 55.7 µmol DIN l^{-1}
337 for high and low irradiances, respectively. Paired comparisons of high vs. low irradiance
338 exposures revealed no difference in DIN tipping points for the respective treatments (Paired
339 Sample Wilcoxon Signed Ranks Test, Z = 0.850, p = 0.933).

340 **Algal Group Tipping Points Comparisons**

341 DIN tipping points for all 7 algal groups were compared after pooling the high and low
342 irradiance responses for each bioassay using a Kruskal-Wallis one-way ANOVA. Tipping points
343 were not different between algal groups for either the CB ($X^2_{5df} = 2.66$, p = 0.085) or WB (X^2_{3df}
344 = 6.95, p = 0.326) sites, likely due to the high variability in nutrient responses. However,
345 pooling all 7 groups for each site, DIN tipping points were higher at the WB site compared to the
346 CB site ($X^2_{1df} = 36.50$, $p < 0.001$). As a final summary, DIN tipping points were pooled (i.e.,
347 high & low irradiance exposures, all dates, all groups) to provide potential target values for
348 nutrient management strategies (Table 4).

349

350 **DISCUSSION**

351 Tipping points have a variety of definitions (Holling 1973; Scheffer et al. 2001; Folke et al.
352 2004; Lenton 2013). In this study, we defined an ecological tipping point as the nutrient
353 concentration (DIN) at which there is a significant change in phytoplankton biomass and group
354 diversity responses to increasing nutrient concentrations (i.e., a change in the response

355 trajectory). Responses varied, sometimes with a decrease in the slope of the fitted line while at
356 other times the slope increased. In some cases, the slope was 0 following the tipping point,
357 indicating a steady state. Regardless of direction and magnitude, the tipping point signaled a
358 change in the response trajectory that resulted in alterations of community structure and, by
359 extension, function. The implications for nutrient management are that these tipping points
360 represent critical values for the determination of water quality nutrient criteria based on
361 fundamental changes in phytoplankton responses to nutrient loading.

362 For our application, tipping point analysis is somewhat analogous to nutrient uptake curves. In
363 curve uptake kinetics, there should be some point at which the uptake rate is maximized and the
364 specific nutrient uptake rate remains constant for increasing concentrations (e.g., V_{max}).
365 However, our data show responses that, in general, do not follow traditional nutrient uptake
366 kinetics. Our approach also differs in that we examine whole community responses to increased
367 nutrient loading. As such, the measured responses of the natural community bioassays include
368 other important contributing factors such as inter- and intraspecific competition, zooplankton
369 grazing, and bacterial uptake. System manipulations using bioassays of natural assemblages
370 offer a much more realistic, ecologically-based, appraisal of how phytoplankton communities are
371 likely to respond to increased nutrient loading and eutrophication.

372 Short-term (72 h) experimental bioassays, using natural communities exposed to *in situ*
373 environmental conditions, allow the detection of changes in phytoplankton photophysiology,
374 metabolism, and community structure in response to manipulations (Gilde and Pinckney 2011;
375 Kline and Pinckney 2014; Pinckney et al. 2017). The approach described in this study represents
376 a realistic appraisal of the potential ecological impacts and risk assessments of DIN loading in
377 estuarine ecosystems (Couture et al. 1989; Clements and Kiffney 1994; Bonilla et al. 1998).

Ecologically-based assessments of nutrient exposures on phytoplankton biomass and community composition should be a primary consideration when developing nutrient criteria for estuaries. Furthermore, the experimental methodologies used in this study could be applied to other estuarine systems.

As expected in this study, phytoplankton community biomass usually increased with increasing DIN loading. Our primary interest was in how the nature of the response *changed* as DIN concentration increased. Total biomass (as chl *a*) responses to increasing nutrient concentrations showed quantifiable tipping points, where biomass increased up to the tipping point. However, responses following tipping points were highly variable and essentially unpredictable. These results illustrate the inherent unpredictability of algal group concentration changes in phytoplankton-nutrient responses (Stanley et al. 1990; Anderson and Garrison 1997; Huisman and Weissing 2001; Smayda and Reynolds 2001; Roelke et al. 2003; Kremp et al. 2008). Thus, DIN tipping points for the phytoplankton community may be interpreted as the DIN concentration at which the effects of the DIN increases become unpredictable.

Exposure irradiance, whether high or low (40% vs. 20% of solar irradiance), did not result in different tipping points for paired samples. The effects of irradiance on nutrient uptake are well documented and the paradigm is that uptake is slower at lower irradiances (Eppley et al. 1969; Geider et al. 1997, 1998). Our results suggest that DIN uptake was not light limited at the exposure levels used in this study. In an unpublished study, the saturating irradiance (PAR) for photosynthesis (E_k)for WB and CB phytoplankton was ca. 700 µmol photons m^{-2} s^{-1} (Pinckney et al., unpubl). Similarly, Lawrenz et al. (2013) found E_k values of ca. 400 µmol photons m^{-2} s^{-1} for North Inlet phytoplankton. Assuming an incoming PAR of 1,500 µmol photons m^{-2} s^{-1}, the low irradiance treatments would have received a PAR of 300 µmol photons m^{-2} s^{-1}. Although

401 Winyah Bay and North Inlet Estuary are relatively turbid environments, phytoplankton growth
402 and nutrient uptake is likely not light-limited due to the high vertical mixing rates driven by tides
403 and winds in the generally shallow waters (< 4 m) of both systems (Yoder and Bishop 1985;
404 Cloern 1987; Alpine and Cloern 1988; Mallin and Paerl 1992; Lawrenz et al. 2013)

405 For the phytoplankton community as a whole, the median DIN tipping points at the WB site
406 were more than double those of the CB site (49.4 vs. 20.3 μmol DIN l^{-1}). This result could
407 reflect differences in phytoplankton species composition between the two sites. At the WB site,
408 the phytoplankton community was composed of mostly freshwater species due to the WB
409 salinity (2 – 4) and freshwater rivers flowing into the bay. As a speculation, the higher tipping
410 points may suggest that the phytoplankton in the upper bay were acclimated to higher nutrient
411 concentrations relative to CB (22 – 36 salinity). Estuarine phytoplankton assemblies may rapidly
412 recover from acute osmotic stress where available nutrients are abundant (Li 2019) and
413 phytoplankton at lower salinities usually have high half-saturation constants for nutrient uptake
414 (Fisher et al. 1988).

415 The initial phytoplankton community composition for the bioassays was represented by 7 algal
416 groups, common at both sites, (chlorophytes, cryptophytes, cyanobacteria, diatoms,
417 euglenophytes, and prasinophytes) that were quantified using ChemTax. Diatoms were the most
418 abundant group in all bioassays, with other groups present in varying concentrations depending
419 on date and location. Similar community compositions have been reported for Winyah Bay and
420 North Inlet estuary in previous studies (Lewitus et al. 2005; Ranhofer et al. 2009; Lawrenz et al.
421 2013; Pinckney et al. 2013). Initial composition (i.e., the seed community) plays a central role in
422 the responses of bioassays, making it difficult to generalize responses of natural systems with
423 different seed communities (Franks 1997; Roelke et al. 1997; Roelke and Buyukates 2001)

Group diversity responses following tipping points were unpredictable. However, tipping points were identified for DIN concentrations at which there was a change, usually in both direction and magnitude of the slopes. In many cases, group diversity declined following tipping points and with increasing DIN concentrations. Phytoplankton group diversity had distinct tipping points, but diversity responses after the tipping point were highly variable between dates. In general, diversity increased up to the tipping point, then increased at a different rate, declined, or remained constant. Although not statistically significant ($p = 0.28$), there was a trend toward higher DIN tipping points for group diversity at the WB site. These results are consistent with the finding that eutrophication usually results in an overall decline in phytoplankton diversity (Revelante and Gilmartin 1980; Reynolds 2006).

All phytoplankton groups (i.e., chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, euglenophytes, and prasinophytes) exhibited a trend toward higher tipping points at WB compared to CB. Incubation irradiances had no effect on DIN tipping points except for cryptophytes and cyanobacteria, where tipping points were higher for the high irradiance exposures relative to low irradiance.

Tipping points were not different for individual algal groups at either the CB or WB sites, likely due to the high variability in nutrient responses. However, when data for groups were pooled, DIN tipping points were higher at WB compared to CB. Although it is very likely that there is a seasonality in tipping points, we were unable to address this confounding factor with our limited data set. Without replication, we cannot draw any conclusions. However, the limited data do suggest a trend toward lower DIN tipping points during the cooler winter months and may imply that phytoplankton are more sensitive to nutrient loading at colder temperatures.

446 All data on DIN tipping points were combined to provide insights into potential limits for water
447 quality management (Table 4). Conservative nutrient estimates for maximum concentrations for
448 the CB site would be ca. 25 μmol DIN l^{-1} and ca. 50 μmol DIN l^{-1} for the WB site. These levels
449 should be below the threshold for major alterations in phytoplankton structure and function in
450 these two systems.

451 Usually, a critical value < 20 μg chl a l^{-1} for phytoplankton concentrations is recommended for
452 the maintenance of water quality in many systems (Van Meerssche and Pinckney 2019). Our
453 results suggest that, during some periods of the year, this critical value is reached above the
454 tipping points for both the low and CB locations examined in this study. The implication is that
455 phytoplankton communities may undergo major changes, possibly detrimental, at concentrations
456 below the 20 μg chl a l^{-1} criterion. Consequently, these marked changes in phytoplankton
457 community assembly and processes may alter the functional role of phytoplankton, with
458 cascading effects throughout the aquatic food web.

459 **CONCLUSIONS**

460 Using nutrient addition bioassays and a range of DIN concentrations, we were able to discern
461 DIN tipping points at which the total community, diversity, and algal group responses shifted
462 from one linear trajectory to another. The implication is that the tipping point signaled a change
463 in responses that may signal a transition from a relatively stable state to an alternate state with
464 marked changes in community structure and, by extension, function. The consequences for
465 nutrient management are that these tipping points may represent critical values for the
466 determination of water quality nutrient criteria based on fundamental changes in phytoplankton
467 responses to nutrient loading. The WB system in our study exhibited higher DIN tipping points
468 (> double) those of the CB site while exposure irradiance (20% vs. 40%) did not have an effect.

469 Conservative nutrient estimates for maximum concentrations for the CB site would be ca. 25
470 µmol DIN l^{-1} and ca. 50 µmol DIN l^{-1} for the WB site. These levels should be below the
471 threshold for major alterations in phytoplankton structure and function in these two systems.

472

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479

480 **REFERENCES**

481 Allen, D.M., W.B. Allen, R.F. Feller, and J.S. Plunket, editors. 2014. Site profile of the North
482 Inlet – Winyah Bay National Estuarine Research Reserve. North Inlet – Winyah Bay
483 National Estuarine Research Reserve. Georgetown, S.C. 432 pgs. (Available on-line at
484 http://northinlet.sc.edu/).

485 Anderson D.M., D.J. Garrison. 1997. The ecology and oceanography of harmful algal blooms.
486 Limnol. Oceanogr. 42: 1009–1013.

487 Alpine, A.E. and J.E. Cloern. 1988. Phytoplankton growth rates in a light-limited environment,
488 San Francisco Bay. Mar. Ecol. Prog. Ser. 44: 167-173.

489 Bergquist, D.C., R.F. Van Dolah, G.H.M Riekerk, M.V. Levisen, S.E Crowe, L. Brock, D.I.
490 Greenfield, D.E. Chestnut, W. McDermott, M.H. Fulton, E. Wirth, and J. Harvey. 2009.
491 The Condition of South Carolina's Estuarine and Coastal Habitats During 2005-2006:
492 Technical Report. Charleston, SC: South Carolina Marine Resources Division. Technical
493 Report No. 103. 74 p.

494 Bonilla, S., D. Conde and H. Blanck. 1998. The photosynthetic responses of marine
495 phytoplankton, periphyton, and epipsammon to the herbicides paraquat and simazine.
496 Ecotoxicol. 7: 99-105.

497 Bricker, S.B., C.G. Clement, D.E. Pirhalla, S.P. Orlando, and D.R.G. Farrow. 1999. National
498 estuarine eutrophication assessment, effects of nutrient enrichment in the nation's
499 estuaries. NOAA, National Ocean Service, Special Projects Office and the National
500 Centers for Coastal Ocean Science, Silver Spring, MD, 71 pp.

501 Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks and J. Woerner.
502 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change.
503 Harmful Algae 8: 21-32.

504 Buzzelli, C., O. Akman, T. Buck, E. Koepfler, J. Morris, and A. Lewitus. 2004. Relationships
505 among Water-Quality Parameters from the North Inlet–Winyah Bay National Estuarine
506 Research Reserve, South Carolina. J. Coastal Res. 45: 59-74.

507 Clarke K.R., R.N. Gorley. 2001. PRIMER v.5: User Manual/Tutorial. PRIMER-E, Plymouth,
508 UK.

509 Clements, W. and P. Kiffney. 1994. Assessing contaminant effects at higher trophic levels of
510 biological organization. Environ. Toxicol. Chem. 14: 357-359.

511 Cloern, J.E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries.
512 Cont. Shelf Res. 7: 1367-1381.

513 Couture, P., C. Thellen, and P. Thompson. 1989. Phytoplankton recovery responses at the
514 population and community levels in hazard and risk assessment study. Hydrobiologia
515 188/189: 269-276.

516 Dame, R., M. Alber, D. Allen, M. Mallin, C. Montague, A. Lewitus, A. Chalmers, R. Gardner,
517 C. Gilman, B. Kjerfve, J. Pinckney, and N. Smith. 2000. Estuaries of the South Atlantic
518 Coast of North America: Their geographical signatures. Estuaries 23: 793-819.

519 DeYoung B., M. Barange, G. Beaugrand, R. Harris, R.I. Perry, M. Scheffer, and F. Werner.
520 2008. Regime shifts in marine ecosystems: detection, prediction and management. Trends
521 Ecol. Evolut. 23: 402-409.

522 Eaton, A.D., L.S Clesceri, E.W. Rice, and A.E. Greenberg. 2005. Standard Methods for the
523 Examination of Water and Wastewater. (21 Ed.). Washington, DC: American Public
524 Health Association.

525 Eppley, R., J. Rogers, and J. McCarthy. 1969. Half-saturation constants for uptake of nitrate and
526 ammonium by marine phytoplankton. Limnol. Oceangr. 14: 912-920.

527 Fisher, T., L. Harding, D. Stanley, and L. Ward. 1988. Phytoplankton, nutrients, and turbidity in
528 the Chesapeake, Delaware, and Hudson estuaries. Est. Coast. Shelf Sci. 27: 61-93.

529 Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C.S. Holling.
530 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annu. Rev.
531 Ecol. Syst. 35: 357-381.

532 Franks, P.J.S. 1997. Models of harmful algal blooms. Limnol. Oceanogr. 42: 1273-1282.

533 Gardner, L.R., B. Kjerfve, and D.M. Petrecca. 2006. Tidal fluxes of dissolved oxygen at the
534 North Inlet – Winyah Bay National Estuarine Research Reserve. Est. Coast. Shelf Sci.
535 67: 450-460.

536 GASCET. 2015. An Approach to Develop Numeric Nutrient Criteria for Georgia and South
537 Carolina Estuaries. A Task Force Report to EPA, GA EPD, and SC DHEC.

538 Geider, R.J., H. MacIntyre, and T. Kana. 1997. Dynamic model of phytoplankton growth and
539 acclimation: responses of the balanced growth rate and the chlorophyll *a*:carbon ratio to
540 light, nutrient-limitation and temperature. Mar. Ecol. Prog. Ser. 148: 187-200.

541 Geider, R.J., H. MacIntyre, and T. Kana. 1998. A dynamic regulatory model of phytoplanktonic
542 acclimation to light, nutrients, and temperature. Limnol. Oceanogr. 43: 679-694.

543 Gilde, K., J.L. Pinckney. 2011. Sublethal effects of crude oil on the community structure of
544 estuarine phytoplankton. Estuar. Coasts 35: 853-861.

545 Glibert, P.M., Z. Mlodzinska, and C.F. D'Elia. 1977. A semi-automated persulfate oxidation
546 technique for simultaneous total nitrogen and total phosphorus determination in natural
547 water samples. Woods Hole Oceanographic Institution Contribution Number 3954.
548 Ocean Industry Program of the Woods Hole Oceanographic Institution, Woods Hole,
549 Massachusetts 02543.

550 Glibert, P.M., D. Hinkel, B. Sturgis, R.V. Jesien. 2014. Eutrophication of a Maryland/Virginia
551 coastal lagoon: a tipping point, ecosystem changes, and potential causes. Estuar. Coasts
552 37: S128-S146.

553 Gross, A. and C.E. Boyd. 1998. A digestion procedure for the simultaneous determination of
554 total nitrogen and total phosphorus in pond water. J. World Aquac. Soc. 29: 300-303

555 Higgins H., S. Wright, and L. Schlüter. 2011. Quantitative interpretation of chemotaxonomic
556 pigment data. In Roy S., Llewellyn C.A., Egeland E.S., Johnsen G. (eds) Phytoplankton
557 pigments. Cambridge University Press, NY.

558 Holling, C.S. 1973. Resilience and stability of ecological systems. Annu. Rev. Ecol. Sys.t 4: 1-
559 23.

560 Hooker, S.B., C.S. Thomas, L. Van Heukelem, L. Schlüter, M.E. Russ, J. Ras, H. Claustre, L.
561 Clementson, E. Canuti, J-F. Berthon, J. Perl, C. Normandeau, J. Cullen, M. Kienast, J.L.
562 Pinckney. 2010. The Fourth SeaWiFS HPLC Analysis Round-Robin Experiment
563 (SeaHARRE-4). NASA/TM–2010–215857. National Aeronautics and Space
564 Administration, Goddard Space Flight Center, Greenbelt, Maryland.

565 Huggett, A.J. 2005. The concept and utility of 'ecological thresholds' in biodiversity
566 conservation. Biol. Conserv. 124: 301-310.

567 Huisman J. and F.J. Weissing. 2001. Fundamental unpredictability in multispecies competition.
568 Am. Nat. 157: 488–494.

569 Kjerfve, B. 1986. Circulation and salt flux in a well mixed estuary. In J. Van de Kreeke (ed.).
570 Physics of Shallow Estuaries and Bays. Springer-Verlag, Berlin.

571 Kline, A. and J.L. Pinckney. 2016. Size-selective toxicity effects of the antimicrobial tylosin on
572 estuarine phytoplankton communities. Environ. Pollut. 216: 806-810.

573 Krebs, C.J. 1999. Ecological Methodology. New York: Harper & Row.

574 Kremp, A., T. Tamminen, and K. Spilling. 2008. Dinoflagellate bloom formation in natural
575 assemblages with diatoms: nutrient competition and growth strategies in Baltic spring
576 phytoplankton. Aquat. Microb. Ecol. 50: 181-196.

577 Lawrenz, E., T. Richardson, and E. Smith. 2013. Spectral irradiance, phytoplankton community
578 composition and primary productivity in a salt marsh estuary, North Inlet, South
579 Carolina, USA. Estuar. Coasts 36: 347-364.

580 Lenton, T.M. 2013. Environmental tipping points. Annu. Rev. Environ. Resour. 38: 1-29.

581 Lewitus A., D. White, R. Tymowski, M. Geesey, S. Hymel, P. Noble. 2005. Adapting the
582 CHEMTAX method for assessing phytoplankton taxonomic composition in Southeastern
583 US estuaries. Estuaries 28: 160-172.

584 Li, G. 2019. Fast acclimation of phytoplankton assemblies to acute salinity stress in the Jiulong
585 River Estuary. Acta Oceanol. Sinica 38: 78-85.

586 Loder, T.C. 1978. A semi-automated total nitrogen and phosphorus method for low volume
587 samples. Ocean Industry Program of the Woods Hole Oceanographic Institution, Woods
588 Hole, MA.

589 Mackey M., D. Mackey, H.W. Higgins, and S. Wright. 1996. CHEMTAX - a program for
590 estimating class abundances from chemical markers: application to HPLC measurements
591 of phytoplankton. Mar. Ecol. Prog. Ser. 144: 265-283

592 Mallin, M.A. and H.W. Paerl. 1992. Effects of variable irradiance on phytoplankton productivity
593 in shallow estuaries. Limnol. Oceanogr. 37: 54-62.

Moore, J.C. 2018. Predicting tipping points in complex environmental conditions. Proc. Natl. Acad. Sci. 115: 635-636.

Muggeo, V.R.M. 2008. Segmented: An R package to fit regression models with broken-line relationships. R News 8: 20-25.

Nixon, S. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41: 199-219.

Paerl, H.W., L. Valdes, J.L. Pinckney, M. Piehler, J. Dyble, and P. Moisander. 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. Biosci. 53: 953-964.

Paerl, H.W., N.S. Hall, B.L. Peierls, and K.L. Rossignol. 2014. Evolving paradigms and challenges in estuarine and coastal eutrophication dynamics in a culturally and climatically stressed world. Est. Coasts 37: 243-258.

Pinckney, J.L., H. Paerl, P. Tester, and T. Richardson. 2001a. The role of nutrient loading and eutrophication in estuarine ecology. Environ. Health Persp. 109: 699-706.

Pinckney J., T. Richardson, D. Millie, and H. Paerl. 2001b. Application of photopigment biomarkers for quantifying microalgal community composition and in situ growth rates. Org. Geochem. 32: 585-595

Pinckney, J.L., L. Thompson, S. Hylton. 2017. Triclosan alterations of estuarine phytoplankton community structure. Mar. Poll. Bull. 119: 162-168.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

615 Ranhofer, M.L., E. Lawrenz, J.L. Pinckney, C.R. Benitez-Nelson, and T.L. Richardson. 2009.
616 Cell-specific alkaline phosphatase expression by phytoplankton in Winyah Bay, South
617 Carolina, USA. Estuar. Coasts 32: 943-957.

618 Reed, M.L., J.L. Pinckney, C.J. Keppler, L.M. Brock, S.B. Hogan, and D.I. Greenfield. 2016.
619 The influence of nitrogen and phosphorus on phytoplankton growth and assemblage
620 composition in four coastal, southeastern USA systems. Est. Coast. Shelf Sci. 177: 71-82.

621 Revelante, N. and M. Gilmartin. 1980. Microplankton diversity indices as indicators of
622 eutrophication in the northern Adriatic Sea. Hydrobiol. 70: 277-286.

623 Reynolds, C.S. 2006. Ecology of phytoplankton. Cambridge Univ. Press.

624 Richardson, T.L., E. Lawrenz, J.L. Pinckney, R.C. Guajardo, E.A. Walker, H.W. Paerl, and H.L.
625 MacIntyre. 2009. Spectral fluorometric characterization of phytoplankton community
626 composition using the algae online analyzer Water Res. 44: 2461-2472.

627 Roelke, D. and Y. Buyukates. 2001. The diversity of harmful algal bloom-triggering mechanisms
628 and the complexity of bloom initiation. Harm. Ecol. Risk Assess. 7: 1347-1362.

629 Roelke, D., L. Cifuentes, and P. Eldridge. 1997. Nutrient and phytoplankton dynamics in
630 sewage-impacted Gulf Coast estuary. A field test of the PEG-model and equilibrium
631 resource competition theory. Estuaries 20: 725-742.

632 Roelke, D., S. Augustine, and Y. Buyukates. 2003. Fundamental predictability in multispecies
633 competition: The influence of large disturbance. Am. Nat. 162: 615-623.

634 Roy. S., C. Llewellyn, E. Egeland, and G. Johnsen. 2011. Phytoplankton pigments. Cambridge
635 Univ. Press, Cambridge.

636 Scheffer, M., S. Carpenter, J.A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in
637 ecosystems. Nature 413: 591-596.

638 Scheffer, M., S.R. Carpenter, T.M. Lenton, J. Bascompte, W. Brock, V. Dakos, J. van de Koppel,
639 I.S. van de Leemput, S.A. Levin, E.H. von Nes, M. Pascual, and J. Verndermeer. 2012.
640 Anticipating critical transitions. Science 338: 344-348.

641 Schlüter L., F. Møhlenberg, H. Havskum, and S. Larsen. 2000. The use of phytoplankton
642 pigments for identifying and quantifying phytoplankton groups in coastal areas: testing
643 the influence of light and nutrients on pigment/chlorophyll *a* ratios. Mar. Ecol. Prog. Ser.
644 192: 49-63.

645 Smayda T.J. and C.S. Reynolds. 2001. Community assembly in marine phytoplankton:
646 application of recent models to harmful dinoflagellate blooms. J. Plankton Res. 23: 447–
647 461.

648 Stanley, E., R. Short, J. Harrison, R. Hall, and R. Wiedenfeld. 1990. Variation in nutrient
649 limitation of lotic and lentic algal communities in a Texas (USA) river. Hydrobiol. 206:
650 61-71.

651 Valderrama, C.J. 1981. The simultaneous analysis of total nitrogen and total phosphorus in
652 natural waters. Mar. Chem. 10: 109-122.

653 Vanacker, M., A. Wezel, V. Payet, and J. Robin. 2015. Determining tipping points in aquatic
654 ecosystems: The case of biodiversity and chlorophyll *a* relations in fish pond systems.
655 Ecol. Indicators 52: 184-193.

656 Van Meerssche, E. and J. Pinckney. 2019. Nutrient loading impacts on estuarine phytoplankton
657 size and community composition: Community-based Indicators of eutrophication. Estuar.
658 Coasts 42: 504-512.

659 Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H.
660 Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle:
661 Sources and consequences. Ecol. Appl. 7: 737-750.

662 Yoder, J.A. and S.S. Bishop. 1985. Effects of mixing-induced irradiance fluctuations on
663 photosynthesis of natural assemblages of coastal phytoplankton. Mar. Biol. 90: 87-93.

Table 1. Summary of bioassay incubation, salinity, temperature, maximum photosynthetically available radiation exposure, and initial nutrient concentrations (means of triplicates). Dashes indicate data not collected.

Site	Date	Salinity (PSU)	Temperature Range (°C)	Max PAR* (15 min^{-1})	NH_4^+ (µmol l^{-1})	$NO_2^- + NO_3^-$ (µmol l^{-1})	PO_4^{3-} (µmol l^{-1})	Total N (µmol l^{-1})	Total P (µmol l^{-1})
Clambank (CB)	May 2018	30	22 - 28	1834	2.88	1.06	0.28	28.01	1.91
	Jun 2018	34	26 - 32	1726	1.33	0.78	0.19	23.82	0.00
	Jul 2018	28	24 - 32	1954	10.48	1.41	0.49	36.90	0.66
	Nov 2018	36	12 - 16	1064	2.72	3.13	-	33.31	-
	Feb 2019	31	14 - 16	1484	3.34	0.57	-	31.99	-
	Apr 2019	22	21 - 24	1725	2.53	3.26	-	64.92	-
Winyah Bay (WB)	Jul 2018	3	24 - 32	1954	6.48	14.41	0.89	69.95	2.74
	Nov 2018	2	12 - 16	1064	2.45	30.65	-	93.56	-
	Feb 2019	4	14 - 16	1484	7.29	37.89	-	77.01	-
	Apr 2019	3	21 - 24	1725	8.24	26.52	-	122.25	-

*(1 mmol photons m^{-2} 15 min^{-1} = 1.11 µmol photons m^{-2} sec^{-1})

Table 2. Dissolved inorganic nitrogen (DIN) concentration tipping points for all phytoplankton, Shannon group diversity, and phytoplankton groups. Values are in units of μmol DIN l^{-1} ± 1 standard error of the estimate. Dashes indicate no tipping point

		Irradiance	All Phytoplankton	Shannon Diversity	Chloro-phytes	Crypto-phytes	Cyano-bacteria	Diatoms	Dino-flagellates	Euglено-phytes	Prasino-phytes
Clambank (CB)	May 18	High	20.7 ± 4.7	13.9 ± 3.6	27.2 ± 2.6	9.5 ± 6.9	30.6 ± 3.4	21.0 ± 4.1	6.3 ± 10.5	33.8 ± 1.34	29.3 ± 3.2
		Low	19.9 ± 1.9	18.7 ± 3.0	10.6 ± 17.5	15.6 ± 1.3	-	20.2 ± 2.1	12.3 ± 3.2	20.6 ± 3.8	20.6 ± 1.6
	Jun 18	High	11.0 ± 7.2	36.2 ± 8.7	32.4 ± 6.7	61.5 ± 1.8	29.5 ± 4.7	55.6 ± 10.5	56.2 ± 8.04	53.4 ± 3.0	9.5 ± 7.3
		Low	33.2 ± 3.2	-	50.4 ± 5.8	42.1 ± 7.7	-	32.7 ± 3.0	15.4 ± 10.9	5.8 ± 2.0	33.3 ± 4.3
	Jul 18	High	45.8 ± 4.7	-	28.0 ± 17.2	50.9 ± 4.1	-	46.4 ± 4.9	58.5 ± 8.8	-	25.8 ± 6.7
		Low	51.9 ± 8.2	-	38.5 ± 11.3	38.5 ± 12.1	25.2 ± 17.9	51.9 ± 8.3	54.6 ± 5.4	38.5 ± 13.9	51.9 ± 10.2
	Nov 18	High	12.5 ± 1.7	-	9.3 ± 1.0	22.8 ± 4.7	12.5 ± 3.3	11.5 ± 1.3	25.7 ± 34.5	51.2 ± 4.3	11.5 ± 2.0
		Low	15.8 ± 5.6	-	39.1 ± 13.7	-	-	-	50.2 ± 7.0	21.5 ± 4.2	-
	Feb 19	High	8.9 ± 1.2	-	10.1 ± 8.2	11.6 ± 1.9	7.3 ± 1.1	8.6 ± 1.1	-	9.7 ± 2.1	13.5 ± 2.4
		Low	9.6 ± 0.7	55.4 ± 7.1	30.6 ± 5.8	13.6 ± 1.6	-	9.4 ± 0.6	30.6 ± 8.9	14.0 ± 1.4	10.6 ± 1.2
	Apr 19	High	64.8 ± 0.7	55.0 ± 6.2	-	61.4 ± 1.4	60.9 ± 2.3	65.2 ± 0.7	39.1 ± 3.5	-	55.2 ± 4.8
		Low	32.6 ± 2.7	15.4 ± 1.4	59.1 ± 9.4	32.4 ± 4.8	30.0 ± 6.6	32.5 ± 3.1	58.3 ± 3.0	20.1 ± 2.3	26.6 ± 5.7
Winyah Bay (WB)	Jul 18	High	54.7 ± 3.8	25.2 ± 0.4	73.8 ± 2.1	37.9 ± 3.1	46.3 ± 3.5	54.8 ± 3.9	54.2 ± 7.6	65.0 ± 4.5	-
		Low	39.2 ± 3.5	29.4 ± 4.4	78.4 ± 3.6	50.0 ± 3.2	34.0 ± 22.3	38.9 ± 3.4	27.5 ± 13.4	60.9 ± 13.3	34.2 ± 9.4
	Nov 18	High	36.9 ± 0.5	59.7 ± 14.5	70.0 ± 5.6	78.2 ± 5.6	83.3 ± 23.6	36.9 ± 0.6	81.4 ± 11.3	39.7 ± 20.4	-
		Low	39.7 ± 21.2	43.9 ± 7.1	66.4 ± 13.2	46.4 ± 5.0	43.5 ± 4.5	39.6 ± 10.3	39.8 ± 8.6	47.4 ± 9.3	36.7 ± 1.8
	Feb 19	High	71.8 ± 9.4	91.8 ± 16.6	71.8 ± 14.7	71.8 ± 9.9	63.1 ± 8.8	71.8 ± 7.9	-	85.1 ± 25.0	71.8 ± 14.1
		Low	49.4 ± 1.2	49.6 ± 1.4	-	49.4 ± 0.9	53.0 ± 2.4	49.1 ± 1.7	58.5 ± 17.7	93.7 ± 5.5	91.1 ± 6.8
	Apr 19	High	93.6 ± 0.7	73.1 ± 5.1	93.5 ± 0.7	81.7 ± 4.0	51.8 ± 3.3	85.8 ± 1.7	36.6 ± 2.1	68.2 ± 9.9	93.7 ± 0.8
		Low	-	48.1 ± 10.1	-	38.6 ± 1.5	38.4 ± 0.2	-	61.4 ± 9.7	48.1 ± 40.2	74.7 ± 15.0

Table 3. Summary statistics for tipping point analyses. Values are μmol DIN l^{-1}.

	Clambank (CB) High Irradiance			Clambank (CB) Low Irradiance			Winyah Bay (WB) High Irradiance			Winyah Bay (WB) Low Irradiance		
Group	Mean	± 1 SD	Median	Mean	± 1 SD	Median	Mean	± 1 SD	Median	Mean	± 1 SD	Median
All												
Phytoplankton	27.3	22.9	16.6	27.1	15.3	26.2	64.2	24.2	63.3	42.8	5.8	39.7
Diversity	35.0	20.6	36.2	29.8	22.2	18.7	62.5	28.1	66.4	42.7	9.2	46.0
Chlorophytes	21.4	10.9	27.3	38.0	16.8	38.8	77.3	10.9	72.8	72.4	8.5	72.4
Cryptophytes	36.3	24.5	36.8	28.4	13.1	32.4	67.4	20.1	75.0	46.1	5.3	47.9
Cyanobacteria	28.2	21.0	29.5	27.6	3.4	27.6	61.1	16.3	57.5	42.2	8.2	40.9
Diatoms	34.7	24.1	33.7	29.4	15.9	32.5	62.3	21.1	63.3	42.5	5.7	39.6
Dinoflagellates	37.2	21.8	39.1	36.9	20.3	40.4	57.4	22.5	54.2	46.8	16.0	49.1
Euglenophytes	37.0	20.2	42.5	20.1	10.8	20.4	64.5	18.7	66.6	62.5	21.7	54.5
Prasinophytes	24.1	17.2	19.6	28.6	15.5	26.6	82.7	15.4	26.6	59.2	28.2	55.7

Table 4. Summary means, standard deviations, and medians for pooled DIN tipping point values for the two sites. Values are μmol DIN l^{-1}

	Clambank (WB)			Winyah Bay (WB)		
Group	Mean	± 1 SD	Median	Mean	± 1 SD	Median
All Phytoplankton	27.2	18.5	20.3	55.0	20.9	49.4
Diversity	32.4	19.4	27.5	52.6	22.0	48.9
Chlorophytes	30.5	16.2	30.6	75.7	9.6	72.8
Cryptophytes	32.7	19.6	32.4	56.8	17.7	49.7
Cyanobacteria	28.0	17.2	29.5	51.7	15.7	49.1
Diatoms	32.3	20.0	32.5	53.8	18.6	49.1
Dinoflagellates	37.0	19.9	39.1	51.3	18.2	54.2
Euglenophytes	26.9	16.7	21.1	63.5	18.8	63.0
Prasinophytes	26.2	15.8	25.8	67.0	26.0	73.3
Grand Means	30.4	17.7	28.8	58.6	19.3	56.6

FIGURE LEGENDS

Figure 1. Map of the two study sites in South Carolina, USA. The Clambank (CB) site was located in the North Inlet Estuary at Clambank Landing and the Winyah Bay (WB) site was in Winyah Bay. Water for the bioassays was collected from these locations.

Figure 2. Bioassay results for the Clambank (CB) site showing the phytoplankton community (total chl *a*) responses to increasing DIN loading. Bioassay dates are indicated on each graph and high vs. low incubation irradiances are shown as separate symbols. The results of tipping point analyses are shown by lines and tipping points are denoted by dashed lines extending from the point to the x-axis. Plots without dashed lines denote no tipping point.

Figure 3. Bioassay results for the Winyah Bay (WB) site showing the phytoplankton community (total chl *a*) responses to increasing DIN loading. Bioassay dates are indicated on each graph and high vs. low incubation irradiances are shown as separate symbols. The results of tipping point analyses are shown by lines and tipping points are denoted by dashed lines extending from the point to the x-axis. Plots without dashed lines denote no tipping point.

Figure 4. Boxplots summarizing the DIN tipping point values for the total phytoplankton community.

Figure 5. Boxplots of initial phytoplankton community composition at the Clambank (CB) site. Bioassay dates are indicated on each graph. Note different scales for x-axis.

Figure 6. Boxplots of initial phytoplankton community composition at the Winyah Bay (WB) site. Bioassay dates are indicated on each graph. Note different scales for x-axis.

Figure 7. Scatterplots for the Clambank (CB) site showing the phytoplankton group diversity (Shannon-Wiener Index) responses to increasing DIN loading. Bioassay dates are indicated on each graph and high vs. low incubation irradiances are shown as separate symbols. The results of tipping point analyses are shown by lines and tipping points are denoted by dashed lines extending from the point to the x-axis. Plots without dashed lines denote no tipping point.

Figure 8. Scatterplots for the Winyah Bay (WB) site showing the phytoplankton group diversity (Shannon-Wiener Index) responses to increasing DIN loading. Bioassay dates are indicated on each graph and high vs. low incubation irradiances are shown as separate symbols. The results of tipping point analyses are shown by lines and tipping points are denoted by dashed lines extending from the point to the x-axis. Plots without dashed lines denote no tipping point.

Figure 9. Boxplots summarizing the DIN tipping point values for group diversity and individual algal groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, euglenophytes, and prasinophytes). The results of *a posteriori* multiple comparisons are indicated by letters above the boxes. Similar letters indicate homogeneous groups.