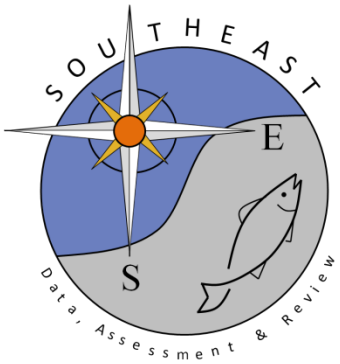
Neuse River Estuary Dataset - Metadata

Hans W. Paerl, Lexia M. Valdes-Weaver, and Benjamin Peierls

SEDAR-PW6-RD34

5 June 2014



Neuse River Estuary Dataset

Metadata Last Updated: **4/13/2011**

For specific information on this dataset and its relevance to laboratory experiments, contact:

Dr. Hans W. Paerl
Institute of Marine Sciences
University of North Carolina at Chapel Hill
3431 Arendell Street
Morehead City, NC 28558
Hans_Paerl@unc.edu

Metadata and Dataset Developer:
Dr. Lexia M. Valdes-Weaver
Post-doctoral Research Associate

Current Data Manager:
Benjamin Peierls
Post-doctoral Research Associate
Institute of Marine Sciences
University of North Carolina at Chapel Hill
3431 Arendell Street
Morehead City, NC 28558
peierls@unc.edu

Dataset Description:	The Neuse River Estuary Water Quality Dataset is a compilation of the biological, chemical and physical water quality data that was collected along the length of the Neuse River Estuary, NC from March 14, 1985 to February 15, 1989 and from January 24, 1994 to the present. The primary purpose of this dataset was to provide long-term environmental information to supplement experimental, process-based research, including the Atlantic Coast Environmental Indicators Consortium (ACE-INC) project as well as other laboratory studies. Bi-weekly water sampling and *in situ* measurements were performed at fixed sampling stations. Water samples and *in situ* measurements were collected at the surface (approximately 0.2 meters) and at the bottom of the water column (approximately 0.5 meters from the sediment layer). These data are included in the worksheet titled "NRE Dataset." *In situ* measurements were also performed throughout the water column in 0.5 meter depth increments. These data are included in the worksheet titled "NRE YSI Profiles." Parameters measured include: temperature, salinity, specific conductivity, dissolved oxygen (DO), pH, chlorophyll fluorescence, photosynthetically active radiation (PAR), turbidity, barometric pressure, secchi depth, colored dissolved organic matter (CDOM), particulate organic carbon (POC) and nitrogen (PN), dissolved organic and inorganic carbon, dissolved inorganic nutrient concentrations (nitrate/nitrite, ammonium, total dissolved nitrogen, phosphate and silicic acid), chlorophyll *a* , primary productivity and diagnostic phytoplankton pigment concentrations (chlorophylls and carotenoids). Calculated parameters include: diffuse light attenuation coefficient (K_d), carbon to nitrogen molar ratio (C:N), dissolved inorganic nitrogen (DIN; nitrate/nitrite plus ammonium), dissolved organic nitrogen (DON; total dissolved nitrogen minus dissolved inroganic nitrogen) and the nitrogen to phosporus molar ratio (N:P).
Quality Assurance/Quality Control Procedures:	

"Data" Sheet:

Column	*Column Title*	*Parameter*	*Parameter Description*	*Methods*
A	Date	Sampling date	Date of water sample collection, filtration, and in situ measurements.	Water sampling was conducted bi-weekly.
B	Year	Year	Year of sampling	
C	Season	Season	The season when the water sample was collected and filtered and when the *in situ* measurements were performed in the field.	Winter refers to sampling during December through February. Spring refers to sampling during March through May. Summer refers to sampling during June through August and Fall refers to sampling during September through November.
D	Station	Station name	The name of the fixed sampling station.	Station names decrease in number (in increments of 10) from 180 (the most downstream station sampled) to 0 (the most upstream station sampled). All stations were located mid-river except stations 95 and 96 which were located close to the nothern and southern shore of the Neuse River Estuary, respectively. Stations were selected to cover the entire length of the Neuse River Estuary from Streets Ferry Bridge (Station 0) to the mouth of the estuary where it flows into Pamlico Sound. When possible, efforts were made to select locations with key stationary features (channel markers, buoys and land markers) to allow easy station identification in the field.
E	Source	Data source	The organization that conducted the sampling.	IMS=Institute of Marine Sciences/Paerl Lab, DWQ=North Carolina Department of Environment and Natural Resources, Division of Water Quality, WEY=Weyerhaeuser Co., RR=DWQ's Rapid Response Team, AVIRIS=Samples collected during AVIRIS Overflight, ECU =East Carolina University, ?=Unknown Data Source.
F	Depth	Depth level	Depth level from which the water sample was collected and where the in situ measurements were made (S=surface, B=bottom).	Surface (S) refers to a surface water sample or *in situ* measurement taken at a depth of approximately 0.2 meters. Bottom (B) refers to a bottom water sample or *in situ* measurement taken at a depth of approximately 0.5 meters above the sediment layer. Surface water samples were collected by submerging 10 liter high-density polyethylene containers just below the water surface or by filling the containers with surface water collected from bucket casts. Bottom water samples were collected with a horizontal plastic Van Dorn sampler. Starting December 2007, all samples collected with diaphragm pump and a weighted, marked hose. All containers were kept in dark coolers at ambient temperature during transport to the laboratory. All filtration was done within a few hours of collection and when conditions permitted, on board the research vessel.
G	YSI_Time	YSI time	Exact time (hours:minutes:seconds) when the in situ measurements were made. This time is an approximate water sampling time.	
H	YSI_Depth	YSI depth	Exact depth (meters) where the in situ measurements were made.	

I	YSI_Temp	YSI temperature	In situ water temperature (degrees Celsius).	Prior to the 09/13/2000 sampling date, *in situ* measurements were performed at discrete depths using a Hydrolab Data Sonde 3 equipped with a multiprobe and SVR3 display logger. Beginning on the 09/13/2000 sampling date, *in situ* measurements were performed at discrete depths on the sunlit side of the research vessel using a Yellow Springs Instruments (YSI Incoporated, Ohio) multiparameter sonde (Model 6600 or 6600 EDS-S Extended Deployment System) equipped with a YSI conductivity/temperature probe (Model 6560), a YSI chlorophyll probe (Model 6025), a YSI pH probe (Model 6561 or 6566), a YSI pulsed dissolved oxygen probe (Model 6562), a self cleaning YSI turbidity probe (Model 6026 or 6136), and beginning on the 07/30/2003 sampling date, a flat Li-Cor sensor (UWQ-PAR 6067). The YSI sonde was coupled to a either a YSI 610 DM datalogger or a YSI 650 MDS Multi-parameter Display System datalogger. *In situ* measurements were performed at the surface (approximately 0.2 meters) and at the bottom of the water column (approximately 0.5 meters from the sediment layer). These data are included in the worksheet titled "NRE Dataset." *In situ* measurements were also performed throughout the water column in 0.5 meter depth increments. These data are included in the worksheet titled "NRE YSI Profiles." The data were stored on the datalogger and downloaded to Ecowin software upon return to the laboratory.
J	YSI_SpecCond	YSI specific conductivity	In situ specific conductivity (milli Siemens per centimeter).	
K	YSI_Salinity	YSI salinity	In situ salinity (parts per thousand).	
L	YSI_DOsat	YSI dissolved oxygen saturation	In situ dissolved oxygen saturation (percent).	
M	YSI_DO	YSI dissolved oxygen	In situ dissolved oxygen concentration (milligrams per liter).	
N	YSI_pH	YSI pH	In situ pH.	
O	YSI_Turbidity	YSI turbidity	In situ turbidity (NTU).	
P	YSI_Chlraw	YSI chlorophyll raw fluorescence	In situ chlorophyll fluorescence (relative fluorescence units).	
Q	YSI_Chl	YSI chlorophyll concentration	In situ chlorophyll concentration from fluorescence (micrograms per liter).	
R	YSI_BP	YSI barometric pressure	Surface barometric pressure (millimeters of mercury).	
S	Secchi	Secchi depth	Depth at which the secchi disk is no longer visible (meters).	The secchi disk was deployed off of the sunlit side of the research vessel. The depth (in meters) at which the secchi disk was no longer visible by the naked eye was recorded as the secchi depth.
T	Kd	Diffuse light attenuation coefficient	Diffuse light attenuation coefficient (per meter).	The diffuse light attenuation coefficient, Kd, was calculated from depth profiles of photosynthetically active radiation (PAR, 400-700 nm). Prior to the 07/30/2003 sampling date, PAR measurements were performed with a spherical underwater quantum sensor (LI-COR LI-193SA) coupled to a LI-COR LI-1000 datalogger. Beginning on the 07/30/2003 sampling date, a flat underwater quantum sensor (LI-COR LI-193SA) attached to a Yellow Springs Instruments YSI 6600 or YSI 6600 EDS-S sonde was used to measure PAR. Measurements of PAR were performed on the sunlit side of the research vessel in 0.5 meter depth increments, beginning just below the water surface. The diffuse attenuation coefficient is the slope of the linear regression between natural log transformed PAR data and depth.
U	CDOM	Colored dissolved organic matter	Colored or chromophoric dissolved organic matter (humic substances) concentration as microgram per liter of quinine sulfate.	Colored dissolved organic matter (CDOM) was measured using a Turner Designs TD-700 fluorometer configured with a near-UV mercury vapour lamp, a 350 nm excitation filter, and a 410–600 nm emission filter. The fluorometer was calibrated to quinine sulfate (QS) solutions made up in 2 N sulfuric acid. Water samples were vacuum filtered (less than 25 kilopascal) using pre-combusted Whatman glass microfibre filters (GF/F) and the filtrate was stored in scintillation vials in the dark at 4 degrees Celsius until fluorometric analysis.
V	POC	Particulate organic carbon	Particulate organic carbon concentration (micrograms of carbon per liter).	Particulate organic carbon (POC) and nitrogen (PN) concentration was determined by elemental analysis of material collected on pre-combusted Whatman glass microfibre filters (GF/F): One hundred milliliters from each water sample was vacuum filtered (less than 25 kilopascal) through the GF/F filters. Two replicates were filtered for each sample. The filters were stored in clear plastic petri dishes until analysis. Carbonates were removed from the filters by vapor phase acidification using concentrated hydrochloric acid (HCl) under the fume hood for at least 6 hours. After drying overnight at 60 degrees Celsius, the filters were folded into tin disks and injected into a Perkin-Elmer 2400 Series II CHNS/O Analyzer (Perkin-Elmer, Norwalk, CT) calibrated with acetanilide.
W	PN	Particulate nitrogen	Particulate nitrogen concentration (micrograms of nitrogen per liter).	
X	CtoN	Carbon to nitrogen molar ratio.	Calculated molar ratio of particulate organic carbon (POC) to particulate nitrogen (PN).	The molar ratio of particulate organic carbon (POC) to particulate nitrogen (PN), or C:N, was calculated by dividing POC by PN.
Y	DOC	Dissolved organic carbon	Dissolved organic carbon concentration (micromolar).	Dissolved organic carbon (DOC) concentration was measured using a Shimadzu TOC-5000A Analyzer: Water samples were vacuum filtered (less than 25 kilopascal) using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in pre-combusted glass scintillation vials with Teflon closures and frozen at -20 degrees Celsius until analysis. The Shimadzu TOC-5000A Analyzer uses high temperature catalytic oxidation followed by non-dispersive infrared analysis of the CO_2 produced. Samples were acidified to a pH less than 2 and sparged with air before they were analyzed for non-volatile organic carbon. DOC values in 1996 were run from previously run nutrient samples.
Z	DIC	Dissolved inorganic carbon	Dissolved inorganic carbon concentration (milligrams of carbon per liter).	Dissolved inorganic carbon (DIC) was measured on samples held overnight in research pond by acidification followed by infrared analysis of carbon dioxide (CO_2) on a Shimadzu Total Organic Carbon Analyzer (TOC-5000A) in IC mode and calibrated with sodium carbonate standards. Prior to 27 Feb 2001, samples were manually acidified and the CO_2 produced was sparged into an argon gas stream, followed by detection using a Beckman 864 infrared gas analyzer (IRGA) or a Li-Cor 6252 CO_2 analyzer, with peak height determined manually from chart recorder printout.
AA	NO3/NO2	Nitrate/Nitrite	Nitrate plus nitrite concentration (micrograms of nitrogen per liter).	Nitrate/nitrite (NO3- / NO2-) concentration was determined using a Lachat/Zellweger Analytics QuikChem 8000 flow injection autoanalyzer (Milwaukee, WI, USA) using method FIA 31-107-04-1-C: Water samples were vacuum filtered (less than 25 kiloPascals) using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in high-density polyethylene bottles and frozen at -20 degrees Celsius until analysis. Two replicates were run from the same bottle. Method detection limits (MDL, µg L-1) were: before 4Nov02 = 1.06; beginning 4Nov02 = 3.68; beginning 11Jul06 = 0.6; beginning 1Dec09 = 0.27.
AB	NH4	Ammonium	Ammonium concentration (micrograms of nitrogen per liter).	Ammonium (NH4+) concentration was determined using a Lachat/Zellweger Analytics QuikChem 8000 flow injection autoanalyzer (Milwaukee, WI) using method FIA 31-107-06-1-A/B: Water samples were vacuum filtered (less than 25 kiloPascals) using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in high-density polyethylene bottles and frozen (-20 degrees Celsius) until analysis. Two replicates were run from the same bottle. Method detection limits (MDL, µg L-1) were: before 4Nov02 = 4.69; beginning 4Nov02 = 4.31; beginning 11Jul06 = 2.55; beginning 1Dec09 = 3.98.
AC	DIN	Dissolved inorganic nitrogen	Calculated dissolved inorganic nitrogen concentration (micrograms of nitrogen per liter).	Dissolved inorganic nitrogen (DIN) concentration was calculated by summing nitrate/nitrite (NO_3^- / NO_2^-) and ammonium (NH_4^+). If either NO_3^- / NO_2^- or NH_4^+ were below the detection limit (-9999), they were taken to be zero for this calculation.
AD	TDN	Total Dissolved Nitrogen	Total dissolved nitrogen concentration (organic plus inorganic species in micrograms of nitrogen per liter).	Total dissolved nitrogen (TDN) was measured by in-line digestion using the Lachat/Zellweger Analytics QuikChem 8000 flow injection autoanalyzer (Milwaukee, WI, USA) using method FIA 31-107-04-3-B for low total nitrogen for brackish/fresh waters (detection level: 0.1 - 5.0 milligrams nitrogen per liter): Water samples were vacuum filtered (less than 25 kiloPascals) using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in high-density polyethylene bottles and frozen at -20 degrees Celsius until analysis. Two replicates were run from the same bottle. Total dissolved nitrogen by in-line digestion works by oxidizing all the nitrogen compounds to nitrate by heating to 100 degrees Celsius and adding energy via UV light. The pH is dropped from 9.1 to 3 during the decomposition. The entire digestion occurs prior to the injection valve. The nitrate/nitrite concentration is then determined using standard colorimetric techniques similar to the strict nitrate/nitrite manifold.

AE	DON	Dissolved Organic Nitrogen	Calculated dissolved organic nitrogen concentration (micrograms of nitrogen per liter).	Dissolved organic nitrogen (DON) was calculated by subtracting dissolved inorganic nitrogen (DIN) from total dissolved nitrogen (TDN). If the DIN value used in the calculation was below the detection limit, it was taken to be zero for this calculation. At one point DON was determined by high temperature oxidation using the Antek 7000N or Antek 7000V analyzer.
AF	PO4	Orthophosphate	Orthophosphate concentration (micrograms of phosphorus per liter).	Orthophosphate (PO43-) was determined using a Lachat/Zellweger Analytics QuikChem 8000 flow injection autoanalyzer (Milwaukee, WI) using method FIA 31-115-01-1-F/G: Water samples were vacuum filtered (less than 25 kiloPascals) using pre-combusted Whatman glass microfibre filters (GF/F). The filtrate was stored in high-density polyethylene bottles and frozen at -20 degrees Celsius until analysis. Two replicates were run from the same bottle. Method detection limits (MDL, µg L-1) were: before 4Nov02 = 0.35; beginning 4Nov02 = 0.74; beginning 1Nov04 = 1.68; beginning 11Jul06 = 1.84; beginning 1Dec09 = 0.62.
AG	NtoP	Nitrogen to phosphorus molar ratio	The calculated molar ratio of nitrogen (N) to phosphorus (P).	The molar ratio of nitrogen (N) to phosphorus (P), or N:P, was calculated by dividing dissolved inorganic nitrogen (DIN) by orthophosphate (PO_4^{3-}) concentrations.
AH	SiO2	Silica	Silica concentration (micromolar).	Silicic acid (SiO2) was measured after vacuum filtration (< 25 kPA) of the collected water samples through pre-combusted (3-4 hours at 450 0C) Whatman GF/F glass fiber filters. The filtrate was stored in high-density polyethylene bottles and frozen (-20 0C) until analysis. Two replicates were run from the same sample bottle. Nitrate plus nitrite concentrations were determined using a Lachat QuikChem 8000 flow injection autoanalyzer (Milwaukee, WI, USA). Method detection limits (MDL, µM) were: before 4Nov02 = 0.18; beginning 4Nov02 =1.24; beginning 1Nov04 = 1.86; beginning 11Jul06 = 0.75.
AI	Chla_InVitro	Chlorophyll a	Chlorophyll a concentration measured by in vitro fluorometry (micrograms per liter).	Chlorophyll *a* (Chl *a*) measurements prior to the 08/17/1999 sampling date were measured on a Shimadzu UV-160U spectrophotometer using the trichromatic equation following sonication (45-60 s) and overnight extraction of glass fiber filters in 90 % acetone. Beginning on the 08/17/1999 sampling date, Chl *a* concentration was measured using the modified in vitro fluorescence technique in EPA Method 445.0 (Welshmeyer 1994, Arar et al. 1997): Fifty milliliters of each water sample was vacuum filtered (less than 25 kilopascals) in duplicate at low ambient light conditions using 25 mm Whatman glass microfibre filters (GF/F). The filters were blotted dry, wrapped in foil and frozen immediately at -20 degrees Celsius until analysis. Chlorophyll *a* was extracted from the filter using a tissue grinder and 10 mL of 90 percent reagent grade aqueous acetone (v/v with deionized water, Fisher Scientific NF/FCC Grade). The samples remained in the acetone overnight at -20 degrees Celsius. The extracts were filter-clarified using a centrifuge and analyzed on a Turner Designs TD-700 fluorometer that was configured for the non-acidification method of Welschmeyer (1994). The value reported is the average chlorophyll a concentration measured from the two filters. The fluorometer was calibrated with a known concentration of pure Chl *a* that was determined using a Shimadzu UV-160U spectrophotometer and the extinction coefficients of Jeffrey and Humphrey (1975). The calibration was checked daily against a solid secondary standard (Turner Designs, proprietary formula). As of August 2010, fluorescence was also measured on a TurnerDesigns Trilogy fluorometer. References: **1.** Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr. 39:1985-1992. **2.** Arar, E.J., W.L. Budde, and T.D. Behymer. 1997. Methods for the determination of chemical substances in marine and environmental matrices. EPA/600/R-97/072. National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. **3.** Jeffrey, S.W., R.F.C. Mantoura, and S.W. Wright. 1997. Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing, Paris, France.
AJ	Chla_IWS	Chlorophyll a integrated water sample	Chlorophyll a concentration measured by in vitro fluorometry (micrograms per liter) integrated throughout the water column to 2x the secchi depth. Water samples for this measurement were collected using the integrated water sampler (IWS) which collects vertically integrated water samples.	
AK	PPR	Primary productivity	Primary productivity by light/dark 14C bicarbonate incorporation (milligrams of C per meter cubed per hour).	Primary Productivity rate was measured using an adaptation of Steeman Nielsen's (1952) ^{14}C bicarbonate method (Paerl et al. 1998). This method of measuring primary productivity allows direct measurement of carbon uptake and measures only net photosynthesis: Water samples were stored in 10 Liter high density polyethylene containers overnight in the research pond, a flow through system that receives water from the adjacent Bogue Sound, thereby simulating ambient water temperatures. The following morning the water samples were removed from the pond and transported to the laboratory for analysis. Water samples (76 milliliters) were added to three clear plastic square bottles to determine light uptake of carbon in triplicate and to 1 dark bottle to determine dark uptake of carbon. A solution of radioactive carbonate (300 microliters) was added to each bottle. The bottles were incubated for 4 hours in the pond. The light bottles were incubated underneath a field light simulator, while the dark bottles were incubated in a covered perforated bucket that was submerged in the pond. The FLS was used to simulate the ambient light conditions that phytoplankton are exposed to in the estuary (mixing conditions). The FLS is comprised of a rotating wheel with varying levels of screening. During the incubation period, photosynthetically active radiation (PAR) measurements were performed using a 2 pi Li-Cor LI-192SA spherical quantum sensor attached to a Li-Cor data logger. After the incubation period, the samples were returned to the laboratory, shaken and the entire contents were gently vacuum filtered (less than 25 kilopascals) using 25 mm Whatman glass microfibre filters (GF/F). The filters were placed in wooden drying trays and treated with concentrated hydrochloric acid fumes for 40 minutes to an hour to remove inorganic ^{14}C. The filters were folded in half and placed in 7 milliliter plastic scintillation vials. Five milliliters of liquid scintillation cocktail (ecolume or cytoscint) was added to the vials. The vials were capped, shaken, stored in the dark for 3-24 hours and then assayed for radioactivity using a Beckman liquid scintillation counter. In addition to the samples, triplicate voucher samples were used to quantify the radioactivity of the ^{14}C added. Voucher samples consisted of 100 microliter of ^{14}C and 100 microliters of phenylethylamine. These vials also received 5 milliliters of liquid scintillation cocktail. A background vial and two ^{14}C background standards were used. The quantity of carbon fixed is proportional to the fraction of radioactive carbon assimilated. (Paerl, H.W., J.L. Pinckney, J.M. Fear, and B.L. Peierls 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA. Marine Ecology Progress Series 166: 17-25; Steemann Nielsen, E. 1952. The use of radio-active carbon (C14) for measuring organic production in the sea. Journal du Conseil permanent international pour L'Exploration de la Mer 18: 117-140)
AL	Chlde_a	Chlorophyllide a	Chlorophyllide a concentration by HPLC analysis (micrograms per liter).	
AM	Chl_c1c2	Chlorophyll c1 and c2	Chlorophyll c1 and c2 concentration by HPLC analysis (micrograms per liter).	
AN	Peridinin	Peridinin	Peridinin concentration by HPLC analysis (micrograms per liter).	

AO	19_but	19'-Butanoyloxyfucoxanthin	19'-Butanoyloxyfucoxanthin concentration by HPLC analysis (micrograms per liter).
AP	Fuco	Fucoxanthin	Fucoxanthin concentration by HPLC analysis (micrograms per liter).
AQ	19_hex	19'-Hexanoyloxyfucoxanthin	19'-Hexanoyloxyfucoxanthin concentration by HPLC analysis (micrograms per liter).
AR	9_cisneo	9' cis-Neoxanthin	9' cis-Neoxanthin concentration by HPLC analysis (micrograms per liter).
AS	Viola	Violaxanthin	Violaxanthin concentration by HPLC analysis (micrograms per liter).
AT	Diadino	Diadinoxanthin	Diadinoxanthin concentration by HPLC analysis (micrograms per liter).
AU	Anthera	Antheraxanthin	Antheraxanthin concentration by HPLC analysis (micrograms per liter).
AV	Myxo	Myxoxanthophyll	Myxoxanthophyll concentration by HPLC analysis (micrograms per liter).
AW	Allo	Alloxanthin	Alloxanthin concentration by HPLC analysis (micrograms per liter).
AX	Diato	Diatoxanthin	Diatoxanthin concentration by HPLC analysis (micrograms per liter).
AY	Monado	Monadoxanthin	Monadoxanthin concentration by HPLC analysis (micrograms per liter).
AZ	Lutein	Lutein	Lutein concentration by HPLC analysis (micrograms per liter).
BA	Zea	Zeaxanthin	Zeaxanthin concentration by HPLC analysis (micrograms per liter).
BB	Gyro	Gyroxanthin	Gyroxanthin concentration by HPLC analysis (micrograms per liter).
BC	Chlb	Chlorophyll b	Chlorophyll b concentration by HPLC analysis (micrograms per liter).

Diagnostic phytoplankton photopigments were identified, separated and quantified by high performance liquid chromatography coupled to an in-line photodiode array spectrophotometer (Jeffrey et al. 1997): Known volumes of water sample (500-1000 milliliters, enough to obtain color on the filter) were vacuum filtered (less than 25 kiloPascals) through 25 or 47 millimeter Whatman glass microfibre filters (GF/F) under reduced light conditions. The filters were blotted dry, folded in half, wrapped in foil and then immediately frozen at -20 degrees Celsius until analysis. The filters were placed in 15 milliliter centrifuge tubes containing 1.5-3.0 milliliters of 100% acetone (HPLC Grade), sonicated for 30-60 seconds using a Fisher Sonic Dismembrator 300 with microtip and extracted at -20 degrees Celsius for 12-24 hours. After extraction the samples were centrifuged at 4500 rpm and the supernatant (i.e.- the combined extracted pigments) collected & filtered into amber glass autosampler vials using Millipex Millipore 0.45 micometer PTFE. Two hundred microliters of extractant from each vial was injected into the HPLC system using a Spectra Physics (now Thermo Separations Products) AS3000 autosampler and SP8800 pump, running a non-linear, 55 minute, 2-solvent gradient adapted from Van Heukelem et.al. 1994 or 1995?. The nonlinear, variable flow, binary gradient consisted of solvent A [80% methanol : 20% ammonium acetate (0.5 M adjusted to pH 7.2)] and B (80% methanol : 20% acetone). The extractant was separated into individual pigments using a series of C18 reverse-phase columns to optimize photopigment separations: The column order was a Rainin Microsorb guard column (0.46 x 1.5 centimeters, 3 micrometer packing) followed by a single monomeric reverse-phase C18 column (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm packing) followed by two polymeric reverse-phase C18 columns (Vydac 201TP5, 0.46 x 25 cm, 5 µm packing). The columns were kept at a constant 52 degrees Celsius in an Alltech 330 column heater. The separated pigments were then passed through an in line Shimadzu SPD-M10AV photodiode array detector which measured the absorbance of the sample/extractant, scanning the range of 350-800 nanometers every 2 seconds. The data was collected and analyzed using Shimadzu's EZChrom software. Individual pigments are identified using a combination of peak retention time and absorbance spectrum shape. Retention times and absorbance spectra are identified for each pigment by analyzing known pigments (either as pure standards or pigments or isolated from algal cultures). Pigments are quantified from their peak areas, calculated at 440nm. A calibration curve is generated by injecting various volumes of a mixed standard composed of known quantities of seven pure pigment standards (fucoxanthin, zeaxanthin, bacteriochlorophyll a, canthaxathin, chlorophyll b, chlorophyll a, echinenone and ß-carotene) and calculating the peak areas of those pigments The peak areas are regressed against the known quantities of each pigment to calculate the slope (Response Factor) for that pigment. Response factors for pigments we do not have reference standards for are calculated using the ratio of absorbance coefficients of each pigment to its closest structurally related reference pigment, multiplying the known pigment's reponse factor by that ratio. Pigments extracted from the samples are then quantified by multiplying the peak areas of a chromatogram at 440nm by the response factors. Pigment values listed as below detection were below the software threshold for peak detection or had spectra below a similarity of 0.9 compared to library spectra. Technician expert judgement was used in difficult cases.

References:

Jeffrey, S.W., R.F.C. Mantoura, and S.W. Wright. 1997. Phytoplankton pigments in oceanography: Guidelines to modern methods. UNESCO Publishing, Paris, France.

Pinckney, J.L., D.F. Millie, K.E. Howe, H.W. Paerl, and J. P. Hurley. 1996. Flow scintillation counting of 14C-labeled microalgal photosynthetic pigments. Journal of Plankton Research 18:1867-1880.

BD	Chla	Chlorophyll a	Chlorophyll a concentration by HPLC analysis (micrograms per liter).	
BE	B_Car	ß-Carotene	ß-Carotene concentration by HPLC analysis (micrograms per liter).	
BF	TotalChla	Total Chlorophyll a	Sum of chlorophyll a and chlorophyllide a concentrations by HPLC analysis (micrograms per liter). Concentrations below detection assumed to by zero for this	
BG	Chlorophytes	Chlorophytes	Chlorophyll a concentration (micrograms per liter) contributed by chlorophytes as determined by ChemTax analysis of HPLC pigment concentrations.	The HPLC derived diagnostic photopigment concentrations were analyzed using the ChemTax matrix factorization program (Mackey 1996). This program uses the steepest decent algorithm to determine the best fit based on an initial estimate of pigment ratios for algal classes. The initial pigment ratio matrix used in the Chemtax analysis was derived from: Mackey M.D., Mackey D.J., Higgins H.W., & Wright S.W. 1996. CHEMTAX- a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Marine Ecology Progress Series 144: 265-283, and consisted of nine photopigments (alloxanthin, antheraxanthin, chlorophyll b, total chlorophyll a (chlorophyll a + chlorophyllide a), fucoxanthin, lutein, peridinin, violaxanthin, and zeaxanthin) for five algal groups that constitute the bulk of the phytoplankton community in the Neuse River and Estuary (chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates). In order to reduce the variation of pigment ratios due to large changes in phytoplankton species composition with depth, season, and salinity regime, homogenous data groupings of the HPLC pigment data were performed prior to running on Chemtax: HPLC pigment data was grouped by Depth Level (surface or bottom) then by Season (winter, spring, summer and fall) then by Salinity regime (oligohaline: <5.0 ppt, mesohaline: 5.01 - 18.0 ppt, polyhaline: >18.01 ppt). When there were less than 10 samples in a given homogenous grouping (Chemtax requires at least 10 samples per run), the data was grouped by oligohaline + mesohaline or mesohaline + polyhaline (This is indicated in the comments section).
BH	Cryptophytes	Cryptophytes	Chlorophyll a concentration (micrograms per liter) contributed by cryptophytes as determined by ChemTax analysis of HPLC pigment concentrations.	
BI	Cyanobacteria	Cyanobacteria	Chlorophyll a concentration (micrograms per liter) contributed by cyanobacetria as determined by ChemTax analysis of HPLC pigment concentrations.	
BJ	Diatoms	Diatoms	Chlorophyll a concentration (micrograms per liter) contributed by diatoms as determined by ChemTax analysis of HPLC pigment concentrations.	
BK	Dinoflagellates	Dinoflagellates	Chlorophyll a concentration (micrograms per liter) contributed by dinoflagellates as determined by ChemTax analysis of HPLC pigment concentrations.	
BL	Total_Chla	Total Chlorophyll a	Total chlorophyll a concentration derived from the sum of Chlorophytes, Cryptophytes, Cyanobacteria, Diatoms, and Dinoflagellates (all in µg L-1). This concentration should equal total chlorophyll a generated from HPLC analysis.	
BM	Comments for:		Lists the parameter for which comments are included for values that are in bold in that row. If there are comments for more than one parameter, there will be multiple columns of this column type.	
BN	Comments		Comments on specific parameter value. If there are comments for more than one parameter, there will be multiple columns of this column type.	

BO	Comments for:		Lists the parameter for which comments are included for values that are in bold in that row. If there are comments for more than one parameter, there will be multiple columns of this column type.	
BP	Comments		Comments on specific parameter value. If there are comments for more than one parameter, there will be multiple columns of this column type.	

"Station List" Sheet:

A	Station	Station name	The name of the fixed sampling station.	Station names decrease in number (in increments of 10) from 180 (the most downstream station sampled) to 0 (the most upstream station sampled). All stations were located mid-river except stations 95 and 96 which were located close to the nothern and southern shore of the Neuse River Estuary, respectively. Stations were selected to cover the entire length of the Neuse River Estuary from Streets Ferry Bridge (Station 0) to the mouth of the estuary where it flows into Pamlico Sound. When possible, efforts were made to select locations with key stationary features (channel markers, buoys and land markers) to allow easy station identification in the field.
B	Station Description	Station description	The physical location of the sampling station, such as at or near a particular river marker, buoy, road or bridge. Lists other names that may also be used to refer to this station.	Not applicable.
C	km0	Kilometers from 0	The distance (in kilometers) of the sampling station from station 0.	Distance (in river kilometers) was calculated using ESRI ArcGIS software. Distances were calculated using projected station locations (North Carolina State Plane 1983 meters projection). Distances from station 0 through 30 (upper river stations) were measured along the main channel of the river. Distances from stations 30 to 180 were measured as straight lines between stations.
D	Lat	Latitude	North latitude of station in decimal degrees	Geographic position was obtained using a North Star differential geographic positioning system (GPS), Model 1505-A.
E	Lon	Longitude	West longitude of station in decimal degrees	
F	AMS_station	AMS station number	NCDENR-DWQ AMS station closest to NRWQ station	Information from NC Department of Environment and Natural Resources - Division of Water Quality Ambient Monitoring System (portal.ncdenr.org/web/wq/ess/eco/ams). Information on discontinued stations retrieved from EPA STORET.
G	AMS_stn_descrip	AMS station descripti	Short description of AMS station location	
H	AMS_Lat	AMS station latitiude	North latitude of AMS station in decimal degrees	
I	AMS_Lon	AMS station longitude	West longitude of AMS station in decimal degrees	
J	Comments			