Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay

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**A B S T R A C T**

Harmful algal blooms are now recognized as a significant threat to the Chesapeake Bay as they can severely compromise the economic viability of important recreational and commercial fisheries in the largest estuary of the United States. This study describes the development of empirical models for the potentially domoic acid-producing *Pseudo-nitzschia* species complex present in the Bay, developed from a 22-year time series of cell abundance and concurrent measurements of hydrographic and chemical properties. Using a logistic Generalized Linear Model (GLM) approach, model parameters and performance were compared over a range of *Pseudo-nitzschia* bloom thresholds relevant to toxin production by different species. Small-threshold blooms (≥10 cells mL−1) are explained by time of year, location, and variability in surface values of phosphate, temperature, nitrate plus nitrite, and freshwater discharge. Medium- (100 cells mL−1) to large-threshold (1000 cells mL−1) blooms are further explained by salinity, silicic acid, dissolved organic carbon, and light attenuation (Secchi) depth. These predictors are similar to other models for *Pseudo-nitzschia* blooms on the west coast, suggesting commonalities across ecosystems. Hindcasts of bloom probabilities at a 1% bloom prediction point yield a Heidke Skill Score of ~53%, a Probability of Detection ~75%, a False Alarm Ratio of ~52%, and a Probability of False Detection ~9%. The implication of possible future changes in Baywide nutrient stoichiometry on *Pseudo-nitzschia* blooms is discussed.

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1. Introduction

The cosmopolitan diatom genus *Pseudo-nitzschia* has emerged as a major player in the global theatre of harmful algal blooms (HABs), occurring on both east and west coasts of the United States (Trainer et al., 2000; Thessen and Stoecker, 2008), the Gulf of Mexico (Dortch et al., 2000; Pan et al., 2001), and throughout Europe and parts of Asia (Bates and Trainer, 2006). In response to a coarsely understood set of environmental conditions, toxigenic *Pseudo-nitzschia* species are known to produce domoic acid (DA), a potent neurotoxin that can be devastating to aquatic life via trophic transfer in the food web (Fritz et al., 1992; Bates et al., 1998; Scholin et al., 2000). In humans, DA exposure manifests itself as amnesic shellfish poisoning (ASP) following the consumption of contaminated filter-feeding mussels (e.g. Bates et al., 1998; Trainer et al., 2007). Samples of *Pseudo-nitzschia* spp. collected from the largest estuary in the USA, the Chesapeake Bay, have recently tested positive for DA (Thessen and Stoecker, 2008; Thessen et al., 2009), yet, to date, there has been no reported incidence of major bird or marine mammal strandings nor ASP in the Bay. However, emerging toxicological and epidemiological research suggests that chronic, sub-acute doses of DA that do not cause any outward signs of intoxication may cause neuropathic injury to vertebrates (Levin et al., 2006; Lefebvre et al., 2007; Ramsdell and Zabka, 2008). For humans, this implies the potential for serious neurological health risks associated with repeated exposure to low levels of DA, for example in coastal communities with an established culture of shellfish consumption (Grattan et al., 2007). Furthermore, the accumulation of DA in sediments after the demise of a surface bloom may reverberate to extending HAB predictions from ecosystem models into the public health arena (Dyble et al., 2008).

Human-driven nutrient enrichment, or cultural eutrophication, is now considered an important factor driving the global increase in HABs, with the Chesapeake Bay as no exception (Grattan et al., 2007). The development of empirical models for toxigenic *Pseudo-nitzschia* blooms in the Bay is part of an ongoing effort to extend HAB predictions from ecosystem models to human impacts, to now address the potential for exposure to DA.
frequency and intensity (Hallegraeff, 1993; VanDolah, 2000; Anderson et al., 2002; Gilbert et al., 2005; Heisler et al., 2008; Anderson et al., 2008). Some of the issues that are relevant to systems where eutrophication may exert an influence on HAB development include the expected effects of nutrient stoichiometry (reviewed in Anderson et al., 2002; Gilbert et al., this issue), potential changes in ratios of dissolved organic carbon to dissolved organic nitrogen (DOC: DON; Paerl, 1988; Anderson et al., 2002), and resultant increases in the probability of toxin production by certain species, such as those within the *Pseudo-nitzschia* genus (Pan et al., 1996; Davidson and Fehling, 2006). With increased nutrient inputs from runoff come reduced Si:N and Si:P ratios (reviewed in Anderson et al., 2002), a factor shown to contribute to and/or be associated with increases of *Pseudo-nitzschia* abundance and DA production (Pan et al., 1996; Fehling et al., 2004; Parsons and Dorch, 2002; Anderson et al., 2006). Whereas most diatoms only thrive under Si-replete conditions, some *Pseudo-nitzschia* species appear to be favored by Si-limitation and certainly tend towards toxicity under extreme Si-limitation (Pan et al., 1996, 1998; Bates et al., 1998).

In the Chesapeake Bay, where HABs are common, diverse, and may be increasing in frequency, Gilbert et al. (2001) documented a direct relationship between nitrogenous fertilizer use in the Bay watershed in spring and the onset of potentially toxic *Provoctrmenum minimum* blooms. *Pseudo-nitzschia* spp. on the west coast generally bloom in response to surface macronutrient increases after natural, coastal upwelling events (Trainer et al., 2000; Kudela et al., 2003; Anderson et al., 2006; Garcia-Mendoza et al., 2009; Lane et al., 2009) and could respond to similar pulses from spring runoff in the Chesapeake Bay (Heisler et al., 2008). Furthermore, recent laboratory and field experiments link organic forms of nitrogen, such as glutamine and urea, to sustained bloom events in an upwelling region and even enhanced production of DA by toxigenic species of *Pseudo-nitzschia* (Howard et al., 2007; Kudela et al., 2008). In the Gulf of Mexico, time series of *Pseudo-nitzschia* spp. from sediment cores document increasing cell abundance in association with increased nutrient input from Mississippi River runoff (Parsons and Dorch, 2002). Nonetheless it remains unclear if highly eutrophied bays and estuaries experience significantly more *Pseudo-nitzschia* blooms or DA events than coastal upwelling systems and whether blooms in the Chesapeake Bay may be associated with anthropogenic nutrient enrichment (Dorch et al., 1997; Cochlan et al., 2008). Long-term hindcasts and forecasts from predictive models of HABs would be useful for understanding climate and land-use change effects on the frequency and magnitude of potentially deleterious algal blooms in the eutrophied Chesapeake Bay region (Kemp et al., 2005).

We present here our efforts to develop a threshold-based, empirical model for predicting the probability of *Pseudo-nitzschia* spp. bloom occurrence (and by extension, the potential for DA production) from a long-term monitoring dataset for the Chesapeake Bay. Currently, no such model exists for *Pseudo-nitzschia* on the east coast, despite the obvious importance of having such predictive capabilities in place. While the methods used to develop statistical models for estimating the distribution of harmful algae and pathogens are not new in scientific application, they are now more widely employed and accepted in ecological forecasting. A fully operational alert system that combines satellite imagery, an ensemble of numerical models, and a rules-based biological model is running in the Gulf of Mexico for blooms of the aerosolized toxin-producing, *Karenia brevis* (Stumpf et al., 2003; Tomlinson et al., 2004, 2009). Accompanying this trend are rapid advances in the development of dynamically-downscaled regional products for forecasting the physical and biogeochemical states of coastal ecosystems (e.g. Moore et al., 2002; Chai et al., 2002; Fennel et al., 2006; Xu and Hood, 2006; Chao et al., 2008; Allen et al., 2008). A system has been implemented that predicts the likely distribution of sea nettles (*Chrysaora quinquecirra*), the toxic dinoflagellate *Karlodinium veneficum*, and *Vibrio cholerae* in the Chesapeake Bay based on habitat suitability and hydrodynamic models (Decker et al., 2007; Constantin de Magny et al., in press; Brown et al., 2010). On the west coast, investigators are building upon earlier statistical models (Blum et al., 2006) for predicting the likelihood and toxicity of *Pseudo-nitzschia* blooms from a suite of hydrographic and nutrient inputs in the Monterey Bay (Lane et al., 2009) and the Santa Barbara Channel (Anderson et al., 2009). The goals of the present study are to 1) identify the physicochemical predictors of regional *Pseudo-nitzschia* spp. blooms, 2) develop and validate a predictive habitat model based on these environmental predictors that could be employed to help predict the probability of occurrence of these blooms in the Chesapeake Bay, and 3) present spatially-explicit hindcasts of past *Pseudo-nitzschia* bloom events.

2. Materials and methods

Long-term data on *surface* phytoplankton abundance and *surface* water quality were acquired from the Maryland Department of Natural Resources (MD DNR) and US EPA Chesapeake Bay Program (US EPA-CBP) for 30 stations within the mainstem Bay and 12 tidal estuarine tributaries covering the period 1985–2007 (N = 6989; Fig. 1). The data subset used for model development included the monthly suite of water quality parameters collected within one day of *Pseudo-nitzschia* spp. cell abundance records at 30 sites across the broad range of salinities represented within the Chesapeake Bay (oligohaline = 0.5–5; mesohaline = 5–18; polyhaline = >18; all salinity values in psu). The majority of observations fall in the salinity range ≤18 (N<sub>non-blooms</sub> = 5957; N<sub>blooms</sub> = 278). Fewer observations are available for salinities >18 (N<sub>non-blooms</sub> = 562, N<sub>blooms</sub> = 199) which are typically found at the four stations in the lower Bay but can also occur seasonally in the mainstem middle Bay and in the lower reaches of tributaries (Fig. 1). Because these monitoring data were not collected with the purpose of creating predictive models, sampling biases make it a less than ideal dataset despite the long time period and sampling effort represented. Observations are heterogeneously distributed in space and time due to the often event-response nature of data collection and our requirement of daily matchups between water quality and cell count data. To help ensure taxonomic fidelity of the microscopic identifications in the available monitoring observations, we limited the majority of observations to those collected by a single taxonomist whenever possible (W. Butler, pers. comm.); this excludes data from the four lower bay sites (CB1.6, CB6.4, CB7.3E, CB7.4) that were required for full Bay coverage. Additionally, due to changes in taxonomic status for the *Pseudo-nitzschia* group over the study period and difficulties with species identification using classic light microscopy, model development (Section 3.1) is focused on *Pseudo-nitzschia* spp. with no distinction between the various individual toxigenic and non-toxigenic species recorded for the Chesapeake Bay (Marshall et al., 2005; Thessen and Stoeker, 2008).

Monthly freshwater discharge data (R<sup>−1</sup>s<sup>−1</sup>) were acquired from seven of the nine sites in the USGS River Input Monitoring (RIM) Program (USGS RIMP Report, 1999) that collects water samples in the non-tidal region of major streams in the Chesapeake Bay watershed. Phytoplankton monitoring stations were grouped with the nearest, relevant RIM site for discharge matchups (Fig. 1). Negative discharge values that are reported from October 1987 to August 1988 for the Mattaponi River were eliminated from this study.

3. Modeling approach

3.1. Model development

A suite of 19 physical and chemical variables publicly available through the MD DNR and US EPA-CBP long-term water quality monitoring programs was chosen to examine conditions associated...
unsuitable model. Instead, we used the Generalized Linear Model (GLM; McCullagh and Nelder, 1989; Fox, 2002; Agresti, 2007), a regression-based approach that allows for several non-Gaussian distributions in addition to OLS linear regression. The GLM for binary data is often referred to as logistic regression and assumes that the binary response follows a Bernoulli distribution or the closely-related binomial distribution. The GLM relates a transformation of the response (bloom occurrence) to a linear combination of the explanatory variables. The transformation is referred to as the logit link function for binary variables and transforms the expectation of the response to the linear predictor as:

$$\log\left[\frac{p}{1-p}\right] = \beta_0 + \beta_1 x_1 + \ldots + \beta_k x_k,$$

where the term $p/(1-p)$ represents the odds of a ‘bloom’ event, $\beta_0$ is the intercept, and $\beta_k$ represents the regression coefficient for the $k$ variables $x$. Thus, the binary GLM simply models the log probability of an event as a linear combination of the explanatory variables. Solving for $p$, the probability of a bloom is then described by:

$$p = P_{\text{bloom}} = e^{\log p} / [e^{\log p} + 1].$$

For each bloom threshold, models were fitted by maximum likelihood using a forward stepwise approach whereby deviance residuals were tested against a Chi-square distribution for assessing the relative significance of parameters in the model (R statistical software, v. 2.7.0). Explanatory variables were added to the model individually, with the parameter leading to the largest statistically significant ($\alpha=0.05$) reduction in deviance being added at each successive step. The final degrees of freedom ($DF$) for a given model is a function of the particular predictors retained in the GLM since there is spatial and temporal heterogeneity in observation matchups between water quality and cell abundance data. To assess the influence of the annual cycle on environmental variables, hypothesis testing was performed twice, once with raw data values, and again with anomalies of all variables whereby monthly climatological means for each station were removed from the observations. Explanatory variables were also lagged from one to three months relative to the response variable in the GLM to test for significant lead/lag relationships.

Table 1: A list of the available environmental parameters tested in model development for the response variable Pseudo-nitzschia spp. abundance (in bold).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Relevant abbreviations</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>Lat</td>
<td>deg</td>
</tr>
<tr>
<td>Longitude</td>
<td>Lon</td>
<td>deg</td>
</tr>
<tr>
<td>Month</td>
<td>Month</td>
<td>n/a</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Chl-a</td>
<td>µg L$^{-1}$</td>
</tr>
<tr>
<td>Temperature</td>
<td>Temp</td>
<td>°C</td>
</tr>
<tr>
<td>Salinity</td>
<td>Sal</td>
<td>psu</td>
</tr>
<tr>
<td>Freshwater discharge</td>
<td>Dis</td>
<td>ft$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>Nitrate</td>
<td>NO$_3$</td>
<td>mg-N L$^{-1}$</td>
</tr>
<tr>
<td>Nitrite</td>
<td>NO$_2$</td>
<td>mg-N L$^{-1}$</td>
</tr>
<tr>
<td>Ammonium</td>
<td>NH$_4$</td>
<td>mg-N L$^{-1}$</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>PO$_4$</td>
<td>mg-P L$^{-1}$</td>
</tr>
<tr>
<td>Silicic acid</td>
<td>Si(OH)$_4$</td>
<td>mg-Si L$^{-1}$</td>
</tr>
<tr>
<td>Nitrate + Orthophosphate</td>
<td>N-P</td>
<td>n/a</td>
</tr>
<tr>
<td>Silicic Acid: Phosphate</td>
<td>Si:PO$_4$</td>
<td>n/a</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>O$_2$</td>
<td>mg L$^{-1}$</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>DOC</td>
<td>mg-C L$^{-1}$</td>
</tr>
<tr>
<td>Secchi depth</td>
<td>Secchi</td>
<td>m</td>
</tr>
<tr>
<td>Pseudo-nitzschia spp. abundance</td>
<td>Pseudo-nitzschia</td>
<td>cells mL$^{-1}$</td>
</tr>
</tbody>
</table>

With regional variability in Pseudo-nitzschia spp. abundance (Table 1). Past studies that focused on Pseudo-nitzschia bloom prediction used Ordinary Least Squares (OLS) regression to model cell abundance and toxin concentration (Blum et al., 2006; Anderson et al., 2009) or logistic regression to model cell abundance (Lane et al., 2009) as a function of physico-chemical and other environmental properties. In this case, as in Lane et al. (2009), we are interested in only the presence or absence of ‘bloom’ levels of Pseudo-nitzschia spp. (hereafter referred to only as Pseudo-nitzschia). The response variable for cell abundance was transformed to a binary variable using several bloom values that fall within the range of threshold levels expected to trigger toxin production. For each bloom threshold, models were fitted by maximum likelihood using a forward stepwise approach whereby deviance residuals were tested against a Chi-square distribution for assessing the relative significance of parameters in the model (R statistical software, v. 2.7.0). Explanatory variables were added to the model individually, with the parameter leading to the largest statistically significant ($\alpha=0.05$) reduction in deviance being added at each successive step. The final degrees of freedom (DF) for a given model is a function of the particular predictors retained in the GLM since there is spatial and temporal heterogeneity in observation matchups between water quality and cell abundance data. To assess the influence of the annual cycle on environmental variables, hypothesis testing was performed twice, once with raw data values, and again with anomalies of all variables whereby monthly climatological means for each station were removed from the observations. Explanatory variables were also lagged from one to three months relative to the response variable in the GLM to test for significant lead/lag relationships.
3.2. Model validation

The resulting logistic GLM was tested using cross-validation. For each year, predictions were made from the model fitted to all years excluding the one for which data were predicted. Predictions from the model were in the form of probabilities, such that a probability threshold is required for determining bloom from non-bloom conditions. Rather than setting this probability (or prediction point as expressed in Lane et al., 2009) to an arbitrary value of 0.5 (i.e. 50%), it may be useful for extreme, epidemic blooms to optimize the threshold in order to determine a relevant probability for defining a possible bloom event. Specifically, we optimized this value relative to the probability of detection (POD), the false alarm ratio (FAR), the probability of false detection (POFD), and the Heidke Skill Score (HSS; Heidke, 1926; Sohn and Park, 2008). These are defined as follows:

\[
\text{POD} = \frac{\text{correct hits}}{\text{correct hits} + \text{false negatives}},
\]

where correct hits are the equivalent of correct bloom predictions, and false negatives are those instances where bloom observations were predicted by the model to be non-blooms. POD is a component of the widely-used Receiver Operating Characteristic (ROC) curve which represents the relationship between specificity and sensitivity (as in Lane et al., 2009).

\[
\text{FAR} = \frac{\text{false positives}}{\text{hits} + \text{false positives}},
\]

where false positives are non-bloom observations that have been overpredicted as blooms by the GLM; and

\[
\text{POFD} = \frac{\text{false positives}}{\text{correct negatives} + \text{false positives}},
\]

where correct negatives are correctly predicted non-bloom observations. POFD is also an important aspect of the ROC curve and describes the proportion of non-bloom events that were falsely predicted to be blooms.

HSS is a generalized skill score that takes into account artificial predictability based on chance statistical relationships between parameters in the model. It is an alternative to the more general ‘true skill’ for describing model performance but more stringent in terms of not giving credit for predicting climatological values (Davis, 1976; Siegel and Dickey, 1986; Song and Haidvogel, 1994; Anderson et al., 2009). HSS values are functionally equivalent to Cohen’s α, ranging from −α to 1, and can be represented by the formula:

\[
\text{HSS} = \frac{|\text{correct hits} + \text{false negatives}|}{(\text{expected correct})_{\text{random}}}/|N-(\text{expected correct})_{\text{random}}|
\]

where the expected number of correct predictions is expressed as:

\[
(\text{expected correct})_{\text{random}} = 1/N|\text{correct hits} + \text{false positives}|
\times(\text{correct hits} + \text{false positives})
+ (\text{correct negatives} + \text{false negatives})
\times(\text{correct negatives} + \text{false positives})|
\]

For each year in the cross-validation, the prediction point is optimized for the POD, FAR, POFD, and HSS. Overall model performance is expressed as the mean of these iterated results in Section 4.2.1.

4. Results

4.1. Observations

Over the 22-year record of *Pseudo-nitzschia* abundance data, very large bloom events were highly episodic in the region (Fig. 2).

Mean abundance was 53 cells mL\(^{-1}\) over the range 0–1.9 × 10\(^4\) cells mL\(^{-1}\) for all 30 stations. This maximum is consistent with other *Pseudo-nitzschia* observations (Thessen and Stoecker, 2008) and is comparable to the largest blooms observed on the west coast where DA poisoning is a recurring threat to living resources and human health. The majority of blooms that exceed 100 cells mL\(^{-1}\) in the record occur in the more meso- and poly-haline middle to lower mainstem Bay (stations CB5.1, CB6.1, CB6.4, CB7.3E, CB7.4). Notable hotspots for bloom development are located in lower tidal tributary estuarine zones (EE3.0, EE3.3, LE1.1, LE1.3, LE2.2, RET1.1; Fig. 1, Table 2). Midbay station CB5.1 experienced a period from 1993 to spring 1998 when *Pseudo-nitzschia* were absent according to this dataset (Fig. 2). Salinities over 18 can occur in these tidal estuarine zones of major rivers entering the Bay, and those intrusions are often associated with high *Pseudo-nitzschia* cell abundance. These patterns are consistent with expected salinity requirements for a marine diatom (Hasle and Svendsen, 1996; Thessen et al., 2005) and corroborate observations associating salinities >5 with the presence of *Pseudo-nitzschia* in the Chesapeake Bay (Thessen and Stoecker, 2008). Locations with the largest variability in bloom occurrence are the middle and lower Bay stations as well as the downstream Patuxent River sites (LE1.1, LE1.3, RET1.1), the downstream Potomac River sites (LE2.2, RET2.1, RET2.2, RET2.4), the eastern Bay estuarine stations (EE3.0 and EE3.3), and the Patapsco River mouth in the north of the Bay (WT5.1; Table 2).

4.2. *Pseudo-nitzschia* bloom likelihood models

The correlation analysis of environmental variables and the response variable, *Pseudo-nitzschia* cell abundance, indicates a weakly significant relationship between previously noted correlates of *Pseudo-nitzschia* blooms: temperature \((r = -0.10)\), salinity \((r = -0.12)\), orthophosphate \((\text{PO}_4; r = -0.10)\), nitrate \((\text{NO}_3; r = -0.07)\), and silicic acid \((\text{Si}(OH)_4; r = -0.08; \alpha = 0.05 \text{ level}; \text{Tables 1 and 3, Fig. 3})\). The correlation between *Pseudo-nitzschia* cell abundance and freshwater discharge is not significant \((r = -0.03)\). However, it is expected that inorganic nutrients and salinity are biologically-relevant proxies for the effects of freshwater runoff into the Bay that incorporate the influence of precipitation, tidal fluxes, and discharge. Cell abundance is generally highest from 5 to 27 °C with an apparent temperature optimum near 10 °C and salinities from 5 to 28 with an apparent salinity optimum near 15. Cell abundance also tends to increase with a decrease in nutrient concentrations (Fig. 3).

However, it should be noted that the observed range of nutrient concentrations (Fig. 3) indicate an enriched system where concentrations are only rarely limiting to phytoplankton.

These correlation values are generally consistent with the logistic GLMs that significantly associate ‘blooms’ of *Pseudo-nitzschia*, tested over a range of bloom thresholds, with PO\(_4\) temperature, salinity, and variability in either NO\(_3\)+ NO\(_2\) or NO\(_2\) alone (Table 4). Blooms starting at 100 cells mL\(^{-1}\) are further controlled or significantly associated with variability in the dissolved organic carbon (DOC) pool and availability of Si(\OH\)\(_4\), while the largest blooms additionally are associated with reductions in light penetration in the water column (Secchi depth).

Despite the low correlation between freshwater discharge and *Pseudo-nitzschia* abundance, discharge also emerges as significant for blooms below 1000 cells mL\(^{-1}\) according to the hypothesis testing. The significant role of latitude and/or longitude (Table 4) suggests a spatially dependent factor that is not explained by any of the chemical or physical variables tested but that is clearly important for bloom formation at different thresholds. Despite the fact that climatological anomalies of all variables were tested during model development to account for seasonality in the parametric effects, the final models that best capture the environmental thresholds driving *Pseudo-nitzschia* blooms are the models built from raw data. The seasonal signal is retained with the inclusion of month of the year as a significant predictor of blooms.

< 1000 cells mL\(^{-1}\) (Table 4), and lag effects were not found to be significant for any of the explanatory variables tested. In agreement with a previous study (Thessen and Stoecker, 2008) and explaining the negative relationship with month of the year, the majority of bloom events above 100 cells mL\(^{-1}\) (Table 2) occurred in winter and spring months.

4.2.1. Model selection and performance

Model fits for a logistic regression cannot be assessed using a coefficient of determination (\(R^2\)) as is often done in the case of OLS linear regression. However, ‘pseudo \(R^2\)’ measures are available such as the one suggested by Nagelkerke (1991) that can be applied to the logistic GLM and very roughly translates to the proportion of deviance explained by the model. The effect of bloom threshold on GLM performance is illustrated in Table 4 where the GLM for blooms \(\geq\) 10 cells mL\(^{-1}\) results in the highest maximum skill (HSS=0.53) and Nagelkerke’s \(R^2\) (\(R^2=0.22\)), in part due to the increased predictability of the more common bloom events. While low, this threshold is currently being employed on the west coast for defining and predicting mixed-assemblage blooms of Pseudo-nitzschia (Lane et al., 2009; Moore et al., 2009) and may be the most likely to capture the onset of potentially deleterious blooms covering the full suite of Pseudo-nitzschia species present in the Chesapeake Bay. Therefore the remainder of our analyses will focus on the evaluation of the small-threshold GLM using the variety of metrics described in the Materials and methods. However, due to this model’s inclusion of discharge as the final, least significant parameter (Table 4) and our current inability to grid discharge data for spatially-explicit predictions of bloom probabilities in the Bay (Section 4.2.3), the final model will be evaluated as it is presented in Table 5, with discharge excluded, according to the algorithm:

\[
\text{logit} = 128 - 1.60 \times \text{Lat} - 68.1 \times \text{PO}_4 - 0.06 \times \text{Temp} - 1.02 \times (\text{NO}_3 + \text{NO}_2) + 0.878 \times \text{Lon} - 0.064 \times \text{Month}
\]

where the probability of a bloom (\(P_{\text{bloom}}\)) follows Eq. (2) (see Table 1 for variables). The Nagelkerke’s \(R^2\) remains effectively the same at 0.226, as does overall model performance (Table 6). Fig. 4 shows the probability of bloom occurrence as predicted by the logit in Eq. (8), split into bloom (\(n=668\); median prob=36%) and non-bloom (\(n=6,106\); median prob=1%) events. Points falling outside the 95th percentile whiskers in the boxplot denote outlier points (\(n=614\)), or in the case of the non-bloom observations, the false positive predictions (i.e. model-predicted blooms where no blooms were observed).

Use of this logistic model to predict blooms requires definition of a threshold (i.e. prediction point) based on the probabilities generated from the GLM. If a default prediction point of 0.50 were employed to define a Pseudo-nitzschia bloom, the POD would be quite low at 0.03, but the POD would also be low at 0.34 (Table 6). While a low POD is desirable, a low POD is highly undesirable, and it is clear from this scenario that a prediction point of 0.5 would lead to an underestimation of bloom events. Since our goal is to maximize model predictive skill (i.e. HSS) and consequently maximize the POD while minimizing the FAR and the POD, we have optimized the prediction threshold data for spatially-explicit predictions of bloom probabilities in the Bay (Section 4.2.3). The final model will be evaluated as it is presented in Table 5, with discharge excluded, according to the algorithm:

\[
\text{logit} = 128 - 1.60 \times \text{Lat} - 68.1 \times \text{PO}_4 - 0.06 \times \text{Temp} - 1.02 \times (\text{NO}_3 + \text{NO}_2) + 0.878 \times \text{Lon} - 0.064 \times \text{Month}
\]

point according to these metrics (Fig. 5), similar to the method of Lane et al. (2009). By doing this, we balance the need to predict potentially harmful blooms with the inconvenience of incorrectly predicting a bloom. Using this method, the maximum POD and potentially harmful blooms with the inconvenience of incorrectly predicting a bloom. Using this method, the maximum POD and

4.2.2. Model cross-validation

In order to maximize skill, the GLMs were developed using all available data ($N_{all}=6,989$) rather than split into training and validation groups as is often done in the absence of an independent validation data set. Cross-validation of the GLM was then performed by excluding a single year of data for the model fitting and making predictions for the excluded year from this model. This process was iterated for each year in the time series, and model performance for each iteration was calculated. The mean performance metrics for the ensemble of 22 cross-validation experiments are: HSS $\sim 0.53$, POD $\sim 0.75$, FAR $\sim 0.52$, and POFD at $\sim 0.09$ (Fig. 5, Table 6).

4.2.3. Pseudo-nitzschia bloom prediction maps

Hindcast maps of baywide bloom probabilities were created using spatial interpolation of physico-chemical observations relevant to the logistic GLM (Table 5) for Pseudo-nitzschia blooms for the period 1990–2006 (Prasad et al., in press). The maps illustrate bloom probabilities

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Mean (cells mL$^{-1}$)</th>
<th>St dev</th>
<th>Max</th>
<th>N</th>
<th>#Non-blooms (&lt;100 cells mL$^{-1}$)</th>
<th>#Blooms (&gt;100 cells mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB2.1</td>
<td>39.44</td>
<td>$-76.03$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>278</td>
<td>278</td>
<td>0</td>
</tr>
<tr>
<td>CB5.1</td>
<td>38.32</td>
<td>$-76.29$</td>
<td>93</td>
<td>489</td>
<td>5691</td>
<td>386</td>
<td>332</td>
<td>54</td>
</tr>
<tr>
<td>CB6.1</td>
<td>37.59</td>
<td>$-76.16$</td>
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<td>1882</td>
<td>19,000</td>
<td>125</td>
<td>80</td>
<td>45</td>
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<tr>
<td>CB6.4</td>
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<td>$-76.21$</td>
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<td>644</td>
<td>6670</td>
<td>229</td>
<td>167</td>
<td>62</td>
</tr>
<tr>
<td>CB7.3E</td>
<td>37.23</td>
<td>$-76.05$</td>
<td>178</td>
<td>536</td>
<td>4330</td>
<td>240</td>
<td>176</td>
<td>64</td>
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<tr>
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<td>10,276</td>
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<td>191</td>
<td>332</td>
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<tr>
<td>EE3.0</td>
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<td>$-76.01$</td>
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<td>877</td>
<td>4373</td>
<td>49</td>
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<td>17</td>
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<tr>
<td>EE3.3</td>
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<td>374</td>
<td>1148</td>
<td>6254</td>
<td>49</td>
<td>33</td>
<td>16</td>
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<td>ET3.1</td>
<td>38.58</td>
<td>$-76.06$</td>
<td>112</td>
<td>1749</td>
<td>361</td>
<td>151</td>
<td>351</td>
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<tr>
<td>ET3.2</td>
<td>38.3</td>
<td>$-76.89$</td>
<td>23</td>
<td>554</td>
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<td>461</td>
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<td>73</td>
<td>1217</td>
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<td>42</td>
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<td>$-75.81$</td>
<td>286</td>
<td>1060</td>
<td>7155</td>
<td>49</td>
<td>37</td>
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<tr>
<td>TF2.2</td>
<td>38.52</td>
<td>$-76.06$</td>
<td>112</td>
<td>1749</td>
<td>361</td>
<td>151</td>
<td>351</td>
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</tr>
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<td>$-77.11$</td>
<td>0</td>
<td>0</td>
<td>318</td>
<td>318</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TF2.4</td>
<td>38.47</td>
<td>$-77.26$</td>
<td>0</td>
<td>0</td>
<td>328</td>
<td>328</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WLD.1</td>
<td>39.30</td>
<td>$-76.40$</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>42</td>
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<td>0</td>
</tr>
<tr>
<td>WLT.1</td>
<td>39.21</td>
<td>$-76.52$</td>
<td>33</td>
<td>530</td>
<td>374</td>
<td>371</td>
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<td>3</td>
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<td>WLT.2</td>
<td>39.08</td>
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<td>0</td>
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<tr>
<td>WLT.4</td>
<td>39.01</td>
<td>$-76.55$</td>
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<td>0</td>
<td>28</td>
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<tr>
<td>WLT.8</td>
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<td>$-76.55$</td>
<td>0</td>
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</tr>
</tbody>
</table>
interpolated according to the same grid that is used in the Chesapeake Bay Regional Ocean Modeling System (ChesROMS) model (see Section 5.2.1) over that time period (Fig. 6). Modeled hindcasts of monthly averaged surface PO4, temperature, and NO3+NO2 were paired with latitude, longitude, and month index to calculate bloom probabilities at 4.5-km² horizontal resolution according to Eqs. (8) and (2). Fig. 6 shows two of these bloom hindcast maps: a large bloom event in March 1998 (see also Figs. 2 and 3) and a non-bloom event in August 2005 when there were no Pseudo-nitzschia cells recorded in the phytoplankton dataset.

Although the hindcasts are generated from monthly means of the environmental parameters, the distribution of observations above the 10 cells mL⁻¹ bloom threshold in March 1998 is reasonably well-captured by the GLM which assigns probabilities between 70 and 80% to the cluster of observations at or above 1000 cells mL⁻¹ in the lower Bay (Fig. 6A). For one bloom observation in the middle mainstem region of the Bay (CB5.1 = 4770 cell mL⁻¹), the GLM assigned a probability just above the optimized 19% prediction point for designating a potentially harmful bloom (Fig. 6A), thus correctly flagging this zone yet underestimating bloom magnitude by assigning fairly low probabilities. Conversely, generally low probabilities were predicted throughout the Bay during the non-bloom event of August 2005, reflecting the absence of Pseudo-nitzschia in the cell abundance records for that month (Fig. 6B). The 19% prediction point appears to cause bloom overprediction in this instance given that much of the lower Bay would have been flagged for a bloom using this threshold. However, in the absence of observations near the mouths of the James and York Rivers, it is not possible to validate the higher probabilities ~50% in that zone.

Future validation will require skill scores computed from concomitant observation-prediction matchups rather than monthly means which cannot capture the high-frequency, temporal variability of bloom dynamics. For a subset of stations, model hindcasted probabilities are compared with monthly averaged observations for the period 1990 to 2006 (Fig. 7) to roughly ascertain differences in model performance across zones. At a consistently freshwater site such as CB2.1 at the mouth of the Susquehanna River (Fig. 1) where no Pseudo-nitzschia are on record (n matchups = 187), the model always correctly predicts low probabilities, far less than the 19% bloom prediction point (Fig. 7A). At a Choptank River tributary station (ET5.2) where blooms do occur but are rare, the model exhibited a tendency to underestimate blooms in this zone by assigning probabilities above the 19% prediction point to only 36% of bloom cases (>10 cells mL⁻¹) but did assign low probabilities to 89% of non-blooms (Fig. 7B). This false negative rate is consistent with the 9% POFD calculated for the GLM (Table 6). Conversely, the model

**Table 4**

Best-fit logistic (logit) algorithms developed over a range of bloom thresholds relevant for toxin production by different Pseudo-nitzschia species (see Section 3.1). Variability in model performance is given by the maximum Heidke Skill Score (HSS) and model fit by the Nagelkerke's $R^2$; bloom probability ($P_{bloom}$) is a function of the GLM logit.

| Bloom threshold (cells mL⁻¹) | Best-fit logistic GLM | DF | Maximum HSS | Nagelkerke's $R^2$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>logit = 125 - 1.65[Lat] - 70.4[PO4] - 0.659[Tmp] - 0.937([NO3 + NO2]) + 0.822[Lon] - 0.056[Month] - 0.00002[Dis]</td>
<td>6682</td>
<td>0.53</td>
<td>0.22</td>
</tr>
<tr>
<td>100</td>
<td>logit = -2.08 - 59.7[PO4] + 0.112[Sal] - 0.091[Tmp] + 0.339[DOC] - 0.724[Si(OH)₄] - 0.00002[Dis] - 0.088[Month] - 32.5[NO2]</td>
<td>5111</td>
<td>0.40</td>
<td>0.10</td>
</tr>
<tr>
<td>1000</td>
<td>logit = 75.4 - 130[PO4] - 0.659[Si(OH)₄] - 0.107[Tmp] + 2.78([NO₃ + NO₂]) - 0.738[Secchi] - 1.93[Lat] - 0.133[Sal]</td>
<td>6694</td>
<td>0.25</td>
<td>0.04</td>
</tr>
</tbody>
</table>

tended to overestimate blooms at the mainstem lower Bay station CB6.1; for those observations below 1000 cells mL$^{-1}$, the FAR is 33% with respect to the 19% prediction point (Fig. 7C). It is important to note, however, that this false positive rate is significantly lower than the overall model FAR of 52% (Table 6), reflecting the model’s tendency to assign high probabilities to the largest blooms in the meso- to polyhaline transition zone.

5. Discussion

Much deserved attention has been given in recent decades to studying the potential influences of cultural nutrient enrichment (eutrophication) on HAB ecology (e.g. reviews by Anderson et al., 2002, 2008; Heisler et al., 2008; Gilbert et al., this issue). While most HAB species fall within various flagellate and cyanobacterial phylogeny groups, the pennate diatom genus, Pseudo-nitzschia, is unique in being the only toxigenic genus of diatoms, the group classically used as the counterpart in descriptions of HAB/flagellate ecophysiology (Smayda, 1997). As a result, our understanding of how a HAB-forming diatom like Pseudo-nitzschia will respond to land-use change and fluxes of both organic and inorganic nutrients in a complex estuarine setting is in its nascent stage. In an evaluation of Chesapeake Bay phytoplankton community composition, Marshall et al. (2005) referenced a post-European settlement shift from diatom assemblages dominated by benthic, pennate diatoms to more planktonic, centric species (Cooper and Brush, 1991), a general decrease in diatoms associated with increased anoxia and P loading (Brush and Davis, 1984), and a shift towards more lightly silicified diatom species. In contrast, the most modern surveys also document a dramatic increase in the magnitude of surface diatom abundance that coincides with an overall biomass enhancement and seasonal fluctuations of potentially toxic phytoplankton in the Bay and its tributaries (Marshall et al., 2005).

According to the time series analyzed in this study, there seems to be an increase in both the abundance and frequency of Pseudo-nitzschia blooms, particularly since the 1990s (Fig. 2), though sampling at many stations also increased during that decade. Although several large blooms were recorded in the lower Bay during the 1980s and 1990s, blooms at the mouths of major tributaries such as the Potomac (LE2.2), Choptank (ET5.2), and Patuxent Rivers (LE1.3) are relatively small and infrequent prior to 1997. After that time, the lower Bay and tidal estuaries appear to become synchronized in terms of bloom timing, but not necessarily intensity (Fig. 2). This potential lag between the lower Bay stations (CB6.1, CB6.4, CB7.3E, CB7.4) and the middle/upper Bay over the study period could signify the introduction of oceanic seeding populations transported through the mouth of the Bay northward into the estuary proper (Marshall et al., 2005) where they may persist at depth until an upwelling or mixing event occurs and brings them to the surface (Garrison, 1981; Mengelt, 2006). Alternatively, the observed relationship could suggest an evolution of conditions desirable for Pseudo-nitzschia blooms, such as eutrophication, in the mesohaline midstream portion of the Bay beginning in the late 1990s (Gilbert et al., 2001; Marshall et al., 2005). Unfortunately, the discontinuous data at many stations and often event-response nature of sampling precludes a reliable trend analysis. On-going time-series analyses using spatio-temporal kriging of the Pseudo-nitzschia abundance data should allow for future identification of such trends and comparison with larger-scale climate indices. While further analysis of existing data is clearly required to statistically establish any increasing trend in Pseudo-nitzschia abundance, it is possible with the models presented in the current

Table 5

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>p-val (Chi)</th>
</tr>
</thead>
<tbody>
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<td>Lat</td>
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</tr>
<tr>
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<td>0.007</td>
<td>&lt;&lt;0.001</td>
</tr>
<tr>
<td>NO3 + NO2</td>
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<td>0.200</td>
<td>&lt;&lt;0.001</td>
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<tr>
<td>Lon</td>
<td>0.878</td>
<td>0.223</td>
<td>&lt;&lt;0.001</td>
</tr>
<tr>
<td>Month</td>
<td>-0.064</td>
<td>0.018</td>
<td>&lt;&lt;0.001</td>
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</table>

Table 6

<table>
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<th>POD</th>
<th>FAR</th>
<th>POFD</th>
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</thead>
<tbody>
<tr>
<td>Absent if $P_{\text{bloom}}&lt;0.50$</td>
<td>0.38</td>
<td>0.34</td>
<td>0.44</td>
</tr>
<tr>
<td>Absent if $P_{\text{bloom}}&lt;0.19$</td>
<td>0.53</td>
<td>0.75</td>
<td>0.52</td>
</tr>
<tr>
<td>Cross-validation $P_{\text{bloom}}=0.19$</td>
<td>0.53</td>
<td>0.75</td>
<td>0.52</td>
</tr>
</tbody>
</table>

5.1. Potential drivers of *Pseudo-nitzschia* blooms in the bay

In all three logistic GLMs, there exist inverse relationships between nutrient parameters and the *Pseudo-nitzschia* bloom index, invoking both concurrent surface nutrient uptake and even the potential onset of nutrient limitation as *Pseudo-nitzschia* cell abundance increases. It is important to note that these relationships to nutrient concentrations are not any more robust when lagged relative to bloom occurrence. Moreover, a meaningful lag time for correlation of the phytoplankton response to nutrient supply is expected to be less than the average monthly sampling frequency available here because of the relatively short timescales for nutrient uptake. Inorganic PO₄ is the most significant nutrient predictor in all GLMs (Table 4). In spring, when the majority of these blooms occur, the Chesapeake Bay is indeed more limited by PO₄ relative to NO₃ (Prasad et al., in press). When the majority of these blooms occur, the Chesapeake Bay is indeed more limited by PO₄ relative to NO₃ (Prasad et al., in press). However, in the absence of direct measurements using nutrient limitation assays, it is not possible to assess the physiological status of the phytoplankton community at the time of sampling. While P-limitation is certainly not an initiator of bloom formation, which of course requires sufficient P availability for cell division, low concentrations in tandem with high cell abundances are captured by the models, indicating surface depletion of PO₄ at the very least. We note that the onset of P-limitation has been shown to stimulate DA production in laboratory cultures (Pan et al., 1998). Unfortunately, the models in the current study cannot address the effects of nutrient drawdown on DA production in the Bay.

As with other *Pseudo-nitzschia* habitat models for the west coast of the U.S.A. (Blum et al., 2006; Anderson et al., 2009; Lane et al., 2009), this nutrient limitation pattern is supported by the GLM results where a significant negative relationship between *Pseudo-nitzschia* blooms and Si(OH)₄ concentrations could indicate a lower Si-requirement for this diatom group. This may suggest a link between runoff-induced reductions in Si:N and Si:P ratios (Smayda, 1990, 1997) and the occurrence of these blooms. The inverse relationship between blooms ≥ 100 cells mL⁻¹ and Si(OH)₄ in the medium-threshold GLM (Table 4) may be related to the mere drawdown of Si(OH)₄. Alternatively, it may reflect the previously observed association between *Pseudo-nitzschia* blooms (P. multiseries and *P. australis*) and the onset of Si-limitation in other regions and experiments where *Pseudo-nitzschia* may be a strong competitor in relatively Si-depleted waters following a large diatom bloom (Sommer, 1994; Marchetti et al., 2004; Anderson et al., 2006; Lane et al., 2009). Perhaps in part due to their (relatively) lightly silicified frustules, *Pseudo-nitzschia* cells seem better adapted to low-Si environments than many other diatoms, often outcompeting other groups during the late stages of a spring diatom bloom when surface waters have been fairly depleted of Si (Anderson et al., 2006; Kudela et al., 2004; Marchetti et al., 2004). However, like all diatoms, *Pseudo-nitzschia* still requires Si, N, and P for maximal growth and will bloom in response to these inputs, particularly during winter and spring months when turbulence and reduced light levels in the water column tend to favor diatoms over flagellates (Margalef, 1978; Smayda, 1997).

A negative relationship with NO₃ + NO₂ availability for the small and large bloom thresholds suggests that N-uptake by surface blooms leads to depletion of both the NO₃ and NO₂ pools, rather than to the NO₂ regeneration that is expected during more N-replete conditions (Lomas et al., 2000; Bode et al., 2002). This association with a reduced NO₂ pool is also seen in the inclusion of the NO₂ parameter in the GLM for blooms ≥ 100 cells mL⁻¹. Rapid NO₃ and NO₂ utilization may be somewhat inconsistent with evidence of a positive association.
Pseudo-nitzschia abundance in this dataset (Table 3) and could useful for predicting the future probabilities of toxic events. Because of the salinity tolerance limits for Pseudo-nitzschia shown in this study (Fig. 3) and elsewhere (Thessen et al., 2005; Thessen and Stoecker, 2008), blooms are expected to be extremely rare and forced predominantly by salinity anomalies (see Section 5.2) in the upper, fresh reaches (salinity < 5) of tributaries where nutrient enrichment from runoff is high. However, this enrichment is manifest in the meso- and polyhaline mainstem portions of the Bay as well (Prasad et al., in press). Given that total N from the Susquehanna River, a major tributary of the Chesapeake Bay, increased 2.5-fold from 1945 to 1990 (Kemp et al., 2005) and that urea usage in agriculture and industry is increasing worldwide (Gilbert et al., 2005), the potential role of NO₃ and urban forms of N runoff in driving Pseudo-nitzschia blooms and perhaps even toxin production should not be ignored, particularly in light of the projected increases in precipitation and population indices for the region (Meehl et al., 2007).

The inclusion of DOC as a significant predictor in the medium-threshold GLM is particularly interesting given its connection to riverine inputs (e.g. Doering et al., 1994; McKenna, 2004) and the previously observed association between Pseudo-nitzschia blooms and river discharge or potential proxies of runoff on the west coast (Kudela et al., 2004; Anderson et al., 2009; Lane et al., 2009) where P. multiseries is a dominant bloom former. Gilbert et al. (2001) found strong correlations between DOC and DON in relation to harmful P. minimum populations in the Bay and underscored the difficulty in ascribing direct or indirect effects to this relationship. One possible indirect effect is the potential for covariation between DOC and DON if Pseudo-nitzschia cells are directly taking up organic N species during blooms as described above. There is some evidence for the uptake of organic substrates by Pseudo-nitzschia after dark adaptation (Mengelt, 2006), but the possibility of interference by extra-cellular or epiphytic bacteria remains (Bates et al., 2004; Stewart, 2008). Studies on the west coast also implicate some kind of allochthonous component to Pseudo-nitzschia blooms from either direct correlation between blooms and rain events (Trainer et al., 2000) or via the inclusion of chlormophoric dissolved organic matter and precipitation/NO₃ relationships in their habitat models (Anderson et al., 2009; Lane et al., 2009). Provided there is a causal relationship here, a positive influence of DOC on Pseudo-nitzschia blooms implies the potential for future changes as precipitation events over the Bay increase in frequency and/or intensity according to current IPCC projections (Meehl et al., 2007). However, the weak correlation between DOC and freshwater discharge ($r = -0.08$, Table 3) and the negative association with discharge in the small- and medium-threshold GLMs (Table 4) may indicate a more autochthonous source of DOC and/or an association between Pseudo-nitzschia blooms and low runoff periods or drier years. Understanding this relationship between DOC, discharge, and Pseudo-nitzschia blooms clearly requires more targeted studies.

The complex nature of environmental change may have opposing effects on Pseudo-nitzschia bloom distributions. Eutrophication is not only manifest in the bottom-up direction with respect to HABs, but is also expected to influence the entire ecosystem with the net result being more favorable conditions for HAB development in general. The timing of nutrient enrichment, for instance, may be de-coupled from predator population increases, resulting in the release of grazing pressure which can be further enhanced by a build-up in anoxia as the bloom decomposes (e.g. Buskey et al., 1997). The present study, however, found no significant relationship between chlorophyll and Pseudo-nitzschia abundance for the Chesapeake Bay (Table 3), suggesting a possible de-coupling of these blooms from the overall rise and fall of phytoplankton biomass. The significant positive relationship with salinity and negative relationship with temperature in the GLMs developed for small- and medium-thresholds implies physical tolerance limits and seasonality that are expected for this

Fig. 7. Observed cell abundance vs. model-predicted probabilities for a Pseudo-nitzschia bloom projected over the period 1990–2006 at stations A) CB21, Susquehanna R., B) ETS2, Choptank R. ($r^2 = 0.10$), and C) CB6, lower mainstem Bay ($r^2 = 0.14$). Trendlines (solid) were determined by linear regression, and the vertical lines (dashed) mark the 0.19 bloom prediction point.

between NO₃, possibly from runoff, and fall Pseudo-nitzschia blooms in the Monterey Bay, CA (Lane et al., 2009). However, the same caveat remains that a negative relationship between a nutrient parameter and the bloom index may reflect more the effects of nutrient drawdown by actively growing phytoplankton rather than suggesting the possible onset of conditions that favor one phytoplankton group over another.

Several studies have now effectively demonstrated the uptake of regenerated forms of nitrogen — ammonium ($\text{NH}_4$), and urea — by Pseudo-nitzschia species (Howard et al., 2007; Kudela et al., 2008; Cochlan et al., 2008; Thessen et al., 2009). An increase in the potential for DA production has been noted when the organism incorporates organic N or NH₄ instead of NO₃ (Howard et al., 2007; Thessen et al., 2009). While NH₄ is not a significant explanatory variable for blooms in the Chesapeake Bay GLMs, it is significantly associated with Pseudo-nitzschia abundance in this dataset (Table 3) and could useful for predicting the future probabilities of toxic events. Because of the salinity tolerance limits for Pseudo-nitzschia shown in this study (Fig. 3) and elsewhere (Thessen et al., 2005; Thessen and Stoecker, 2008), blooms are expected to be extremely rare and forced predominantly by salinity anomalies (see Section 5.2) in the upper, fresh reaches (salinity < 5) of tributaries where nutrient enrichment from runoff is high. However, this enrichment is manifest in the meso- and polyhaline mainstem portions of the Bay as well (Prasad et al., in press). Given that total N from the Susquehanna River, a major tributary of the Chesapeake Bay, increased 2.5-fold from 1945 to 1990 (Kemp et al., 2005) and that urea usage in agriculture and industry is increasing worldwide (Gilbert et al., 2005), the potential role of NO₃ and urban forms of N runoff in driving Pseudo-nitzschia blooms and perhaps even toxin production should not be ignored, particularly in light of the projected increases in precipitation and population indices for the region (Meehl et al., 2007).

The inclusion of DOC as a significant predictor in the medium-threshold GLM is particularly interesting given its connection to riverine inputs (e.g. Doering et al., 1994; McKenna, 2004) and the previously observed association between Pseudo-nitzschia blooms and river discharge or potential proxies of runoff on the west coast (Kudela et al., 2004; Anderson et al., 2009; Lane et al., 2009) where P. multiseries is a dominant bloom former. Gilbert et al. (2001) found strong correlations between DOC and DON in relation to harmful P. minimum populations in the Bay and underscored the difficulty in ascribing direct or indirect effects to this relationship. One possible indirect effect is the potential for covariation between DOC and DON if Pseudo-nitzschia cells are directly taking up organic N species during blooms as described above. There is some evidence for the uptake of organic substrates by Pseudo-nitzschia after dark adaptation (Mengelt, 2006), but the possibility of interference by extra-cellular or epiphytic bacteria remains (Bates et al., 2004; Stewart, 2008). Studies on the west coast also implicate some kind of allochthonous component to Pseudo-nitzschia blooms from either direct correlation between blooms and rain events (Trainer et al., 2000) or via the inclusion of chlormophoric dissolved organic matter and precipitation/NO₃ relationships in their habitat models (Anderson et al., 2009; Lane et al., 2009). Provided there is a causal relationship here, a positive influence of DOC on Pseudo-nitzschia blooms implies the potential for future changes as precipitation events over the Bay increase in frequency and/or intensity according to current IPCC projections (Meehl et al., 2007). However, the weak correlation between DOC and freshwater discharge ($r = -0.08$, Table 3) and the negative association with discharge in the small- and medium-threshold GLMs (Table 4) may indicate a more autochthonous source of DOC and/or an association between Pseudo-nitzschia blooms and low runoff periods or drier years. Understanding this relationship between DOC, discharge, and Pseudo-nitzschia blooms clearly requires more targeted studies.

The complex nature of environmental change may have opposing effects on Pseudo-nitzschia bloom distributions. Eutrophication is not only manifest in the bottom-up direction with respect to HABs, but is also expected to influence the entire ecosystem with the net result being more favorable conditions for HAB development in general. The timing of nutrient enrichment, for instance, may be de-coupled from predator population increases, resulting in the release of grazing pressure which can be further enhanced by a build-up in anoxia as the bloom decomposes (e.g. Buskey et al., 1997). The present study, however, found no significant relationship between chlorophyll and Pseudo-nitzschia abundance for the Chesapeake Bay (Table 3), suggesting a possible de-coupling of these blooms from the overall rise and fall of phytoplankton biomass. The significant positive relationship with salinity and negative relationship with temperature in the GLMs developed for small- and medium-thresholds implies physical tolerance limits and seasonality that are expected for this
diatom group and have been observed for *Pseudo-nitzschia* in the Chesapeake Bay when high cell abundances occur with colder, relatively salty waters (Table 4) (Pan et al., 1993; Thessen and Stoeger, 2008). In contrast, the weakly significant negative association with salinity in the GLM for the largest, rarest blooms is not easily explained but may be due to the fact that the majority of very large blooms fall within the 10–15 salinity range (Fig. 3). Another possibility is that since some large blooms do occur at lower salinities in the lower tidal tributary estuarine zones, two modes of bloom formation might exist in the Bay: one stemming from vertical mixing where high salinities, low temperatures, and high nutrient concentrations are in phase, and a second mode where river runoff is associated with lower salinities and high nutrients. Given the sum of these relationships, expected increases in precipitation for the region could restrict *Pseudo-nitzschia* blooms to a smaller portion of the Bay if the size of the meso- and polyhaline zones were to contract, although subsurface blooms are still probable, particularly in the lower Bay.

5.2. Performance of the *Pseudo-nitzschia* bloom GLM

Model results presented here for *Pseudo-nitzschia* blooms in the Chesapeake Bay join the growing effort to create and validate empirical habitat suitability models for species or groups of biogeochronologically important or potentially deleterious pathogens, microbes, zoonotic diseases, and planktonic organisms (e.g. Iglesias-Rodriguez et al., 2002; Blaauw et al., 2006; Decker et al., 2007; Patz et al., 2008; Constantin de Magny et al., 2008). An inherent constraint of these ecological forecasting models is the explanatory power of the available predictor variables, which in this study were limited to a standard array of hydrographic properties and chemical constituents from long-term water quality monitoring programs in an ecologically complex estuarine system. This complexity presents challenges for forecasting biological processes in the absence of strong physiological data to support mechanistic modeling. Great strides have been made in this direction for at least one group of HABs in the Gulf of Maine (McGillicuddy et al., 2003, 2005). However, pressing human health issues drive the need for statistical methods that take advantage of data currently available to glean process and predictive understanding as well as build models for a variety of HABs and toxic agents (Moore et al., 2008). To that end, the approach described in this paper marks the first such attempt for the *Pseudo-nitzschia* genus on the U.S. eastern seaboard.

Predictive success for the GLM is dependent on (1) the chosen threshold for defining a ‘bloom’, and in creating the binary variable for the logistic regression (Table 4); (2) determining a relevant prediction point for forecasting blooms from model output (Fig. 5); and (3) the location along the Bay’s salinity gradient (Fig. 7). As the bloom threshold increases, bloom events decrease in frequency and model skill is reduced, as can be seen when the bloom threshold increases from 10 to 1000 cells mL\(^{-1}\) (Table 4). Use of a single threshold for the final model (10 cells mL\(^{-1}\) GLM; Tables 5 and 6; Figs. 5–7) then assumes that all resident species of *Pseudo-nitzschia* will bloom in response to similar ecological conditions or occupy the same niche space (Smyda, 1963; Margalef, 1978; Bidigare et al., 1990). However, since such a large proportion of resident species are potentially toxigenic and were only included in the analysis when collected from surface samples, it is reasonable to assume they are similarly adapted or that a given sample is dominated by only one or two *Pseudo-nitzschia* species most adapted to the ambient conditions (Smyda, 1963; Margalef, 1978; Bidigare et al., 1990). This implies wider utility in a model that predicts *Pseudo-nitzschia* blooms from a relatively low threshold (10 cells mL\(^{-1}\)).

We note that the likelihood of DA production at or just above this density begins to increase (on the U.S. west coast) for the toxigenic *P. fraudulenta* group and for other members of the indigenous toxigenic *Pseudo-nitzschia* assemblage (*P. pungens*, *P. calliantha*, *P. cuspidata*, *P. multiseries*; Thessen and Stoeger, 2008) at even higher densities (Trainier and Saddleson, 2005). The important caveat still remains that genetic diversity at the species and strain level can strongly regulate toxicity and cannot be accounted for in the generic GLM (Orsini et al., 2004; Evan et al., 2005; Erdner et al., 2008; Thessen et al., 2009). Any niche-space flexibility offered by such diversity introduces further complications for predicting *Pseudo-nitzschia* distributions based on habitat suitability models.

The optimization method for choosing a prediction point (Fig. 5) allows the user to adjust the operational probability according to the relative weightings of the various performance metrics. In any operational or routine monitoring context, probability maps of *Pseudo-nitzschia* blooms must be presented with annotated instructions for interpretation of these contingencies. The applicability of our optimized prediction point of 19% varies with location and date in the Bay according to our hindcasts, and without future validation and tuning, may prove to overestimate blooms in the mesohaline mainstem and underestimate the rare bloom events that tend to occur in tidal tributary zones (Figs. 6 and 7). These differences in predictability in different portions of the Bay might then be influenced by the uneven distribution of observations in the dataset with respect to salinity since much of the data used for model development fall in zones fresher than the salinity tolerance for *Pseudo-nitzschia*.

To account for any bias in model performance caused by increased skill in oligohaline regions where few blooms occur (see Fig. 7A), we tested separate models for the three different salinity zones. The results (not shown) do not display improvement in model skill for any individual zone but do, in fact, exhibit an expected decrease in skill (max HSS=0.48) compared with our final GLM when salinities <5 are excluded from model development. It turns out to be difficult to correctly predict blooms in the oligohaline zone alone (max HSS=0.18) because of the low frequency of bloom observations below a salinity of 5 (i.e. rare events). Salinity is indeed an important factor selecting for *Pseudo-nitzschia* blooms in the Bay, and this is accounted for in development of the final GLM (Eq. (8)) which considers salinity and location (but not an interaction term). By using all available observations, we have tuned model sensitivity at various thresholds (Table 4) to those conditions suitable for blooms as well as for the infrequent salinity anomalies in fresher zones that lead to rare bloom events in tributaries. However, future validation efforts may support the use of separate models for the different thresholds and salinity zones based on location or time of year.

Relative to *Pseudo-nitzschia*-specific predictive models for the west coast, our final 10 cells mL\(^{-1}\) GLM in Table 5 performs comparably in its ability to correctly predict blooms for the Chesapeake Bay with a 75% probability of detection (cf. 75–89%) and a 52% false positive rate (cf. 25–62%) (Anderson et al., 2009; Lane et al., 2009). In terms of error type, minimizing the POD far over FAR is arguably more desirable in the context of protecting human health since the perceived or actual damage from missing a potential health threat may be deemed worse than a false alarm scenario. In that sense, this model performs quite well at the optimized bloom prediction point. The relatively low value of Nagelkerke’s $R^2 \sim 0.23$ (Table 5) brings into question the influence of environmental variables absent from this analysis, in particular, measures of organic nutrients and trace metals. Use of these models by resource managers will ultimately require a keen understanding of model uncertainty (see Fig. 5) along with an adaptive strategy for implementation that takes into account any model and threshold sensitivity within different zones of the Bay as well as emerging data on the risks of both acute and chronic exposure to DA toxins (Erdner et al., 2008).

5.2.1. Forecasting *Pseudo-nitzschia* blooms

Producing nowcasts or forecasts of *Pseudo-nitzschia* blooms in the Bay would be facilitated by assimilation of environmental predictions from numerical models into the diagnostic GLM. This is possible in conjunction with the Chesapeake Bay Forecast System (CBFS) which...
currently uses dynamical downscaling with the Weather Research and Forecasting Model (WRF, 2010) to provide high resolution forcing of the ChesROMS ocean model and Soil and Water Assessment Tool (SWAT, 2010) river basin-scale model (Murtugudde, 2009). The forecast is generated every three days using a six to eight member ensemble forced with the National Center for Environmental Prediction – Global Ensemble System (NCEP GENPS Products, 2008) and can provide accurate estimates of sea surface temperature and salinity (used only in higher threshold GLMs). The ChesROMS model is currently being tuned with an embedded Nitrogen–Phytoplankton–Zooplankton–Detritus (NPZD) ecosystem model for generating forecasts of macronutrient and chlorophyll concentrations, including a recently implemented DON component (Fennel et al., 2006). Together, the ChesROMS and NPZD models will be able to predict the six explanatory variables used in the final GLM (Table 5). However, use of higher-threshold GLMs (Table 4) will require estimates of Si(OH)₄ and DOC which are not predicted from the ecosystem model currently coupled to ChesROMS. Present research involves diagnostic coupling of alternative ecosystem models to ChesROMS that include Si(OH)₄ in their formulation (e.g. Moore et al., 2002; Chai et al., 2002). Unfortunately, it may not be possible to generate accurate DOC predictions from existing models which at best include a “small detritus” carbon fraction (Christian et al., 2002; Moore et al., 2002), in which case, alternative GLMs may prove more practical for making Pseudo-nitzschia bloom nowcasts and forecasts.

6. Concluding remarks

This modeling exercise identifies several environmental variables associated with Pseudo-nitzschia blooms in the Chesapeake Bay but unfortunately cannot identify environmental indicators of the presence of its associated toxic agent, DA. Indeed, defining the distinction between factors that initiate bloom development and those that trigger toxin production is a recurring theme in HAB ecology, particularly for Pseudo-nitzschia. The issue of toxigenesis is highly relevant in a region such as the Chesapeake Bay which, to date, has experienced no known outbreaks of DA poisoning but whose populations may still be affected by chronic exposure to moderate toxin levels (Grattan et al., 2007; Thessen and Stoecker, 2008). Forecasting endeavors that follow should not only relate these uncertainties to end-users but also continually revisit bloom thresholds and model formulation with the addition of new validation data and input from resource managers and shellfish harvesters alike. Lastly, future efforts to model HABs must look closely at the combined effects of inorganic and organic nutrient uptake, trace metal utilization (e.g. Rue and Bruland, 2001; Maldonado et al., 2005), allelopathic competition (e.g. Adolf et al., 2008), and grazing (e.g. Bargar et al., 2003) to move beyond statistics-based habitat models and incorporate a mechanistic understanding of the complex processes regulating HAB development and toxicity.

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