

Non-monotonic Responses of Phytoplankton Biomass Accumulation to Hydrologic Variability: A Comparison of Two Coastal Plain North Carolina Estuaries

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Abstract Freshwater inputs often play a more direct role in estuarine phytoplankton biomass (chlorophyll *a*) accumulation than nitrogen (N) inputs, since discharge simultaneously controls both phytoplankton residence time and N loading. Understanding this link is critical, given potential changes in climate and human activities that may affect discharge and watershed N supply. Chlorophyll *a* (*chl_a*) relationships with hydrologic variability were examined in 3-year time series from two neighboring, shallow (<5 m), microtidal estuaries (New and Neuse River estuaries, NC, USA) influenced by the same climatic conditions and events. Under conditions ranging from drought to floods, N concentration and salinity showed direct positive and negative responses, respectively, to discharge for both estuaries. The response of *chl_a* to discharge was more complex, but was elucidated through conversion of discharge to freshwater flushing time, an estimate of transport time scale. Non-linear fits of *chl_a* to flushing time revealed non-monotonic, unimodal relationships that reflected the changing balance between intrinsic growth and losses through time and along the axis of each estuary. Maximum biomass occurred at approximately 10-day flushing times for both systems. Residual analysis of the fitted data revealed positive relationships between *chl_a* and temperature, suggesting enhanced growth rates at higher temperatures. N loading and system-wide, volume-weighted *chl_a* were positively correlated, and biomass yields per N load were greater than other marine systems. When combined with information on loss processes, these results on the hydrologic control of phytoplankton biomass will help formulate mechanistic

models necessary to predict ecosystem responses to future climate and anthropogenic changes.

Keywords Estuary · Flushing time · Phytoplankton · Nitrogen · Neuse River · New River

Introduction

Understanding and predicting estuarine phytoplankton dynamics is an elusive yet critical goal for researchers and water quality managers, especially in systems affected by cultural eutrophication. Nitrogen (N) has long been known to be the major nutrient limiting phytoplankton biomass production in coastal systems (Ryther and Dunstan 1971), and it is usually the target for management actions taken to reverse estuarine eutrophication (Nixon 2009). Evidence showing the direct relationship between N loading and phytoplankton biomass and production, however, is often weak, and other factors such as tidal, wind, and hydrologic forcing must be considered when trying to predict or manage system behavior (Cloern 2001; Borsuk et al. 2004).

Complex physical dynamics are inherent in estuarine systems, and much of that is driven by freshwater inputs. River discharge covaries with nutrient loading, but also controls plankton residence time in many estuaries, which confounds the nutrient–phytoplankton relationship. At extreme discharge rates, for example, phytoplankton may not reside long enough in a system to experience significant accumulation, despite an abundance of nutrients. Success of phytoplankton populations depends on the net balance between biomass gains (transport in+growth) and losses (transport out+sedimentation+grazing) (Ketchum 1954). The hydrologic characteristics of a system, therefore, play a crucial role in controlling resident phytoplankton bloom potential. This, in turn, affects the sensitivity of

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an estuary to anthropogenic nutrient enrichment and climate (precipitation) variation.

In a recent review, Lucas et al. (2009) discussed variations in the relationships between phytoplankton biomass and transport time scales such as flushing time and residence time, which can be related to freshwater discharge. They reported on systems where biomass increases, decreases, or remains the same with increasing transport time (e.g., decreasing discharge, for systems strongly influenced by freshwater inflow). Using a conceptual model, they showed that the particular relationship present depends on the net balance between phytoplankton growth and loss. The phytoplankton–transport time relationships can also vary within a single system over time, and space and may be used to infer the relative magnitudes of the growth and loss terms.

Phytoplankton–transport time relationships were investigated in two neighboring, coastal plain North Carolina estuaries: the New River Estuary (NewRE) and the Neuse River Estuary (NRE). In both systems, phytoplankton dominate primary production, and high rates of anthropogenic nutrient loading have led to eutrophic conditions accompanied by frequent algal blooms, reduced water clarity, and bottom water hypoxia (Paerl et al. 1998; Mallin et al. 2005). Long-term monitoring programs were started in both estuaries in response to the water quality concerns (Luettich et al. 2000; Paerl et al. 2004; Mallin et al. 2005). Nitrogen is the principal limiting nutrient, particularly during the late spring through fall when productivity is highest (Paerl et al. 2004; Mallin et al. 2005). Average residence time within both micro-tidal systems (>1 month) is much greater than phytoplankton generation times. However, during river flooding events (e.g., following tropical cyclones and frequent summer thunderstorms), transport times can be drastically reduced to the point where residence time limits productivity.

The goal of this study was to compare water quality responses to varying freshwater inputs and to compare the phytoplankton biomass–transport time relationships for both estuaries using 3 years of parallel monitoring data. The close proximity (~30 km) of the two systems means that they both experienced similar meteorological forcing (e.g., temperatures, periods of thunderstorms, tropical cyclones, and local droughts), which produced conditions during the study ranging from extreme drought to flood events. Borsuk et al. (2004) documented a non-monotonic response of phytoplankton biomass to river discharge in the NRE and attributed this response to the dual role of discharge in controlling N delivery and advective transport. We build upon these findings by converting discharge into a transport time that scales discharge to the size of the receiving estuary and is therefore appropriate for comparing phytoplankton biomass responses to hydrological variability between systems. The transport time scale was represented by the date-specific

freshwater flushing time (Alber and Sheldon 1999), which represents average time freshwater spends in the estuary and avoids the problem of variable time lags between river gage and downstream locations. A companion paper investigates the community compositional responses to changes in flushing time within the NewRE (Hall et al. 2012).

Methods

Study Sites

The New River originates within the coastal plain of North Carolina and traverses ~70 km of mostly forested and agricultural land before forming the NewRE near the city of Jacksonville, NC, USA. Throughout its length, the New River is impacted by nutrient inputs from row crop agriculture, silviculture, small municipal wastewater discharges, and the recent expansion of confined animal feeding operations. Prior to 1998, the City of Jacksonville and the Marine Corp Base Camp Lejeune (MCBCL) discharged partially treated sewage directly into the NewRE, and the legacy of this organic matter within the sediments remains a substantial, unquantified source of nutrients to the upper estuary (Tomas et al. 2007). The NewRE empties directly into the Atlantic Ocean, although circulation near the New River Inlet is complicated by water exchange with the intra-coastal waterway (Fig. 1). The intermittently mixed NewRE is microtidal (~0.25 m tidal amplitude), and salinity varies with riverine discharge, but conditions are generally oligohaline near the head of the estuary and polyhaline near the inlet (Ensign et al. 2004). Stratification intensity, as measured by salinity difference between surface and bottom, ranges from 0 to 16 with a mean and median of 2.6 and 1.4, respectively. Pycnocline depth varies throughout the water column depending on location, discharge, and time since mixing. Mixing events are wind driven and occur at time scales of frontal systems and diurnal wind variation. Maximum depths along the axis of the estuary are generally around 4 m throughout the estuary, and average depth is ~1.5 m (Ensign et al. 2004). The NewRE is mesotrophic to eutrophic and also has a multi-decadal history of algal blooms, hypoxia, and fish kills associated with anthropogenic nutrient loading (Mallin et al. 2005; Tomas et al. 2007). The National Atmospheric and Oceanic Administration (NOAA) listed the NewRE as one of the four most eutrophic estuaries within the south Atlantic region (Bricker et al. 1999). Since sewage treatment improvements were made by the city of Jacksonville and MCBCL, water quality has improved substantially with lower concentrations of nutrients, suspended sediment, and phytoplankton biomass (Mallin et al. 2005). However, phytoplankton blooms, including harmful algal blooms (HABs), and bottom water

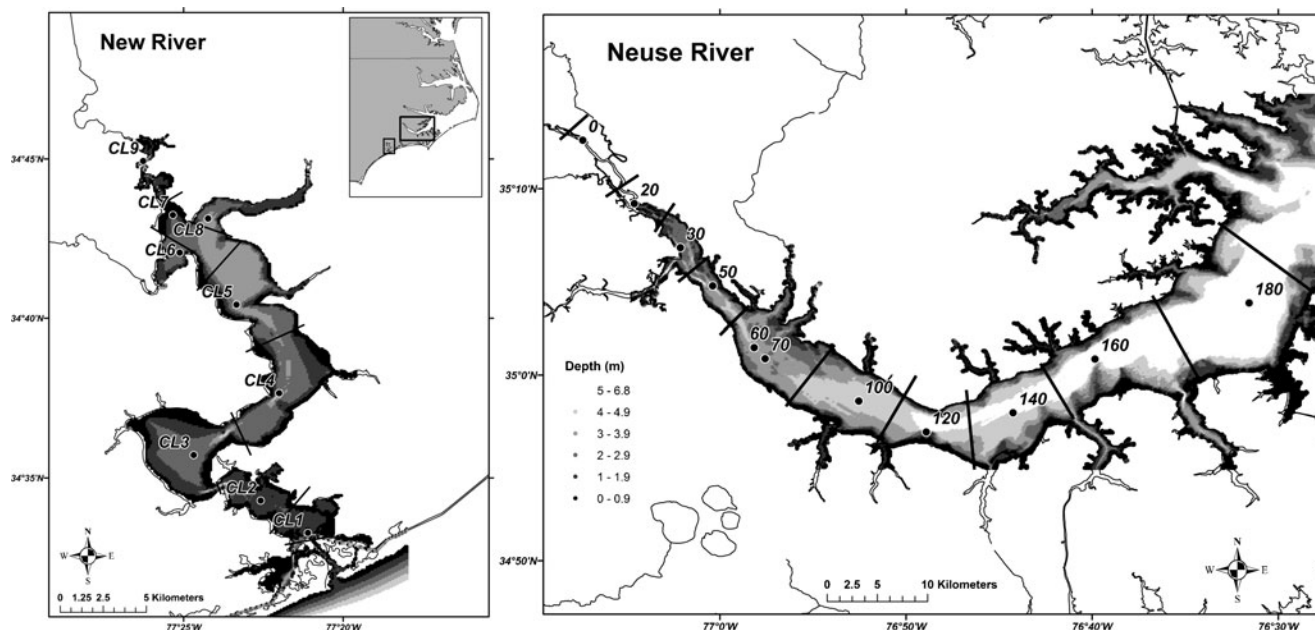


Fig. 1 Map of NewRE and NRE showing sampling stations and zones used in flushing time and volume-weighted average calculations

hypoxia are still regular occurrences within the estuary and threaten its ecological integrity and value as a fisheries and recreational resource (Tomas et al. 2007).

The Neuse River originates within the Piedmont, and its watershed encompasses the urban centers of Raleigh, Durham, and numerous smaller cities. The NRE begins over 350 km downstream near New Bern, NC, USA, and is one of three major tributary estuaries of the Albemarle-Pamlico Sound estuarine system (Paerl et al. 1998; Peierls et al. 2003). The few narrow inlets in the Outer Banks of North Carolina restrict shelf water exchange with Pamlico Sound and, as a result, astronomical tides are negligible, <4 cm (Luettich et al. 2002). Like the NewRE, the NRE is intermittently mixed (Reynolds-Fleming and Luettich 2004), and the salinity regime ranges from oligohaline to polyhaline, largely depending on riverine discharge (Christian et al. 1991). Stratification intensity (mean and median salinity difference of 4.2 and 3.5, respectively) is somewhat greater than in the NewRE, but wind-driven water column mixing patterns are similar (Reynolds-Fleming and Luettich 2004). Maximum depths along the axis of the estuary increase from approximately 4 m at the head of the estuary to near 7 m where the estuary discharges to Pamlico Sound. Average depth is only 3.5 m due to the extensive shoals that rim the estuary (Boyer et al. 1993). The NRE is also mesotrophic to eutrophic (Boyer et al. 1993), and has a multi-decadal history of algal blooms, bottom water hypoxia, and fish kills associated with excessive anthropogenic nutrient loading (Paerl et al. 1998, 2004, 2007). It was also one of the four most eutrophic estuaries in the south Atlantic region

(Bricker et al. 1999). Interest in understanding the relationships between water quality and environmental drivers resulted in establishment of the Neuse River Estuary Modeling and Monitoring (ModMon) Program (<http://www.unc.edu/ims/neuse/modmon>) (Luettich et al. 2000) that has collected biweekly to monthly water quality data within the NRE since 1994.

Stations and Sample Collection

Sampling of each estuary was done biweekly to monthly from October 2007 through December 2010. For each sample date, a network of 11 sites in the NRE and nine sites in the NewRE were visited for sample collection and in situ measurements (Fig. 1). Water samples for determination of chl a and nutrients were collected from both 0.2 m below the surface and 0.5 m above the bottom into 5-L polyethylene bottles using a non-destructive diaphragm pump. Water samples were maintained at ambient temperature in the dark for <6 h prior to returning to the Institute of Marine Sciences.

Physical Data

Profiles of temperature, salinity, and photosynthetically active radiation (PAR) at 0.5-m depth intervals were made at each station using a YSI 6600 multiparameter water quality sonde (Yellow Springs Inc., Yellow Springs, OH, USA) linked to a LI-COR LI-192 quantum sensor (LI-COR Biosciences, Lincoln, NE, USA). Diffuse attenuation

coefficients (K_d) were calculated as the slope of the least square fit of natural log-transformed PAR measurements versus depth. Historical drought conditions were retrieved from the NC Department of Environment and Natural Resources, Division of Water Resources Drought Monitoring site (www.ncwater.org/Drought_Monitoring/dmhistory). River discharge data were downloaded using the USGS National Water Information System web interface for sites closest to the estuary. For the Neuse River, this was Fort Barnwell (site #02091814), which is about 24 km upstream of station 0. For the New River, discharge data came from site #02093000 (New River Near Gum Branch, Jacksonville, NC, USA), about 20 km upstream of station CL9.

Chlorophyll *a* and Nutrient Analyses

For *chl a* measurements, duplicate aliquots of 50 mL were filtered separately through Whatman 25 mm GF/F filters (nominal pore size, 0.7 μm). Filters were folded in half (content side faced inward), blotted with a paper towel to remove excess water, and stored in foil packets at -20°C . For nutrient analyses, 100 mL aliquots of each sample were filtered through pre-combusted (4 h at 450°C) GF/F filters and stored in acid-washed and sample-rinsed 150-mL polyethylene bottles frozen at -20°C . Frozen *chl a* samples were analyzed within a week, and nutrient samples were analyzed within 4 weeks of each sampling trip.

For *chl a* analysis, filters were extracted using a tissue grinder in 90 % acetone (EPA method 445.0; Arar et al. 1997). *Chl a* concentration of the extracts was measured using the non-acidification method of Welschmeyer (1994) on a Turner Designs TD-700 fluorometer calibrated with pure, liquid chlorophyll *a* standards (Turner Designs, Sunnyvale, CA, USA). Frozen nutrient samples were quick-thawed and nitrate/nitrite ($\text{NO}_2^- + \text{NO}_3^-$, reported as NO_3^-), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}) concentrations immediately determined using a Lachat Quick-chem 8000 auto-analyzer (Lachat, Milwaukee, WI, USA; Lachat Quick-chem methods 31-107-04-1-C, 31-107-06-1-B, and 31-115-01-3-C, respectively). Detection limits for NO_3^- , NH_4^+ , and PO_4^{3-} were 0.03, 0.2, and 0.04 μM , respectively. Daily dissolved inorganic N ($\text{DIN} = \text{NO}_3^- + \text{NH}_4^+$) loading was calculated as the product of daily average discharge, measured at Gum Branch (NewRE) and Fort Barnwell (NRE), and daily DIN concentration measured at Gum Branch (NewRE) and station 0 (NRE), where daily concentration was estimated by linear interpolation between measurements dates. For each estuarine sampling date, DIN load was calculated as the mean over the period prior to sampling equivalent to the calculated flushing time for that date (see below); this was done to account for the lag between N inputs and *chl a* response.

Flushing Time Calculations

Estuarine freshwater flushing time was calculated using the date-specific fraction of freshwater method as described in Alber and Sheldon (1999) and using estuarine and freshwater volumes determined from digital bathymetric data (Ensign et al. 2004). Raster bathymetric data were downloaded from the NOAA National Geophysical Data Center using the US Coastal Relief Model database. For both systems, the grid files were ASCII formatted, 3-s cell size, tenth of meter precision, set to 0 for empty cells, and selected as sea cells only. The bounds for the selected grid areas were $35^\circ 15' \text{N} - 76^\circ 28' \text{W} - 34^\circ 55' \text{N} - 77^\circ 8' \text{W}$ for the NRE and $34^\circ 48' \text{N} - 77^\circ 30' \text{W} - 34^\circ 30' \text{N} - 77^\circ 18' \text{W}$ for the NewRE. Grids were imported into ArcGIS (ver. 9.3, ESRI Inc.).

Each estuary was divided into segments with midpoints located at the monitoring stations, except for the upper estuarine stations (Fig. 1). Polygons were drawn around segments so as to eliminate minor tributaries and subsequently converted to shape files. Gridded bathymetry data were converted to volume using cell size, and cell volumes were summed within each segment. For upstream NRE where bathymetry data were not available (segments 0 and part of 20), segment volume was estimated using average soundings from NOAA chart #11552 and average channel width measured with the distance tool in Google Earth.

Discharge data (see above) was divided by the ratio of gaged to total watershed area (0.69 and 0.22 for NRE and NewRE, respectively) as a correction for ungaged watershed discharge. Freshwater volumes by date and segment were calculated using mean water column salinity, total segment volume, and assuming a seawater salinity of 36.2 and 35 for NewRE and NRE, respectively. For station 9 in the NewRE, salinity values came from one depth (~ 1 m), which was assumed to represent mean water column salinity. Freshwater flushing time was calculated for each segment by dividing the cumulative freshwater volume upstream of and including each segment by the date-specific average discharge (Alber and Sheldon 1999). The date-specific method is an iterative calculation of flushing time, where the period of time-averaged antecedent discharge is increased until the averaging period equals the flushing time. The fraction of freshwater calculation for flushing time was chosen because these systems for the most part are dominated by river discharge and are heavily impacted by river-borne dissolved and particulate matter. Splitting the estuary into segments produced more data and a wider range of flushing times than when using the whole estuary as a single estimate.

Statistical Analyses

Volume-weighted averages for environmental variables were calculated by summing the products of each segment

mean value (of surface and bottom measurements) and segment volume, then dividing the sum by the total estuarine volume. The relationship between natural log-transformed *chl*a and freshwater flushing time was modeled using a non-linear least squares fit of a power law variation known as the Shepherd function:

$$f(x) = \frac{ax}{(b + x^c)}$$

using “nls” in R ver. 2.13.2 (R Development Core Team 2011). Confidence intervals for the flushing time that maximized the function (FTmax) were estimated by the bootstrap standard method (Hall et al. 2004), where FTmax was determined for fits of 1,000 resampled data sets, each the same size as the original. The upper and lower bounds for the 95 % confidence interval were then defined as the 2.5th and 97.5th percentile ranking of the FTmax estimate. The same technique was used to produce confidence intervals for the coefficients of the fitted Shepherd functions. We utilized these confidence intervals to test the statistical robustness of differences in the flushing time–*chl*a relationships between estuaries and between high and low (greater than and less than median) temperature conditions within each estuary, where terms without overlapping intervals were considered significantly different. Correlation analysis was performed using the non-parametric Spearman’s rank test, reported as ρ with the significance level set at 0.05.

Results

Time Series Comparison

Regional climate trends left the area under severe drought conditions from 2007 through September 2008, with some relief in spring 2008. There were some dry periods during 2009 as well, but a very wet period began in the late fall and extended through April 2010. In late September/early October 2010, the remnants of Tropical Storm Nicole combined with a stationary low pressure system to produce record rainfall over the region (National Climatic Data Center 2010). Discharge at the two gage sites mostly reflected the precipitation trends and events (Fig. 2), with 2007 and 2008 mean discharge below the long-term annual means (New, $3.2 \text{ m}^3 \text{ s}^{-1}$, $n=46$; Neuse, $108.8 \text{ m}^3 \text{ s}^{-1}$, $n=14$). Annual mean discharge for 2009 and 2010 were close to the long-term average for each system. The drought did not show up as clearly on the Neuse River hydrograph since it represents about tenfold more watershed area than for the New River and precipitation trends can vary dramatically across a larger basin. The late September 2010 rain event produced record discharge for the New River and the Neuse River (respectively ranked as 99.9th percentile since 1949 and 97.5th percentile

since 1996). The New River hydrograph showed more rapid responses to rain events than the Neuse River hydrograph.

Over the duration of the study, 40 and 69 visits were made to the NewRE and the NRE, respectively. Time series data from those trips showed very similar temperature conditions for the two systems (Fig. 2) ranging from about 3 °C to greater than 31 °C. Volume-weighted average salinity reflected discharge patterns for both estuaries, although absolute salinities were higher in the NewRE. Volume-weighted average dissolved inorganic N (DIN) patterns were similar between systems in that the peaks (mostly nitrate) followed discharge pulses. The DIN concentration range was somewhat larger in the NewRE than the NRE, with a peak of $31.9 \mu\text{mol L}^{-1}$. Volume-weighted average dissolved inorganic phosphorus (DIP) in the NewRE paralleled the DIN pattern and peaked during discharge events, except for April 2008. In the NRE, however, DIP peaked (maximum, $6.8 \mu\text{mol L}^{-1}$) during the summer months of each year and did not track discharge. Both systems had a similar range of volume-weighted average *chl*a concentrations, but the two time series showed significant differences. *Chl*a in the NewRE showed greater variability overall, with peaks in late winter and fall 2009, spring 2010, and a month after the late September 2010 rain event. NRE volume-weighted average *chl*a showed less variability, but did show a peak in spring 2010. Both estuaries showed a rapid decline in *chl*a immediately following the September 2010 rain event, but unlike the NewRE, there was no post-event peak in *chl*a in the NRE.

The volume-weighted average time series represented overall system behavior, but variability along the salinity gradient can be significant. Example longitudinal transects of surface *chl*a, salinity, and nitrate for both systems showed similar variation with changes in freshwater discharge (Fig. 3). The gradient of salinity and nitrate moved downstream with increasing discharge. Peak *chl*a also tended to move downstream as discharge increased and was located near the steepest N gradient, which is always obvious in NRE, but only at high flow in the NewRE. There were occasions when *chl*a concentrations exceeded the NC state standard for “acceptable” water quality conditions ($40 \mu\text{g L}^{-1}$); when considering only surface samples, both systems were considered impaired in 2009 based on the criterion of 10 % of samples exceeding the standard (Table 1).

Flushing Times

Calculated flushing time ranged from a minimum of less than 2 h to a maximum of greater than 7 months, depending on discharge conditions and location in the estuary; flushing time always increased with distance downstream. The NewRE had greater median and mean flushing time than the NRE (41.6 and 51.0 vs. 18.2 and 34.1 days), but had a

lower maximum flushing time (150 vs. 221 days). Flushing time showed weak but significant positive correlation with temperature ($\rho=0.16$, NewRE and 0.12, NRE; $p<0.01$), which was driven by the trend for river discharge (inversely proportional to flushing time) to be lower during warm periods (Fig. 2).

For both estuaries, natural log-transformed chl a showed a non-monotonic, unimodal response to variation in flushing times (Fig. 4). Chl a was minimum at the shortest flushing

times and rapidly increased with flushing time until a maximum was reached at about 10 days. With longer flushing times, chl a decreased, but generally stayed above minimum concentrations. The data were fit to the non-linear Shepherd function that was chosen based on shape and not on a priori knowledge of the relationship. Adjusted r^2 values for the non-linear regressions were 0.245 (357, df) and 0.454 (671, df) for NewRE and NRE, respectively. The maximum of the function (FTmax) was reached at 9.36 days for the NewRE

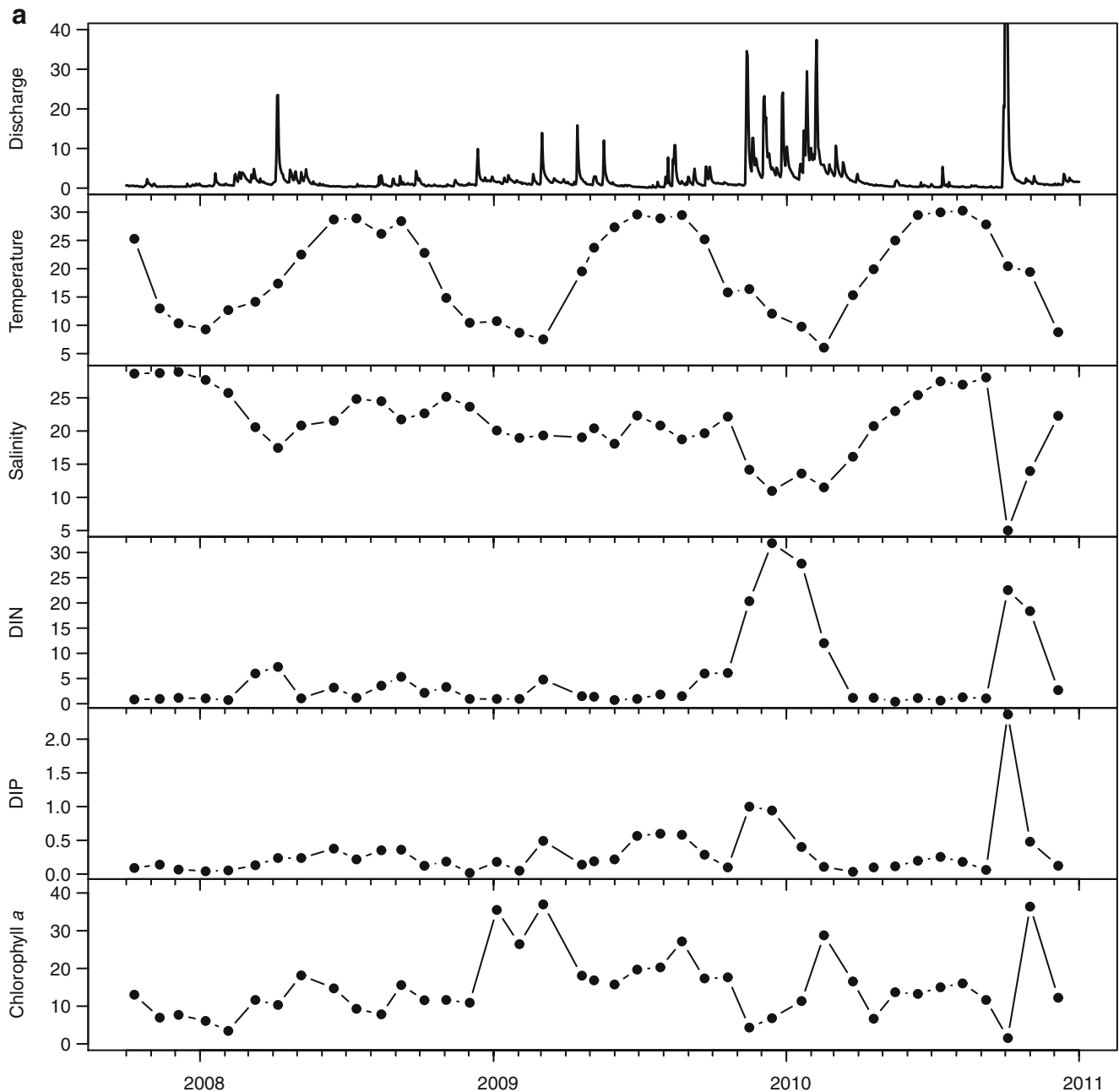


Fig. 2 Time series of discharge (cubic meter per second), volume-weighted average temperature (degrees Celsius), salinity (micrograms per liter), dissolved inorganic N (DIN, micromoles per liter), dissolved

inorganic P (DIP, micromoles per liter), and chlorophyll a (micrograms per liter) for NewRE (a) and NRE (b). See text for volume-weighted average calculation. Peak discharge to NewRE was off scale at 135 m³ s⁻¹

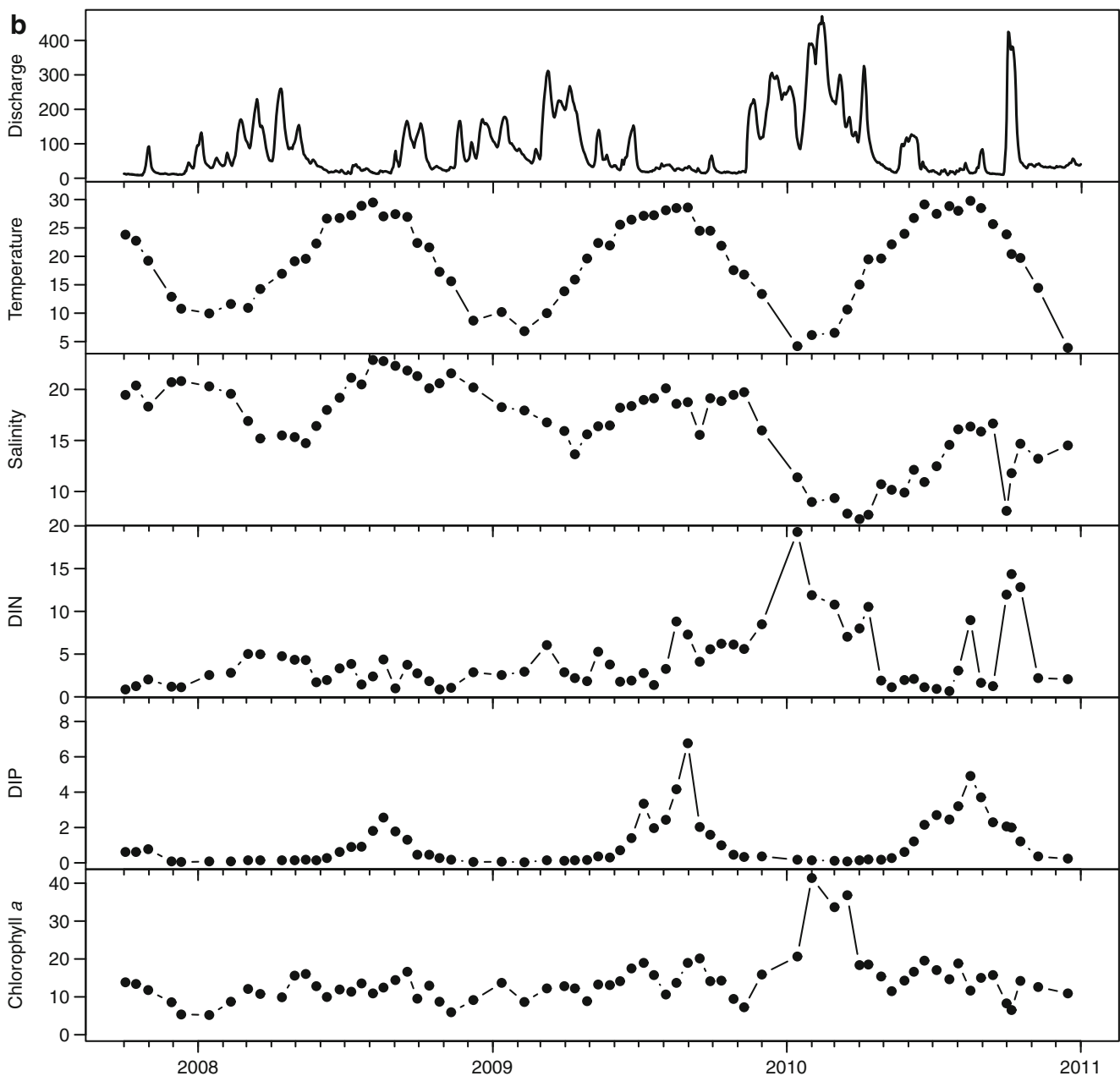


Fig. 2 (continued)

and at 10.1 days for the NRE, which were not significantly different based on overlapping 95 % confidence intervals generated by the bootstrap method (Table 2). The regression coefficients were also similar for both systems based on overlapping confidence intervals, and this was evident in the generally similar shape and limits of the functions.

Residuals from the *chl a*-flushing time regression fits were compared to other variables potentially contributing to variance in *chl a*. DIN concentrations had no significant correlation with the residuals for either system. Diffuse attenuation coefficients (K_d) were positively correlated with the residuals ($\rho=0.19$ and 0.31 for NewRE and NRE,

respectively; $p<0.001$), which is most likely due to the attenuation caused by phytoplankton biomass (Woodruff et al. 1999). Mean water column temperature also correlated positively and significantly with the *chl a*-flushing time residuals in both estuaries ($\rho=0.35$ and 0.21 for NewRE and NRE, respectively; $p<0.001$).

The effect of temperature was examined further by repeating the regression analysis and bootstrap estimation on data binned into samples above and below the median temperature (Fig. 5). For the NRE, the fitted functions were significantly different between high and low temperature bins based on non-overlapping confidence intervals of the

Shepherd function coefficients (Table 2). The positive initial slope was steeper, and the negative slope beyond FTmax was shallower under higher temperatures (Fig. 5). There were no significant differences between temperature conditions in the NewRE. FTmax was not significantly different for either system when comparing the data binned by temperature.

Mean nitrate concentrations throughout both systems were also compared to calculated flushing times (Fig. 6). Most of the highest concentrations were found when flushing times were less than 10 days (FTmax). When flushing time increased beyond 10 days, nitrate concentrations decreased to below detection. Deviations from this trend generally occurred when water temperatures were less than the median (about 20 °C). Dividing the nitrate data into bins using 10 days as the split point shows the dramatic and significant difference (based on

lack of overlap in confidence intervals for medians) between concentrations below and above FTmax (Fig. 6, inset).

Chlorophyll *a* and N Inputs

Volume-weighted *chl a* concentration by sampling date for the entire extent of each estuary was compared to an estimate of average daily, volume-normalized DIN loading calculated from concentration and daily discharge data near the head of each estuary. Volume-weighted *chl a* concentration showed a positive correlation with average DIN loading for both the NRE ($\rho=0.27, p=0.029$) and the NewRE ($\rho=0.35, p=0.032$), but only without the high loading/low *chl a* outlier caused by the September 2010 rain event in the NewRE (Fig. 7). The overall magnitudes of *chl a* and DIN

Fig. 3 Examples of surface chlorophyll *a*, salinity, and nitrate spatial patterns for NewRE (a) and NRE (b). Distance downstream is measured from stations CL7 (NewRE) and 0 (NRE). Dates represent low, medium, and high flow periods. Nitrate values are divided by 2 for the NRE

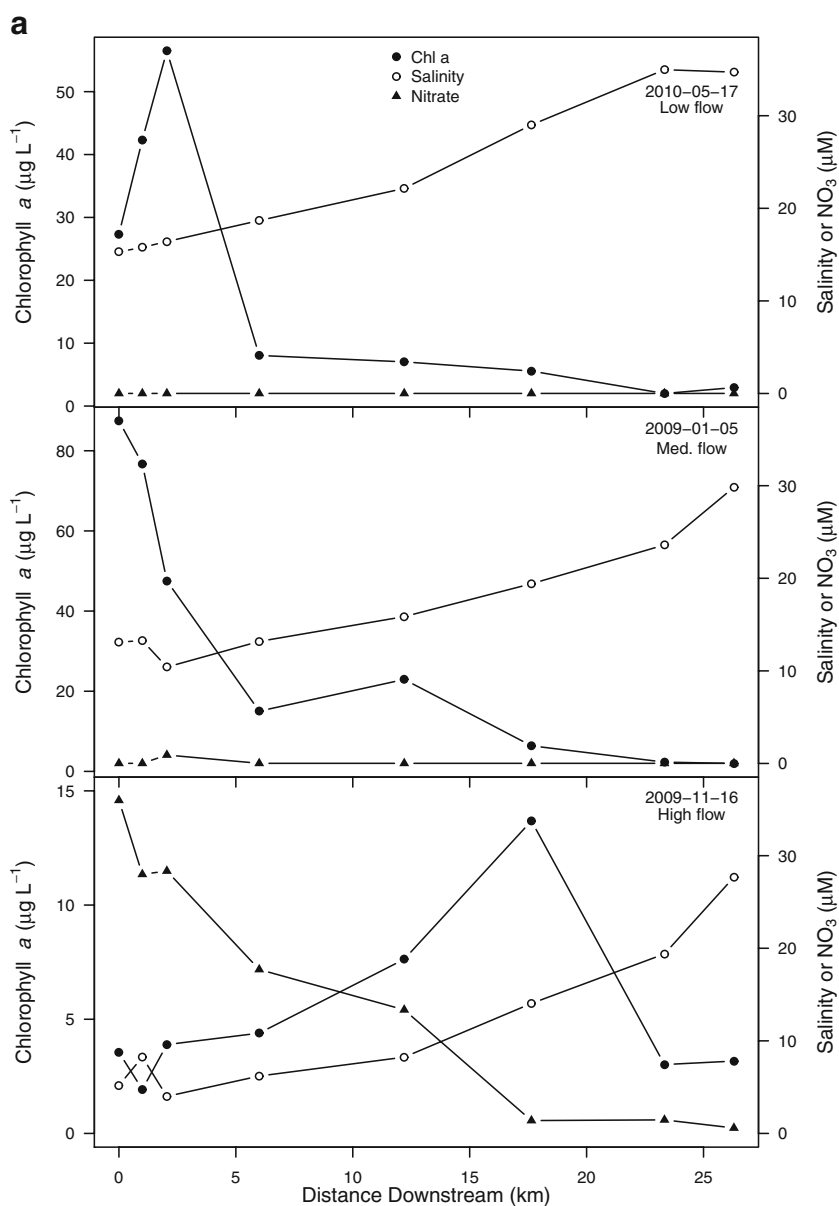
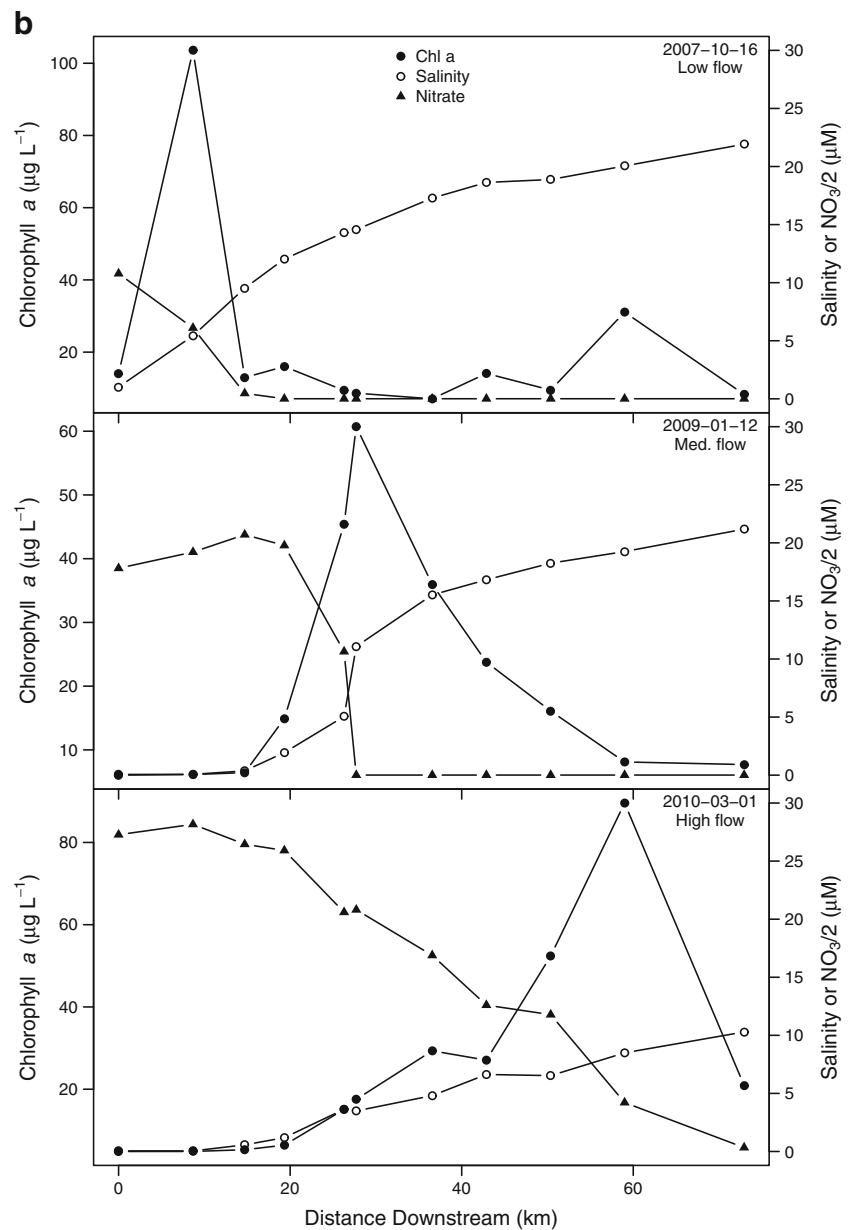


Fig. 3 (continued)



loading were quite similar for both systems, and most of the variation in DIN loading was explained by flushing time-averaged river discharge, which was similarly correlated to *chl_a*. The *chl_a*-DIN load relationships from this study were examined in context with the cross-system comparison of

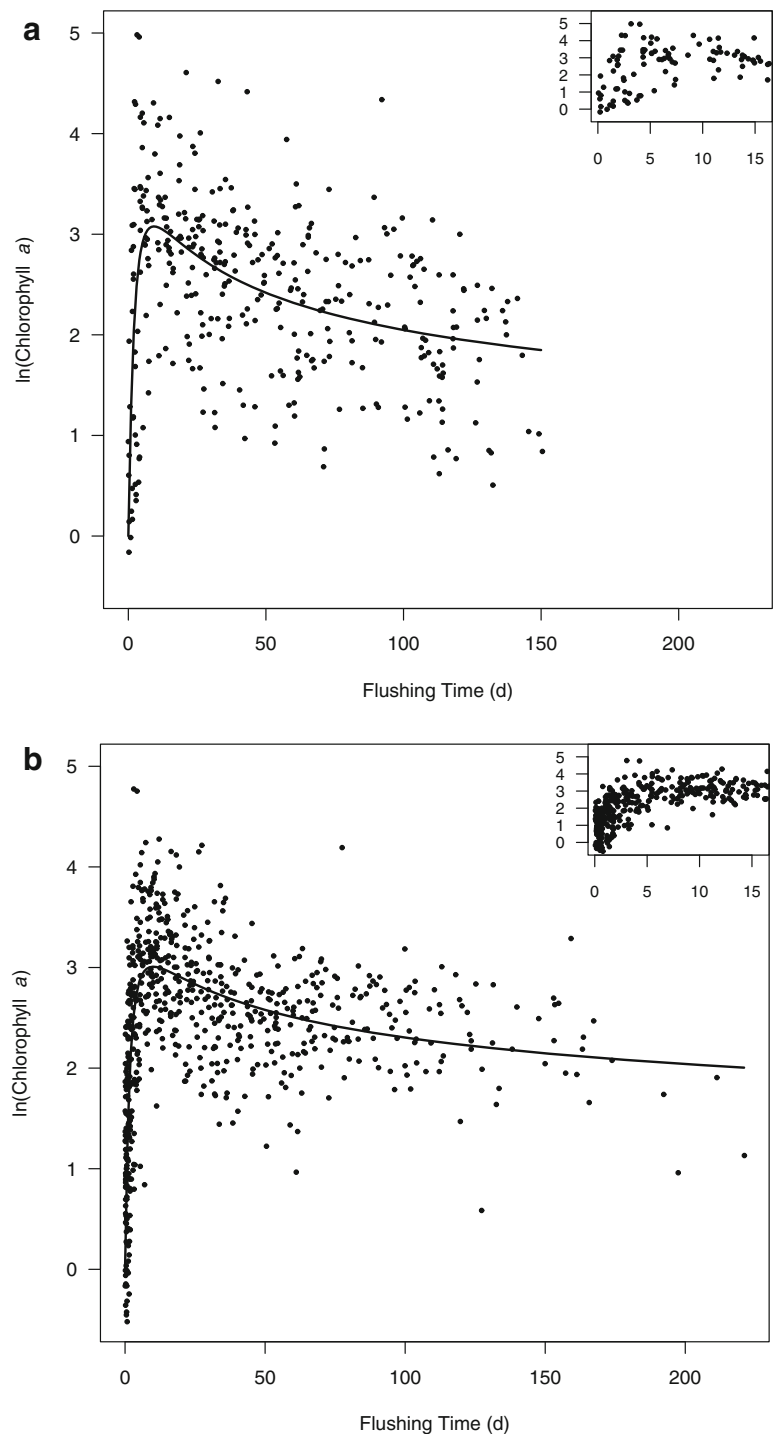
Table 1 Percent of chlorophyll *a* samples in NewRE and NRE exceeding NC state standard (40 µg L⁻¹) using all or just surface (S) samples

Year	NewRE	NewRE-S only	NRE	NRE-S only
2008	1.6 (192)	3.1 (96)	4.2 (452)	6.6 (226)
2009	8.7 (208)	10.6 (104)	9.0 (458)	14.8(229)
2010	5.2 (192)	7.3 (96)	6.6 (468)	8.1 (234)

Sample N shown in parentheses

Nixon (1992) that used annual means of a wide range of oceanic, coastal, and estuarine systems, including the MERL mesocosms (Fig. 7). The *chl_a*-DIN loading data for both the NewRE and NRE generally agreed with the cross-system comparison in that there was a positive and non-linear increase in *chl_a* with increasing DIN load. Many of the data points from this study, including the overall averages, were above the prediction limits for the cross-system regression line, although there was some overlap. It appears that at low to medium loading rates, there was a greater average yield of *chl_a* per DIN load in the NewRE and NRE than in the other ecosystems. It should be noted that temporal and spatial scale averaging for the NewRE/NRE analysis was different than the annual means used in the cross-system comparison (Nixon 1992).

Fig. 4 Natural log-transformed chlorophyll *a* (micrograms per liter) as a function of freshwater flushing time (days) for the NewRE (**a**) and NRE (**b**). Each point is the mean of surface and bottom concentrations at each station. Line is non-linear regression fit of data using the Shepherd function. Inset shows the response at short flushing times expanded for clarity



Discussion

Time Series Comparison

The climate in eastern North Carolina during the study period provided a wide range of hydrologic conditions with which to examine estuarine nutrient and phytoplankton dynamics. Both estuaries experienced the effects of extreme drought and flood events, though even for systems so close

to one another, there were differences in meteorological forcing. The hydrographs show times when one system received precipitation that affected discharge while the other did not, for example, June 2009 and May 2010. The hydrograph for the New River was quite different from that of the Neuse River, both in absolute discharge amount (lower) and shape of peaks (sharper). These features are probably best explained by the relative size differential between the watersheds, although there are many factors which play a role in

Table 2 Non-linear regression results of $\ln(\text{chlorophyll } a)$ versus flushing time in NewRE and NRE

System	FTmax (days)	a	b	c
NewRE	9.36 (6.92–12.2)	7.06 (5.53–9.21)	4.51 (2.34–8.67)	1.27 (1.21–1.33)
NewRE<20.0 °C	7.60 (8.12–11.0)	7.88 (5.82–9.40)	4.82 (3.26–8.25)	1.33 (1.23–1.35)
NewRE>20.0 °C	12.1 (6.35–16.6)	7.90 (4.57–11.8)	6.20 (1.18–14.8)	1.27 (1.14–1.36)
NRE	10.1 (8.84–11.3)	5.46 (4.72–6.24)	2.85 (1.98–3.81)	1.18 (1.15–1.22)
NRE<21.9 °C	9.51 (8.14–11.0)	7.41 (5.82–9.40)	5.29 (3.26–8.25)	1.29 (1.23–1.35)
NRE>21.9 °C	10.2 (8.74–11.9)	4.96 (4.39–5.62)	2.07 (1.48–2.81)	1.15 (1.12–1.18)

FTmax is the flushing time (days) that yields the maximum fitted chlorophyll a value. The fitted coefficients of the Shepherd function (a, b, and c) are also given. *Numbers in parentheses* represent 95 % confidence intervals based on the bootstrap method

producing hydrographs (Singh 1997). The Neuse River watershed is about ten times the size of the New River watershed, and therefore receives more and responds more slowly to changes in freshwater inputs.

Despite being an order of magnitude different in size, both estuarine systems showed similar behavior to hydrologic events. Temperature patterns were essentially the same, although the higher frequency sampling in the NRE caught some more extreme values. Volume-weighted average salinity tracked discharge in both systems, with increases in salinity during drought periods and decreases in salinity during floods, especially the large events like the late September 2010 storm. For this event, however, the system responses did differ, with salinity dropping fivefold after the storm in the NewRE, but less than twofold in the NRE. The reason for the larger salinity drop in the NewRE was the combination of a smaller watershed area, smaller estuarine volume, and track of the storm. Water yield (discharge per watershed area) was an order of magnitude higher in the New River. The track of the mostly coastal storm covered the entire NewRE watershed, but missed the western portion of the NRE watershed. This is yet another example of the overwhelming importance of the track rather than strength of tropical systems in determining estuarine water quality impacts (Paerl et al. 2010).

River discharge appeared to control concentrations of DIN in both systems and DIP in the NewRE, especially when discharge was high. Some rainstorms occurred more locally to the estuaries and did not register on the hydrograph, and yet still contributed to DIN loading. A peak in average DIN concentration was recorded in the NRE in August 2010, even though gaged discharge remained low. In both estuaries, there was often a gradient of decreasing DIN with increasing salinity (e.g., Fig. 3). Since we used volume-weighted concentrations, the large volume of the N-depleted downstream segments in the NRE made the DIN time series similar to the NewRE. In the NRE, DIP concentrations peaked in the summer, in response to increased flux from the sediments associated with higher temperatures and increased hypoxia (Fisher et al. 1982). It is not clear why this pattern was not

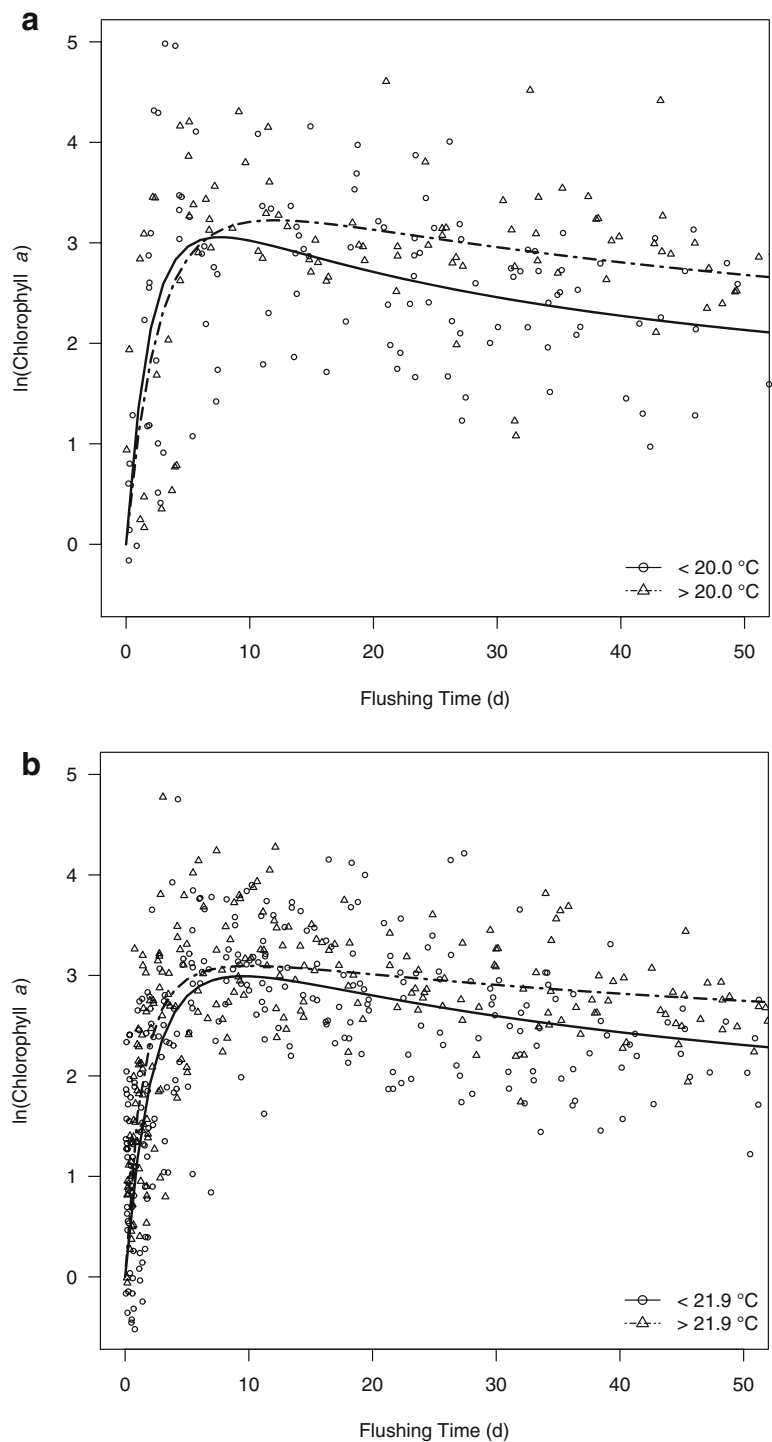
seen in the NewRE, but the relationship to discharge suggests that it was watershed sources driving DIP concentrations. The nutrient time series reported here are quite similar to earlier time series in the NewRE (Mallin et al. 2005) and the NRE (Paerl et al. 2007), suggesting little change.

The two estuarine time series for $chl a$ also resemble previous work on these systems. As would be expected for N-limited systems (Paerl et al. 1998; Mallin et al. 2005), pulses of DIN (mostly nitrate) caused the biggest response in phytoplankton biomass. In the NewRE, however, the time series for $chl a$ showed periods (winter/spring 2009) of high biomass without a preceding rise in DIN concentration. One explanation is that phytoplankton growth occurred upstream of the sampling stations and was advected downstream (Pinckney et al. 1997), utilizing most of the DIN pool in the process. There were also times when DIN pulses did not result in peaks in volume-weighted $chl a$, such as August to October 2010 in the NRE. The lack of a peak in the volume-weighted $chl a$ time series does not indicate a lack of response, since high $chl a$ concentrations may be at a single location (i.e., the $chl a$ max; Paerl et al. 2007; Fig. 3); high volume segments with low $chl a$ concentrations drive down volume-weighted means. The location of the $chl a$ max is often near the head of the estuary, but that location varies with discharge (Fig. 3).

Flushing Times

River discharge or flow has long been recognized as an important variable driving phytoplankton biomass and productivity patterns through its effect on nutrient delivery, residence time, and light availability (Ketchum 1954; Cloern 2001; Borsuk et al. 2004). Relating discharge to variables measured along the estuarine gradient is difficult because of the inherent and changing lag time between the discharge measurement and estuarine variables. Therefore, we opted to use freshwater flushing time as an explanatory variable instead of discharge, since flushing time is a proxy

Fig. 5 Same as Fig. 4, but with non-linear fit done separately for data below (*circles/solid line*) and above (*triangles/dashed line*) median water temperature. Flushing time scale truncated at 50 days to help visualize regression differences

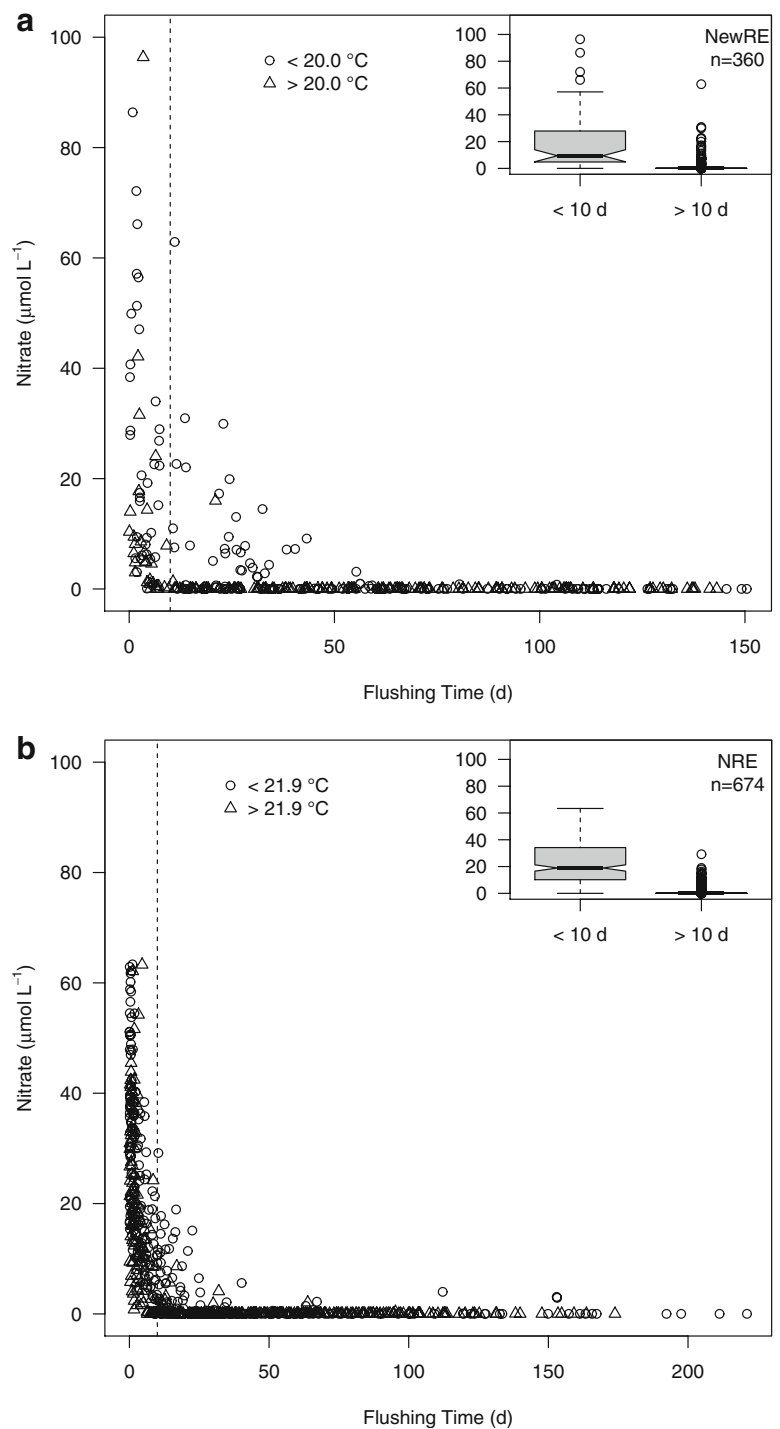


for transport time scales at each estuarine segment and can readily be compared between estuarine systems. Segmented analysis was chosen to widen the range of flushing times; only a few periods of short flushing time would have been found if the calculation was based on the whole estuary. No transport time scale can fully represent the existing processes (Monsen et al. 2002), but the flushing time measurement seemed a reasonable method given the data available and the river-dominated nature of the study sites. The calculated

flushing times for this study were quite similar to those already reported for the same systems (Christian et al. 1991; Ensign et al. 2004). Both systems have relatively long flushing times, which may explain some of the similarities in nutrient and phytoplankton patterns in these estuaries.

The observed, unimodal relationship between flushing time and *chl a* indicates a changing balance between growth and loss over time and space (Lucas et al. 2009). The positive relationship between *chl a* and flushing time up to

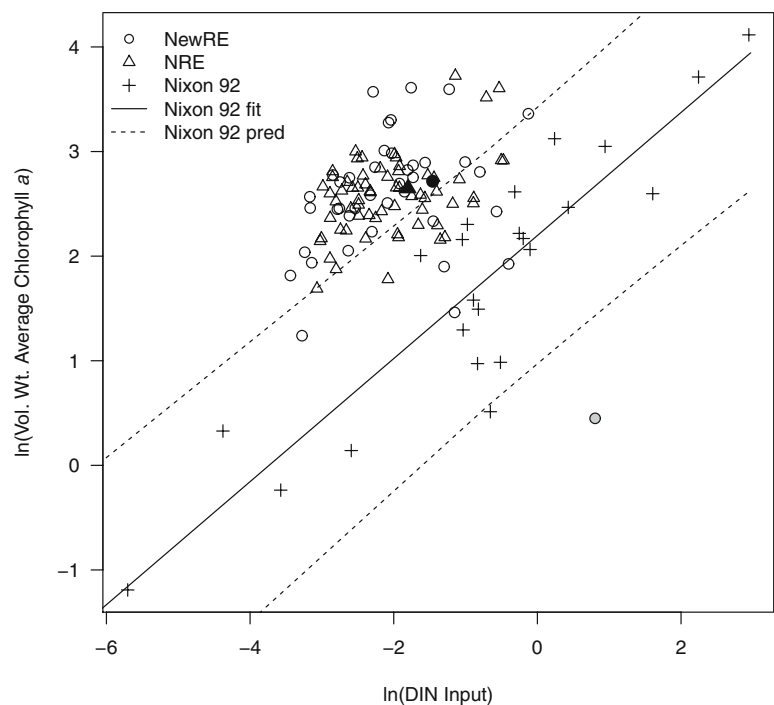
Fig. 6 Mean nitrate concentration (micromoles per liter) versus flushing time (days) for NewRE (a) and NRE (b). Data are binned into below (circles) and above (triangles) median water temperature. Vertical dashed line indicates flushing time of 10 days. Inset plots are the box plots of the same data binned into below and above 10 days flushing time. For box plots, heavy line indicates median, boxes indicate interquartile range, whiskers indicate 1.5 times range from boxes, and circles indicate outliers. Notches in boxes indicate 95 % confidence interval of median



the FTmax indicates that net growth (growth–losses) is positive and biomass increases with increasing transport time (Lucas et al. 2009). At very short flushing times, even though net growth is positive, the transport time scale is small enough that little biomass accumulates. Beyond the FTmax threshold, the slope of the relationship decreases, indicating that loss processes (zooplankton and bivalve grazing, mortality due to infections, sedimentation, etc.) are greater than intrinsic growth, yielding negative net

growth. Nutrient limitation likely leads to decreased growth rates at longer flushing times (Rudek et al. 1991; Mallin et al. 2005). The disappearance of nitrate as flushing time increases (Fig. 6) is evidence that riverine N supplies are quickly utilized in these systems, leading to N limitation of growth rates at long flushing times. Like phytoplankton, short flushing times can limit zooplankton abundance, and therefore, zooplankton grazing may be expected to increase under longer flushing time conditions (Walz and Welker

Fig. 7 Volume-weighted average chlorophyll *a* concentration (micrograms per liter) versus average daily DIN load (millimoles per cubic meter per day) for NewRE (circles) and NRE (triangles). Both variables were natural log-transformed (see “Methods” for average load calculation). Grey circle indicates an outlier not used in correlation tests. Solid symbols indicate average of all values by system. Crosses are the digitized, natural log-transformed data from the cross-system comparison of mean annual chlorophyll *a* and annual DIN input (converted to daily input) in Nixon (1992) (his Fig. 7a). Solid line is the least squares fit of the cross-system data, and dashed lines represent the 95 % prediction intervals for the regression



1998). Impacts of viruses and benthic grazers on controlling phytoplankton biomass are unknown in these estuaries, but are important sources of phytoplankton mortality in other coastal and estuarine systems (Brussaard 2004; Cloern 2001). Combined, the decreased growth rate associated with nutrient limitation and enhanced mortality contributes to net negative growth and thus a negative phytoplankton–transport time relationship at longer flushing times. Data on intrinsic growth and mortality rates in the study systems are not easily available, so this will be an important future effort to help resolve residual variability in the *chl**a*–flushing time relationships.

Salinity-based water column stratification is common within both systems and is also a source of unquantified variability within the *chl**a*–flushing time relationships. During periods of stratification, the vertical distribution of phytoplankton within these systems can be quite patchy at times (Hall and Paerl 2011; Hall et al. 2012), creating error in estimates of average water column *chl**a* derived from discrete surface and bottom water samples. Additionally, interactions between vertical patchiness of *chl**a* and estuarine circulation have been demonstrated to impact downstream phytoplankton transport in other systems (Tyler and Seliger 1978; Anderson and Stolzenbach 1985). Vertical averages present a simplified approximation of complex estuarine processes. Fully exploring the ramifications of vertical heterogeneity of these systems on phytoplankton transport processes is beyond the scope of the present work.

Other than longer flushing times in the NRE and greater variability in *chl**a* in the NewRE, the shape and limits of the fitted lines corresponded quite well, and the FTmax values

were essentially the same. This was somewhat surprising given the differences in size, geomorphology, freshwater inputs, and tidal amplitude of each estuary. Observations of unimodal patterns in estuaries are rare in the literature, but they have been reported for highly flushed lakes and rivers (e.g., Walz and Welker 1998; Hein et al. 2003) and have been predicted with simple eutrophication models (Swaney et al. 2008). Comparisons of the flushing time/phytoplankton biomass response across a wider selection of estuaries with greater differences in climate, geomorphology, tidal energy, and resident phytoplankton populations are warranted. These unimodal patterns would be expected to occur in river-dominated systems where autochthonous production is primarily fueled by riverine nutrients and flushing times range from a few phytoplankton doublings to a time span sufficient for complete assimilation of the riverine load (<1 to 10's of days). Unimodal relationships may not exist in estuaries where allochthonous riverine or oceanic inputs of phytoplankton are important, where major nutrient sources exist within the estuary itself, or in systems where residence time is strongly impacted by tidal flushing.

Temperature explained some of the residual variation in the phytoplankton–transport time curve. With the data divided by the observed median temperature, the initial slope was more positive and the negative slope less negative for temperatures greater than the median in the NRE (Fig. 5). No significant differences in model fits were observed between temperature bins in the NewRE, probably due to the smaller amount of data. However, a weak positive relationship between phytoplankton biomass and temperature has been demonstrated for the NewRE (Hall et al. 2012). The

observed relationship in the NRE may be predicted based on the generally positive response of phytoplankton intrinsic growth rates to changes in temperature over the observed range, 3 °C to ~30 °C (Raven and Geider 1988). However, other drivers, including light availability, nutrient limitation, and grazing, must also be considered as possible covariates of temperature.

Higher temperature generally leads to increased grazing rates in crustacean and protistan zooplankton and bivalve molluscs (Keller et al. 1999). Therefore, under warmer conditions, intrinsic growth of phytoplankton must be stimulated to a greater degree than grazing, with resultant higher biomass accumulation. At short flushing times, nutrients are generally replete (Pinckney et al. 1997). However, at flushing times greater than FT_{max}, nutrient concentrations are reduced to levels that limit net growth in the NRE (Fig. 6; Rudek et al. 1991). Under these long residence time conditions, the flux of nutrients from the sediments is the major source maintaining biomass and productivity (Christian et al. 1991). Fluxes of both N and P show strong positive relationships to temperature (Fisher et al. 1982). Thus, temperature enhancement of remineralization may explain the observed higher phytoplankton biomass at longer flushing times under warmer conditions. It is important to note that while the temperature effects are consistent with well-documented physiological responses of phytoplankton and benthic nutrient fluxes, light availability and temperature covary strongly within the study region (Litaker et al. 2002), and we were unable to separate light and temperature effects.

Chlorophyll *a* and N Inputs

Evidence for the direct linkage between N inputs and phytoplankton biomass has been less clear for estuaries than for lakes (Cloern 2001). For instance, the high degree of coupling between pelagic productivity and benthic nutrient fluxes in shallow (<5 m) systems such as the NewRE and NRE may obscure the linkage between phytoplankton biomass and short-term variations in riverine N loads (Fisher et al. 1982). However, this relationship has been illustrated using cross-system analyses with annual averages (Nixon 1992). We did find a positive relationship between chl_a concentration and average DIN loading for both the NRE and the NewRE. Some of the variability in the relationships could be caused by downstream areas that were uncoupled from external N loading, which was driving upstream biomass production instead. The one outlier driven by extreme low flushing time suggested the presence of a unimodal relationship in the NewRE data. Since N loading is closely tied to discharge, it could be another variant of the phytoplankton–transport time relationships already shown.

Comparing our discrete data to the trend based on averages in the Nixon cross-system study (Fig. 7) revealed that

the NewRE and NRE had higher yields of chl_a per N input than a variety of temperate marine systems. One possible explanation is that the NC estuaries are shallow and have long flushing times, which allows more complete utilization of N inputs. We do not exclude the possibility that differences in estimating DIN load and phytoplankton biomass could also have caused the discrepancy. From a management perspective, the comparison of these systems shows that increases in N loading will in general yield more phytoplankton biomass, except at extreme discharge rates when transport times are too short to allow biomass accumulation. Much of the variability in N loading to the study estuaries is driven by discharge (climate), which complicates management action. Understanding an estuary's response to hydrologic variability, however, will help separate out the system's response to natural versus anthropogenic stressors.

Summary

Two distinct, neighboring, estuaries were compared over similar climatic and hydrologic conditions. Despite being different in size, shape, tidal regime, and discharge, both showed similarities in the response of nutrients and phytoplankton biomass to events ranging from drought to floods. Phytoplankton–transport time relationships combined over space and time turned out to be non-monotonic, unimodal, and very similar between the two systems. The conceptual model of Lucas et al. (2009) was acknowledged to be simple and assumed that physical conditions were constant. Those authors suggested that under varying conditions, an obvious phytoplankton–transport time relationship might be obscured. Under the varying conditions of the present study, however, a unique relationship between phytoplankton and flushing time with regions of both net growth and net loss was clearly demonstrated. Also of significance was that similar, robust relationships spanning two different systems were found. Temperature was determined to control some of the residual variation for these functions. The importance of N inputs was also shown through correlation with aggregated phytoplankton biomass data, but yields of biomass per N input were higher than expected based on published meta-analysis. This fundamental understanding of hydrologic and nutrient control of estuarine phytoplankton biomass is one step in explaining phytoplankton variability that, when combined with information on other explanatory factors such as temperature, grazing, and viral lysis, will allow the formulation and parameterization of mechanistic models. These models will be critical for forecasting and managing (e.g., through nutrient controls) future behavior of these estuaries, especially in light of variable anthropogenic nutrient inputs and predicted climate and hence hydrologic change.

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