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Water Quality Relationships to Concentrations of *Pfiesteria*-Like Organisms in Virginia Estuaries for 1998

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ABSTRACT

A series of statistical analyses were performed to identify the relationship between abundance of dinoflagellates grouped as *Pfiesteria*-like organisms and a set of 25 water quality variables from May through October of 1998 at 41 estuarine locations. Although regions were identified in relation to seasonal density of cells present, there were no strong relationships to specific water quality variables. Factors that may have influenced these results included: a) several species were included in the group analyzed and this composite did not respond as a unit to changing environmental conditions; b) cell concentrations were low and there were a large number of zero counts; and; c) there were no marked changes involving increasing abundance during the study that could be related to environmental factors.

INTRODUCTION

Phytoplankton populations in Virginia estuaries include an assemblage of many diatoms, chlorophytes, cyanobacteria, dinoflagellates, and other less dominant algal components (Marshall, 1994; Marshall and Burchardt, 1998). Included among the dinoflagellates are those species that are recognized as *Pfiesteria*-like organisms (PLO). They have motile cells (e.g. zoospores) that are similar in size and morphology to the toxin producing dinoflagellate *Pfiesteria piscicida* (Burkholder and Glasgow, 1997; Steidinger et al., 1997). This category may include members of the genera *Pfiesteria*, *Gymnodinium*, *Gyrodinium*, and others.

The most favorable environmental conditions that have been associated with *Pfiesteria piscicida* have been nutrient rich waters, salinities around 15 ppt, temperatures >26 °C, and in estuaries with low flushing rates (Burkholder et al., 1995; Magnien et al., 1999). Other direct and indirect relationships to nitrogen and phosphorus concentrations have been discussed, including the influence high nutrient levels will have on the development of algae preyed upon by *Pfiesteria* (Burkholder et al., 1992; Fensin and Burkholder, 1996; Burkholder and Glasgow, 1997).

To date *Pfiesteria piscicida* has been reported in Virginia from the York River (Burkholder et al., 1995) and from Mosquito Creek, located on the Virginia ocean side of the Delmarva peninsula (Parke Rublee, personal communication). The 1998 presence of *Pfiesteria*-like organisms (PLO) in Virginia estuaries is presented by Marshall et al. (1999). Since PLO organisms have been found in the water column when *Pfeisteria piscicida* is present, their general relationships to water quality parameters gain additional significance in identifying conditions that may favor the development of *P. piscicida*. In 1998, an extensive six month survey regarding the abundance and distribution of PLO in Virginia estuaries was conducted. At the same time, water quality parameters were also determined. The objectives of this study were to apply a

series of regression analysis procedures to identify relationships that existed between PLO abundance and specific water quality parameters.

METHODS

This study is based on the use of regression analysis statistics to identify relationships of water quality parameters to concentrations of *Pfiesteria*-like organisms. Water samples for the PLO analysis were collected by personnel from the Virginia Department of Environmental Quality (VDEQ) from June through October 1998 at widely distributed stations in Virginia estuarine rivers, creeks (Table 1). These stations included 20 which were sampled twice a month as part of the Virginia Department of Health COHORT monitoring program, with another 14 stations sampled monthly as a component of the VDEQ monitoring plan. During each sampling date, water samples were collected for water quality measurements that included 25 chemical and physical parameters to be analyzed by VDEQ (Table 2).

Two sets of water samples were collected at each site for the PLO analysis, one set was preserved with Lugol's solution, the other set did not have a preservative added. In this study, only the preserved sample PLO data are included. It will be noted later that a comparison of the station data for the two sets indicated slightly higher cell concentrations were in samples preserved with Lugol's solution. The lower cell concentrations in the non-preserved samples are likely due to the transformation of many of the motile zoospores present to either cyst or amoeba stages of their life cycle. This change may be easily accomplished during the transport period from the water site to the laboratory by any agitation to the water sample. The preserved water samples provided a more accurate cell count estimate of the motile zoospores at the time of collection.

From each water sample, an aliquot was placed in a plankton counting cell, and only those recognizable PLO cells were counted using light microscopy at 400x magnification. Concentrations were given as numbers of cells/mL (Marshall et al., 1999). The VDEQ provided results of the water quality analysis. To facilitate analysis and to generate broader conclusions of the data, the sites were divided into categories based upon location (Figure 1). Table 1 lists each of the stations by river code, DEQ site number, location, station type, and co-ordinates. The primary divisions were by river basin and include the James (JW), Piankatank (PKW), Potomac (PW), Rappahannock (RW), and York Rivers (YW). The Chesapeake Bay was divided into the eastern (CBE) and western (CBW) Bay. The stations listed as Chesapeake Bay east, or Chesapeake Bay west were stations within minor tributaries and bays. There were also two larger bay categories listed as Ingram Bay (IBW) and Mobjack Bay (MBW). There was also one site located along the Atlantic coastline of the Eastern Shore of Virginia (AT).

Data Analysis

A ranked correlation matrix was made to determine relationships between cell abundance and water quality variables. The individual variables were chosen based upon their significance, their correlation with other variables, and the number of observations for the variable. Regressions were run on the variables with significant correlations against PLO concentrations. If a variable was correlated with another variable, it was considered unlikely to provide information in the regression model and would likely result in multi-collinearity. The presence of missing values decreased the total number of observations used in the regression model. To resolve this condition, an arbitrary limit of 2500 water quality observations for the variable of interest was made for inclusion of a variable into the model. As variables are added to a regression model, the ability of the model to predict the dependent variable improves and

TABLE 1. Water Quality and Cohort Station Locations, with River code and coordinates

River[1] Code	Station Abreviation	DEQ station id	Location	Station Type	Latitude[2]	Longitude
AT	AT 2	7FLL00050	Folly Creek	WQ	37.68444	-75.6058
CBE	CBE1	7NSS00060	Nassawadox Creek	COHORT	37.47417	-75.9517
CBE	CBE2	7OCH00160	Occahanock Creek	COHORT	37.55111	-75.9106
CBE	CBE3	7OCN00192	Onancock Creek	COHORT	37.72833	-75.8047
CBE	CBE4	7POC00000	Pocomoke River	COHORT	37.96389	-75.6478
CBE	CBE7	7KNS00040	Kings Creek	WQ	37.27944	-76.0097
CBE	CBE8	7PUN00212	Pungoteague Creek	WQ	37.66472	-75.8289
CBW	CBW1	1ALIS00420	Little Wicomico	COHORT	37.8975	-76.3011
CBW	CBW2	1ALIS00200	Little Wicomico	COHORT	37.88861	-76.2686
CBW	CBW3	1ALIS00200	Little Wicomico	COHORT	37.88861	-76.2686
CBW	CBW6	7IND00050	Indian Creek	WQ	37.68389	-76.3306
CBW	CBW7	7IND00261	Indian Creek	WQ	37.70333	-76.3539
CBW	CBW8	7BBY00288	Lynnhaven River	WQ	36.8975	-76.0378
IBW	IBW14	7BLS00073	Balls Cr / Gr Wicomico R Trib	WQ	37.84556	-76.3822
IBW	IBW15	7COC00161	Cockrell Cr / Gr Wicomico R Trib	WQ	37.83722	-76.2794
IBW	IBW19	7GWR00889	Great Wicomico River	WQ	37.87028	-76.4197
IBW	IBW20	7GWR00485	Great Wicomico River	WQ	37.84833	-76.3672
JW	JW 1	2WWK00000	Warwick River	COHORT	37.0725	-76.5414
JW	JW2	2WBE00444	Western Branch Elizabeth River	WQ	36.82917	-76.3958
JW	JW3	2JMS03259	James River	WQ	37.20667	-76.6517
JW	JW4	2PGN00119	Pagan River	WQ	36.99639	-76.5842
MBW	MBW1	7NOR00638	North River	COHORT	37.43944	-76.4431
MBW	MBW2	7NOR00269	North River	COHORT	37.415	-76.4106
MBW	MBW3	7NOR00676	North River	COHORT	37.44444	-76.4458
MBW	MBW4	7WAR00282	Ware River	COHORT	37.38583	-76.4492
MBW	MBW5	7WAR00577	Ware River	COHORT	37.40333	-76.4897
PKW	PKW2	7PNK01549	Piankatank River	WQ	37.54806	-76.5089
PKW	PKW3	7PNK00536	Piankatank River	WQ	37.52972	-76.3728
PW	PW 1	1ALOW00473	Lower Machodoc Creek	COHORT	38.09861	-76.6539
PW	PW 2	1ALOW00135	Lower Machodoc Creek	COHORT	38.13944	-76.6492
PW	PW 3	1ANOM00472	Nomini Creek	COHORT	38.10222	-76.7172
PW	PW 4	1ANOM00162	Nomini Creek	COHORT	38.14028	-76.7244
PW	PW13	1AMON00191	Monroe Bay	WQ	38.24278	-76.9678
RW	RW 1	3CRR00338	Corrotoman River	COHORT	37.69333	-76.4733
RW	RW 2	3CRR00138	Corrotoman River	COHORT	37.66583	-76.4797
RW	RW 3	3LAN00000	Lancaster Creek	COHORT	37.79264	-76.6456
RW	RW 4	3RPP04302	Rappahannock River	COHORT	37.92194	-76.8353
RW	RW15	3URB00100	Urbanna Creek, Rt 227	WQ	37.62931	-76.5698
RW	RW16	3URB00150	Urbanna Creek	WQ	37.62278	-76.5819
RW	RW7	3CTR00106	Carter Creek	WQ	37.66472	-76.4356
YW	YW 1	8SRH00000	Sarah Creek	COHORT	37.25361	-76.4828

1 AT=Atlantic sites, MBW=MobJack Bay sites, JW = James River sites, YW=York River sites, CBE=CHesapeake Bay East sites, IBW=Ingram Bay sites, PKW=Piankatank sites, RW=Rappahannock River sites, CBW=Chesapeake Bay West Sites, PW=Potomac River sites.

2 Latitude and Longitude are in decimal degrees.

TABLE 2. List of environmental parameters analyzed.

Long Field Name	Short Field Name	Storet field number
WATER TEMP CENT	TEMP	10
WEATHER WMO CODE 4501	WEATHER_CODE	41
TIDE STAGE CODE	TIDE	67
TURB TRBIDMTR HACH FTU	TURB	76
TRANSP SECCHI METERS	SECCHI	78
CNDUCTVY FIELD MICROMHO	CND_FLD	94
CNDUCTVY AT 25C MICROMHO	CND_25	95
SALINITY AT 25C MG/ML	SALINITY	96
DO PROBE MG/L	DO_PROBE	299
DO SATUR PERCENT	DO_SAT	301
BOD 5 DAY MG/L	BOD	310
PH SU	PH	400
PH LAB SU	PH_LAB	403
T ALK CACO3 MG/L	ALK	410
RESIDUE TOTAL MG/L	RES_TOT	500
RESIDUE TOT VOL MG/L	RES_TOT_VOL	505
RESIDUE TOT FIX MG/L	RES_TOT_FIX	510
RESIDUE TOT NFLT MG/L	RES_TOT_NFT	530
RESIDUE VOL NFLT MG/L	RES_VOL_NFT	535
RESIDUE FIX NFLT MG/L	RES_FIX_NFT	540
NH3+NH4- N DISS MG/L	NH34_DISS	608
NH3+NH4- N TOTAL MG/L	NH34_TOT	610
NO2-N DISS MG/L	NO2_DISS	613
NO2-N TOTAL MG/L	NO2_TOT	615
NO3-N DISS MG/L	NO3_DISS	618
NO3-N TOTAL MG/L	NO3_TOT	620
TOT KJEL N MG/L	N_KJEL	625
NO2&NO3 N-DISS MG/L	NO23_DISS	631
PHOS-TOT MG/L P	P_TOT	665
PHOS-DIS ORTHO MG/L P	P_ORTHO_DISS	671
T ORG C C MG/L	ORG_C	680
CHLORIDE TOTAL MG/L	CL	940
SULFATE SO4-TOT MG/L	SO4	945
SILICA DISOLVED MG/L	SILICA	955
FEC COLI MPNECMED /100ML	COLIFORM	31615
PHOSPHUS PATCSUSP WTR MG/L	P_SUS	49567
CARBON PATCSUSP WTR MG/L	CARBON	49569
NITROGEN PATCSUSP WTR MG/L	N_SUS	49570
NITROTOT DISSLOVD WTR MG/L	N_TOT_DISS	49571
CHLRPHYL A UG/L CORRECTD	CLR_A	32211
PHEOPHTN A UG/L	PHEO_A	32218
PHEOPHTN RATIO SPECTRO	PHEO_RAT	32219
PHOSHTOT DISSLOVD WTR MG/L	P_TOT_DISS	49572
PHOS-T ORTHO MG/L P	P_ORTHO_TOT	70507
Presumed *Pfiesteria* Count (Cells per L)	PLO	

results in a higher R^2. Decisions relating to variable inclusion, or exclusion were based on the adjusted R^2, which compensates for this increased resolution (Draper and Smith, 1981). In this modeling process the goal was to obtain the most parsimonious model with the highest possible resolution measured by adjusted R^2.

Different models were developed to describe the PLO concentrations. The first was a river model, which included only the categorical variable of river, described earlier and listed in

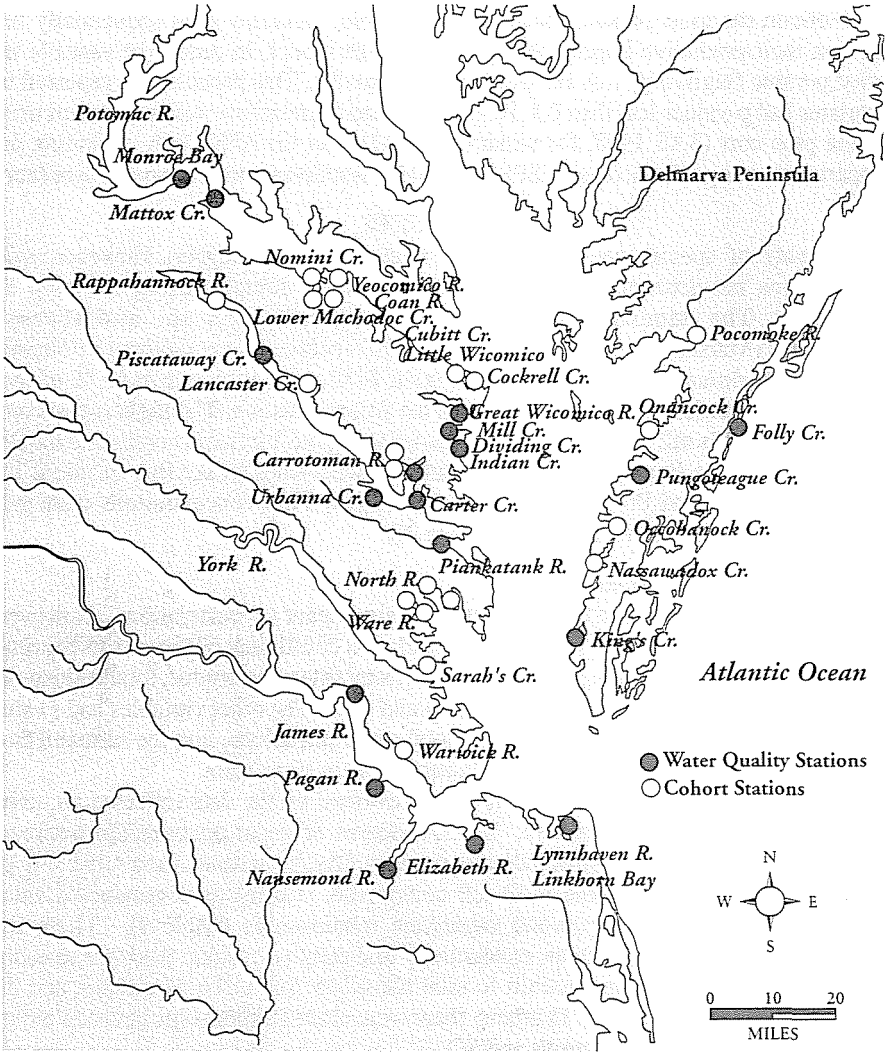

Figure 1. Station locations.

Table 1. The second model was a river/month model, which accounted for time of year by including month as a categorical variable in addition to location. The design of the river/month model was factorial, with month and river crossed in addition to the inclusion of river and month as main effects. The selected environmental variables were initially placed into the models as covariates in descending order, from those with the highest R^2 first. Covariates were added to the model for two reasons. First, to identify significant factors for determining PLO counts, and second for the included covariates to adjust the means to allow comparisons between sites as if they had the same environmental conditions.

To obtain the most parsimonious model possible, variables were sequentially removed based on their predictive importance (p-value) to the model. In order, the variable with the lowest p-value (highest p) was removed from the model. This process was repeated until all covariates had p-values less than 0.1. Regressions and correlations were performed using proc reg and proc corr (SAS 1998, for windows v6.12), and the ANCOVA, including pairwise comparisons, was performed using SPSS (1998) for Windows univariate procedure (ver. 9.00).

RESULTS

A variety of species were identified within the PLO category. However, *Pfiesteria piscicida* was not detected in any of the representative samples using scanning electron microscopy. The dominant species were *Cryptoperidiniopsis* sp. and *Gymnodinium galatheanum* (Marshall et al. 1999). Additional PLO included several others belonging to the genera *Gymnodinium*, *Amphidinium*, and others. PLO were observed in 52 % of the water samples, and recorded at least once at all but the Atlantic station. The highest cell concentrations (300-400 cells mL^{-1}) were in small estuaries along the Virginia shoreline of the Potomac River, at sites in the Rappahannock River, and Western Chesapeake Bay locations between the Rappahannock and Potomac Rivers. These Higher PLO concentrations were generally associated with mid-day field collections (Fig 2.).

Correlation of the variables

Table 3 is a ranked list of the variables with significant (p) correlations. However, none of the correlations are strong. The highest correlation coefficient (R) was 0.15 for time. This was followed by the DO probe, conductivity, pH, total filterable residue, total residue, salinity, and DO saturation, with r values between 0.14 and 0.12. The other variables had r values less than 0.1. These included total phosphate, total ortho-phosphate, and the nitrogen fractions. Neither total nitrogen, nor dissolved total nitrogen were significant.

A principle components analysis (PCA) performed on the data reflected an inability to separate the sites by these variables. The low degree of linear relationships between these variables and PLO concentrations indicates neither of these variables were reliable in predicting the concentrations of these PLO cell composites. Correlations conducted among the environmental variables had several significant relationships (Table 4). These included several expected linkages, such as, conductivity and salinity, residue fractions to each other, the non-combustible residue fractions to total phosphate and ortho-phosphate, and the total residue to NH_3 - NH_4 together. In others, there were close relationships between oxygen and pH, and salinity to residue fractions and SO_4.

Figure 2 is a scatter plot of time versus PLO abundance including its regression line. Time was the strongest regressor with PLO concentrations and is representative of the other variables. An analysis of the residuals did not find any particularly influential points with significant leverage. High counts did not separate well from the low counts and usually occurred at concentrations of the variable of interest where the largest number of low counts occurred. This was true for all variables.

As indicated in the correlations, none of the regressions were strong. Attempts at transformation failed to significantly improve the fit. The highest R^2 was 0.022 with the time of day. The lowest R^2 was with total phosphate ($R^2 = 0.0027$). Other nutrients (total ortho-phosphate, total phosphorous, NH_3 and NH_4, and SO_4), the residue variables (total residue, total filterable residue, and non-combustible filterable residue), and salinity, all had negative slopes indicating that as the variable of interest increased, the cell counts decreased. In contrast pH, dissolved

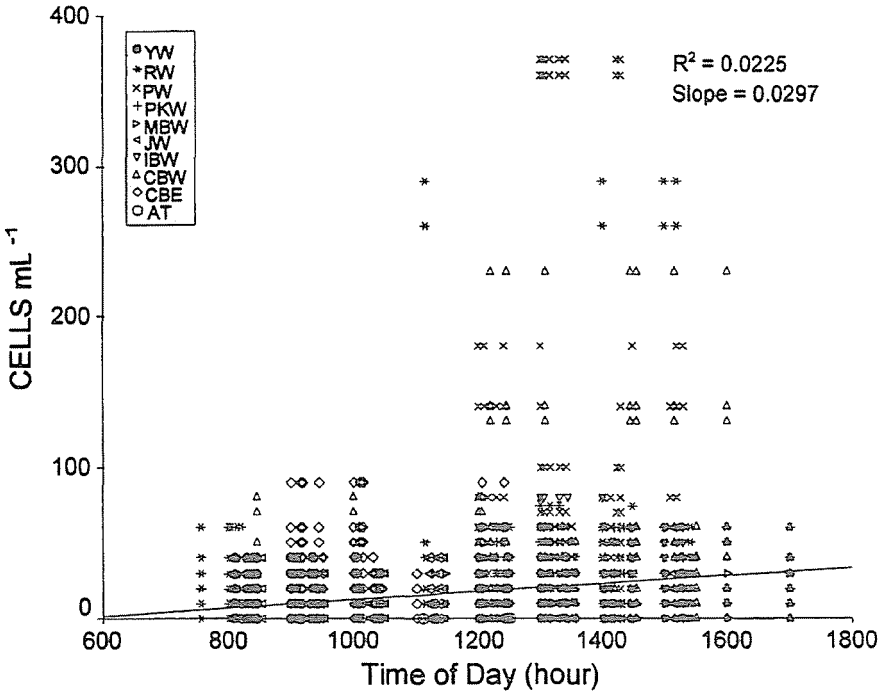

Figure 2. Scater Plot of Cells by Time of Day.

oxygen, chlorophyll *a*, and time all had positive slopes indicating that as the variable of interest increased so did the cell concentrations.

Figure 3 is a box plot of PLO concentrations by station. The box includes all data from the 25th to the 75th percentile and the line in the box is the median value. The whiskers (error bars) are 95% confidence intervals. Circles represent outliers and asterisks are extreme outliers. Two stations in the Potomac River (PW2, 13), 2 stations on the Rappahannock (RW7,16) and one station on the Chesapeake Bay West (CBW2) had greater than 75% of their counts above 0. The Chesapeake Bay east, Mobjack Bay, James River, and York River had over half of their counts at 0. The Ingram Bay and Piankatank River had half of their Stations with counts of 0. The stations with particularly high concentrations (>100) are in order: PW2, RW16, CB2, PW3, and PW1. All of these stations were within geographic regions where stations have greater than 75% of their counts greater than 0. These stations are located in Chesapeake Bay west estuaries, and in the Potomac and Rappahannock Rivers.

Advanced Regression Analysis

Time, *in situ* DO, pH, total residue, salinity, chlorophyll *a*, total NH_3 and NH_4, SO_4, total ortho-phosphate, and total phosphate were chosen for the initial ANCOVA model. Of the variables with significant correlations described earlier, silica and dissolved ortho-phosphate were eliminated because they had fewer than 2500 obser-

TABLE 3. Ranked significant correlations with PLO concentrations from dataset.

PLO

Var.	Time	DO Probe	Cond. 25	pH	Total Filt. Residue	Total Residue	Salinity	DO Sat.	Chlor *a*
R	0.15008	0.14052	-0.13914	0.13861	-0.13146	-0.13134	-0.11743	0.115545	0.08913
sig.	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
N	3153	3076	3057	3062	3050	3050	3061	3076	2806

TABLE 3.(*continued*).

Var.	Total NH_3+NH_4	SO_4	Total Ortho P	Silica	Filt. Noncom. Residue	Total P	Dissolv. Ortho P	Total Nonfilt. Residue
R	-0.08295	-0.07683	-0.06823	0.06658	-0.06225	-0.05233	-0.04552	-0.04537
sig.	0.0001	0.0001	0.0002	0.0017	0.0005	0.0044	0.0317	0.0118
N	3016	3092	3016	2217	3092	2956	2227	3082

TABLE 4. Ranked significant correlations greater than 0.5. Only those variables which were significantly correlated with PLO were included. The variables are ordered by their correlation with PLO. If a variable does not occur in this table, there are no correlations greater than 0.5 with other variables correlated with PLO.

DO Probe

Var.	Do Sat.	pH
R	0.92293	0.53591
sig.	0.0001	0.0001
N	3076	3062

Conductivity at 25 deg. C

Var.	Total Filt. Rsidue	Salinity	Total Residue	Total NH_3+NH_4	SO_4
R	0.97482	0.96444	0.96296	0.63599	0.51651
sig.	0.0001	0.0001	0.0001	0.0001	0.0001
N	3015	3012	3015	2950	3057

pH

Var.	Do Sat.	DO Probe
R	0.66444	0.53591
sig.	0.0001	0.0001
N	3062	3062

Total Nonfilterable Residue

Var.	Total Residue	Cond. at 25 deg C	Salinity	Total NH_3 +NH_4	SO_4
R	0.98884	0.97482	0.96449	0.66294	0.51964
sig.	0.0001	0.0001	0.0001	0.0001	0.0001
N	3050	3015	3005	2913	3050

Total Residue

Var.	Total Filt. Residue	Cond. at 25 deg C	Salinity	Total NH_3 +NH_4	SO_4
R	0.98884	0.96296	0.95531	0.62150	0.51643
sig.	0.0001	0.0001	0.0001	0.0001	0.0001
N	3050	3015	3005	2913	3050

Salinity

Var.	Total Filt. Residue	Cond. at 25 deg C	Total Residue	Total NH_3 +NH_4	SO_4
R	0.96449	0.96444	0.95531	0.65296	0.50236
sig.	0.0001	0.0001	0.0001	0.0001	0.0001
N	3061	3005	3005	2924	3047

DO Saturation

Var.	DO Probe	pH
R	0.92293	0.66444
sig.	0.0001	0.0001
N	3076	3062

Total NH_3 and NH_4

Var.	Total Filt. Residue	Salinity	Cond. at 25 deg C	Total Residue
R	0.66294	0.65296	0.63599	0.62150
sig.	0.0001	0.0001	0.0001	0.0001
N	2913	2924	2950	2096

continued on next page

TABLE 4. *continued*

SO_4

Var.	Total Filt. Residue	Cond. at 25 deg C	Total Residue	Salinity
R	0.51964	0.51964	0.51643	0.50236
sig.	0.0001	0.0001	0.0001	0.0001
N.	3050	3057	3050	3047

Total Ortho-Phosphate

Var.	Total N-comb. Residue	Filt. N-comb. Residue
R	0.65554	0.63962
sig.	0.0001	0.0001
N	2985	2995

Filterable Non-combustible Residue

Var.	Total N-comb Residue	Total Ortho P
R	0.98557	0.63962
sig.	0.0001	0.0001
N	3082	2995

Total Non-Combustible Residue

Var.	Filt. N-comb Residue	Total Ortho P
R	0.98557	0.65554
sig.	0.0001	0.0001
N	3082	2985

vations (Table 3). *In situ* DO was chosen over DO saturation because it had a higher correlation. Salinity was selected over conductivity because it was a more informative variable. Although total residue was highly correlated with salinity, it measured a fundamentally different parameter and was therefore included in the model.

There was little change in the R^2, or adjusted R^2, with the removal of any of the variables during the variable selection process. The R^2 remained constant, or declined slightly, while the adjusted R^2 increased slightly. Both models resulted in R^2s around 0.1 and because of the low final variable number and low R^2, there was little difference between the adjusted R^2 and the R^2 for either model. The removed variables with their p values are given in Tables 5 and 6. The final models are shown in Tables 7 and 8. Salinity and combined NH_3 and NH_4 were the only significant covariates left in the model after selection when only the river effect is a fixed effect. Total ortho-phosphate was the only variable left in the model when month was included in the model.

Table 9 shows significant differences in Sidak pairwise comparisons of the adjusted means. The Atlantic , Mobjack Bay , James River, York River, Chesapeake Bay east, Ingram Bay, and Piankatank River did not have significantly different counts under either of the models. The means ranged from a low of -3.089 at the Atlantic site, to a high of 12.483 using the river model, and 2.760 to 12.024 using the river/month model. These sites represented the low PLO cell concentration areas. If the counts had not been adjusted, the Atlantic site would most likely have separated out as having the lowest PLO abundance. The adjusted mean of the Rappahannock was 20.182 and 18.377 for the river and river/month models respectively. The

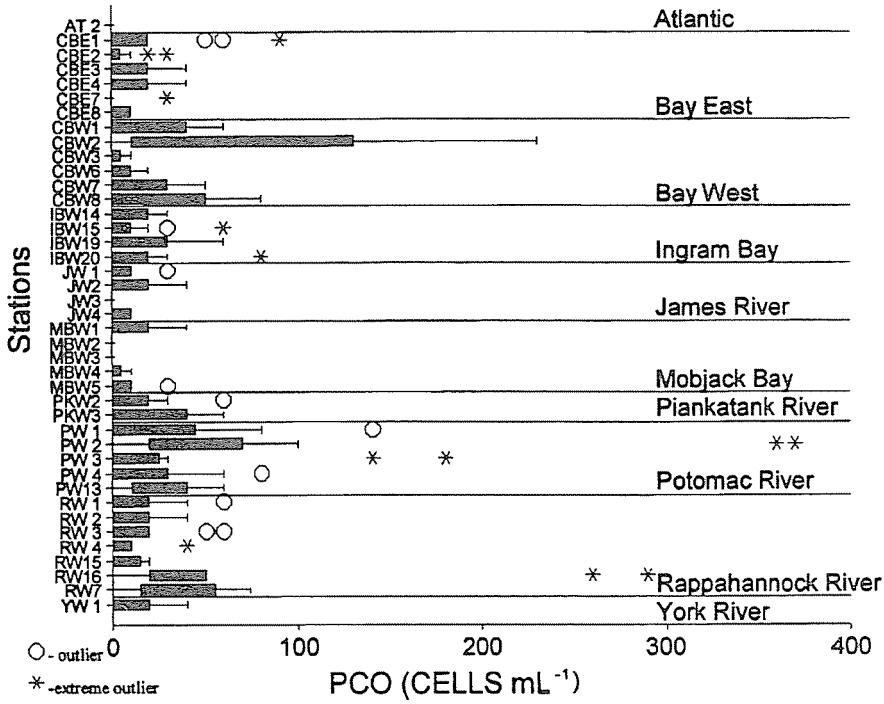

FIGURE 3. Wisker box plot of PCO Cell Count by Station.

TABLE 5. River model order of variable removal with R^2 and adjusted R^2 prior to removal

Variable	Sig.	R^2	Adj. R^2
Res_tot	0.979	0.107	0.1
DO_probe	0.842	0.107	0.101
res_f_n	0.819	0.107	0.101
SO4	0.599	0.107	0.101
time	0.482	0.107	0.102
CLR_a	0.208	0.107	0.102
p_ortho_t	0.146	0.107	0.103
Variables left in model			
Salinity	0.043		
NH34_tot	0.05	0.107	0.103

TABLE 6. River/month model order of variable removal with R^2 and adjusted R^2 prior to removal.

Variable	Sig.	R^2	Adj. R^2
Res_Tot	0.851	0.111	0.092
P_tot	0.797	0.111	0.093
time	0.773	0.111	0.093
DO_probe	0.443	0.11	0.094
Res_F_N	0.537	0.11	0.094
SO4	0.403	0.11	0.094
NH34_tot	0.123	0.11	0.095
CLR_a	0.104	0.11	0.095
Variables left in model			
P_ortho_T	0.023	0.108	0.094

TABLE 7. ANOVA table for River model.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	476965.805[a]	11	43360.528	30.503	0.000
Intercept	5836.717	1	5836.717	4.106	0.043
Salinity	10347.273	1	10347.273	7.279	0.007
NH34_tot	5467.247	1	5467.247	3.846	0.050
River	410093.144	9	45565.905	32.054	0.000
Error	3990194.294	2807	1421.516		
Total	5436204.000	2819			
Corrected Total	4467160.099	2818			

a. R^2 =0.108 (Adjusted R^2 = 0.094)

TABLE 8. ANOVA table for river/month model.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	499990.285[a]	45	11110.895	7.624	0.000
Intercept	80702.833	1	80702.833	55.375	0.000
P_Ortho_T	7488.319	1	7488.319	5.138	0.023
River	248021.890	9	27557.988	18.909	0.000
Month	2632.330	4	658.083	0.452	0.771
River*Month	8970.982	31	289.387	0.199	1.000
Error	4131698.895	2835	1457.389		
Total	5652701.000	2881			
Corrected Total	4634389.180	2880			

[a]. R^2 = 0.108 (Adjusted R^2 = 0.094)

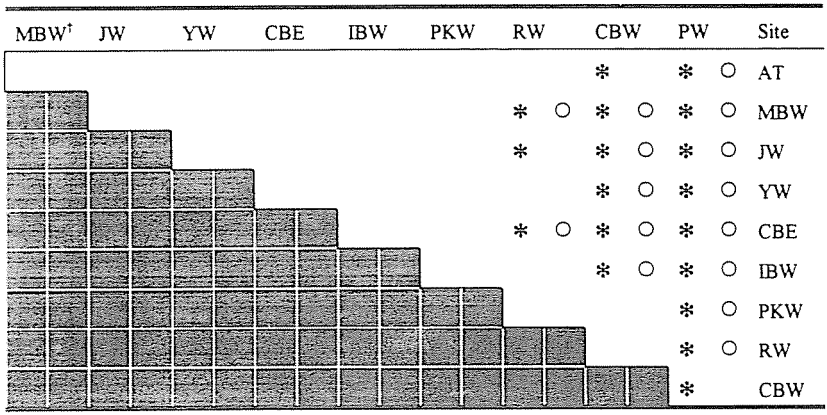

FIGURE 4. Pairwise comparisons of adjusted means (Sidak).(✻) Represents significant ($p < 0.05$) pairwise differences between adjusted means for sites in the river model. (O) Represents significant ($p < 0.05$) pairwise differences between adjusted means for sites in the river/month model. Areas without symbolds are not significantly different. Gray area is redundant and therefore intentionally left blank. (AT=Atlantic sites, MBW=MobJack Bay sites, JW = James River sites, YW=York River sites, CBE=CHesapeake Bay East sites, IBW=Ingram Bay sites, PKW=Piankatank sites, RW=Rappahannock River sites, CBW=Chesapeake Bay West Sites, PW=Potomac River sites.)

TABLE 9. Test for parallel regresions between preserved and unpreserved PLO samples.

Source	SS	df	ms	f	p-value
Parallel	348	2	172	0.24	0.7871
error	186765	1082	727		

TABLE 10. Test for preservation effect in PLO counts.

Source	SS	df	MS	f	p-value
Regression	103073	2	51537	11.0	0.0001
Treatment	1546	1	1546	2.13	0.145
error	787112	1084	726		

Rappahannock River was significantly different from Mobjack Bay , James River, and Chesapeake Bay east and the Potomac River. The Rappahannock River appears to be a transition from the lower tributaries of Chesapeake Bay to the high counts of the Chesapeake Bay west and Potomac River sites.

The Chesapeake Bay west (28.899 river model, 27.079 river/month model) was significantly different from all other river categories aside from the Piankatank River, the Rappahannock River and the Atlantic sites. The Potomac (45.570 river model, 41.270 river/month model) was significantly different from all other river categories.

ANCOVA on Preservation effect

The effect of preservation on cell count was initially analyzed with the covariate of pH and salinity. The regressions for both treatments had equivalent slopes (Table 10). It was therefore possible to perform an analysis of covariance. As indicated in Table 11, the regression was significant ($p < 0.0001$), but preservation did not significantly effect cell count if one adjusts for salinity and pH ($p = 0.145$). The adjusted means of cell abundance with their associated standard errors for the unpreserved versus the preserved samples are respectively 9.32 (1.17) and 11.7 (1.15). Although the difference was not statistically significant, there were higher cell counts and a lower standard error in the preserved samples.

SUMMARY

1) The regressions applied to the PLO concentrations in relation to the environmental variables had very low R^2's and therefore conclusions based upon them are tenuous. There were no significant correlations of the composite PLO concentrations over this time period to nutrients at these stations.

2) This study found significant geographic differences in PLO concentrations in Virginia estuaries and some weak relationships between environmental variables and PLO counts. The Rappahannock River, Potomac River, and Chesapeake Bay west all had significantly higher PLO counts than the other sites. However, the low number of high PLO events, hampered the ability to develop stronger relationships between the environmental variables and the PLO. Nitrogen, phosphate and salinity were the only covariates which survived the selection process, however removal of these parameters resulted in only moderate improvement of the R^2.

3) The other geographic regions had moderate PLO concentrations. There was little variation in environmental variables between sites other than time of sampling. This was reflected in a weak separation in a PCA. There was also high multi-collinearity between the environmental variables.

4) The PLO concentrations in these samples had a negative relationship with nutrients, residues, and salinity, and a positive relationship with dissolved oxygen, Chlorophyll *a*, pH, and time of day. When placed in a larger regression model with site as the first variable, salinity and NH_3 and NH_4 were significant covariates. When month of sampling was also included, only phosphate was significant.

5) Concurrent laboratory studies on the cells of the *Pfiesteria* like organisms from these collections by Marshall et al. (1999) and Seaborn et al. (1999) indicate several different species were identified within this complex. These included *Cryptoperidiniopsis* spp., *Gymnodinium*

spp., *Amphidinium* spp., *Gyrodinium* spp. and others. *Pfiesteria piscicida* was not observed in these 1998 samples. Additional laboratory observations indicate a possible temporal succession in several of these species over this study period. Although these species may mimic *Pfiesteria* in general size, appearance, and life stages, their development may be determined by other sets of environmental conditions. This may explain the lack of closer correlations between the water quality parameters and the group of different species that were present within this complex, in contrast to relationships that may exist when emphasis would be placed on one, or fewer species.

6) Another deterrent in establishing closer environmental relationships to the PLO are the low concentrations of these cells in the water samples. The PLO have multiple stages in their life cycle and this analysis is based only on the presence of their motile zoospore stage. Not included in these abundance studies are amoeboid and cyst stages. The multiplicity of the life stages, and the possible variations in the responses of each life stage to the environmental variables that can enhance or inhibit their development, can complicate specific ecological relationships (Burkholder and Glasgow, 1997). Since these motile cells may follow a heterotrophic, or mixotrophic life style, close relationships to nutrient levels also become difficult to ascertain.

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