NUTRIENT COMPOSITION AND PHYTOPLANKTON COMMUNITY DYNAMICS IN THE NEW RIVER ESTUARY

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ABSTRACT

JULIA CHARLOTTE ALTMAN: Nutrient composition and phytoplankton community dynamics in the New River Estuary
(Under the direction of Hans Paerl)

Inorganic and organic nitrogen loading may be linked to the persistence of algal blooms in the New River estuary. Characterizing these loads and investigating their role in shaping natural phytoplankton assemblages was the focus of this thesis. Water samples from a 10 station transect were analyzed for urea, nitrate, ammonium, orthophosphate, dissolved organic nitrogen, chlorophyll *a*, salinity, and temperature. Nutrient addition bioassays were run to examine the effects of varying nutrient loads on an estuarine site. Bioassays indicated that dual enrichment by inorganic N and P led to maximum increases in phytoplankton biomass, but additional organics may promote the growth of potentially toxic phytoplankton, including dinoflagellates and cyanobacteria. Evaluating the impact of varying nutrient forms on phytoplankton dynamics is necessary in order to develop strategies to avoid changes in community structure and larger-scale changes in ecosystem health.

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LIST OF ABBREVIATIONS AND SYMBOLS

^{14}C	carbon isotope 14
°C	Celsius degrees
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
DON	dissolved organic nitrogen
HPLC	high performance liquid chromatography
L	Liter
ml	Milliliter
M	molar
N	nitrogen
NC	North Carolina
NH_4^+	ammonium
NO_3^-	nitrate
NO_x^-	nitrate plus nitrite
NRE	New River Estuary
P	phosphorous

PO_4^{-3} phosphate

psu practical salinity unit

TDN total dissolved nitrogen

UNC-IMS University of North Carolina at Chapel Hill Institute of Marine Science

USGS United States Geologic Survey

YSI Yellow Systems Instruments

µg Microgram

µM Micromolar

µmol Micromole

INTRODUCTION

The abundance and species composition of phytoplankton has been shown to be strongly controlled by resources available such as nutrients and light (Cloern 1999; Reynolds 2006). Inorganic nutrient controls of phytoplankton abundance and species composition have been widely studied (Harrison and Turpin 1982; Sanders et al. 1987; Fisher et al. 1999). However, organic nutrient influence has been explored mostly in terms of utilization of specific organic compounds by phytoplankton isolates in cultures (Wheeler et al. 1974; Palenik and Morel 1990; Antia et al. 1991; Moore et al. 2002). The effects of a bulk DON mixture on natural phytoplankton communities have been examined in only a few studies (Peierls and Paerl 1997; Seitzinger and Sanders 1999). Here we use an *in situ* bioassay approach to examine the regulation of a natural phytoplankton community in estuarine water, and its production by both inorganic and bulk organic nitrogen resources.

Nutrient enrichment in coastal waters is an increasingly important problem. Nitrogen (N) is of particular concern, because it is most often the nutrient controlling primary production and promoting eutrophication of these waters (Ryther and Dunstan 1971; Paerl 1988; Boesch et al. 2001; Conley et al. 2009). The microtidal New River Estuary (NRE), NC is an example of a strongly N-limited system that has experienced negative effects of anthropogenic nutrient enrichment, including toxic algal blooms, hypoxia, fish kills and benthic habitat degradation (Mallin et al. 1997; 2005; Tomas et al. 2007). The NRE receives external N inputs from a variety of sources; including surface runoff, municipal wastewater, the atmosphere, and groundwater

(Mallin et al. 1997; 2005). These sources supply varying forms of N, including nitrate, nitrite, ammonium, and dissolved organic nitrogen (DON) compounds.

DON is the largest pool of fixed nitrogen in most aquatic environments (Bronk 2002). Due to the extremely heterogeneous and cryptic nature of the bulk DON pool, only a small portion of it has been identified and quantified. Sources of the DON bulk pool are of both abiotic and biotic origin. Sources include surface runoff, and riverine discharge, groundwater, and atmospheric deposition (Seitzinger and Sanders 1999; Bronk et al. 2007). Dissolved combined amino acids (DCAAs) may in part be made up of amino acids bound to humics or adsorbed to clays. Biologically-produced DCAA and dissolved free amino acid inputs include microbial (microautotrophs and heterotrophs), zooplankton grazing and excretion activities, and release from cell death. Humic acids, which can variably account for up to 70% of the DON pool (Bronk et al. 2007) arise from microbial degradation and leaching of plant materials. Another DON compound, urea, is a by-product of biotic metabolism. It is of particular interest because of the increasing amounts of urea in fertilizers and animal feed (Glibert et al. 2006), and is of particular concern in eastern North Carolina watersheds since it is also a waste product from rapidly-proliferating livestock operations in this region.

In the past, DON had been considered to be largely unavailable for phytoplankton growth because of observations of consistently high, invariant concentrations of DON in aquatic systems (Bronk et al. 2007). Furthermore, its role was thought to be purely in supporting bacterial production and it was not considered directly available to phytoplankton (Bronk et al. 2007). However, more recent studies have shown that DON compounds may be a source of bioavailable N for phytoplankton. Phytoplankton were also shown to be able to use labeled humic compounds in riverine and coastal environments (Bronk et al. 2007). Humics may be a source of

usable N to some toxic dinoflagellate species, including *Alexandrium tamarense* (Gagnon et al. 2005) and *Alexandrium catenella* (Doblin et al. 2000). Urea uptake and stimulation of phytoplankton biomass has been shown in numerous studies (Antia et al. 1991; Glibert et al. 1991, 2001, 2004; Twomey et al. 2005). Ammonium (NH_4^+) is the most energetically favorable source of inorganic N, and direct evidence of nitrate (NO_3^-) uptake pathways present in phytoplankton cells support its role as a major available source to algae (Owens and Esais 1976), but inorganic N forms are not always the exclusive forms of N utilized. Enzymatic activity on the cell surface may enable phytoplankton to access the N in organic molecules (Palenik and Morel 1990). Phytoplankton can act as photo-heterotrophs and take up organic molecules via several pathways including active transport, use of proteolytic enzymes, pinocytosis, and phagocytosis (Bronk et al. 2007). They may utilize DON during high activity summer months when nitrate is depleted (Paerl 1991). Lastly, phytoplankton can compete with bacteria for nutrients when they are scarce, and species that can exploit organic nutrient sources may have a competitive advantage (Bronk et al. 2007). The role of riverine DON in driving phytoplankton production therefore warrants addressing.

Most water quality management practices stress reducing total N inputs to N-sensitive estuaries (Bricker et al. 1999; Boesch et al. 2001). However, this approach does not distinguish between different N forms. The first research objective of this study was to quantify the concentrations of all major forms of dissolved N in the NRE and its proximal major freshwater tributary, the New River. Previously, investigations of spatial and temporal trends of nutrients in the NRE have been largely focused on inorganic nutrient species (Mallin et al. 1997), though land use in the watershed suggests that DON and urea may be important components of the total dissolved nitrogen pool (Mallin et al. 2005). DON and urea have not yet been delineated in the

NRE. This study aimed to examine the different dissolved nutrient concentrations and forms over a one year period and complete seasonal *in-situ* bioassays designed to mimic nutrient loading events observed in the monitoring data. The second objective of this study was to examine the relationship between the chemical composition of nutrients entering the system (organic and inorganic N forms and inorganic P (PO_4^{3-}) and phytoplankton community composition and growth in the controlled bioassay experiments. The research question addressed was: How do upstream-derived supplies of DON, DIN, and DIP impact downstream estuarine phytoplankton productivity, biomass, and community composition?

MATERIALS AND METHODS

Study Site

The New River Estuary (NRE) is a relatively small, mid-Atlantic coastal plain estuary located in Onslow County, south-eastern North Carolina (Fig. 1). Most of the estuary resides within the United States Marine Corps Base Camp Lejeune (MCBCL). Jacksonville, a moderate sized city (2009 Population: 80,500), is located on the upper part of the estuary on Wilson Bay (Fig. 1). With a surface area of 88 km^2 and an average depth of 3m (NOAA 1999), the NRE is a relatively broad and shallow series of lagoons, that is confined by barrier islands at the mouth, restricting water exchange with the Atlantic Ocean (Mallin et al. 2005). Fresh water flushing time in the NRE varies seasonally with storm and runoff events, ranging from 8 to 187 days, with an average of 70 days (Ensign et al. 2004). In comparison, the average flushing time of neighboring Cape Fear Estuary just to the south is only 7 days (Ensign et al. 2004). The semi-lagoonal nature of the NRE plays a significant role in its relatively long residence time and sensitivity to nutrient inputs, since long residence times allow more time for algal nutrient assimilation, growth, and "internal" nutrient recycling. Similar to its neighboring semi-lagoonal estuaries to the north- the Neuse River Estuary and Pamlico Sound- the NRE experiences periodic phytoplankton blooms(including harmful species, Tomas et al. 2007) and periods of bottom water hypoxia on an annual basis (Mallin et al. 2005, Paerl et al. 2007, 2010).

Watershed-generated, non-point nutrient sources discharged via the New River control nutrient loading to the estuary (Paerl et al., unpub data). The NRE watershed is dominated by agricultural activities, including row crop and concentrated feeding operations (CAFOs) (Mallin et al. 2005). Downstream, in the area of Wilson Bay (Fig. 1), there is a history of nutrient inputs

from the Jacksonville waste-water treatment plant, which promoted highly eutrophic conditions into the late 1990s (Mallin et al. 1997, 2005). Upstream, non-point source nutrient inputs associated with burgeoning CAFO and rowcrop operations have increased significantly over the past several decades, leading to sustained eutrophication in the NRE, including exceedances of the State of North Carolina's "acceptable" chlorophyll *a* concentration (40 μg L^{-1}) (NCDENR 2005), and harmful algal bloom outbreaks (Mallin et al. 2005; Tomas et al. 2007).

Sampling

Physical, chemical, and biological water quality parameters were measured monthly at stations 1-8 along a downstream transect in the New River estuary from June 2010 to September 2011(Fig. 1). Surface (0.2m) samples were collected using a diaphragm pump and dispensed into 4L bottles. Bottles were stored in the dark and brought back to UNC-Chapel Hill Institute of Marine Sciences (IMS) for nutrient (total dissolved nitrogen (TDN), NO_x, NH_4^+, and PO_4^{3-}) and phytoplankton biomass analysis. Surface temperature and salinity were measured at stations 1-8 using a YSI 6600 sonde.

Water samples were collected monthly for nutrients and phytoplankton biomass at station 9 and 10, which are two United States Geologic Survey (USGS) gaging stations located upstream of the estuary (Fig.1). These gaging stations monitor temperature, salinity, and flow (data available at http://waterdata.usgs.gov/nwis/qw). Station 10 was furthest upstream, which corresponds to USGS Gum Branch gaging station #02093000 (34°50'57" N, 77°31'10" W). Station 9 is further downstream at the approximate head of the estuary and corresponds to USGS New River Below Hwy17 gaging station #0209303205 (34°44'56" N, 77°26'16" W).

Urea was measured monthly at stations 1-8 from May to September 2011. Urea was measured monthly at stations 9 and 10 from January to July 2011. The discrepancy in sampling periods is due to variations in sample collection and analysis schedules between IMS and USGS.

Bioassay Water Collection

Water for the nutrient enrichment bioassays was collected from station "7" (34˚43'14" N, 77˚25'20" W) on the NRE (Fig.1). Surface water temperatures for station 7 ranged from 8.0˚C to 32.4˚C with an annual average of 23.6 ˚C during the study period. Salinity ranged from 2.7 to 24 psu with an annual average of 14.8 psu. River water used in the bioassays was collected from station "10" (Fig. 1). Surface water temperatures ranged from 5.5˚C to 25.7˚C with an annual average of 15.6 ˚C. This site was virtually fresh year round (psu<0.25).

Bioassay Design

The effects of upstream-derived DON and inorganic nutrients on phytoplankton growth responses were examined using five nutrient addition bioassays completed in June 2010, September 2010, December 2010, April 2011, and July 2011. On the initial day of each experiment, water for the bioassay was collected from near the surface of NRE station 7 (Fig. 1). Water was immediately filtered through a 200µm mesh screen on the sampling boat to remove zooplankton and particulate debris. Filtered water was dispensed into 20 L polypropylene carboys, returned to UNC-CH Institute of Marine Sciences (IMS), and transferred to a set of 4L transparent polyethylene Cubitainers (Hedwin Inc.). Cubitainers are 80% transparent to photosynthetically active radiation (PAR; 400-700nm). Water was also collected from station 10 on the initial day of each experiment and brought back to the lab for immediate nutrient analysis (described below). Nutrient treatments were added as follows in replicates of four: Control (no

additions), DIN (ammonium and nitrate); DIP (orthophosphate), DIN+DIP (nitrate, ammonium, orthophosphate), River filtrate (upstream river water), and Urea (Fig. 2). The upstream river water addition was made up of 0.8 L of Gum Branch water filtered through pre-cleaned 25 mm diameter Whatman glass fiber (GF/F) filters (0.7 µm pore size).

To determine if natural phytoplankton communities showed a response to heightened organic N levels, initial levels of inorganic nutrients measured at station 10 were used to formulate the N and P additions in the other treatments. For the urea treatment, urea was added at the same molar N concentration as the DIN treatment in order to compare responses. Inorganic nutrients and urea were added to Cubitainers with 0.8 L of Major Ion Solution (MIS; Paerl and Bowles 1987) in order to replicate the ionic properties of the river water filtrate addition. Bioassays were incubated under natural light and temperature conditions by suspending the Cubitainers in the experimental ponds at IMS under a layer of neutral density screening (~ 60% of surface irradiance) to avoid photoinhibition. Cubitainers were incubated for 8 days and subsampled for phytoplankton growth parameters on days 0, 2, 4, and 8. At sampling time points, approximately 500 mL was collected from each Cubitainer in polyethylene bottles. In the laboratory, water was partitioned into appropriate volumes for (1) nutrient analysis, (2) phytoplankton biomass analysis, (3) primary productivity determination, and (4) phytoplankton community composition analysis

Laboratory Protocols and Analysis

Nutrient Analysis: Sample water was gently filtered through pre-cleaned 25 mm diameter Whatman GF/F filters (0.7 µm pore size). Filtrate was analyzed for total dissolved nitrogen (TDN), NO_x, NH_4^+, and PO_4^{3-} using the Lachat/Zellweger Analytics QuickChem 8000 flow

injection autoanalyzer using standard protocol (Lachat method numbers 31-107-04-1-C, 31-107-06-1-B, and 31-115-01-3-C, respectively). Bulk DON concentrations were calculated by subtracting DIN from TDN. Urea concentration was determined using the room temperature calorimetric diacetylmonoxime method as described by Goeyens et al. (1998).

Phytoplankton Biomass (Fluorometric Chlorophyll a Analysis): Approximately 150 mL of sample water were vacuum filtered onto pre-combusted 25 mm GF/F filters. Filters were stored at -4˚C until they were sonicated and extracted overnight in 90% acetone. Extracted chlorophyll samples were analyzed using a Turner Designs TD-700 fluorometer.

Primary Productivity: Primary productivity measurements were made on days 2 and 4 of the bioassays using the ^{14}C method according to Paerl (2002). Four light and one dark 76 ml polyethylene bottle per treatment type were filled with sample water and 0.3 ml ^{14}C-$NaHCO_3$ (ICN Radiochemicals; 58μCi μmol^{-1} specific activity). The bottles were incubated in the IMS outdoor pond for approximately 4 hours under neutral density screening. Following incubation, samples were filtered onto 25mm GF/F filters, which were then fumed for at least 2 hours in a plastic container with concentrated HCl in order to remove abiotically precipitated ^{14}C-$NaHCO_3$. Filters were then placed in vials with 5ml of CytoScint scintillation cocktail, and counted on a Beckman Coulter LS 6500 liquid scintillation counter. Dissolved inorganic carbon DIC) content of the samples was determined using a Shimadzu Total Organic Carbon Analyzer (TOC-5000A0. Using the method of Paerl (2002), we converted counts to total CO_2 fixed.

Community Composition Analysis: Major algal taxonomic groups were partitioned and identified by using high performance liquid chromatography (HPLC; Shimadzu model LC-20AB) equipped with a photodiode array spectrophotometric detector (Shimadzu SPD-M20AC) (Pinckney et al. 1996, 1999). Approximately 100ml aliquots were filtered onto pre-cleaned

25mm diameter Whatman GF/F filters and frozen until extraction. Filters were extracted in 100% acetone via sonication and stored at 20˚C for approximately 24 h. Pigment extracts (200 µl) were autoinjected and separated using column configurations described by Pinckney et al. 2001. Pigments were identified and quantified based on their absorption spectra calibrated against pure pigment standards (DHI, Denmark). HPLC results were periodically verified using inverted microscopy (Utermöhl 1958) of preserved (1% Lugol's solution) water samples.

Statistical Analysis

JMP statistical software program was used for all statistical tests conducted on the data. To determine if environmental factors influenced variations in DON, urea, and chlorophyll *a*, linear regression models were fitted to response variables. Significance was indicated by a p value < 0.05. Bioassay results were analyzed using analysis of variance (ANOVA). Chlorophyll a, ^{14}C fixation, and accessory pigment concentration data were used to determine significant differences between response in control and five treatments. If a difference in response means existed among the treatments (p value < 0.05), we followed up with Dunnett's post-hoc test (for comparisons with control).

(*Author's Note:* for December and April bioassay experiments, responses observed in River filtrate treatment were excluded from statistical analysis due to laboratory error. Incorrect baseline nutrient calculations lead to this treatment having disproportionately lower dissolved nutrients than the other treatments).

RESULTS

Monitoring

Abiotic Environmental Parameters

During the period observed (June 2010 to September 2011) surface water temperatures varied little across stations on the New River Estuary (stations 1-8) at any given time (Fig. 3) The estuary as a whole was coldest in the winter, with a whole estuary average of 8.2˚C. The minimum temperature observed during the study period was 6.6˚C, which was recorded on January 4, 2011 at station 5. Spring was considerably warmer with an average water temperature of 21˚C, though colder water temperatures persisted slightly longer into the spring at the lower stations close to the ocean. Fall was slightly warmer than spring with an average surface water temperature of 24˚C. Water temperatures measured during the summer months were warmest, averaging at 29.6˚C. The warmest temperature recorded was 32.6˚C on August 8, 2011 at station 8.

Salinity was typically lowest upstream and increased with distance downstream (Fig. 4). Station 8 had slightly higher salinities than station 7 because of its location, which is actually slightly more downstream than station 7. Averaged seasonally, salinity was highest in the summer, followed by the winter. Spring had lower salinities, and fall had the lowest salinities. Low fall salinity values during the study period were likely influenced by two major rain events: a 100 year flood event in October 2010 and Hurricane Irene in late August 2011.

Mean NO_x, NH_4^+, PO_4^{3-}, DON, and urea concentrations for stations 10, 9, 7, 5, and 1 during the sampling periods are shown in Table 1. For all seasons, N-NO_x was highest in the riverine stations and decreased rapidly in the estuarine stations (Table 1, Fig. 5). The highest N-

NO_x concentrations were measured upstream at stations 9 and 10 and were observed during the winter. Downstream, within the estuary, the highest N-NO_x concentrations were observed in the fall, which was concurrent with periods of high discharge. N-NO_x values were extremely low in the estuarine stations during the rest of the year. At station 7, N-NO_x was below detection limit for all of the summer sampling dates and portion of fall and spring as well. N-NH_4^+ concentrations showed similar spatial trends as N-NO_x (Table 1, Fig. 6). Concentrations decreased from upstream to downstream in all seasons except the winter, when N-NH_4^+ remained fairly constant across all stations (Fig. 6). Highest N-NH_4^+ concentrations upstream in the river were observed during the fall whereas highest N-NH_4^+ concentrations downstream in the estuary were observed during the winter. N-NH_4^+ was only below detection on one occasion July 12, 2010, compared to chronic below detection levels of N-NO_x in the estuary. Dissolved inorganic phosphorus (P-PO_4^{3-}) followed the spatial trend of decreasing with distance from the upstream to downstream throughout the year (Table 1, Fig. 7). P-PO_4^{3-} concentrations were highest in the river and the estuary in the fall and summer. During the spring and winter P-PO_4^{3-} levels from station 7 seaward were generally extremely low (less than 10 μgL^{-1} P-PO_4^{3-}).

Bulk DON had different patterns across space and time than the inorganic N and P species. Bulk DON concentrations were the highest across all the stations during fall and summer (Fig. 8). During these two seasons, DON increased downstream from station 10, peaked at station 7, and decreased continuing downstream. In the winter and spring however, bulk DON was highest in the river and decreased with distance downstream. Urea was measured monthly at the riverine stations from January through July 2011, and in the estuarine stations from May to October 2011. Overall mean urea measured in the river from January to July 2011 was 26.6 μgL^{-1} N-$CO(NH_2)_2$ and median was 14.04 μgL^{-1} N-$CO(NH_2)_2$. Mean urea measured in the

estuary from May 2011 to October 2011 was 6.8 μgL^{-1} $N\text{-}CO(NH_2)_2$ and median was 5.4 μgL^{-1} $N\text{-}CO(NH_2)_2$. Spatially, there were no consistent observable patterns in urea concentration from upstream to downstream except during the spring (Table 1, Fig.8). In the spring, urea concentrations showed a general trend of decreasing with distance downstream, but the trend did not hold for the summer and fall sampling times. Temporally, urea was highest in the spring at station 9 and 10, but there was no clear temporal pattern at other stations. The maximum urea value detected in the river was 163 μgL^{-1} $N\text{-}CO(NH_2)_2$ at station 10 on April 27, 2011.

Factors influencing variation in DON and Urea

In order to examine environmental factors that might influence variation in DON and urea concentrations that were observed in the NRE stations 1-10, we used JMP statistical software to perform linear regressions. Results are summarized in Table 2. The models regressed DON and urea separately against temperature, salinity, NO_x, NH_4^+, PO_4^{3-}, chlorophyll *a*, and each other (interaction). The models indicated that DON was positively correlated with temperature, NO_x, NH_4^+, PO_4^{3-}, chlorophyll *a*, and urea. The model indicated that DON was negatively correlated with salinity. The second set of regression models indicated that urea was positively correlated with NO_x, NH_4^+, PO_4^{3-}, and DON. Temperature, salinity, and chlorophyll *a* content did not appear to correlate with urea concentration.

Chlorophyll a

Mean chl *a* concentrations for stations 10, 9, 7, 5, and 1 during the sampling period are shown in Table 1. Phytoplankton biomass as chlorophyll *a* varied greatly over space in the NRE (Table 1, Fig. 9). During all seasons, chlorophyll *a* was virtually absent from station 10, but rapidly increased with distance downstream until peaking at station 7 and then again decreasing

with distance downstream. Seasonally, average chlorophyll *a* concentrations were highest in the fall and spring, but high chlorophyll *a* values at station 7 were observed during all seasons. Notable bloom events included a fall bloom on November 1, 2010 at station 7 (149 μgL^{-1}), a winter bloom on February 1, 2011 at station 7 (68 μgL^{-1}), and a spring bloom on May 31, 2011 at station 8 (86 μgL^{-1}).

Factors influencing variation in phytoplankton biomass

Linear regression model results for chlorophyll *a* against various environmental parameters are summarized in Table 3. Only chlorophyll *a* was positively correlated with DON. Chlorophyll *a* was negatively correlated with salinity, NO_x, and PO_4^{3-}. Temperature and urea did not appear to correlate with chlorophyll *a* in the NRE.

Bioassays

Summary of nutrient additions

Initial concentrations of nutrients present in the control and five treatments varied for each experiment, since concentrations at sampling sites varied. Background nutrient conditions at sample sites are shown in figure 10. Mean ambient nutrient concentrations in Cubitainers on initial day of experiment in the treatments are listed in Table 4. External molar inorganic N:P ratios varied for each bioassay (Fig. 11). In the control, the external molar N:P ratio was less than 16 for all time points except for T8 of the June 2010 bioassay and T0,T2,T4 of the December 2010 bioassay.

Summer I Bioassay- June 2010

Primary productivity was observed at significantly elevated levels compared to the control in each of the treatments, at different time-points (Fig. 12). At T2, primary productivity was significantly higher than control in the DIN, DIN+DIP, River Filtrate, and Urea treatments. At T4 none of the treatments had primary productivity rates greater than controls. At T8, only the DIP treatment yielded a primary productivity rate significantly greater than the control (Fig. 12). Initial chlorophyll *a* concentration was 31.3 μgL^{-1} in June 2010 (Fig. 13). Chlorophyll *a* concentrations were significantly elevated for 67% of the time sampled in all of the treatments except for DIN, which had no responses that were significantly greater than the control (Fig. 14). The initial estuarine phytoplankton community was dominated by diatoms (fucoxanthin), cyanobacteria (zeaxanthin), and dinoflagellates (peridinin) respectively (Fig. 15). Phytoplankton community shifted during the course of the experiment to a cyanobacteria dominated community in all treatments (Fig.16); however some treatments stimulated certain major diagnostic pigments more frequently than others over the whole bioassay. Dinoflagellate (peridinin) biomass was greater than the control in the River Filtrate addition 67% of the time (2 out of 3 time points) and was greater in all of the other treatments only 1 out of the three time points (Fig. 16). DIN, DIN+DIP, River Filtrate, and Urea yielded significant cyanobacteria (zeaxanthin) and chlorophyte (chlorophyll b) biomass responses an equal number of times. Diatom biomass (fucoxanthin) was significantly higher than the control in the Urea treatment 100% of the times sampled and 67% of the time in the DIN, DIN+DIP, and River Filtrate treatments (Fig. 16). Myxoxanthophyll (cyanobacterial pigment) was only greater than the control one time: T8 in the DIP treatment. On T8, cyanobacterial pigments were greater in the DIP treatment than all other treatments and chlorophyll *a* was measured at over 64 μgL^{-1} (Fig. 16).

Fall Bioassay - September 2010

Significant responses in primary productivity rates were seen only on T2, but were observed in all N-containing treatments (Fig. 12). The DIP treatment did not yield significant responses in primary productivity or chlorophyll *a*. Initial chlorophyll *a* levels were low (6.9 μgL^{-1}) and remained low throughout the experiment (Figs. 13 and 17). Nonetheless, chlorophyll a was significantly higher than the control in the DIN, DIN+DIP, River Filtrate, and Urea treatments at both T2 and T4. At T8, no treatments yielded a significant response (Fig. 17). Initial phytoplankton community was dominated by cyanobacteria (zeaxanthin) and diatoms (fucoxanthin) respectively (Fig. 18). Dinoflagellates (peridinin) and chlorophytes (chlorophyll *b*) were also present in smaller proportions. The phytoplankton community at the end of the experiment was similar to the initial and had the same dominant groups (Fig. 19). Cyanobacteria biomass (zeaxanthin) was observed at significantly elevated levels compared to the control in the DIN treatment 100% of the times sampled and in the DIN+DIP and Urea treatments 33% of the time sampled (Fig. 19). Another cyanobacterial pigment, myxoxanthophyll, was significantly elevated most frequently in the River Filtrate addition. Peridinin and chlorophyll b were also stimulated most frequently by the River Filtrate addition. Fucoxanthin was stimulated most frequently by DIN (Fig. 11).

Winter Bioassay - December 2010

Initial chlorophyll *a* concentration was 15.8 μgL^{-1} in the December experiment (Fig. 13) and grew to 25 μgL^{-1} at the end of the experiment in the dual DIN+DIP addition. Primary productivity rates were not higher than the control in any treatments until 96 hours (T4), when they were greater in only the DIP and DIN+DIP treatments (Fig. 12). On T8, primary productivity was only greater in the DIN+DIP treatment. Biomass as chlorophyll *a* however, was elevated in the DIP treatment during all time-points and in DIN+DIP on T2 and T4, but was not

greater in any other treatments on any time-points (Fig. 20). Initial phytoplankton community had a relatively even distribution of cyanobacteria, diatoms, cryptophytes (alloxanthin), and dinoflagellates. (Fig. 21). Chlorophytes were also present in small concentrations initially. By the end of the experiment, the same groups remained (except chlorophytes, which completely diminished) in varying proportions depending on treatment type (Fig. 22). There were no significant responses of any of the major pigments in any treatments until T4. Pigments peridinin, alloxanthin, and fucoxanthin were all significantly higher than the control most frequently in the DIN+DIP treatment. Zeaxanthin was only significantly greater than the control once, at T4 in the DIP treatment. Chlorophyll b was also only significantly elevated compared to the control in the DIP and Urea treatments (Fig. 22).

Spring Bioassay - April 2011

Initial chlorophyll *a* concentration was low (5.2 μgL^{-1}) but peaked at 25 μgL^{-1} on T8 in DIN+DIP treatment (Figs. 13 &23). Primary productivity rates were significantly elevated most frequently by the DIN+DIP treatment, but were also elevated one time each in the DIN and Urea treatments (Fig. 12). Significant chlorophyll *a* responses mirrored primary productivity responses point for point (Fig. 23). Initial phytoplankton community was dominated by dinoflagellates and cryptophytes respectively (Fig. 24). However, community shifted to diatom dominance by T8 (Fig. 25). All the pigments were greater than the control most frequently in the DIN+DIP treatment. Diatoms and dinoflagellates were also stimulated by Urea and DIN treatments. DIP did not stimulate and pigments significantly from the control.

Summer II Bioassay - July 2011

Initial chlorophyll *a* concentration was 12.6 μgL^{-1} (Fig. 13) and peaked at 16 μgL^{-1} on T2 before declining in all treatments (Fig. 26). Primary productivity rates were significantly greater than the control in all the treatments except DIP on T2 and all the treatments except DIP and Urea on T4 (Fig. 12). There were no significant responses on T8. Chlorophyll *a* was most frequently stimulated by DIN+DIP during this bioassay (Fig. 26). Initial phytoplankton community was dominated by cyanobacteria and chlorophytes and these two groups remained prevalent throughout (Figs. 27 & 28). Zeaxanthin was most frequently elevated in the DIN and Urea treatments, where-as myxoxanthophyll was most frequently elevated in DIN, DIN+DIP, and River Filtrate. Chlorophyte responses mirrored that of myxoxanthophyll, and diatoms were most frequently stimulated by DIN+DIP and River Filtrate.

Summary of Bioassay Results

Percentiles of total significant responses of growth parameters for each treatment are shown in Fig. 29. Across all 5 bioassay and all 15 time points, DIN+DIP was the most stimulatory treatment of primary productivity and total biomass. Chlorophyll *a* was greater than the control in DIN+DIP 80% of the time sampled followed by 67% in the River Filtrate treatment. DIP yielded significant responses the least number of times. Maximum primary productivity and phytoplankton biomass values for all five experiments occurred on day 8 of the June 2010 experiment in the DIP treatment (Figs. 12 and 14). Primary productivity was measured at 463.41 mg C m^{-3} h^{-1} and mean chlorophyll *a* was 64.6 μgL^{-1}.

Major phytoplankton pigments were also stimulated significantly more often in some treatments. Peridinin (dinoflagellate) was greater in the River Filtrate treatment 56% of the times sampled followed by 40% in the DIN+DIP treatment. Myxoxanthophyll (cyanobacteria) was

similar to peridinin in that it was most frequently stimulated by River Filtrate (44%) followed by DIN+DIP (26%). Chlorophyll b (chlorophyte) was also most stimulated by River Filtrate (55%) followed by DIN+DIP (40%) . Zeaxanthin (cyanobacteria) was most frequently stimulated by DIN+DIP (60% of the time) followed by DIN (47%). Fucoxanthin (diatoms) and alloxanthin (crptophytes) were most often stimulated by DIN+DIP, then River Filtrate.

DISCUSSION

Dissolved nutrients in the NRE

In the current study concentrations of inorganic N and P were greater above the estuary in the oligohaline section than in the mesohaline sections of the mid and lower estuary (Figs. 5-8). This pattern of decreasing nutrient concentrations with distance downstream has been established by studies monitoring estuarine processes in the area including the nearby Neuse River Estuary (Mallin et al. 1991, Twomey et al. 2005). This discussion will focus primarily on DON and urea dynamics, since less is known about their patterns in the New River Estuary. Average DON concentrations obtained in this study were similar to those reported for other estuaries in the US. A collection of estuarine DON values are reported by Bronk (2002; Table 1). The mean of these values is approximately 315 μgL^{-1}, while the mean DON value we observed in the NRE was approximately 345 μgL^{-1}. Bronk (2002) also reports various urea values ascertained from estuarine monitoring. An approximate average of the values reported is 28 μgL^{-1}. The mean we obtained for the NRE from January to September 2011 was 14.8 μgL^{-1}. However, urea at station 10 tended to be higher and had a mean of 34.9 μgL^{-1}. The range of urea values observed in this study is greater than the range that Twomey et al. (2005) observed in the Neuse River estuary. Our maximum detected value of 163 μgL^{-1} was over three times higher than the maximum value he detected. Using the data from this study we can conclude that urea is a small source of N in the New River estuary, making up to 30% of the DON pool on some sampling points. Given that DON is the largest pool of nitrogen in most estuarine systems (Bronk et al. 2007), and makes up an average 40% of the total dissolved N pool in this system (not shown), urea merits being considered a significant available source of nitrogen in the NRE.

Linear regression models were used to analyze potential correlations between DON and urea and various other parameters. DON and urea were both significantly positively correlated with NO_x, PO_4^{3-}, and NH_4^+. Nitrate is commonly referred to "external N" since it originates from sources outside the river via runoff, groundwater, wastewater, and rainfall (Paerl et al. 1995). Thus positive correlations with NO_x suggest that DON and urea may be allochthonous and arrive via similar mechanisms. However, ammonium and phosphate were also positively correlated with DON and urea. While NH_4^+ and PO_4^{3-}, may originate from some external sources (waste, rainfall), these nutrients are mainly considered to be internally supplied in estuaries, by reminalization in the sediments and water column (Paerl et al. 1995, Twomey et al. 2005). Thus this positive correlation suggests that DON and urea may be internally supplied. Both scenarios are plausible given that DON and urea can have both internal and external origins. Externally supplied DON is transported via runoff, groundwater, wastewater, and rainfall as humics, amino acids adsorbed to clay particles, or other unknown compounds (Bronk et al. 2007). Internally, DON can originate from phytoplankton and bacteria as metabolites, excretions, and cell releases during senescence. Urea, which was also positively correlated to NO_x, NH_4^+ and PO_4^{3-}, can be both internally and externally supplied as well. Urea is produced *in situ* by fish and zooplankton excretions, bacterial metabolism, and sediment release (Glibert et al. 2004). Externally, urea may come from animal waste runoff and fertilizer runoff. Urea's inclusion in fertilizers is a trend that is escalating on a global scale (Glibert et al. 2005). Although urea was not significantly influenced by temperature or salinity in this study, DON was positively correlated to temperature and negatively correlated to salinity. Since DON may be supplied by biological activity and metabolism, a positive correlation with temperature is likely, given that biological activity increases with temperature. The negative correlation with salinity

is evident in the monitoring data (Fig. 8). This spatial pattern of decreasing with distance downstream is similar to patterns seen in concentrations of inorganic nutrients in the NRE (Figs. 5-7). This correlation suggests that the estuary functions as a net sink of DON.

Phytoplankton biomass in the NRE

Total Chl *a* concentrations obtained in this study were similar to those previously reported in the NRE (Mallin et al. 2005, Tomas et al. 2007). These results also show similar chl *a* distribution trends with the chl *a* maximum occurring in the mesohaline section of the estuary. In the present study chl *a* maximum was station 7. At station 7, chl *a,* was highest in the fall. Given that fall was a period of high discharge during our study, this agrees with other findings that phytoplankton abundance is controlled by external nutrient loading (Paerl et al. 2003). For the rest of the stations, chl *a* varied minimally seasonally (Fig. 9). This suggests that chlorophyll *a* did not necessarily respond to growth condition factors such as light and temperature. Our regression analysis results showed that temperature did not significantly influence chl *a* (Table 3), which corroborates our observations of minimal variation in seasonal trends for chl *a* in the NRE. However, these results differ from widely documented trends that show temperature to be an influential factor controlling phytoplankton abundance (Eppley, 1972).

Chorophyll *a* was negatively correlated with salinity in the NRE. Mallin et al. (1991) also observed an inverse relationship between chl *a* and salinity in the Neuse River estuary. Chl *a* was negatively correlated with NO_x and PO_4^{3-} (Table 3). However, chronic low concentrations of these nutrients in the estuary during this study period may have affected these results. Rapid uptake of nitrate and phosphate by phytoplankton make relationships between chl *a* and these nutrients difficult to interpret. Thus the negative correlations observed must not be

interpreted as evidence that these nutrients are not linked to phytoplankton abundance. Quite to the contrary, many studies have established the occurrence of high phytoplankton densities following nutrient loading events (Paerl et al. 2007). Higher resolution sampling regimes and models that account for time-lags might provide more insight into correlations between these parameters. The regression model also showed that chl *a* was positively correlated with DON (Table 3). This suggests that phytoplankton abundances are high when DON levels are high. Moreover, the DON maximum in the estuary occurred station 7; the same station as the chl *a* maximum (Figs. 8 and 9). Phytoplankton have been shown to utilize and simultaneously produce DON (Bronk et al. 2007). Thus the potential for phytoplankton to serve as both a source and a sink for DON may influence the correlation observed.

Total phytoplankton biomass response during bioassays

Riverine loading is the dominant source of new nutrients to the NRE (Hall et al., personal communication). Thus, elevated nutrient loads from upstream are transported downstream to the estuary where physical conditions including lowered turbidity, relatively long residence time, and periodic vertical stratification are favorable for excessive algal growth. This study demonstrated that nutrient enrichment was likely an important factor promoting phytoplankton growth, but there were substantial differences in terms of the magnitudes and types of phytoplankton community stimulation promoted by individual and combined forms of N and P. Total biomass was most frequently stimulated by DIN+DIP, followed by River filtrate, Urea, DIN, and DIP respectively. Total phytoplankton biomass response to nutrient enrichment may have been linked to nutrient limitation as indicted by external molar N:P ratios. Molar ratios of N:P less than 16 indicate nitrogen limitation (Redfield 1958). The N:P ratios were consistently less than 16 in the water during the majority of the five experiments (Fig. 11), which indicates a

trend of N-limitation in NRE estuary. This observation agrees with other findings that the NRE is strongly N-limited (Mallin et al., 1997, Hall et al. in prep.). In the present experiment, production of new biomass was more often stimulated by nutrient additions containing additional N and P rather than N alone (Fig. 29). An explanation for the strong response to combined N and P may be that single nutrient enrichments by either N or P alone may quickly induce limitation by the nutrient not supplied (Elser et al. 2007). P limitation is generally indicated by N:P ratios greater than 16 (Redfield 1958; Smith 1990; Justić et al. 1995). The molar N:P ratio of the control water was greater than 16 on day 8 of the June 2010 bioassay. The only treatment that elicited a significant chlorophyll response at this time point was DIP, which backs up that it was a P limited environment as indicated by the molar ratio**.** Moreover, high chlorophyll *a* and cyanobacterial pigment concentrations indicated a cyanobacterial bloom at this sampling point. Microscopic examination(not shown) revealed the bloom to be composed of a heterocystous cyanobacterium (*Anabaenopsis* sp.). Since the phytoplankton in these Cubitainers had been supplied with extra P, *Anabaenopsis* sp. was likely able to grow and supply itself with N through N_2-fixation (a high frequency of heterocysts was observed, indicative of N_2 fixation; Horne 1979). Cyanobacteria likely did not thrive in the other Cubitainers due to constraints on P supply.

This series of experiments reiterated similar findings by other studies that urea additions may stimulate phytoplankton growth (Twomey et al. 2005; Glibert et al., 2006). Elevated chlorophyll *a* levels were seen more often in the Urea treatment than in the DIN treatment. This observation is notable considering combined inorganic sources of N are considered to be the preferred forms of N for phytoplankton (Owens and Esaias 1976). These results create compelling reason to further the investigation of urea and its sources in our rivers and estuaries

since urea may be a significant source of N to some nuisance algal bloom taxa (Glibert et al. 2006). Due to increased use of urease inhibitors in fertilizers, which can delay the hydrolysis reaction to ammonium carbonate by many weeks, urea may be making it into our waterways in substantial concentrations (Glibert et al. 2006). Our study may agree with this phenomenon since we found urea to make up a portion of the N pool in the New River estuary.

Implications for bloom formations of specific taxa

Phytoplankton groups that were promoted by specific nutrient forms and combinations varied. The most commonly identified photopigments were peridinin, zeaxanthin, fucoxanthin, myxoxanthophyll, and chlorophyll *b*. One pigment that showed a distinct connection to a particular treatment was peridinin, which was observed at elevated levels in the River filtrate addition over half of the time sampled (Fig. 29). The link between organic N supply and dinoflagellate productivity has been shown in other studies in recent years (Palenik and Morel, 1990; Doblin et al., 2000; Dyhrman and Anderson, 2003; Gagnon et al., 2005). Myxoxanthophyll (cyanobacteria) and chlorophyll b (chlorophytes) were also more frequently stimulated by River filtrate, indicating that phytoplankton from these groups were able to exploit some portion of the DON in the addition. A recent study by Wawrik et al. (2009) demonstrated the ability for a cyanobacterium, *Synechococcus* spp. to actively incorporate N from a variety of organic compounds. In contrast, zeaxanthin (cyanobacteria), fucoxanthin (diatoms), and alloxanthin (cyptophytes) did not appear to be significantly stimulated by the extra organic nitrogen in the River filtrate addition. The same study by Wawrick et al. (2009) found that diatoms did to take up amino acids or glutamate, suggesting that diatoms may not exploit DON. These pigments were most frequently stimulated by the inorganic N & P addition.

The findings presented here of a possible link between organic N enrichment and the growth of specific taxa such as dinoflagellates may have significant implications for eutrophication in nutrient-sensitive waters such as the NRE. Dinoflagellate blooms have been observed in the NRE on numerous occasions (Mallin et al. 2005; Tomas et al. 2007). Blooms comprised of this group are linked to the production of toxic metabolites, bottom water oxygen depletions, reductions in water clarity, and successional adverse effects on fish and shellfish habitat (NCDENR 2005, Diaz and Rosenberg 2008).

Synthesis and Future Work

In this study, which involved both monitoring and experimental components, we investigated the trends of multiple nutrient forms in the NRE, delineated urea for the first time in this system, and assessed phytoplankton response to additions that mimicked nutrient loading events from the river. In five *in-situ* bioassay experiments, we evaluated phytoplankton response to inorganic N & P, a known organic N compound (urea), and a bulk DON mixture made up of river water. However, individual organic compounds present in the bulk organic mixture were not measured. This makes interpretation of the effects of DON enrichment challenging, since organic compounds may go through a series of biochemical transformations before being used by phytoplankton and the role of bacteria may be important in mediating these processes. A somewhat unexpected finding in our study may be attributed to the role of bacteria. We found that total phytoplankton biomass was less frequently stimulated by the River filtrate addition than by the DIN+DIP addition despite the two treatments having equal concentrations of inorganic nutrients and the River filtrate having more TDN (Table 4, Fig. 29). Since we accounted for dilutions in all of our treatments with major ion solution, the explanation left is

that there may have been something inhibitory to phytoplankton growth in the river water on some occasions. This inhibition may be attributed to competition for nutrients with bacteria in the River filtrate. Competition for inorganic N resources between phytoplankton and bacteria and resulting suppression of phytoplankton growth is reviewed by Bronk (2007). Although it was filtered through clean GF/Fs, the river filtrate likely contained a population of bacteria. The major ion solution, which was made up with deionized water and assorted minerals, did not contain additional bacteria.

In estuaries, where nutrient loading is often dominated by river discharge, nutrients arrive in various forms and magnitudes, which can shape phytoplankton community structure (Valdes-Weaver et al. 2006; Paerl et al. 2007). Evaluating the potential for these various nutrient forms to promote phytoplankton growth, especially by HAB species, is important in order to develop targeted, nutrient-specific coastal management strategies. Future work evaluating nutrient resource use should continue to use naturally occurring phytoplankton communities rather than axenic cultures since they may not be representative of the ecosystem population. Additionally, as advanced techniques have been developed that can characterize more components of the DON pool, these compounds should be delineated in eutrophic environments and evaluated for availability to estuarine primary producers.

Table 1. Sampling period means of dissolved nutrients and Chl *a* at stations 10, 9, 7, 5, and 1. All values listed in μgL^{-1}

Station	**N-NO_x**	**N-NH_4^+**	**P-PO_4^{3-}**	**N-DON**	**N-Urea**	**Chl *a***
10	1271.7	83.2	147	377.3	34.9	1.3
9	260.7	55.4	49.2	432.9	15.5	21.6
7	44.6	22.4	24.4	431.9	10	38.8
5	18.8	26.2	13.6	375.7	4.9	23.5
1	7.7	32.3	7	110	8.5	6.6
Mean	320.7	43.9	48.2	345.6	14.8	18.4

Table 2. Linear regression results expressed as p values for factors correlated to DON and urea concentrations. Significance indicated by asterisks, and positive or negative correlations indicated by + and -, respectfully.

Parameter	DON	Urea
Temperature	.0052* +	.6257
Salinity	<.0001* -	.0526
NO_x	<.0001* +	<.001* +
NH_4^+	<.0001* +	<.0001* +
PO_4^{3-}	<.0001* +	<.01* +
Interaction	<.01* +	

Table 3. Linear regression results expressed at p values for factors correlated to Chlorophyll *a*. Significance indicated by asterisks, and positive or negative correlations indicated by + and -, respectfully.

Parameter	Chlorophyll *a*
Temperature	.3514
Salinity	<.0001* -
NO_x	.0253* -
NH_4^+	.0927
PO_4^{3-}	.0111* -
DON	<.001* +
Urea	.7978

Table 4. Mean ambient nutrient concentrations in Cubitainers for each treatment on initial day of experiment. Concentrations measured in Control and River filtrate, then extrapolated to other treatments based on treatment. BDL = below detection limit. All concentrations are reported in μgL^{-1}. Dash indicates value not measured or reported. *Note: some responses to river filtrate addition not analyzed due to set-up error. See methods.*

		Treatment					
		Control	DIN	DIP	DIN+DIP	River Filtrate	Urea
JUNE	$N\text{-}NO_x$-	BDL	338	BDL	338	338	BDL
	$N\text{-}NH_4$+	8	14	8	14	14	8
	$P\text{-}PO_4^{-3}$	3	3	27	27	27	3
	N-Urea	-	-	-	-	-	338
	N-DON	379	379	379	379	451	379
SEPT	$N\text{-}NO_x$-	BDL	121	BDL	121	121	BDL
	$N\text{-}NH_4$+	10	14	10	14	14	10
	$P\text{-}PO_4^{-3}$	6	6	19	19	19	6
	N-Urea	-	-	-	-	-	121
	N-DON	321	321	321	321	341	321
DEC	$N\text{-}NO_x$-	48	391	48	391	-	48
	$N\text{-}NH_4$+	25	145	25	145	-	25
	$P\text{-}PO_4^{-3}$	6	6	20	20	-	6
	N-Urea	-	-	-	-	-	391
	N-DON	219	219	219	219	-	219
APR	$N\text{-}NO_x$-	1	244	1	244	-	1
	$N\text{-}NH_4$+	21	46	21	46	-	21
	$P\text{-}PO_4^{-3}$	7	7	17	17	-	7
	N-Urea	-	-	-	-	-	244
	N-DON	270	270	270	270	-	270
JULY	$N\text{-}NO_x$-	1	201	1	201	201	1
	$N\text{-}NH_4$+	13	24	13	24	24	13
	$P\text{-}PO_4^{-3}$	3	3	35	35	35	3
	N-Urea	-	-	-	-	-	225
	N-DON	283	283	283	283	363	283

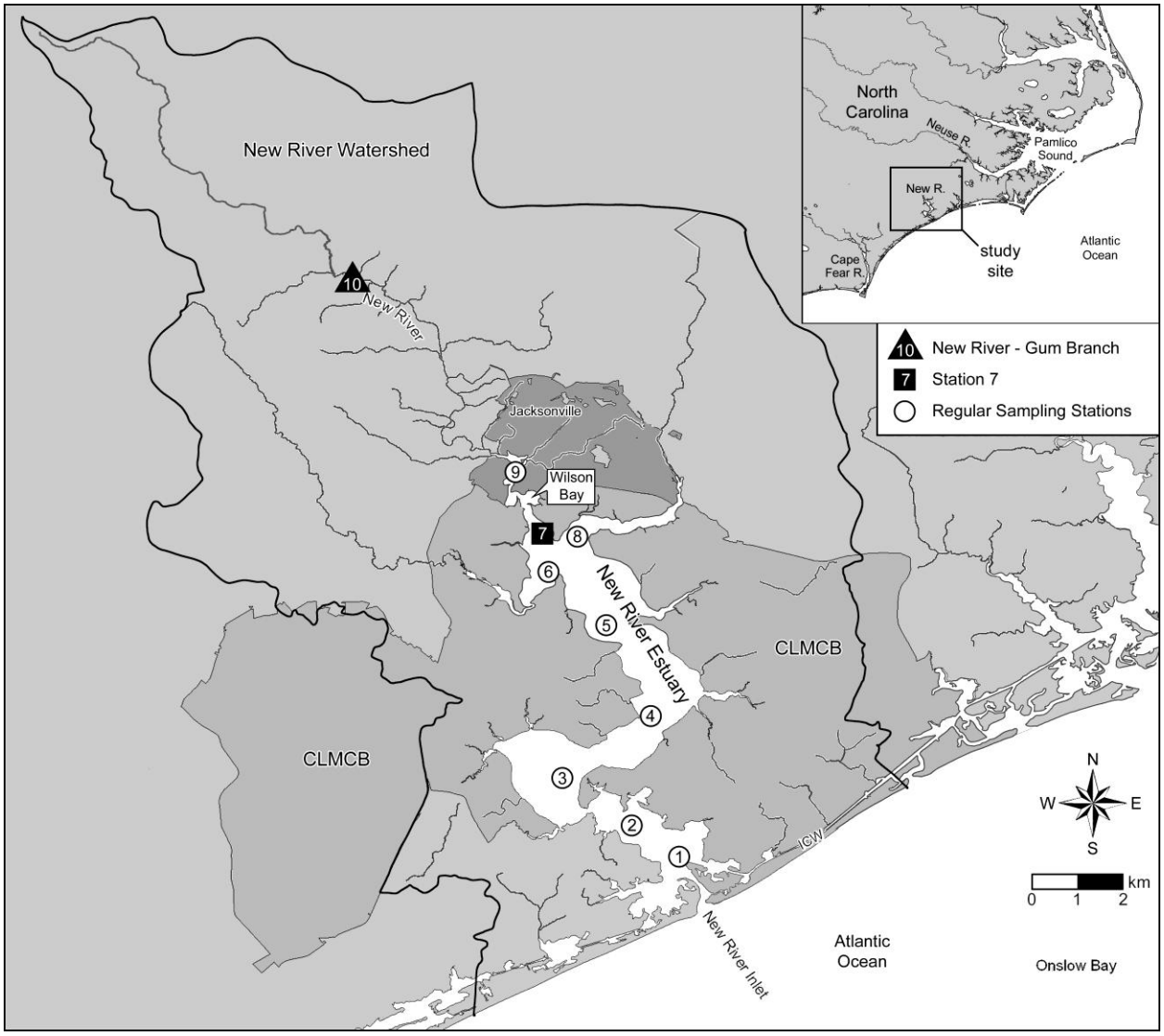

Figure 1. Map of the New River Estuary, North Carolina, USA and its watershed. All numbered shapes represent stations sampled monthly. Square symbol marks station 7, the bioassay sample site for estuary water. Triangle marks the river water collection site used in bioassays.

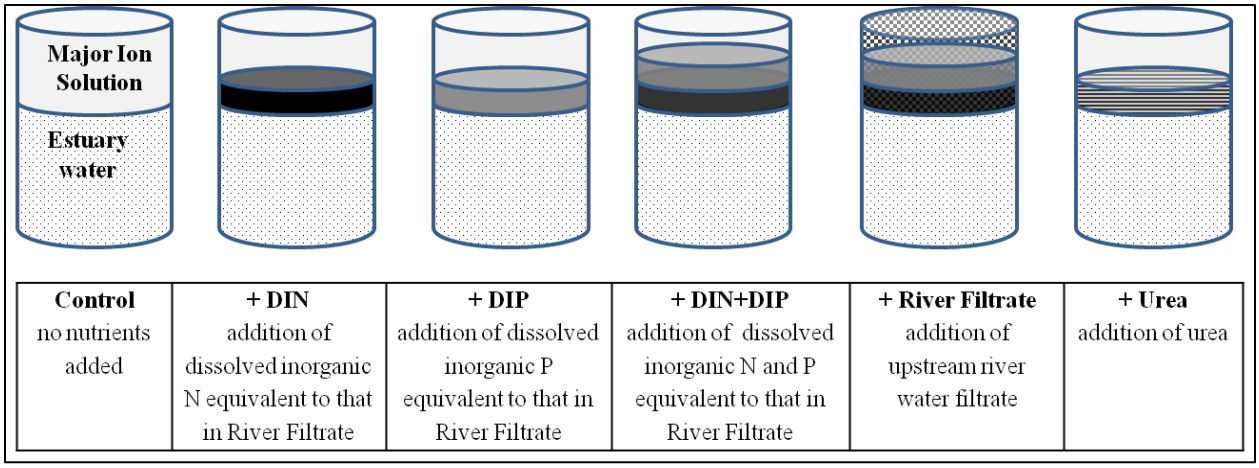

Figure 2. Schematic diagram of bioassay treatments.

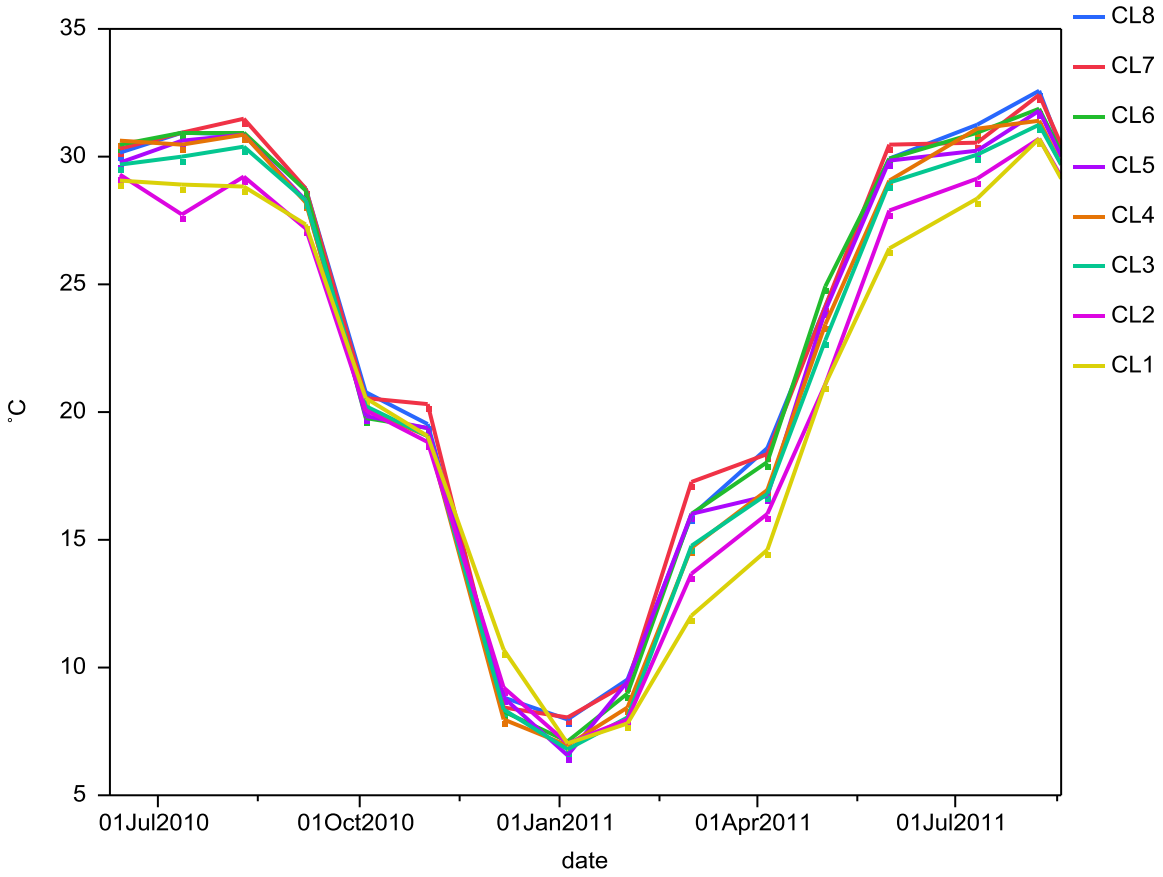

Figure 3. Time series plot of temperature measured at stations 1 to 8 through the entire time period of the study (June 2010 – September 2011).

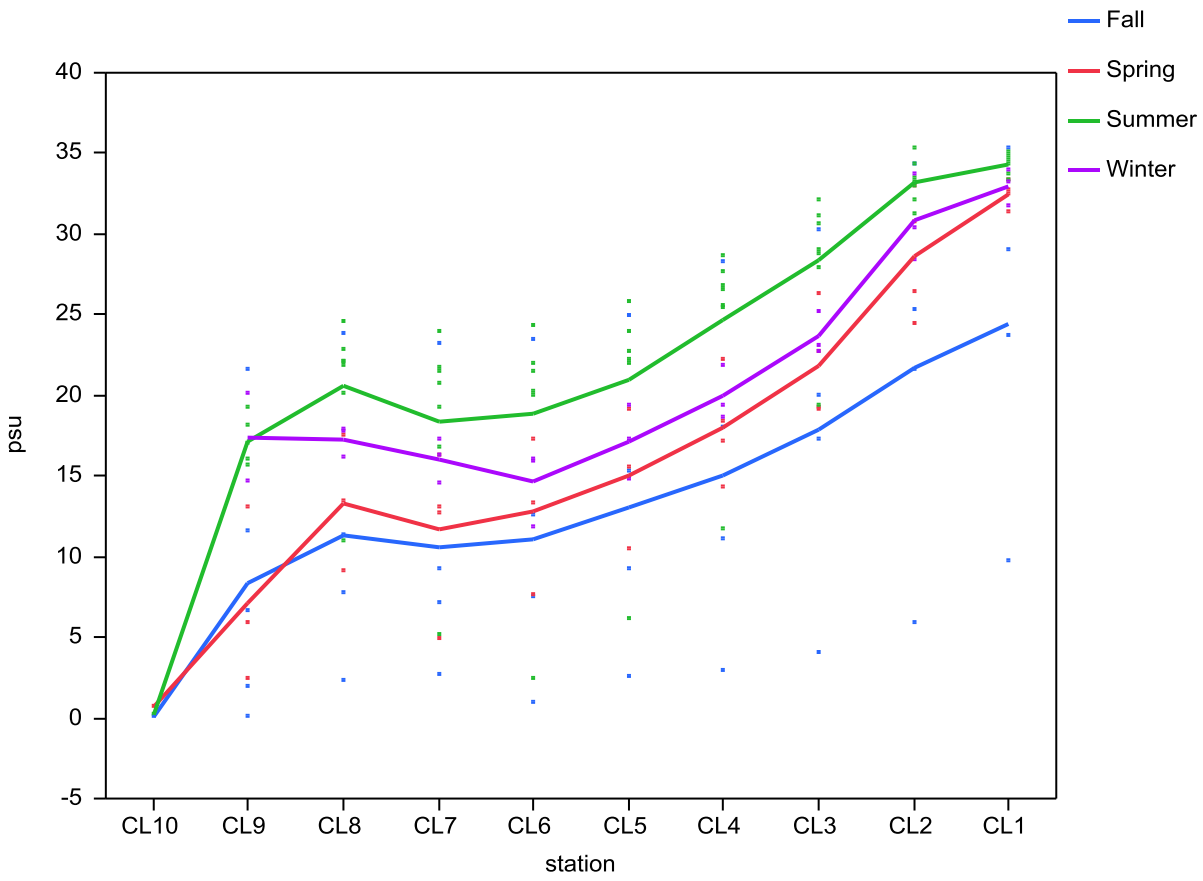

Figure 4. Salinities measured at stations 1 to 10 over the study period. Colored lines represent seasonal average salinities. Station numbers increase from river to mouth.

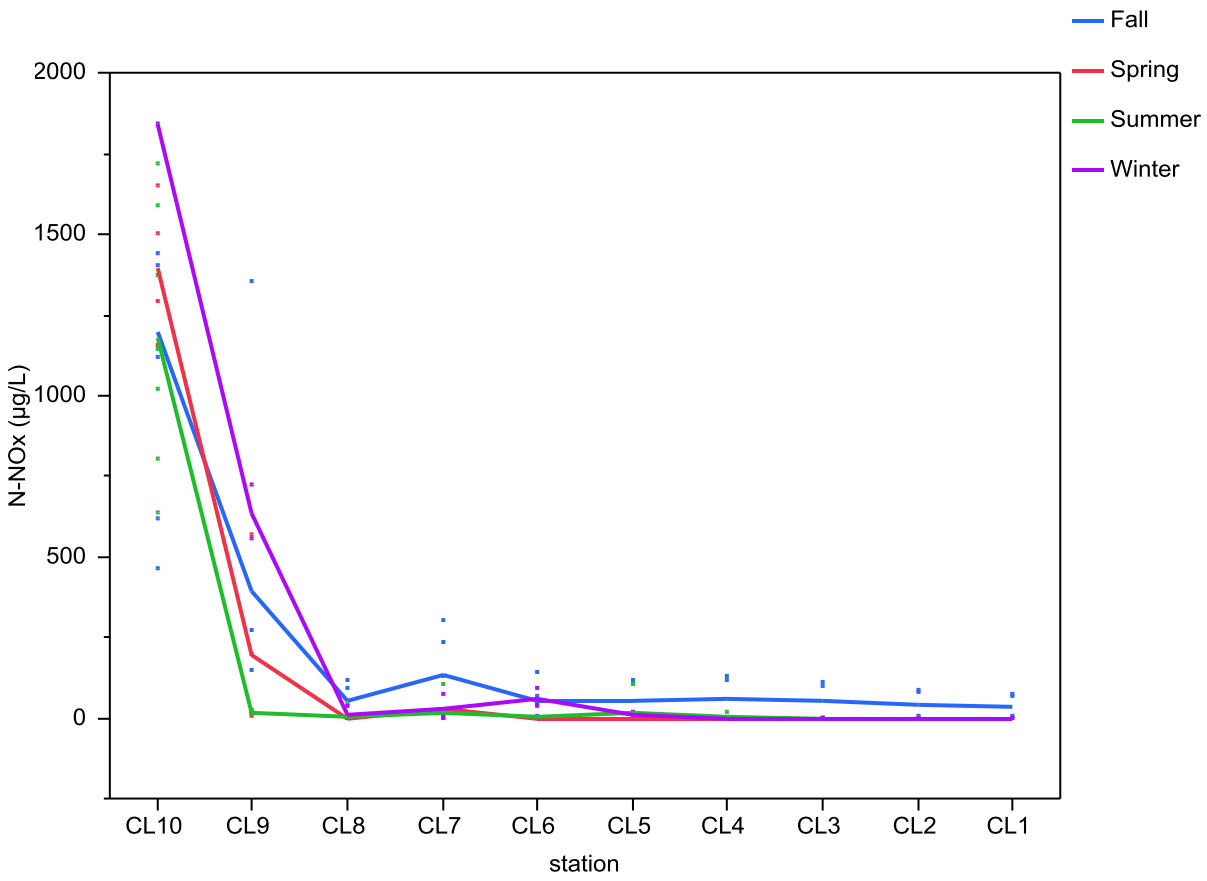

Figure 5. N-NO_x concentrations measured at stations 1 to 10 over the study period. Colored lines represent seasonal averages.

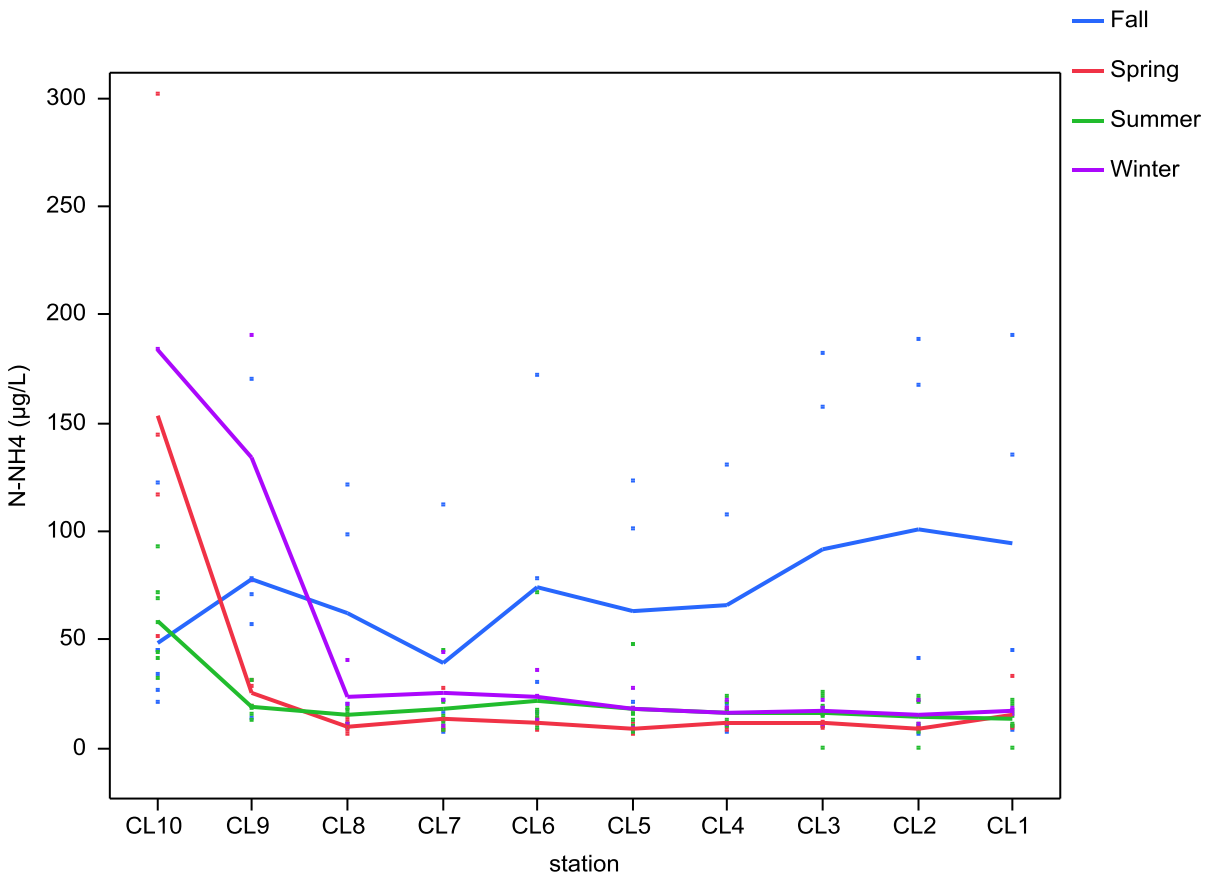

Figure 6. N-NH_4^+ concentrations measured at stations 1 to 10 over the study period. Colored lines represent seasonal averages.

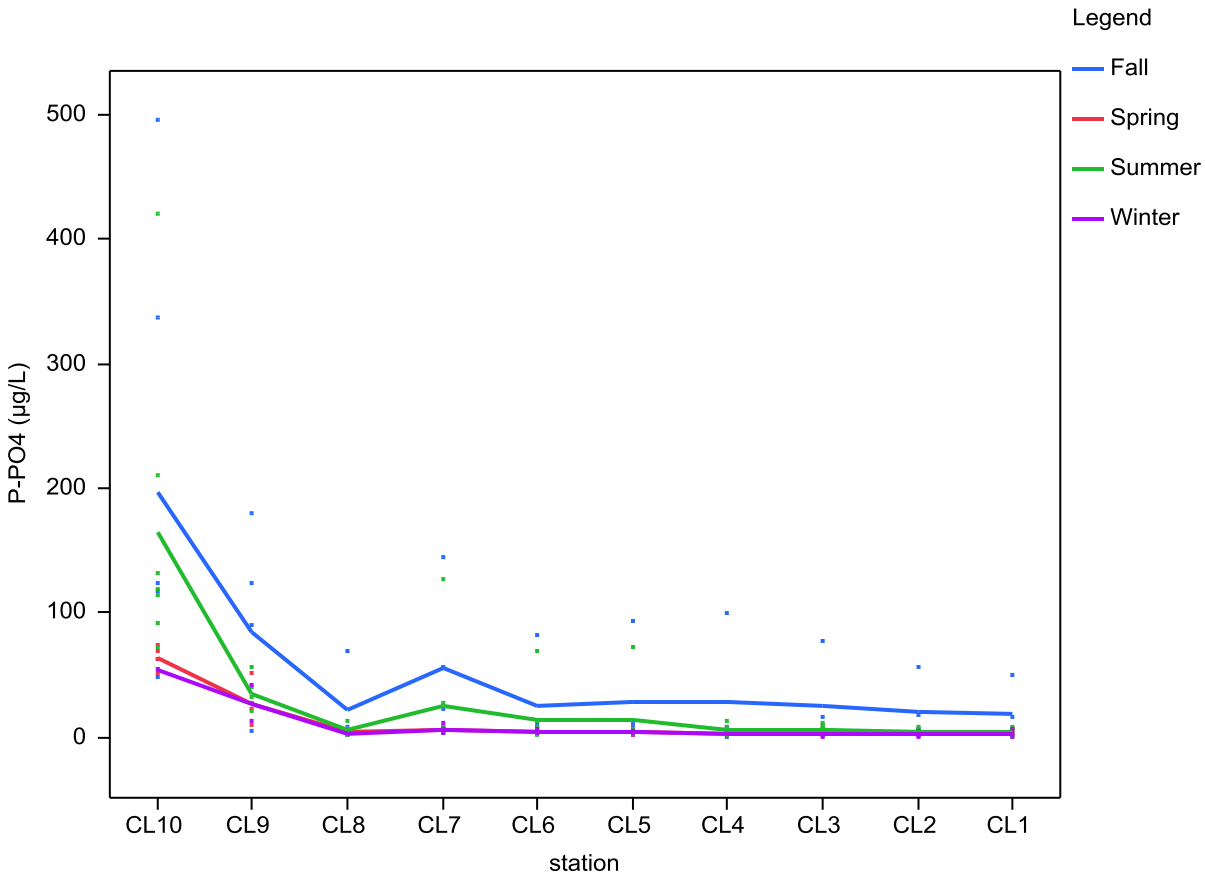

Figure 7. P-PO_4^{3-} concentrations measured at stations 1 to 10 over the study period. Colored lines represent seasonal averages.

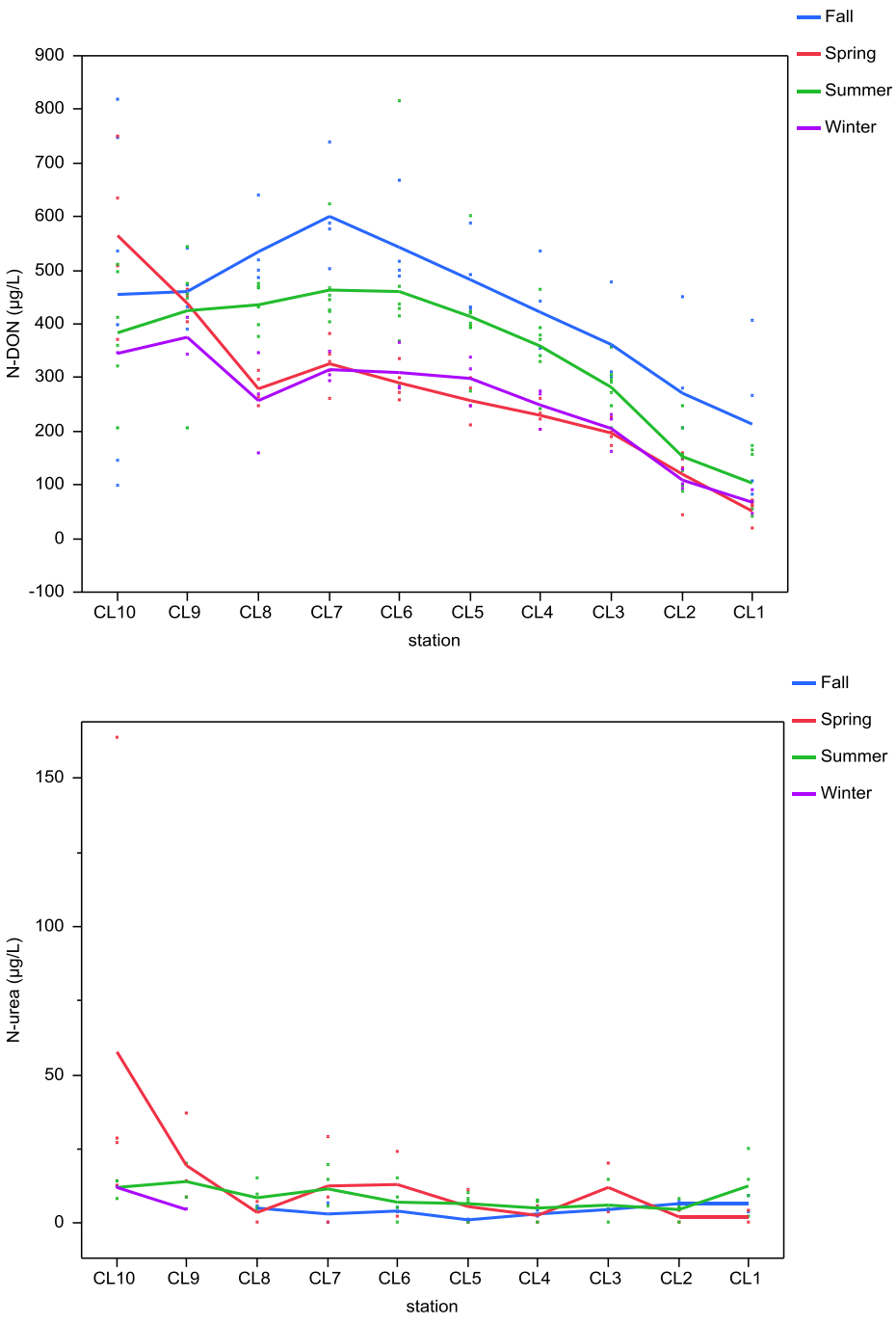

Figure 8. DON (top) and urea (bottom) concentrations measured at stations 1 to 10 over the study period. Colored lines represent seasonal averages.

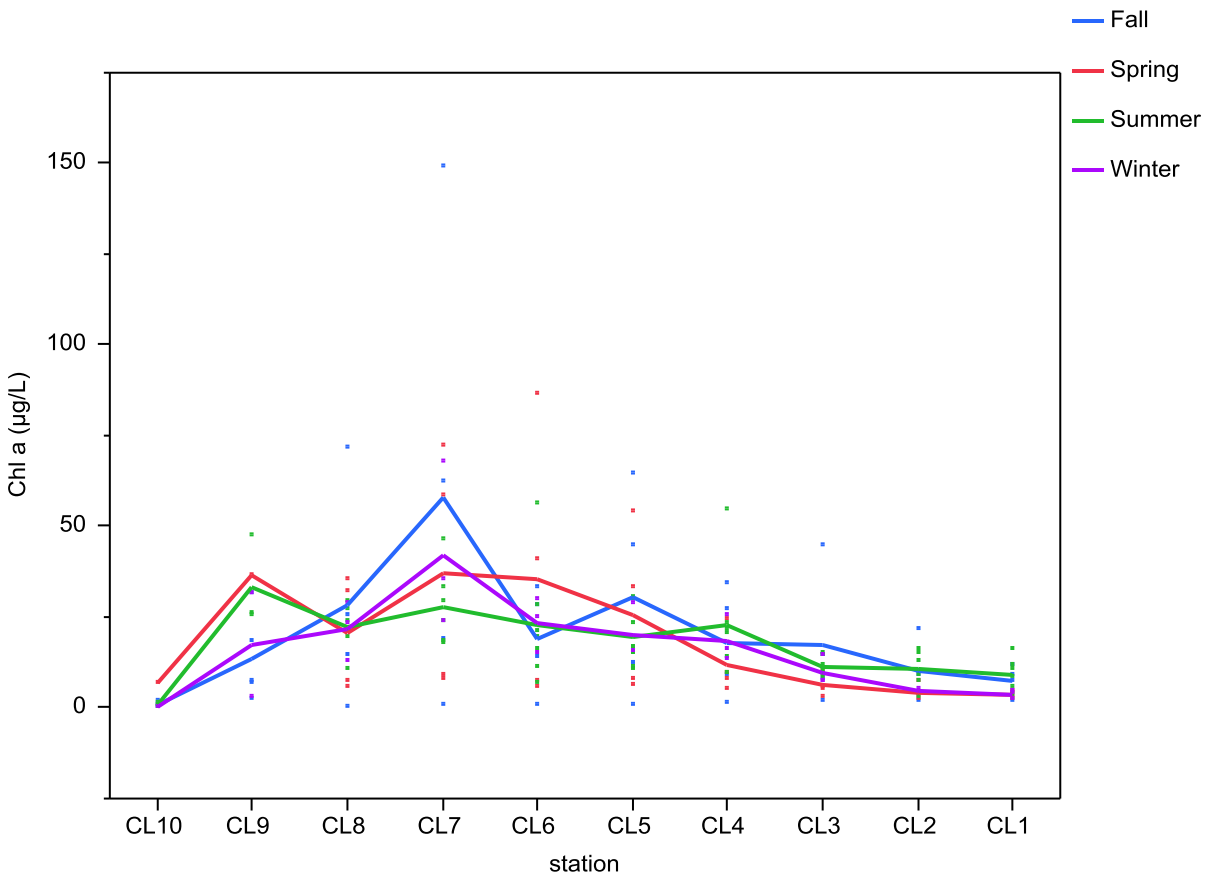

Figure 9. Chlorophyll *a* concentrations measured at stations 1 to 10 over the study period. Colored lines represent seasonal averages.

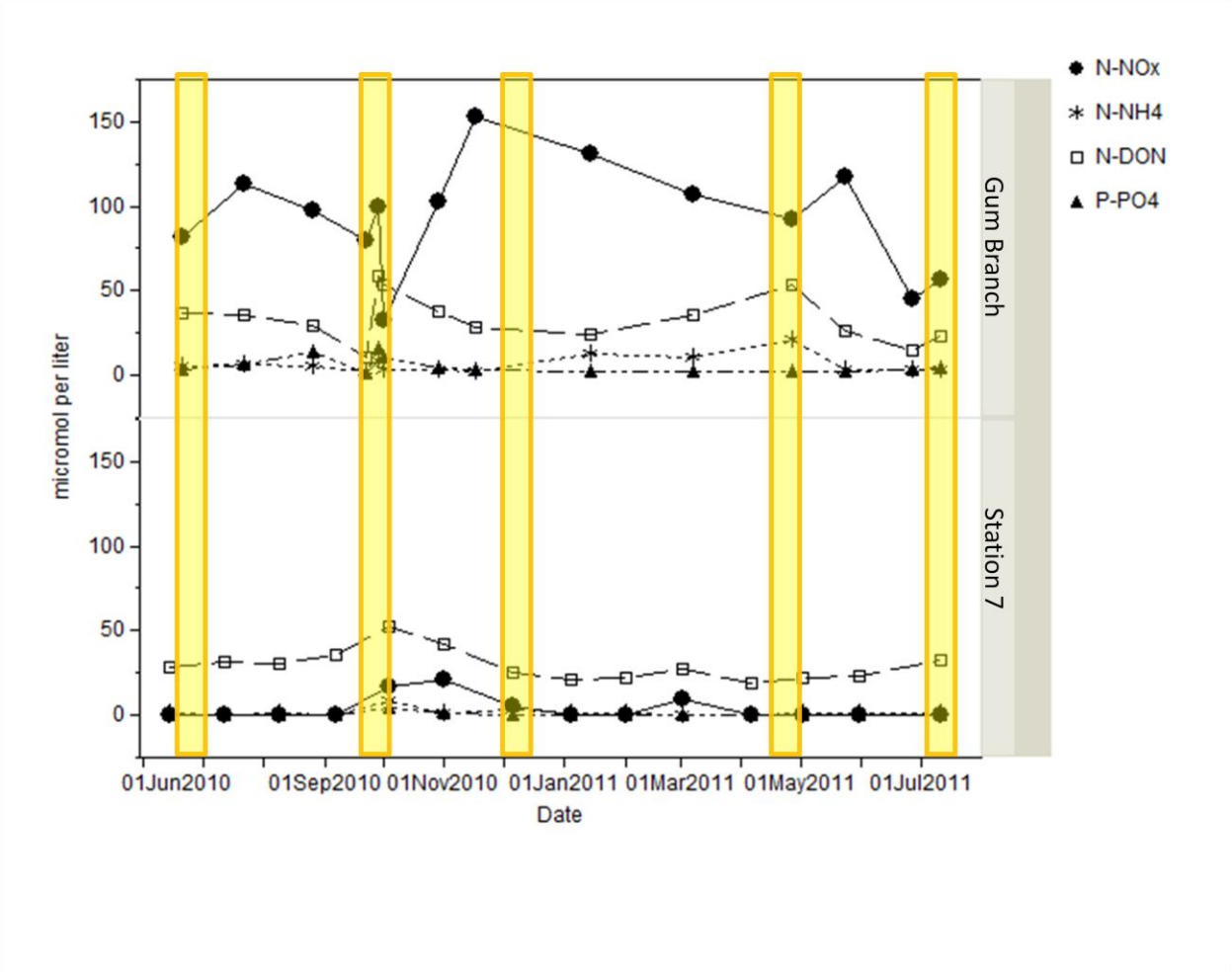

Figure 10. Nutrient concentrations at Station 10 (Gum Branch) and Station 7 on the New River June 2010 through July 2011. Times of bioassay experiments are highlighted by yellow rectangles.

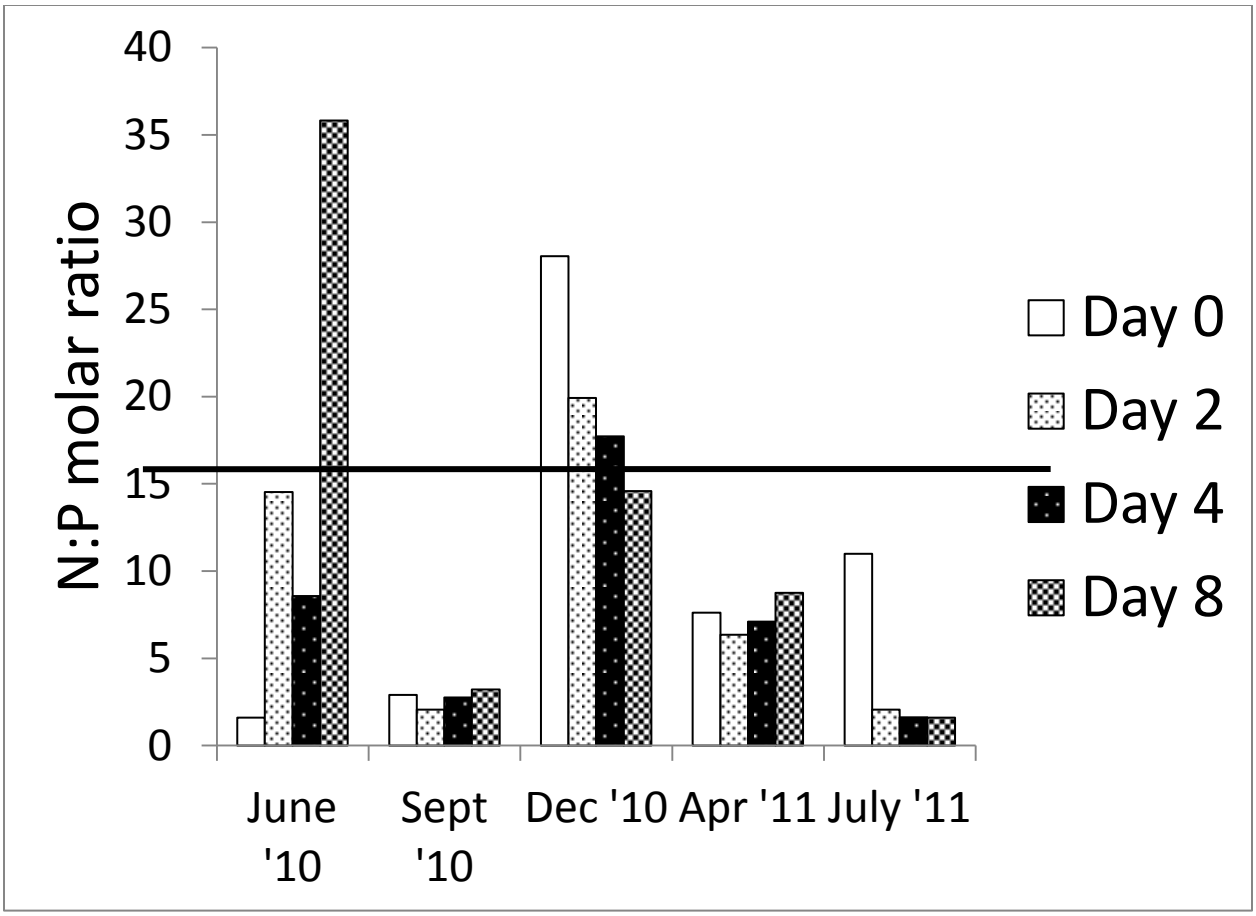

Figure 11. Mean dissolved inorganic N/P molar ratios in Control cubitainers on sampling days. Horizontal line indicates N:P = 16

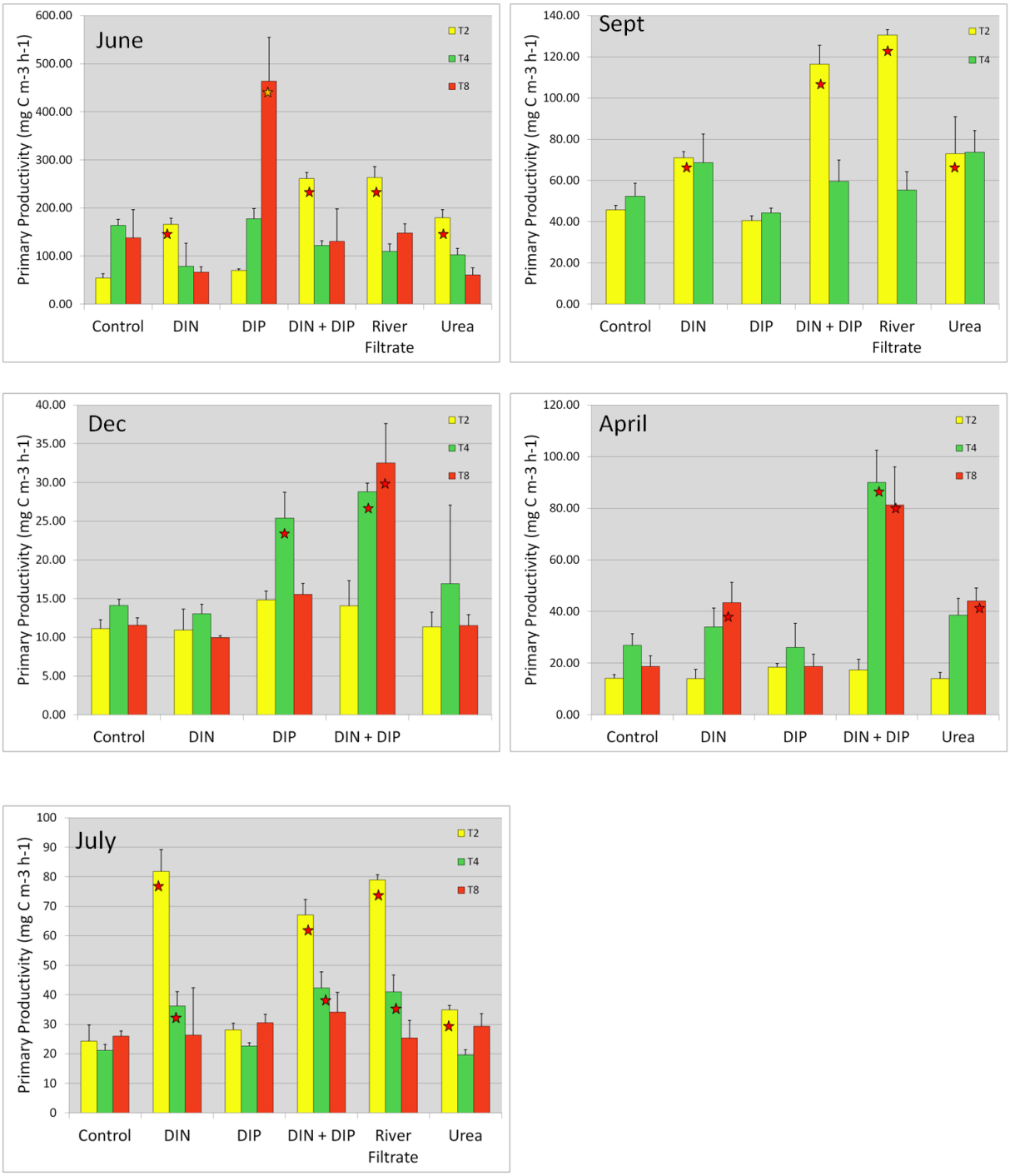

Figure 12. Primary productivity rates measured throughout each bioassay. Red star indicates significantly greater than control ($p < 0.05$).

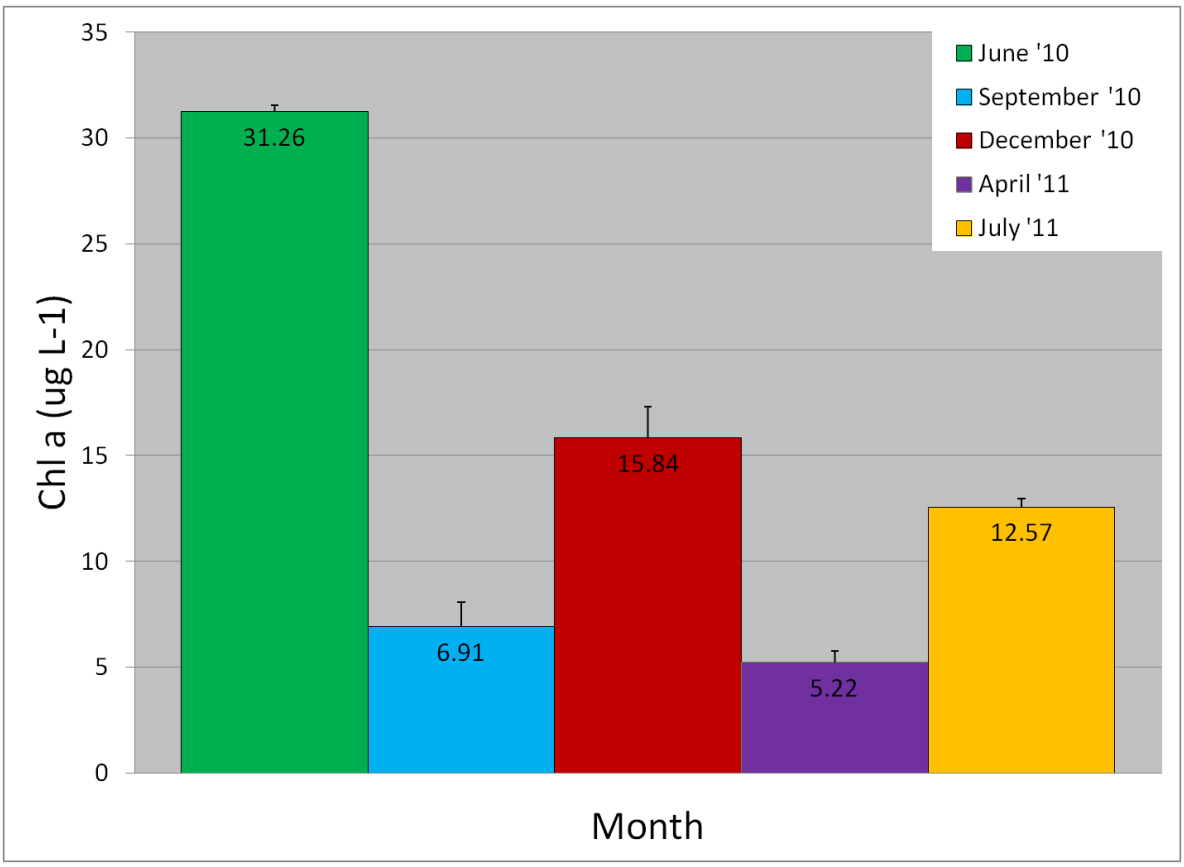

Figure 13. Initial (TO) chlorophyll *a* values for each bioassay .

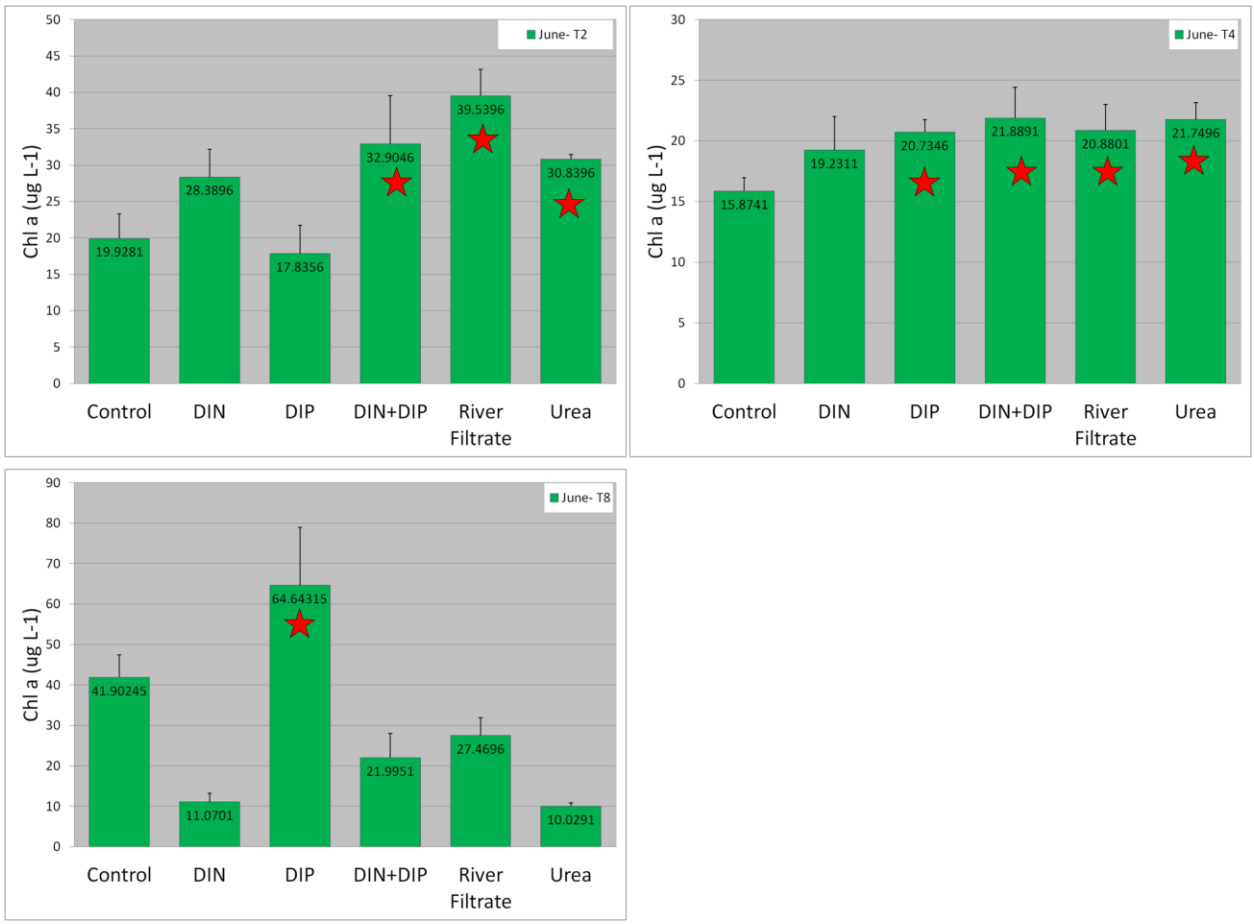

Figure 14. June bioassay T2, T4, and T8 chlorophyll *a* results. Red star indicates significantly greater than control ($p < 0.05$).

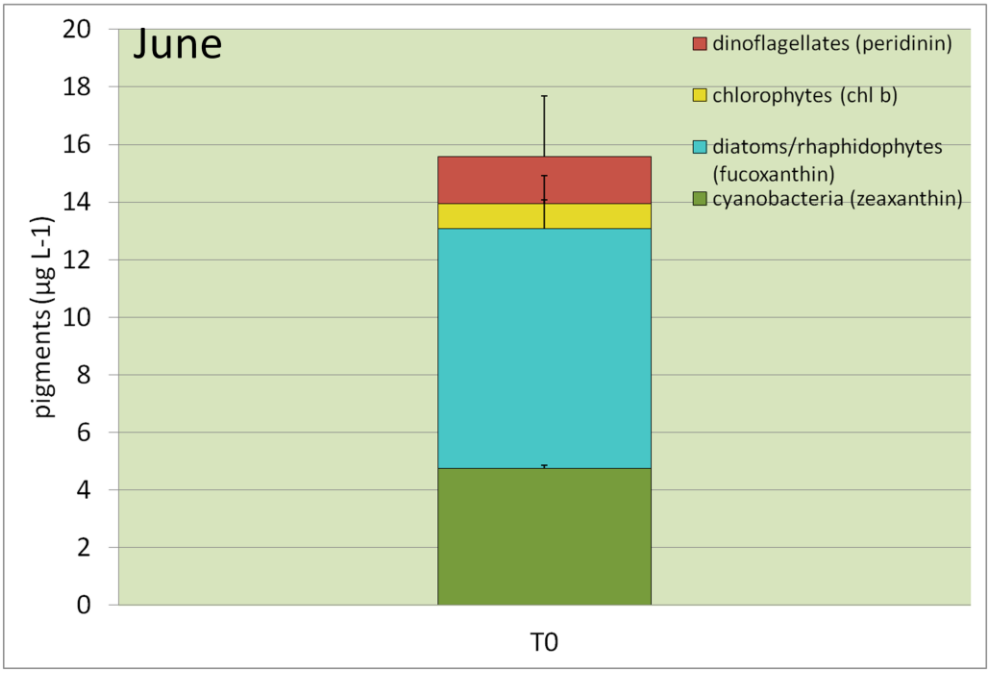

Figure 15. June bioassay T0 major diagnostic photopigments.

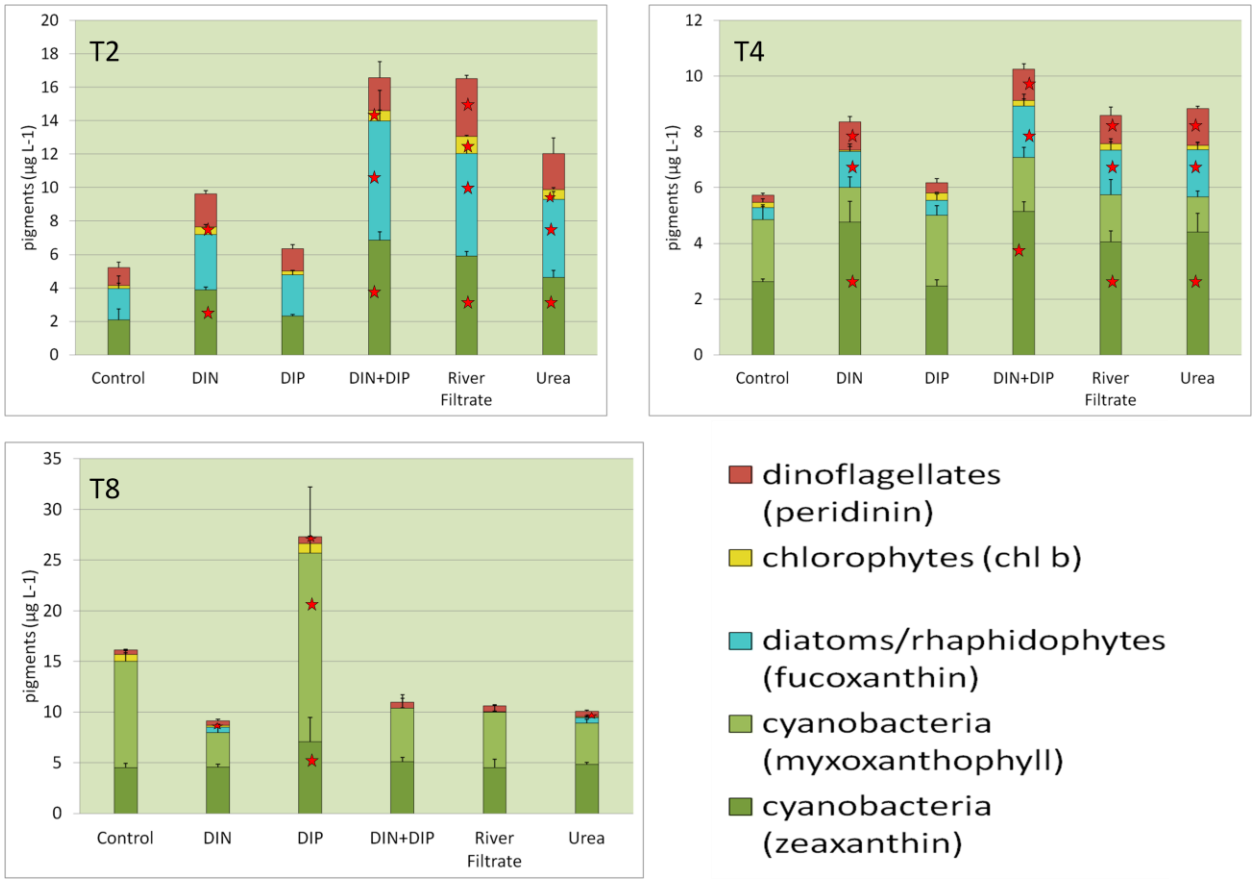

Figure 16. June bioassay T2, T4, and T8 HPLC photopigment results. Red star indicates significantly greater than control ($p < 0.05$).

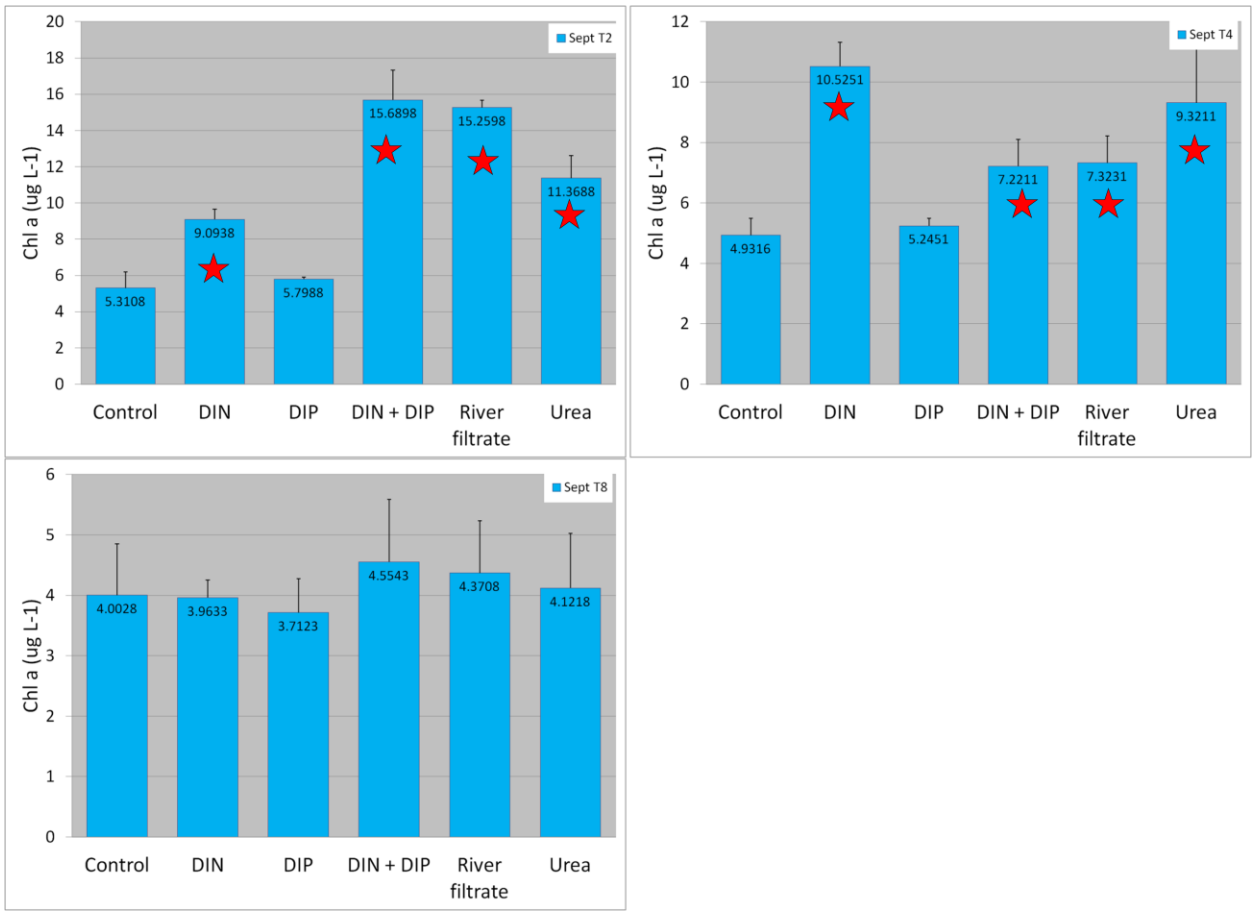

Figure 17. September bioassay T2, T4, and T8 chlorophyll *a* results. Red star indicates significantly greater than control ($p < 0.05$).

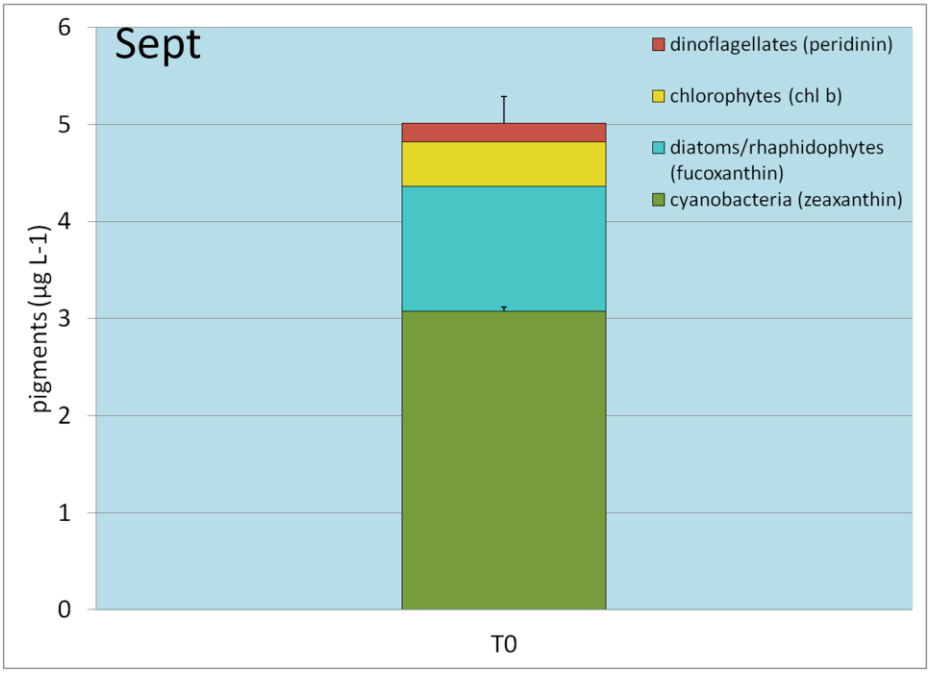

Figure 18. September bioassay T0 major diagnostic photopigments.

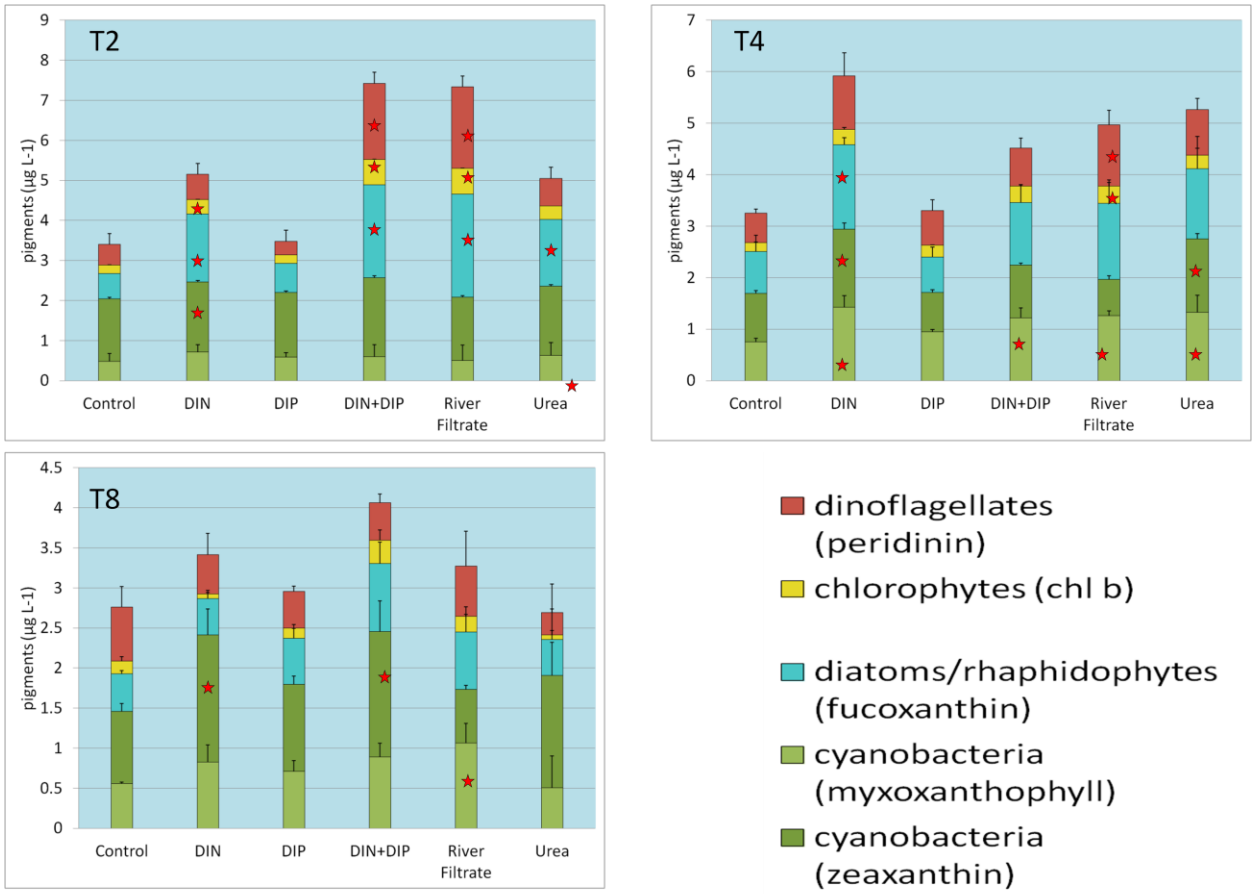

Figure 19. September bioassay T2, T4, and T8 HPLC photopigment results. Red star indicates significantly greater than control ($p < 0.05$).

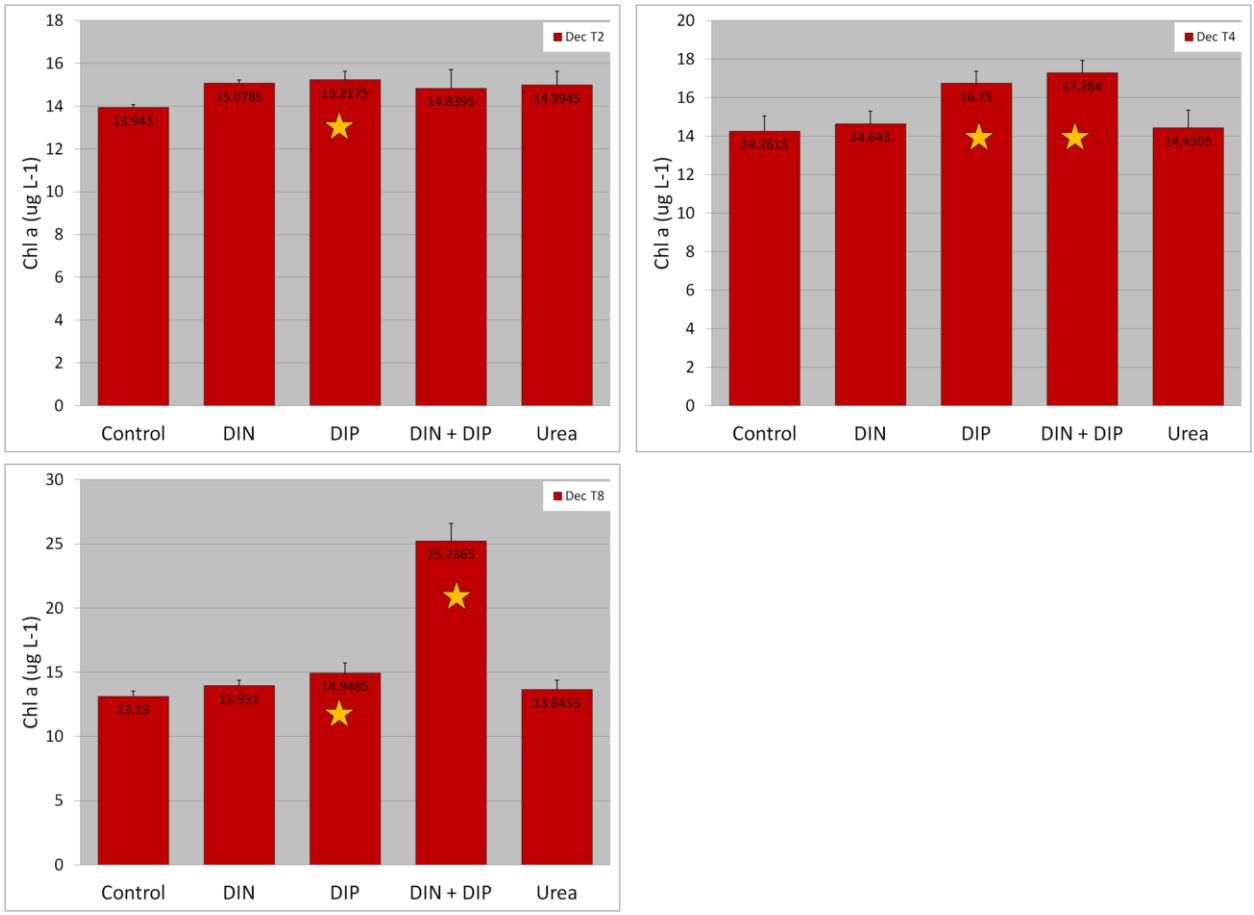

Figure 20. December bioassay T2, T4, and T8 chlorophyll *a* results. Red star indicates significantly greater than control ($p < 0.05$).

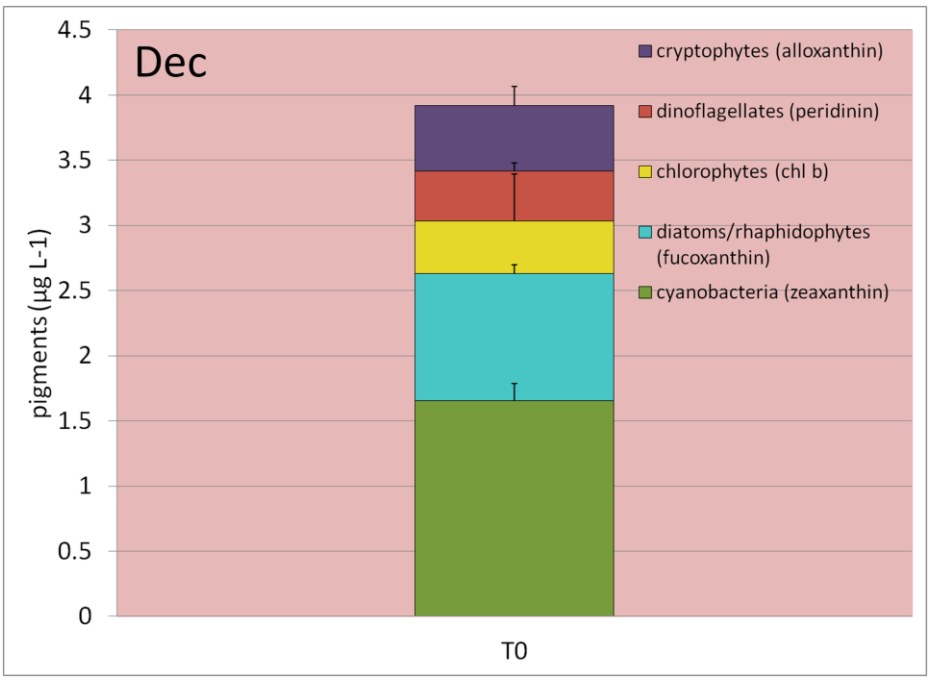

Figure 21. December bioassay T0 major diagnostic photopigments.

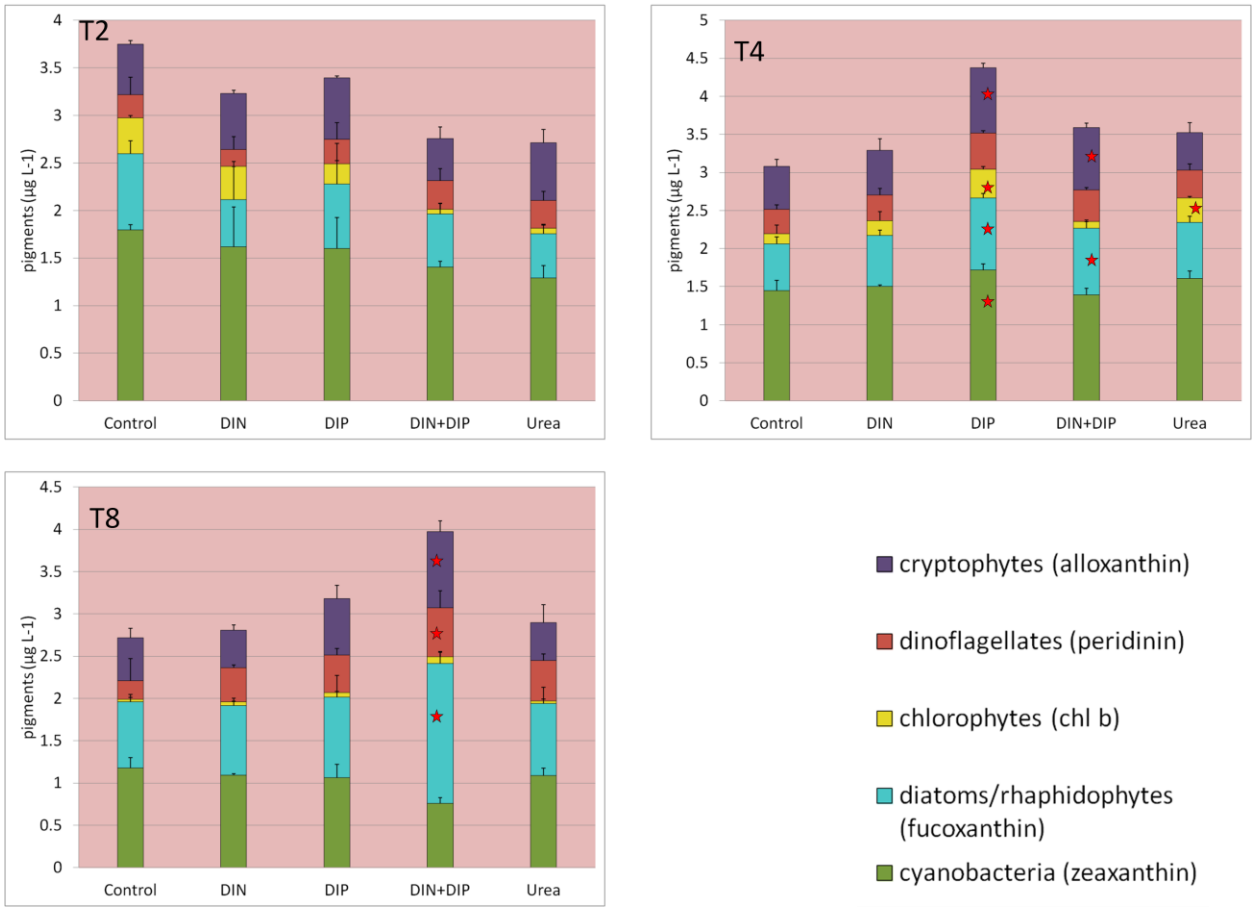

Figure 22. December bioassay T2, T4, and T8 HPLC photopigment results. Red star indicates significantly greater than control ($p < 0.05$).

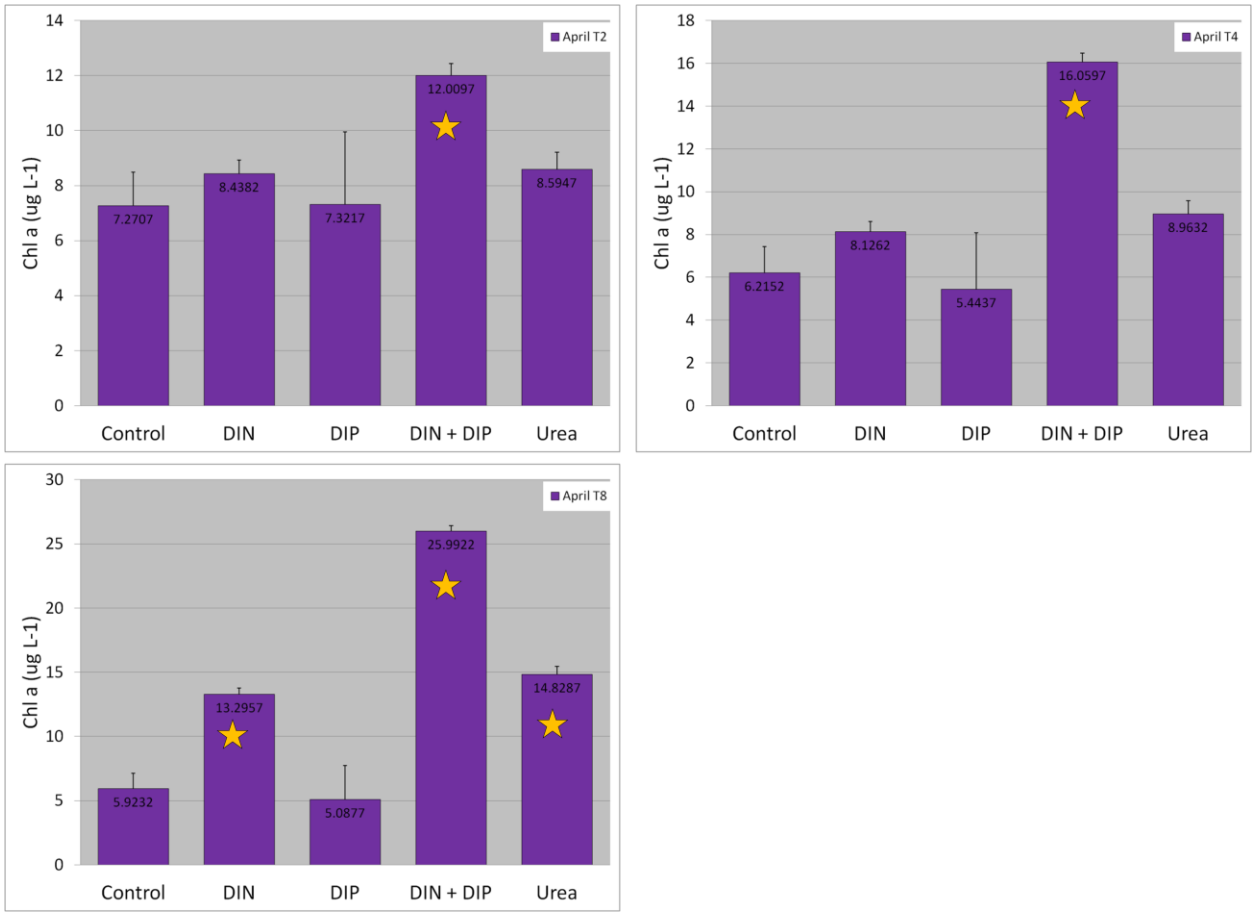

Figure 23. April bioassay T2, T4, and T8 chlorophyll *a* results. Red star indicates significantly greater than control ($p < 0.05$).

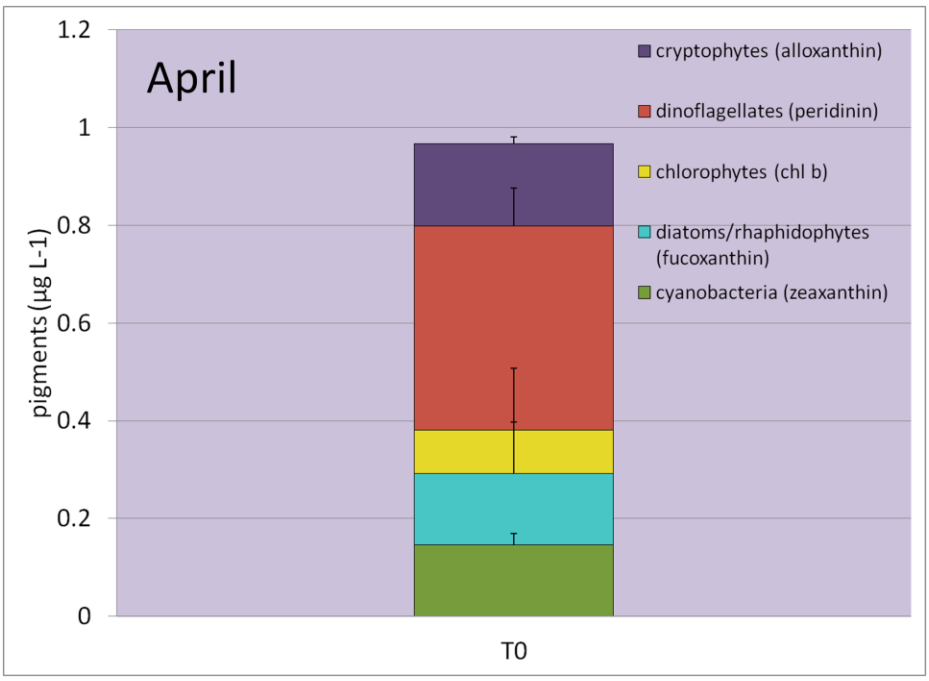

Figure 24. April bioassay T0 major diagnostic photopigments.

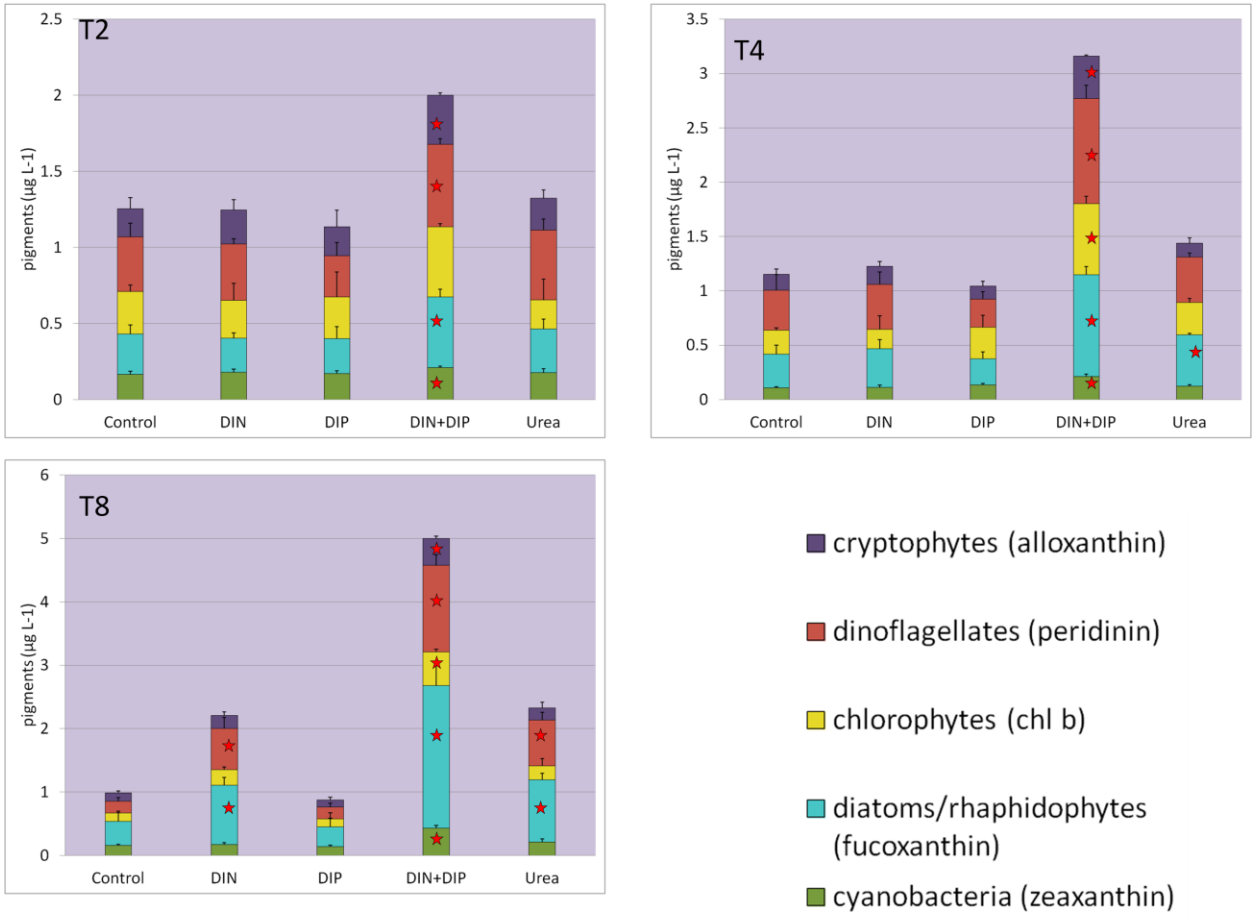

Figure 25. April bioassay T2, T4, and T8 HPLC photopigment results. Red star indicates significantly greater than control ($p < 0.05$).

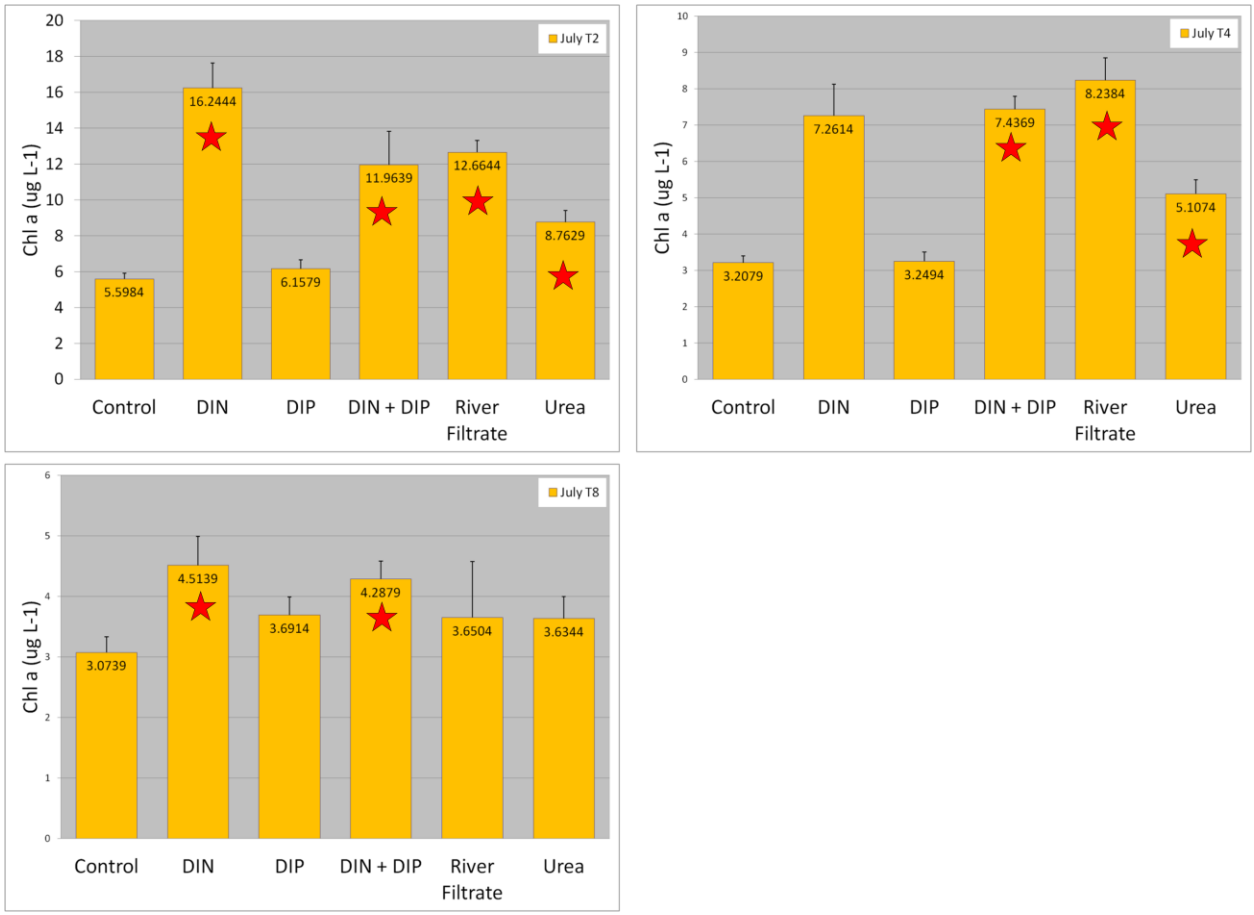

Figure 26. July bioassay T2, T4, and T8 chlorophyll *a* results. Red star indicates significantly greater than control ($p < 0.05$).

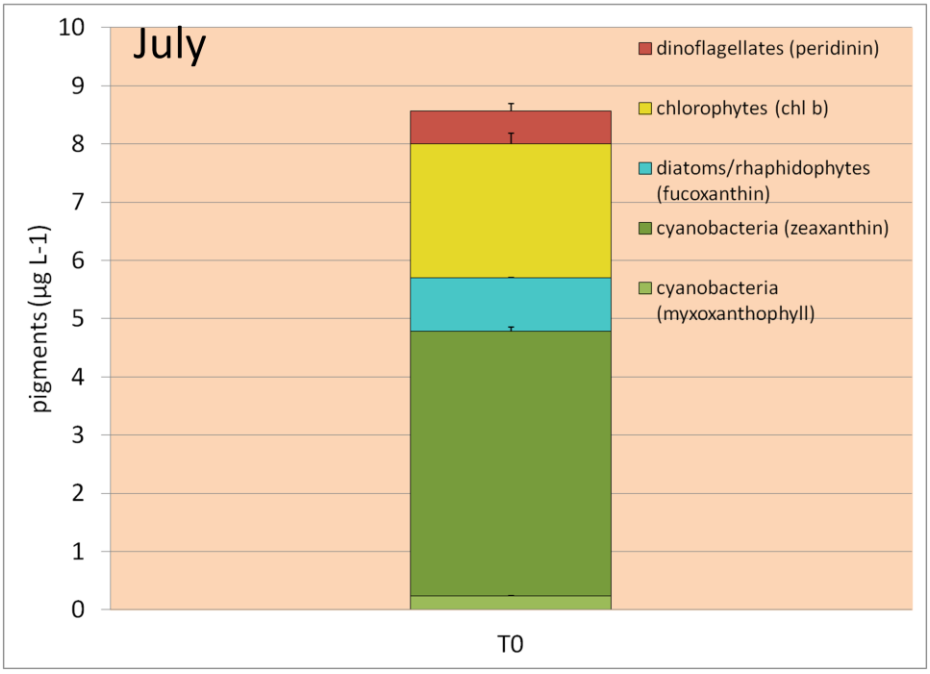

Figure 27. July bioassay T0 major diagnostic photopigments.

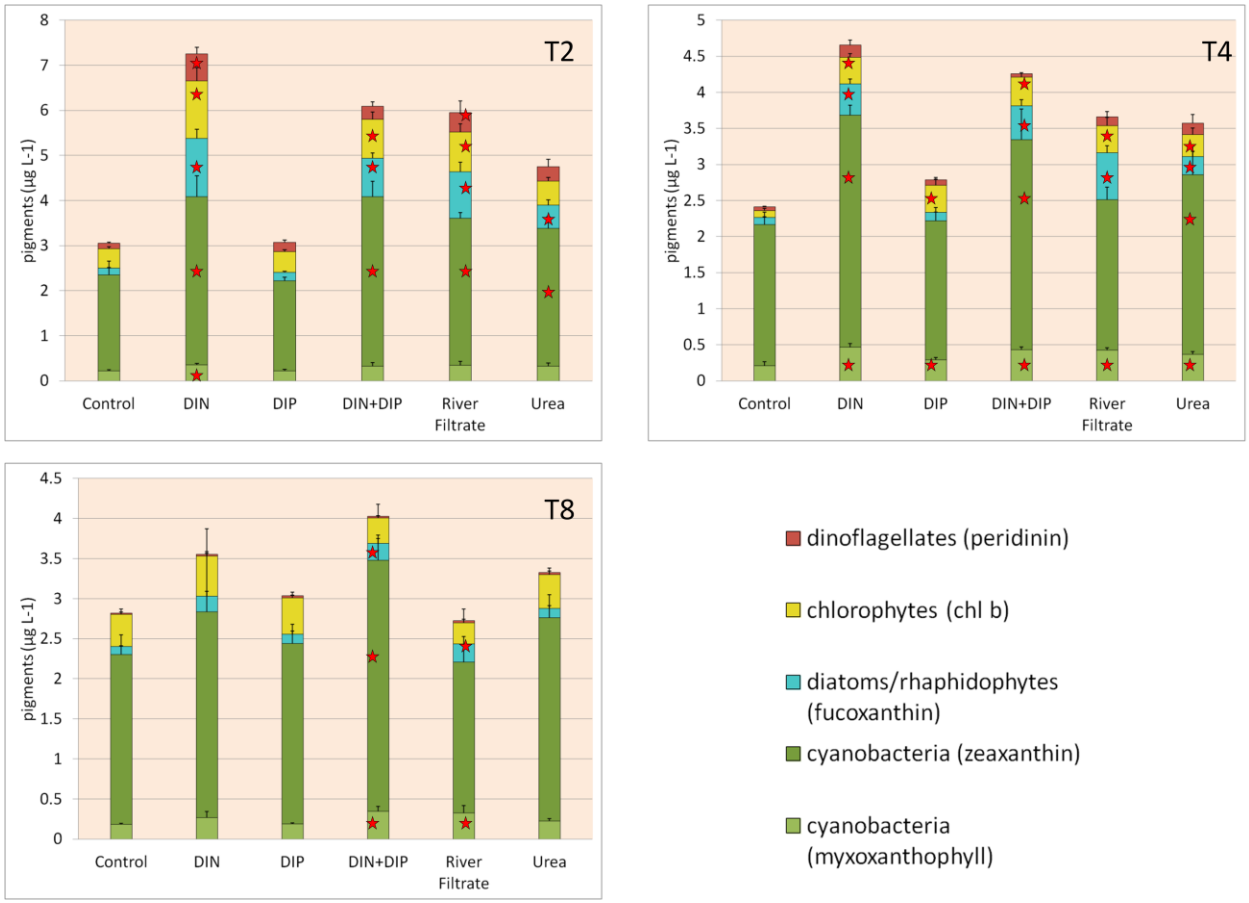

Figure 28. July bioassay T2, T4, and T8 HPLC photopigment results. Red star indicates significantly greater than control ($p < 0.05$).

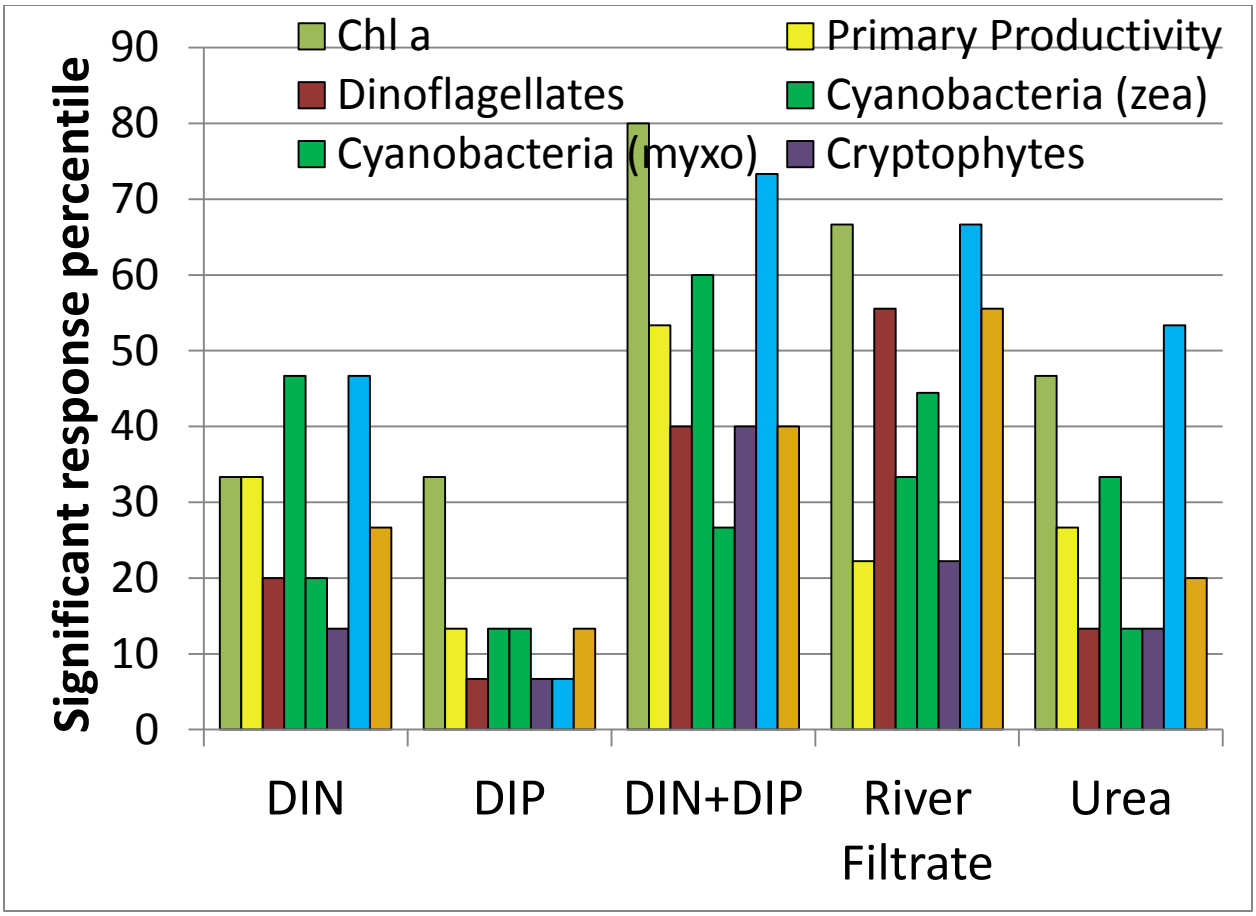

Figure 29. Frequency of significant responses of growth parameters for each treatment expressed as percent of occurrence out of all sub-sampling time-points. *Note: percent of significant responses for river filtrate addition only out of 9 time-points.*

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