Nutrient and phytoplankton dynamics of the Hunter River estuary

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Abstract

Observational studies and nutrient amendment experiments were conducted to better understand the nutrient and phytoplankton dynamics of the Hunter River estuary. Eutrophic conditions above ANZECC guidelines for estuaries dominate the Hunter River estuary. The upper Hunter estuary, upstream of its confluence with the Williams River, had the highest concentrations of nutrients and chlorophyll *a*. The major source of nutrients appears to be riverine discharge. Discharge from WWTP in the upper Hunter potentially contributes an important secondary source of phosphorus. Processes such as bank erosion and resuspension may also be important in explaining variation in nutrient concentrations. Light and turbidity were the main factors limiting phytoplankton growth in the upper estuary. The nutrient

amendment experiments showed that when light limitation was alleviated, phytoplankton were either nitrogen limited or remained unlimited by nutrients (suggesting nutrients were in surplus for growth). The expression of nitrogen limitation is likely due to low N:P in the estuary. Organic nitrogen dominates the nitrogen pool within the Hunter estuary. The bioavailability of organic nitrogen in the estuary is unknown which may explain the lack of relationship between phytoplankton and nitrogen concentrations within the estuary. Diatoms and green algae dominated phytoplankton. There were occasions when toxic cyanobacteria was in high abundance in the upper estuary, however a longer data set of phytoplankton assemblage is needed to more adequately assess the risk of toxic cyanobacteria. Comparison of data from the monthly, twice-weekly, and hourly sampling intervals demonstrated the five-year monthly sampling data appeared to mostly capture the variability of nutrient and chlorophyll *a* concentrations in relation to their main explanatory factors (discharge and light). There were some examples of chlorophyll *a* and nitrogen concentrations that fell outside of predicted ranges. Overall the results suggest any increase in nitrogen loads to the estuary may lead to increased phytoplankton growth. Improved light climate may also lead to increased phytoplankton growth. Reducing inputs of both nitrogen and phosphorus to the upper Hunter estuary should be a priority action to increase ecosystem health.

1. Background

To understand the major water quality and ecological processes of the Hunter River estuary a range of observational and experimental work has been conducted. Observational studies have been conducted at three different temporal scales. The initial long-term monthly monitoring program has been conducted by UTS and NSW Department of Planning, Industry, and Environment Water. This long-term sampling program was designed to assess the ecological impacts of freshwater inflows to the estuary, though is also useful in understanding the potential impacts of WWTP discharge. The study provides the most comprehensive recent data set on the water quality and in-stream ecology of the estuary. In order to validate this data set and characterise the variation at different temporal scales, an additional observational study has been conducted at the twice-weekly and hourly (tidal-cycle) scales between the monthly sampling.

Seasonal nutrient amendment experiments were conducted to provide an understanding of phytoplankton responses to potential changes in nutrient inputs to the estuary. The change in phytoplankton biomass and community composition in response to nutrient additions can demonstrate which nutrients may be limiting algal growth and this can inform on the risk of excessive algal growth and blooms and potentially determine tipping points (nutrient concentrations and stoichiometry) when these may occur. The experiments are useful in testing hypothesis related to algae growth developed from the observational studies, in an environment where other factors such as light and grazing are controlled. The experiments were conducted adjacent to selected WWTP outfalls in the Hunter River estuary and its tributaries to provide insight to potential ecological responses to changes in WWTP nutrient loads associated with future management scenarios.

This technical report provides an overview of the nutrient and phytoplankton dynamics within the Hunter estuary. The results are pertinent to understanding both the potential impacts of the WWTP discharge, and to guiding refinements of the Hunter estuary water quality model.

2. Methods

2.1 Observational studies

Mitrovic and Westhorpe’s pre-existing data set of monthly sampling consisted of 48 sampling occasions between April 2010 and December 2014. To test the variation and accuracy of this data at predicting ambient water quality conditions additional sampling was conducted at a finer time-scale. Twice-weekly sampling was conducted on 16 occasions split evenly between November 2016, and February 2017. Seven sampling stations were used, this included five stations on the Hunter River, at Morpeth, Rowers

Club (Rowers), Casuarina Corner, Raymond Terrace, and Hexam (Fig. 1). Sampling was also conducted on the primary tributaries, the Paterson River at Dunmore Bridge, and the Williams River at Seaham (downstream of the weir wall). Hourly tidal-cycle sampling was conducted on the Hunter River at Morpeth and Raymond Terrace from high tide to high tide on two occasions, 22/23 November 2016 and 14 February 2017.

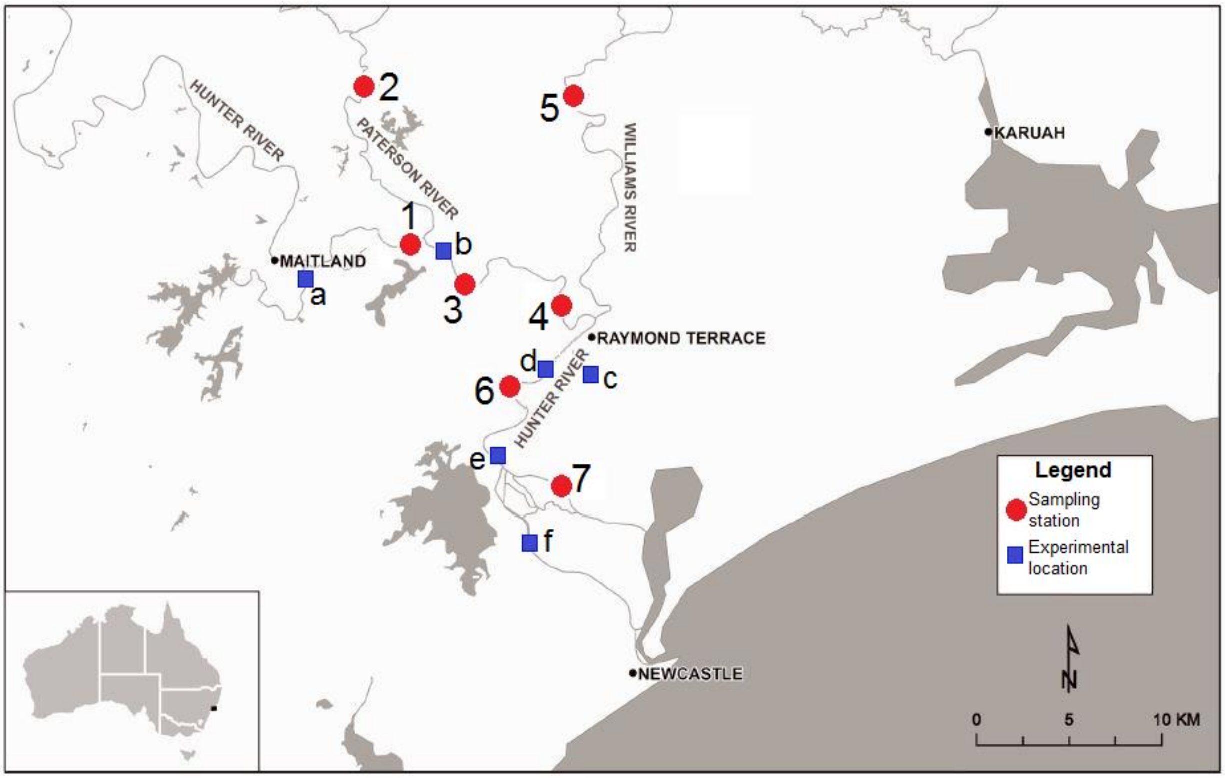

Figure 1. Hunter river estuary. Locations of sampling stations (circles and numbers) and experimental studies (squares and letter). Sampling stations are 1) Hunter River at Morpeth, 2) Paterson River at Dunmore Bridge, 3) Hunter River at Rowers Club, 4) Hunter River at Casuarina Corner, 5) Williams River at Seaham, 6) Hunter River at Raymond Terrace, and 7) Hunter River at Hexam. Experimental Locations are a) Wallis Creek at Maitland, b) Hunter River adjacent to Morpeth outfall, c) Windeyers Creek at Raymond Terrace, d) Hunter River adjacent to Windeyers Creek confluence at Raymond Terrace, e) Hunter River at Hexam, f) Hunter River adjacent to the Shortland outfall.

A full range of nutrient and physiochemical sampling was conducted including: total nitrogen (TN), total phosphorus (TP), filtered reactive phosphorus (FRP), silica (Si), nitrate/nitrite (NOx), ammonium (NH_4), dissolved organic nitrogen (DON), dissolved total nitrogen (DTN), dissolved total phosphorus (DTP), dissolved organic carbon (DOC), and total organic carbon (TOC), temperature, conductivity, dissolved oxygen, turbidity, pH, and secchi depth. TOC was only sampled for the first two years of the long-term monitoring program. DON, DTN, DTP were sampled from 2012 onwards. Biological samples were taken for bacterial abundance and biomass, chlorophyll *a*, phytoplankton and zooplankton.

All nutrient samples were collected in 50ml PET containers in either triplicate (monthly sampling) or duplicate (twice-weekly and hourly sampling) and stored on ice before being frozen until analysis. Samples for dissolved nutrients were filtered in the field with 0.45 µm polycarbonate filters. Organic carbon samples were analysed in the laboratory by the High Temperature Combustion Method (APHA 2005). Nitrogen and phosphorus samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA 2005). Physiochemical measurements were taken for temperature, conductivity, dissolved oxygen and pH with a Hydrolab Surveyor and MS5 Sonde probe; depth profiles were completed for the majority of occasions. Turbidity was measured in the field with a Hach 2100 Turbidimeter. Salinity was calculated as a function of conductivity and temperature (Fofonoff and Millard Jr 1983). Light penetration depth (1% Z_{EU}) was recorded during four separate sampling occasions during monthly sampling at all stations using a Licor light meter. This data was then used to create a model (polinormal inverse third order regression) of 1% Z_{EU} as a function of turbidity (NTU), where 1% Z_{eu} (m) =

$$0.2389+(42.5718/NTU)+(-186.579/NTU^\wedge 2)+(304.1844/NTU^\wedge 3)$$

Data presented here is for Zeu:Zm, the ratio between light penetration and depth at the sampling station. This value is useful in indicating the proportion of the water column with light available to primary producers.

Samples for chlorophyll *a* were determined by filtering 250 ml of water onto GF/C filters. Filters were frozen until subsequent determination by Standard Methods (APHA 2005) using the grinding technique and acetone as a solute with correction for phaeophytin. A detection limit of 1 µg L^{-1} was used for chlorophyll *a* analysis. Phytoplankton samples were preserved with Lugols iodine and subsequently counted using a calibrated Lund cell (monthly sampling) or Sedgewick-Rafter counting chamber (microcosms) and compound microscope after concentration by sedimentation in a measuring cylinder (APHA 1998). Counting precision was ± 20% (Hötzel and Croome 1999). Phytoplankton were identified to genus level (Prescott 1984). Phytoplankton assemblage data have currently only been determined between 2010 – 2011.

Bacterial abundance and biomass were sampled for the long-term data set and analysed at stations 1, 2, and 6. Samples (10 mL) were collected in sterile centrifuge tubes and fixed with 0.4 mL of concentrated 0.2 µm filtered formalin (37% Formaldehyde) and stored at 4°C. In the laboratory, subsamples (2 mL) were stained with DAPI (4’6-diamindion-2-phenylindole) at a final concentration of 1 mg mL^{-1} for 15 minutes, and filtered through a polycarbonate black 0.2 µm pore-sized filter (Porter and Feig 1980). Polycarbonate filters were mounted onto microscope slides and non-fluorescence immersion oil used. Slides were examined at ×100 using a fluorescence-equipped Olympus BX41 compound microscope. For each slide ≥500 total cells were captured using an Olympus DP72 camera and cellSens Standard software (version 1.3). Images were analysed for cell abundance and volume using CellC software (Selinummi et al. 2005). Bacterial biomass was calculated using the formula given by (Romanova and Sazhin 2010). Samples for zooplankton enumeration were taken in duplicate at each station by vertical tows using a 30 cm diameter 35 µm plankton net and preserved with >50% ethanol. Zooplankton density (individuals m^3) was estimated by counting consecutive aliquots using a Sedgewick-Rafter counting chamber until 100 specimens of a class specific taxon (micro or mesozooplankton) were counted or until 50% of the sample was counted. Organisms were identified to the highest taxonomic resolution feasible. Analysis of bacteria and zooplankton data are outside the scope of this report (results for major zooplankton group abundance is included in Appendix C).

Discharge data was obtained from two gauging stations, the Hunter River at Greta and the Paterson River at Gostwyck. For all Hunter River sampling stations a combined discharge of these two stations were used to explore relationships between the parameters and flow. Daily nutrient loads were calculated for WWTP outfalls at Morpeth and Raymond Terrace (Windeyers Creek). Daily discharge rates were multiplied by the nutrient concentration measured on that day (or the closest day measured, at most 2 days prior or after). Data for loads was only available from July 2012 to Dec 2014 at the time of this report.

Significant differences between sampling stations were determined via Permanova using Primer (Ver. 6). Resemblance matrix were calculated using Euclidean distance for all nutrient data (excluding DON, DTP, DTN, TOC), turbidity, and chlorophyll *a*. Data was first log transformed (Ln[x+1]), and normalised. Pair-wise tests were used to test differences between sampling stations. The same procedure was used to test differences between the monthly and twice-weekly sampling. As the twice-weekly and hourly sampling took place during a low flow period, a subset of monthly data was used that was sampled during a similar period (September to March) and that fell within the same range of 10 day antecedent discharge conditions. Correlation and regression analysis were conducted using Sigmaplot (Ver.12). Where data failed the Sharpio-Wilk normality test it was log transformed (Ln[x+1]). For significant regression models 95% predictive intervals were calculated. For all statistical analysis data collected on 13 March 2013 was excluded as an outlier; this sampling date was during a hypoxic event following large scale flooding.

2.2 Nutrient amendment experiments

To test potential nutrient limitation of phytoplankton communities, amendment experiments were conducted four times in 2017 (February, May, August, November). Experiments were conducted *in-situ* using 1L microcosms (Fig. 2). Water used in the microcosm was filtered through a 63 µm zooplankton net to exclude large bodied zooplankton. Microcosm bottles were filled at each site, and amendments added of nitrogen (KNO_3, 0.5 mg L^{-1}), phosphorus (KH_2PO_4 0.3 mg L^{-1}), and nitrogen and phosphorus (KNO_3, 0.5 mg $L^{-1,}$ and KH_2PO_4 0.3 mg L^{-1}), as well as controls (no additions) (Fig. 2). Triplicates of all amendments were performed. The experiment was conducted over 72 hours and sampling performed at 0 and 72 hours. Samples were taken for phytoplankton biomass (chlorophyll *a*) and species composition, TN, TP, FRP, NO_x, NH_4, DO, and temperature.

The experiments were conducted at four locations on the Hunter River, downstream of Morpeth adjacent to the outfall, adjacent to its tributary with Windeyers Creek downstream of Raymond Terrace, at Hexam, and adjacent to the Shortland outfall at the rail bridge. In addition, Windeyers Creek on the eastside of Adelaide St Raymond Terrace (receiving water from the Raymond terrace WWTP), and Wallis Creek downstream of the bridge on High St Maitland (receiving water from Farley and Kurri Kurri WWTP).

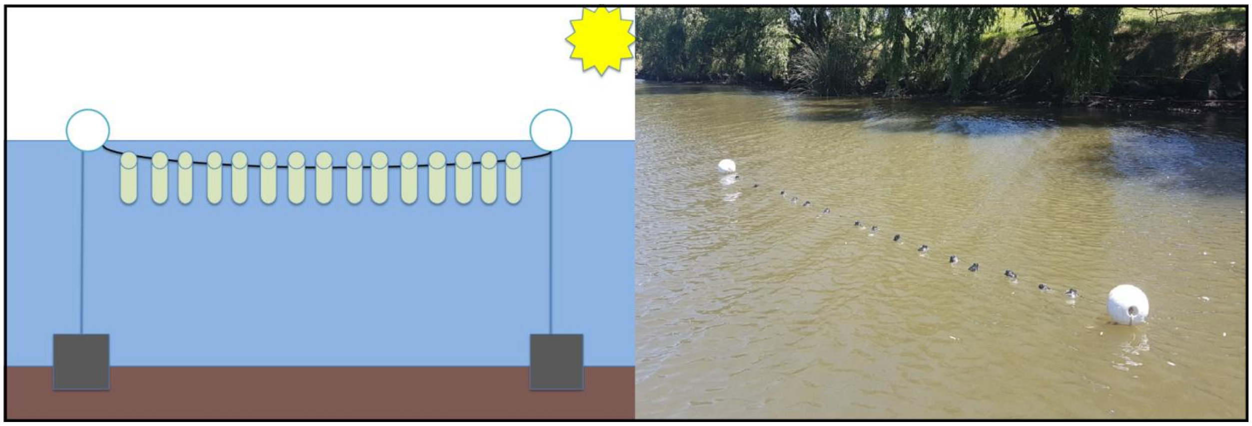

Figure 2. Design and set-up for the nutrient amendment experiments.

Chlorophyll *a* samples (200 ml) were filtered via vacuum filtration onto glass fibre filters on site. Filters were frozen until subsequent determination by Standard Methods using ethanol extraction (APHA 2005). Phytoplankton samples were preserved with Lugols iodine and subsequently concentrated, identified and enumerated at 200 times magnification using a light microscope and Sedgwick-Rafter counting chamber. Phytoplankton taxa were identified to a genus level using identification material by Prescott (1978).

All nutrient samples were collected in 50ml PET containers in either triplicate (monthly sampling) or duplicate (twice-weekly and hourly sampling) and stored on ice before being frozen until analysis. Samples for dissolved nutrients were filtered in the field with 0.45 µm polycarbonate filters. Nitrogen and phosphorus samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA 2005).

We conceptualised a nutrient to be limiting if chlorophyll *a* concentrations were significantly higher in a treatment compared to the control. Where both N and P treatments were higher than the control, then both N and P were deemed to be equally limiting. Where only the N+P treatment was higher than the control it was deemed co-limited. Differences between treatments were tested via Permanova using Primer (ver.6). Chlorophyll *a* data was first log transformed (Ln[x+1]) and resemblance matrix created using Euclidian distance. Where Permanova detected a significant difference (< 0.05) pair-wise tests were used to test differences between treatments.

3. Results and Discussion

3.1 Nutrient and Chlorophyll *a* dynamics

The sampling stations for the monitoring study were selected to represent the longitudinal changes in the Hunter estuary. This included five stations along the Hunter River, as well as two additional stations on the major tributaries, the Paterson and Williams River. During the 2010 – 2014 period the upper most stations at Morpeth, Rowers, and Paterson River remained fresh at all times (Fig.3 A). The middle Hunter station at Casuarina Cnr and the Williams River station were oligohaline with salinity <3 for most of the period. The station at Raymond Terrace was mesohaline for most of the study, whilst the Hexam station was generally polyhaline. Salinity at these sites was strongest during low flow; during floods all sites became freshwater.

We compared differences between the sampling stations based on their nutrient, DOC, turbidity, light, and chlorophyll *a* concentrations over five years (2010-2014). The results indicated there were significant differences between sampling stations (Appendix A Table 1). Pair-wise tests were used to determine differences between stations. The results showed longitudinal groupings of sites that were similar (i.e. not statistically different from each other) including the upper Hunter River sites at Morpeth, Rowers, and Casuarina Cnr, the middle Hunter sites at Casuarina Cnr and Raymond Terrace, and the lower Hunter sites at Raymond Terrace and Hexam (Fig.3 B). The Williams and Paterson River sites were statistically different from all stations. These results illustrate the changes in water quality and ecological function that likely occur along the estuarine continuum. They support the conceptualization of the estuary into distinct but related zones for the purpose of modelling water quality dynamics.

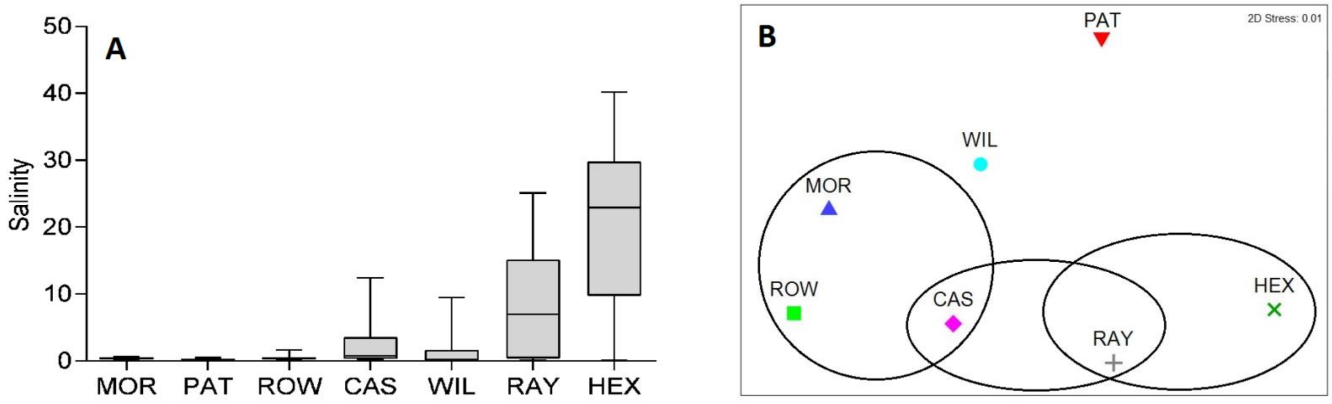

Figure 3: A) Boxplots for salinity at the seven Hunter Estuary sites 2010-2014. MOR = Hunter River at Morpeth, PAT = Paterson River at Dunmore Bridge, ROW = Hunter River at Rowers Club, CAS = Hunter River at Casuarina Corner, WIL = Williams River at Seaham Weir, RAY = Hunter River at Raymond Terrace, HEX = Hunter River at Raymond Terrace, HEX = Hunter River at Hexam. B) Non-metric MDS of the seven Hunter River sampling stations. Distance between centroids was determined from the Resemblance matrix. The circles indicate groups of stations that were not significantly different ($P < 0.05$) from each other.

The Hunter estuary displayed eutrophic conditions throughout much of the 2010 – 2014 period (Fig. 4). Chlorophyll *a* and nutrients exceeded recommended ANZECC water quality guidelines for South-East Australian estuaries at almost all occasions. The upstream stations had much higher chlorophyll *a* concentrations than the downstream stations. Concentrations of NOx, TP, and FRP were moderately lower on the Paterson and Williams sampling stations compared to the Hunter River stations, whilst NH$_4^+$ was higher at the lower estuary stations. Eutrophic conditions are consistent with analysis of historical (1972 – 2000) water quality data (Sanderson and Redden 2001), as well as more recent short term (August2 – March 2015) water quality monitoring (Swanson et al. 2017). Turbidity generally decreased downstream, consistent with increasing salinity (Appendix A Fig. 1 G). Dissolved oxygen remained with normal ranges for most of the time, though following large flood in 2013 most of the middle and lower estuary experienced prolonged hypoxic conditions (Appendix A Fig. 1 C-).

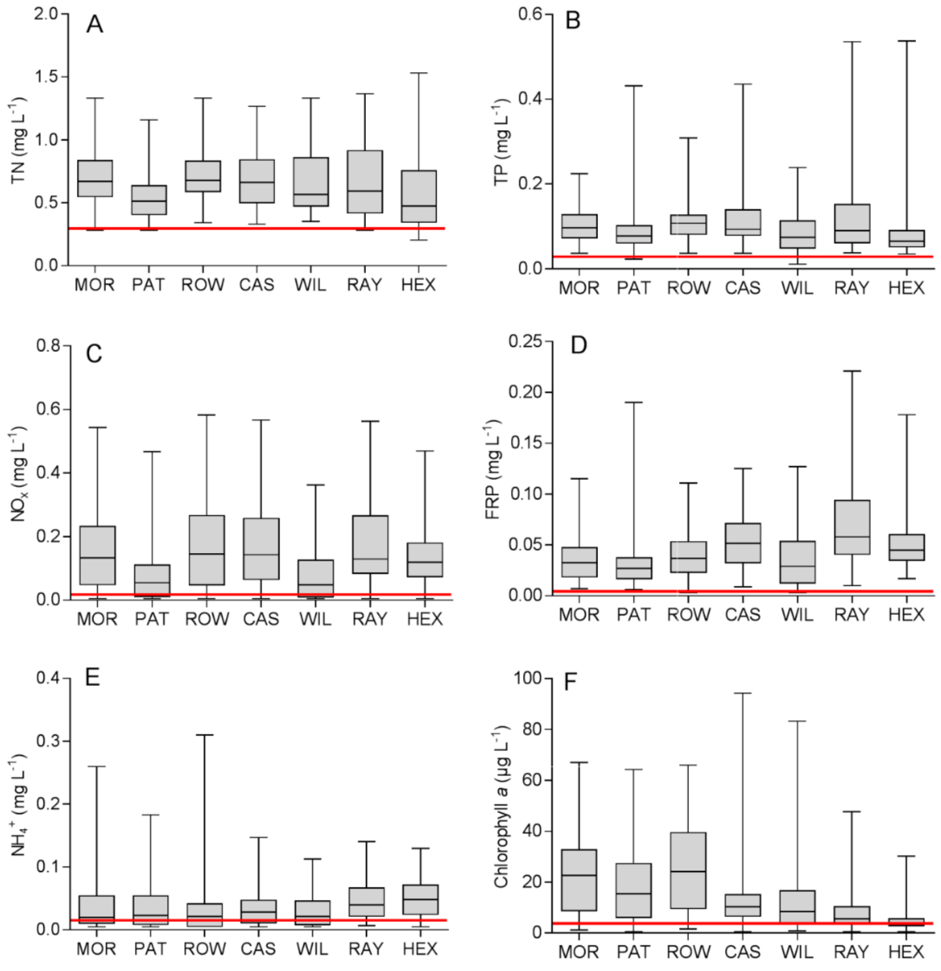

Figure 4: Boxplots for chlorophyll *a* and nutrient parameters. The boxes represent the median, 25th and 75th percentile ranges, whilst the error bars indicate the maximum and minimum values recorded. The red line indicates ANECC water quality guideline for South-East Australian estuaries.

The relative composition of the total nutrient pools varied between stations (Fig. 5A, Appendix 1, Table 2). DON comprised the majority of the nitrogen pool at all stations varying between 47-63%. The relative proportion of ammonia increased from 4% upstream to 10% at downstream Hexam station. At the Paterson and Williams River stations the relative proportion of NOx was around half that of the Hunter River stations at approximately 11%.

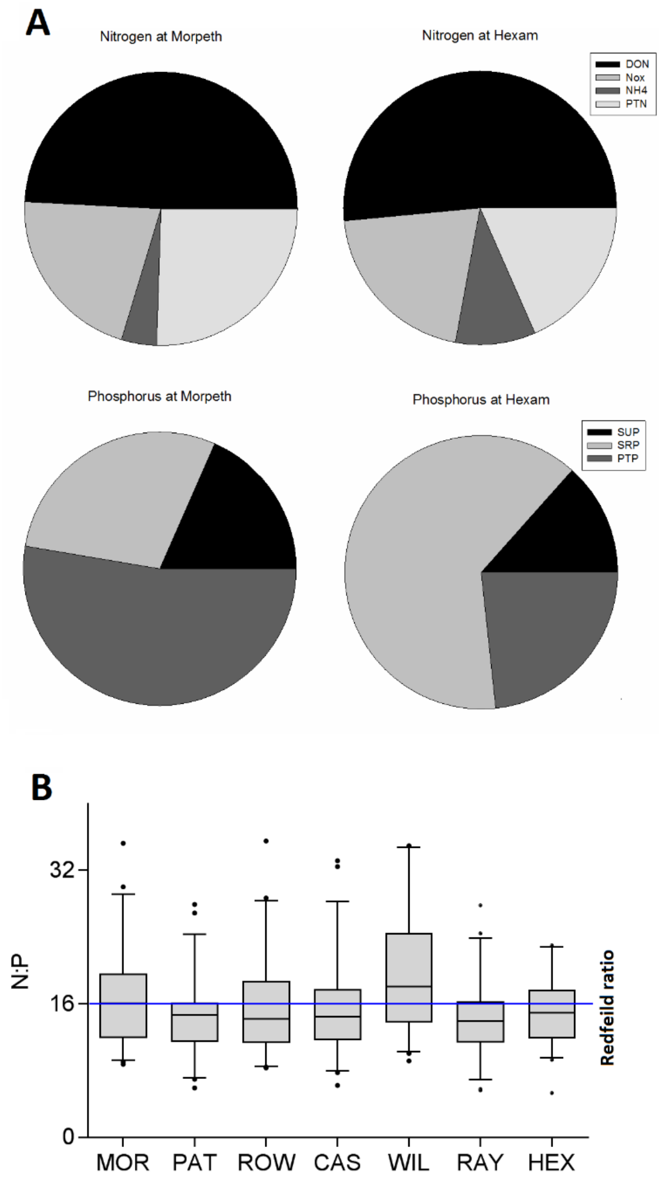

Figure 5: A) Mean relative percent composition of total nitrogen and phosphorus pool. SUP = soluble unreactive phosphorus, PTN = particulate total nitrogen, PTP = particulate total phosphorus. B) N:P ratio (TN and TP) on the Hunter estuary. Boxplots show the median, 25th and 7th percentile, and the error the bars the 5th and 95th percentile. Two outlying values (high N:P) on the Williams River and Hunter River are excluded from the plot.

The relative proportion of particulate nitrogen and phosphorus (i.e > 0.45 µm) reduced with distance downstream. Particulate nitrogen comprised 28% of the total pool at Morpeth but only 18% at Hexam. Similarly, particulate phosphorus comprised 52.6% of the total phosphorus pool at Morpeth, whilst at Hexam accounted for 23% to the total pool. This longitudinal relationship is likely to due to larger or heavy particles dropping or flocculating out of the water column with distance downstream.

Whilst inorganic nitrogen and SRP are generally considered bioavailable, the proportion of DON and soluble unreactive phosphorus (SUP) that is bioavailable is variable. This factor may be important in understanding phytoplankton responses to nutrient conditions and accounting for losses of nutrients within the water quality model.

The ratio of the nitrogen to phosphorus can provide an indicator of the nutrient that may be limiting algal growth. As the N:P declines from 16:1 (Redfield ratio) there is generally an increasing chance of nitrogen limitation, whilst N:P > 16:1 has an increasing chance of phosphorus limitation. Most of sampling stations showed N:P ratios <16:1 for the majority of the 2010 - 2014 period indicating potential N limitation. Under N limited conditions it may be possible for cyanobacteria to dominate the phytoplankton community as some are able to supplement nitrogen requirements through N-fixation. The high concentrations of both nitrogen and phosphorus in the Hunter estuary suggest that nutrients may not be limiting phytoplankton growth under most circumstances. Nutrient concentrations varied seasonally (Fig. 6, Appendix A Fig. 2-4). At all sampling stations there was a pattern of lower concentrations of TN and TP over the winter period from June to September. In many cases the highest concentrations of nutrients occurred in Autumn which is mostly likely due seasonal rain and inflow events that occurred during these months. There were no clear seasonal patterns in chlorophyll *a* at any sampling stations. This contrasts from Sanderson and Redden (2001) who found peaks in chlorophyll *a* in late summer and early spring.

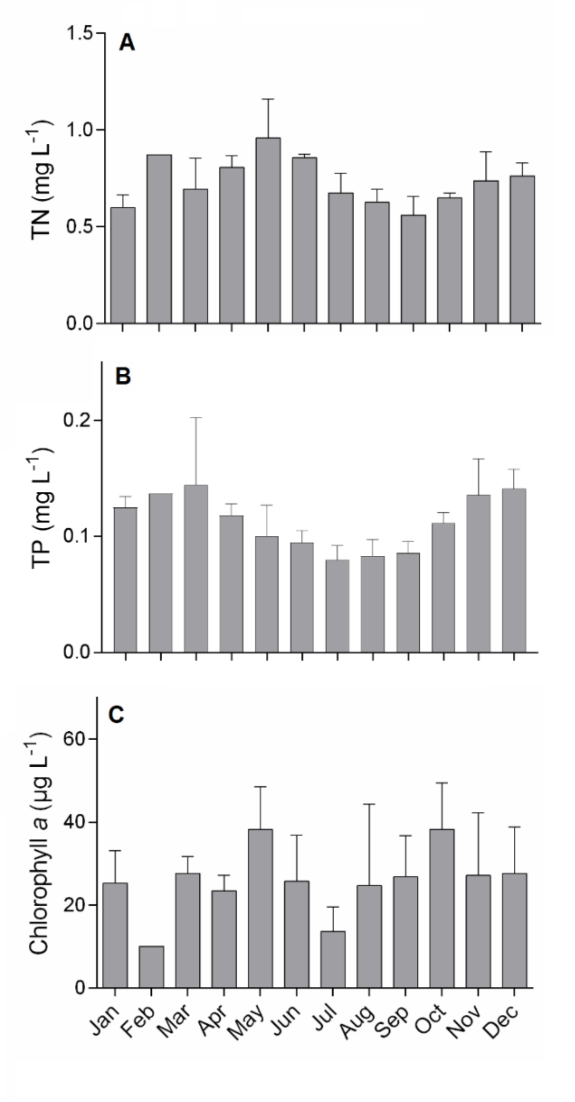

Figure 6 Monthly trends for Hunter River at Rowers for A) TN, B) TP, C), Chlorophyll a. Error bars are standard error.

We calculated differences in TN and TP concentrations between sampling stations on the Hunter River to provide an indication of whether mixing was conservative. The results showed changes in TN and TP from upstream to downstream stations was highly variable in the upper and middle sections of the

Hunter estuary (Fig. 7). In the lower Hunter, between Raymond Terrace and Hexam, nutrients decreased at most times. The longitudinal increase in nutrients can indicate a significant source of nutrient input within these areas. Sources of nutrients include WWTP outfalls at Morpeth and Windeyers Creek at Raymond Terrace, inputs from industry, stormwater runoff, runoff from agricultural areas, and bank erosion. Major tributaries the Paterson and Williams River are unlikely to be responsible for the longitudinal increases in nutrients present as on most occasions nutrient concentrations were lower at these stations, than the stations immediately upstream and downstream of their confluences. The other factor influencing these results is variable flushing/residence times. The average residence time within the estuary is around 30 days, however this time greatly decreases during flood, and increases during periods of low inflow (MHL 2003).

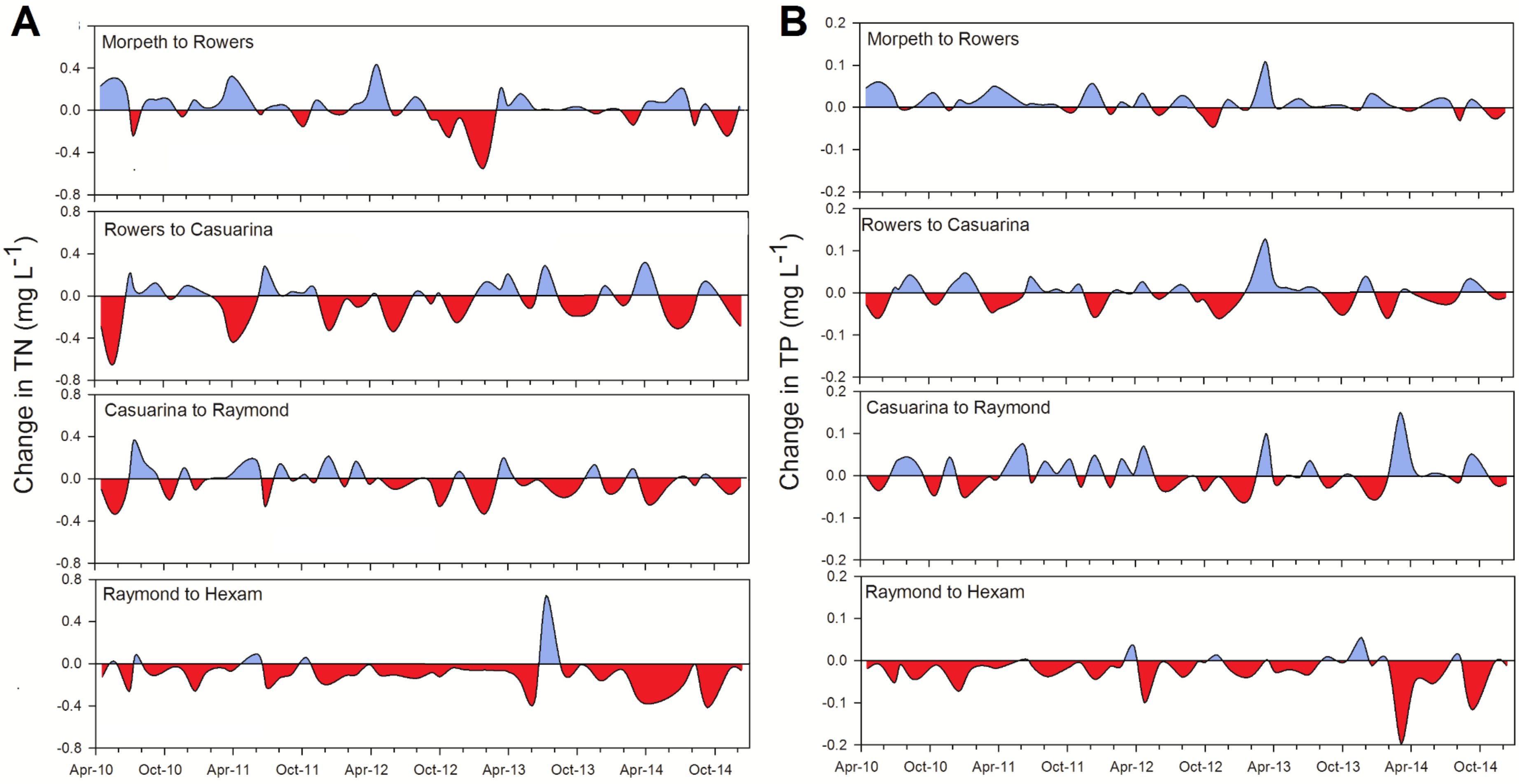

Figure 7. Change in nutrients between sampling stations on the Hunter River estuary for a) TN, and B) TP. The blue sections indicate periods where nutrient concentrations increased form upstream to downstream, and red sections periods when nutrients decreased form upstream to downstream.

To explore relationships between nutrients, chlorophyll *a* and other variables we conducted an exploratory analysis process using scatterplots, correlation and regression analysis (Appendix A Fig. 5–10). TN and TP concentrations were positively correlated with discharge at all sampling stations on the Hunter River, whilst only TP was related to discharge on the Paterson (Fig. 8 A, B). We did not test the relationships between nutrients and discharge on the Williams River due to the available discharge data being derived from a gauging station upstream of the Seaham Weir. These results indicate riverine discharge is a major source of nutrients to the estuary. TN and TP loads from riverine discharge are estimated to be orders of magnitude larger than localised diffuse or points sources (MHL 2003). The fact that the relationships between discharge and nutrients are not strong suggests other localised inputs may be present. Both TN and TP were positively correlated to turbidity at all times which indicates the processes controlling suspended sediment (erosion, flocculation, resuspension) may also be important factors influencing nutrient concentrations.

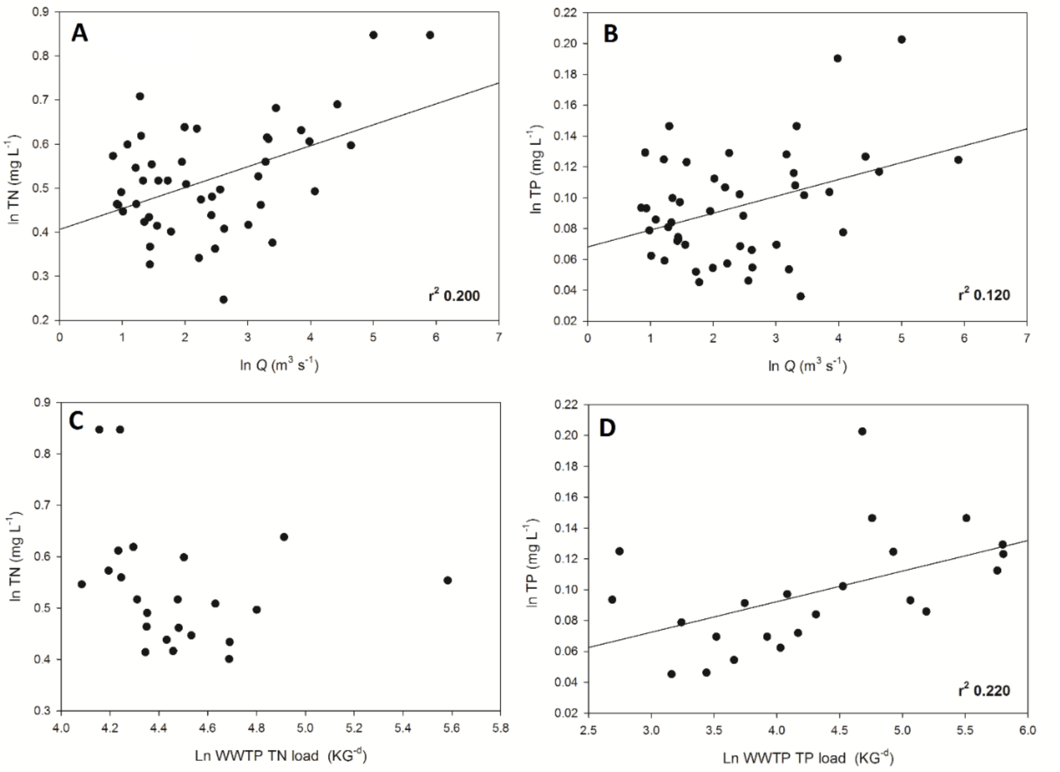

Figure 8. Relationships at Hunter River sampling station at Morpeth for A) TN vs discharge, B) TP vs discharge, C) TN vs WWTP TN load, and D) WWTP TP load. WWTP Nutrient loads are those for the Morpeth outfall.

There was no relationship between TN concentrations and nutrient loads released from Morpeth or Raymond Terrace WTTP (Fig. 8 C). This indicates the increasing concentrations of TN may be related to localised runoff or other significant point sources such as the fertilizer manufacturing or chicken processing plants in the lower estuary (MHL 2003). There was a positive relationship between TP concentrations and TP loads from the WWTP outfall at Morpeth on the Hunter River at the Morpeth and Rowers stations (Fig. 8 D). These stations are the closest located to the outfall, so if the WWTP is contributing to variation in estuarine TP concentrations it would be expected to witness it at these locations. TP Inputs from this outfall may explain the increase in TP between Morpeth and Raymond Terrace sampling stations. These results support the contention by Sanderson and Redden (2001) of a possible point source of TP between Morpeth and Raymond Terrace.

There was no relationship between nutrients and chlorophyll *a* at any sampling stations. This may be because nutrients were generally high at all times and more than met phytoplankton requirements. Chlorophyll *a* was positively related to Z_{eu}:Z_m ratio and negatively related to discharge at the upper Hunter estuary stations, and on the Paterson and Williams Rivers (Fig. 9, Appendix A Fig. 11, 12). Higher turbidity in these upper stations is exerting a strong control on light availability. At the lower stations turbidity was lower, and in turn light penetration higher, likely due to higher salinities causing sediment to flocculate from the water column. The negative relationship between discharge and chlorophyll *a* may be due to advection, or through higher discharges creating turbulence in the water column disrupting any stratification present. Separating the influence of these variables is difficult due to the collinear nature of discharge and turbidity/light availability. In the lower Hunter estuary there was no variables that were able to explain the variation in the chlorophyll *a*.

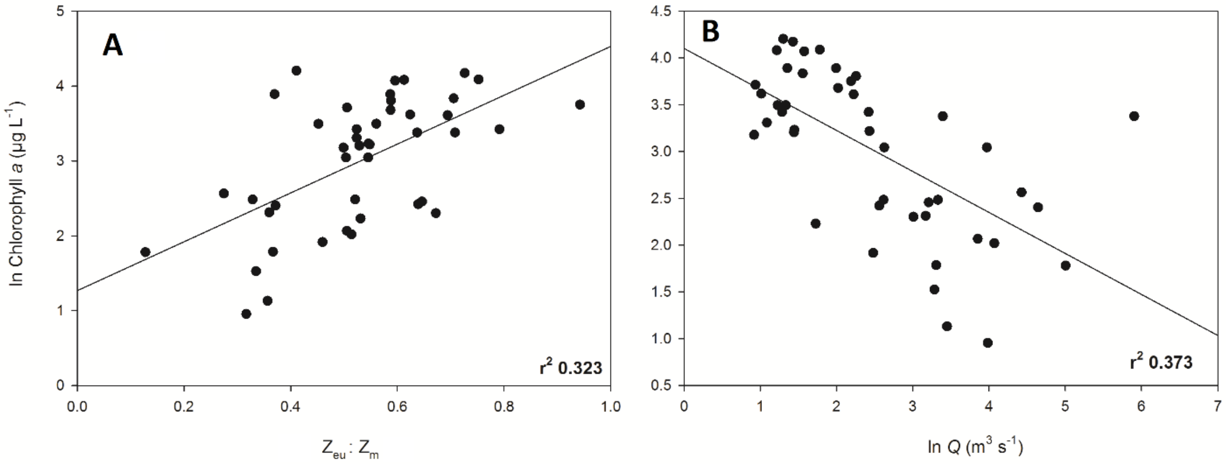

Figure 9. Relationship between Chlorophyll *a* and A) Z_{eu}:Z_m, and B) Discharge for Hunter River at Rowers sampling station

Phytoplankton assemblages for the 2010-2011 period were dominated by green algae (Chlorophyceae) and diatoms (Bacillariophyceae) at all sampling stations (Fig. 10, Appendix Fig. 13, 14). The most common green algae genera were *Scenedesmus*, *Oocystis*, *Ankistrodesmus*, and the most common diatom genera *Cyclotella*, *Skeletonema*, *Nitzschia*. Potentially toxic cyanobacteria (*Anabaena circinalis, Anabaena flos-aquae, Microcystis aeruginosa, Microcystis flos-aquae*) were present at Morpeth, Paterson, Rowers, Casuarina cnr, and Williams sampling stations. During May/June 2010 and January 2011 biolvolumes of potentially toxic cynoabcteria reached levels that would trigger an amber alert under NSW algal management guidelines (biovolume 0.4mm^3 L^{-1} – 4mm^3 L^{-1}) at Morpeth, Paterson, and Rowers. At all times potentially toxic cyanobacteria remained below recreational water guidelines. A longer time series of phytoplankton assemblage data is needed to adequately assess the risks of toxic cyanobacteria in the estuary.

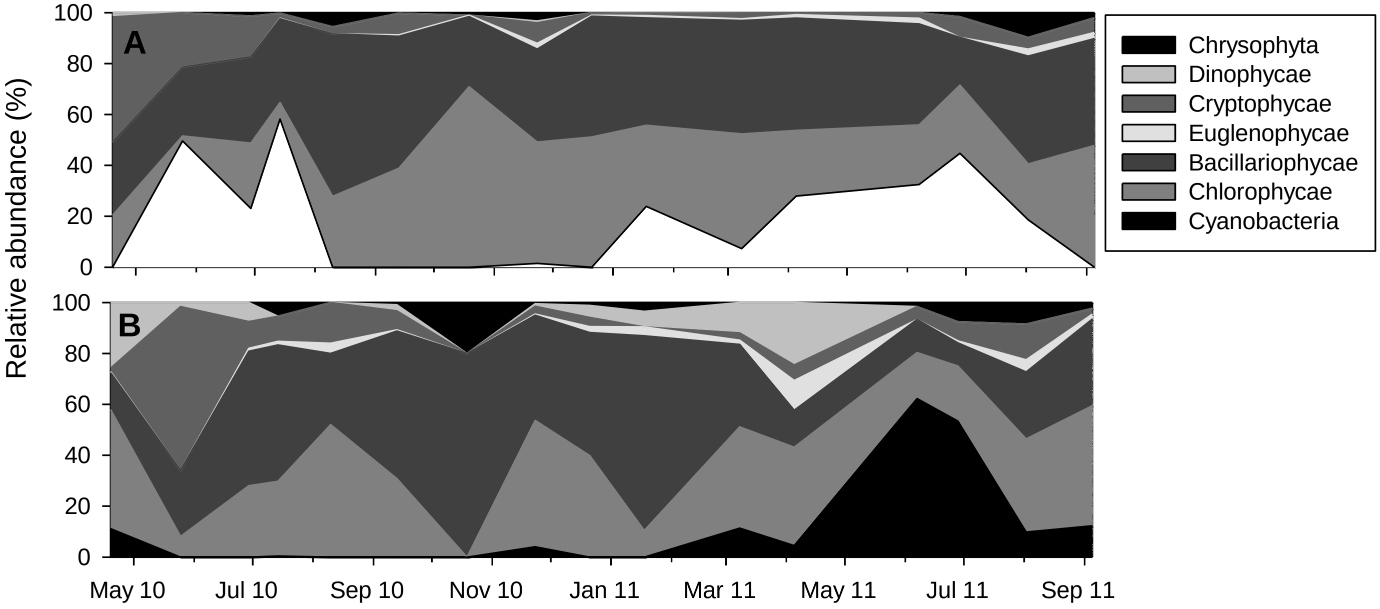

Figure 10. Relative phytoplankton abundance at A) Hunter River at Morpeth, and B) Hunter River at Raymond Terrace.

Comparrison of the nutrient, chlorophyll *a*, and turbity data showed no signficant differences between the monthly and twice-weekly data at the Hunter River at Morpeth, Raymond Terrace, Hexam, and the Paterson and Williams Rivers (Appendix A Table 2). There were however significant differences at the middle estuary sites of Rowers and Casuarina cnr. Distance based redundnacy analysis indicated these differences were likely due to chlorophyll *a* and turbidity.

As discharge appeared to be a strong explanitory factor in explaining nutrient variation, and Z_{eu}:Z_m in explaining chlorophyll *a* concentration we assessed if the twice-weekly and hourly data would fit within the 95% predicition intervals of the regression models developed from the monthly data (Fig. 11, Appendix A Fig. 15-17). All weekly data fit within these 95% prediction intervals. The exception of chlorophyll *a* vs Z_{eu}:Z_m at rowers which had a number of chlorophyll *a* samples below predicted concentrations. Simiraily the hourly data, collected at Morpeth and Ryamond Terrace, fell witin the 95% prediction intervals, though there were a few values for TN vs discharge at Morpeth that did not.

The hourly sampling over the tidal cycle showed predicted patterns for some parameters (Appendix A Fig. 18-21). For example conductivty decreased from high tide to low, and increased from low tide to high tide, dissolved oxygen increased from the morning to afternoon. There was no apparent pattern in nutrient concentrations over the tidal cycle. Chlorophyll *a* decreased from high tide to low tide, and increased from low to high tide on both occasions at Morpeth, and during Feburary sampling at Raymond Terrace; during November sampling at Raymond Terrace chlorophyll *a* increased throughout the day. Sampling was conducted at the same time each month during the five year observational study, sampling from the lower estuary to hunter estuary starting at high tide in the morning, to control for any variation over the tidal cycle at sampling stations.

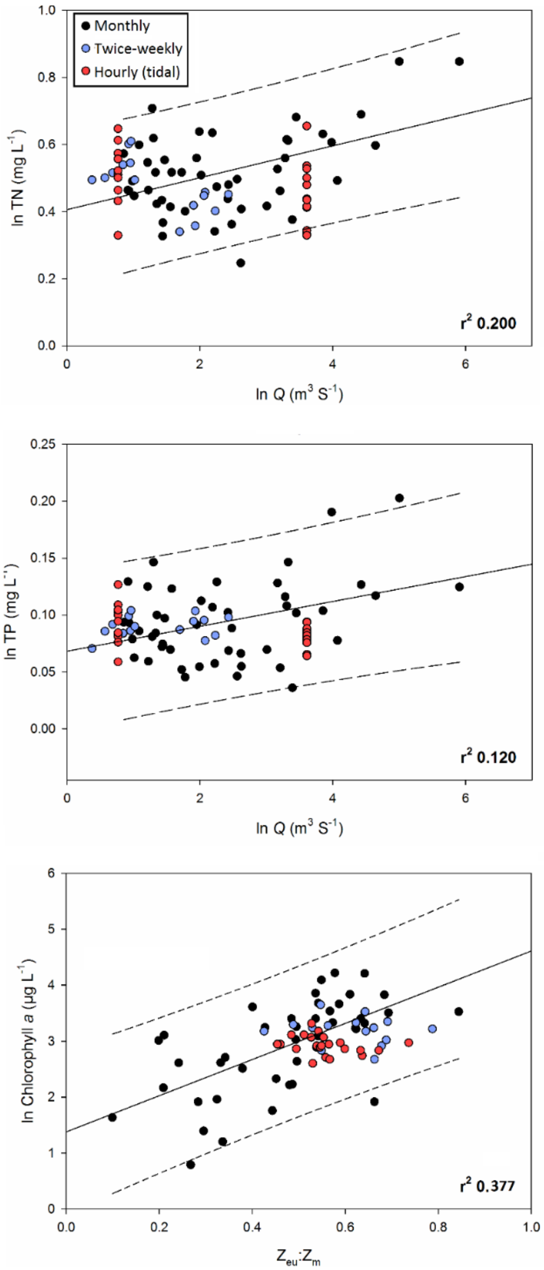

Figure 11 Comparison of monthly, twice-weekly, and hourly sampling data for TN, TP, and chlorophyll a for the Hunter River at Morpeth.

These results indicate that the five year observatoinal study has largely captured the range in variation of nutrients and chlorophyll *a* as they relate to their explanitory variables. For the most part the twice-weekly and hourly sampling will increase the predictive strength of the regression models. As the twice-weekly and hourly sampling occurred under low flow conditions it may not account for the variation under higher flow conditions. A priority for future studies should be capturing nutrient concentations during high inflow events at the hourly, daily and weekly time scales.

3.2 Nutrient amendment experiments

Results from the nutrient amendment experiments showed phytoplankton were generally nitrogen limited or not limited by major nutrients during 2017 (Table 2, Appendix B Table 1, Fig 1-10). These results support our hypothesis that because nutrient concentrations within the estuary are very high, they are likely in excess, and not limiting growth. The results also support our hypothesis that if nutrient were limiting, nitrogen was more likely to be limiting than phosphorus due to N:P being <16:1 for the majority of the time. The results align with previous experiments on the Hunter estuary indicating phytoplankton are likely nitrogen limited (Hitchcock et al. 2010). The observational studies indicated light to be the main factor limiting phytoplankton growth within the upper estuary. This experiment controlled for light by conducting the experiments within the surface layer, even with adequate light phytoplankton growth was routinely not nutrient limited.

Table 1 Limiting nutrients during seasonal amendment experiments. Limiting nutrients where determined comparing chlorophyll *a* results at 72 hours between treatments and control. *not significantly different from control though chlorophyll a and phytoplankton biovolume higher in N treatment.

	Summer	**Autumn**	**Winter**	**Spring**
Hunter River at Morpeth Outfall	N+P	none	none	none
Hunter River at Windeyers Creek	N*	none	none	none
Hunter River at Hexam	N	none	N	N
Hunter River at Shortland Outfall	N	none	N+P	none
Wallis Creek	N	none	none	N
Windeyers Creek	N,P	none	none	none

The highest chlorophyll *a* concentrations were at Wallis Creek and at the Hunter River at Morpeth, displaying hypereutrophic responses during the experiments (Fig. 12 C, Appendix B Fig. 1, 2). These responses were due in part to high initial phytoplankton biomass, as well as likely the nutrient rich discharges from Farley and Kurri Kurri WWTP in Wallis Creek, and from Morpeth outfall to the Hunter

River. The responses are supported by the observational study, which showed highest chlorophyll *a* concentrations to be in the upper estuary. In the lower estuary at Hexam and Shortland, nutrient limitation was more prevalent. This is likely because nutrient concentrations are lower in this part of the estuary compared to upstream.

Windeyers Creek, which receives discharge from the Raymond Terrace WWTP had the lowest chlorophyll *a* concentration of all experiments and showed the least response to nutrient additions. These low results are supported by HunterWater monitoring data within the Windeyers Creek which showed average chlorophyll *a* concentrations of <5 µg L-1 between 2005-2016. Possible reasons for a lack of response may mean limitation by micronutrients (e.g. Fe, Cu, Zn). Current work by UTS (not reported here) suggests metals may be an important factor in understanding phytoplankton growth dynamics in the Hunter estuary. Silica is also an important nutrient that can commonly limit diatom growth. We found no evidence of potential silica limitation within the Hunter estuary during the observational experiment.

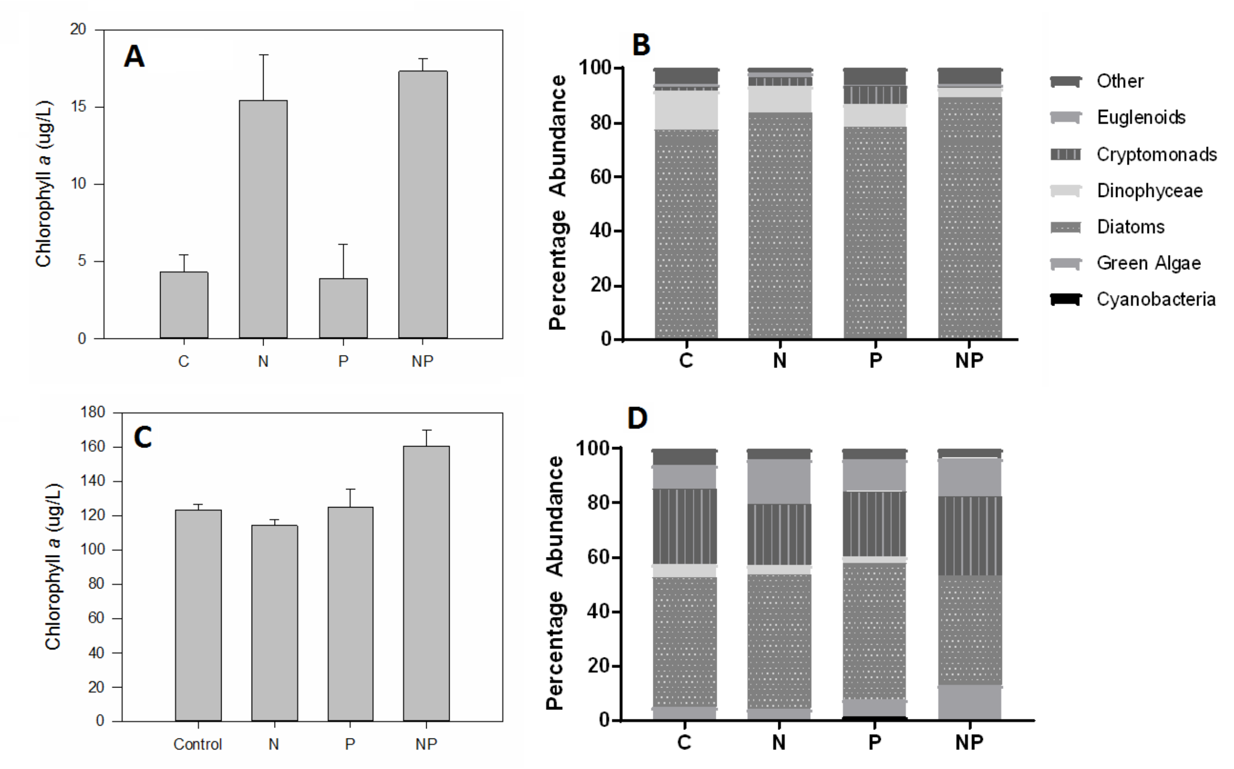

Figure 12. Results for nutrient amendment experiments conducted in summer. A) Chlorophyll a results at Morpeth, B) relative phytoplankton family abundance at Morpeth, C) chlorophyll a results at Wallis Creek, and D) relative phytoplankton family abundance at Wallis Creek. Error bars are standard error.

4. Conclusions

Eutrophic conditions dominate the Hunter River estuary, with the upper estuary, upstream of its confluence with the Williams River, most eutrophic. Riverine discharge appears to be the major source of nutrients, though discharge from WWTP in the upper Hunter potentially contributes an important secondary source of phosphorus. Processes such as bank erosion and resuspension are also important, as both a potential local source of nutrients, and also a factor likely influencing turbidity and light dynamics. Light and turbidity were the main factors limiting phytoplankton growth in the upper estuary. Light also covaried with discharge which may have also supressed phytoplankton growth through advection and mixing. The nutrient amendment experiments showed that when light limitation was alleviated, phytoplankton were either nitrogen limited or remained unlimited by nutrients (suggesting nutrients were in surplus for growth). The expression of nitrogen limitation is likely due to low N:P in the estuary. The total nitrogen pool is dominated by organic nitrogen; the bioavailability of organic nitrogen is variable which may also explain the lack of relationship between phytoplankton and nitrogen concentrations within the estuary. Diatoms and green algae dominated the phytoplankton though there were occasions when toxic cyanobacteria was in high abundance in the upper estuary. As phytoplankton assemblage data was limited, the potential risks of toxic cyanobacteria under different conditions are hard to define.

Comparison of data from the monthly, twice-weekly, and hourly sampling intervals demonstrated the five-year monthly sampling data appeared to mostly capture the variability of nutrient and chlorophyll *a* concentrations in relation to their main explanatory factors (discharge and light). There were some examples of chlorophyll *a* and nitrogen concentrations that fell outside of predicted ranges. The hourly sampling also showed that nutrient concentrations can vary throughout the day; these are controlled in the monthly data as sampling was conducted at the same time/tidal conditions on each occasion.

The results suggest that increases in nitrogen loads have the potential to increase phytoplankton growth. As light limited growth within the upper estuary much of the time, reductions in turbidity and increases in light penetration also have the potential to increase phytoplankton growth. These results should not hamper efforts to reduce erosion and suspended solids in the estuary as these they also likely lead to concomitant reductions in nutrients (as well as broader ecosystem health outcomes). Overall, as loads of both nitrogen and phosphorus are very high, reducing inputs of both nutrients, at the local and catchment scale, will be important in improving the health of the estuary and avoiding potential algal blooms.

5. References

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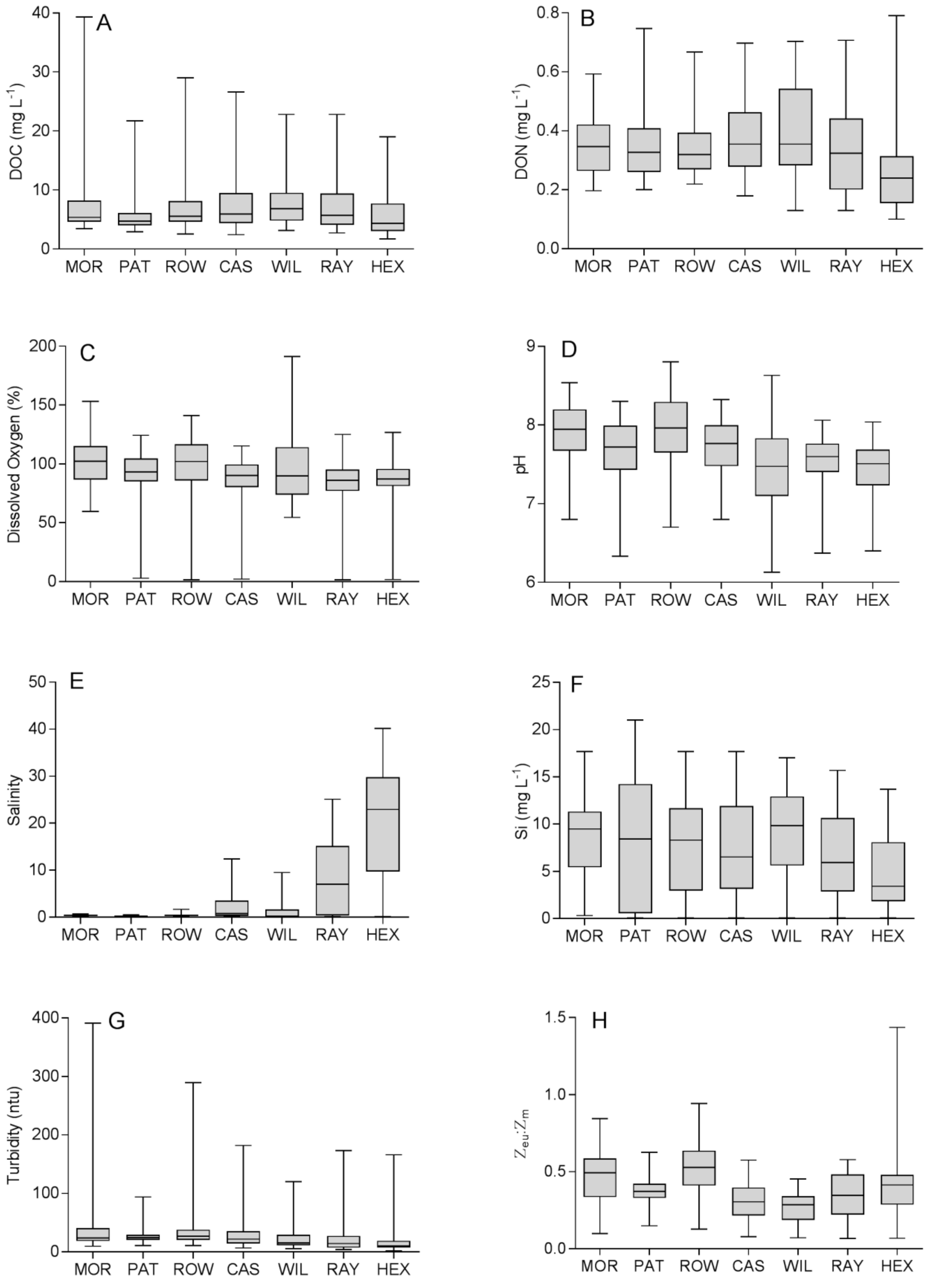
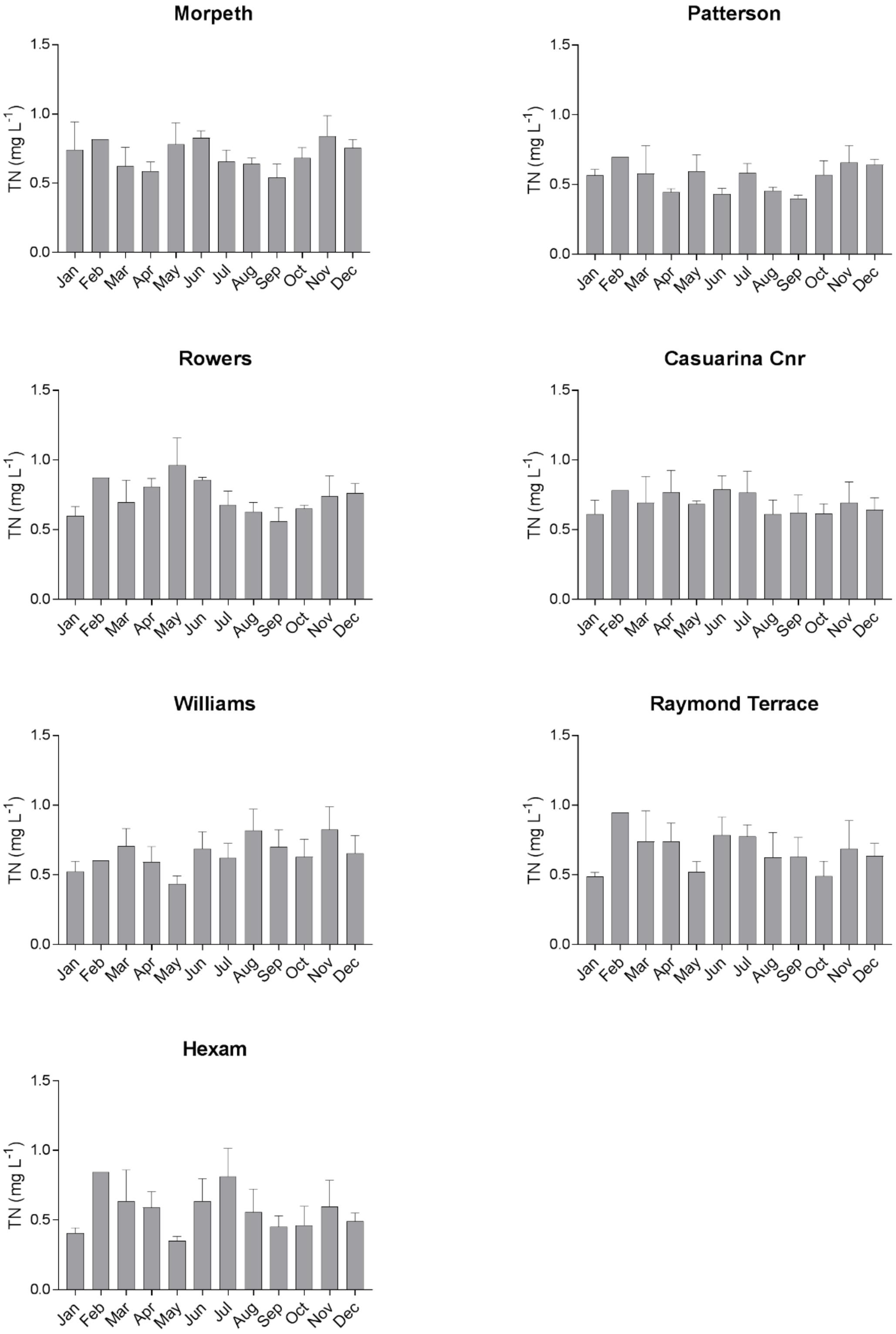
Appendix A. Observational study results



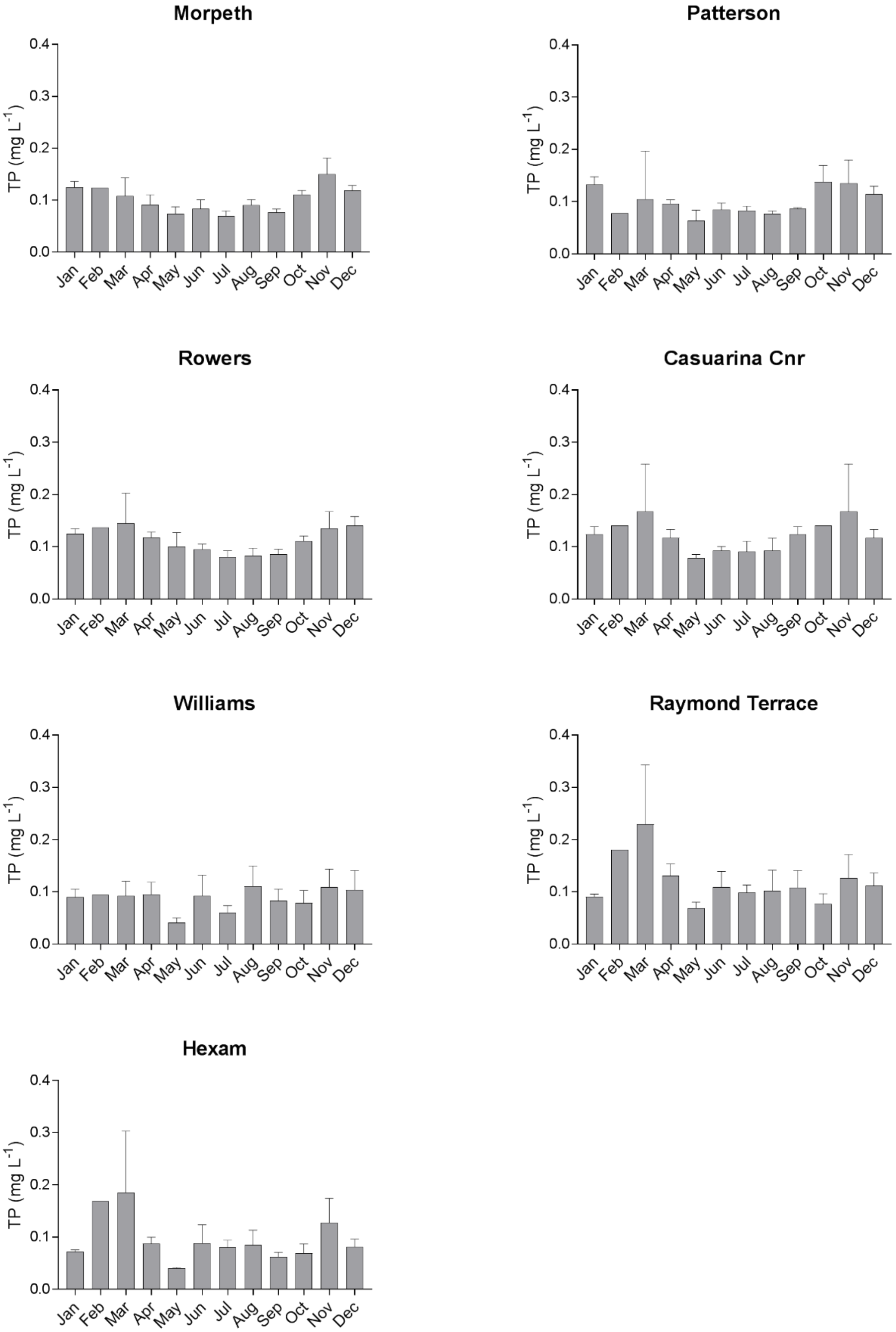
Appendix A Figure 1. Boxplots for water quality parameters during 2010 - 2014 parameters. A) DOC, B) DON, C) dissolved oxygen, D) pH, E) Salinity, F) Silica, G) turbidity, and H) Z_{eu}:Z_{m}. The boxes represent the median, 25th and 75th percentile ranges, whilst the error bars indicate the maximum and minimum values recorded. Error bars are standard error.

Appendix A Table 1. Mean relative percentage composition of nitrogen and phosphorus of the total nutrient pool. DON, NOx, NH4, SUP, SRP are all dissolved (< 0.45 µm). Soluble unreactive phosphorus (SUP) is calculated by subtracting SRP from DTP. SRP may contain both organic and unreactive inorganic phosphorus. Particulate TN (PTN) and particulate TP (PTP) are calculated by subtracting the total nutrients (unfiltered) from the dissolved total nutrients; this fraction may contain organic and inorganic nutrients. There were a handful of occasions where dissolved SRP returned results higher than dissolved total phosphorus in which case we assumed 100% of the dissolved phosphorus pool was SRP. ± is standard error.

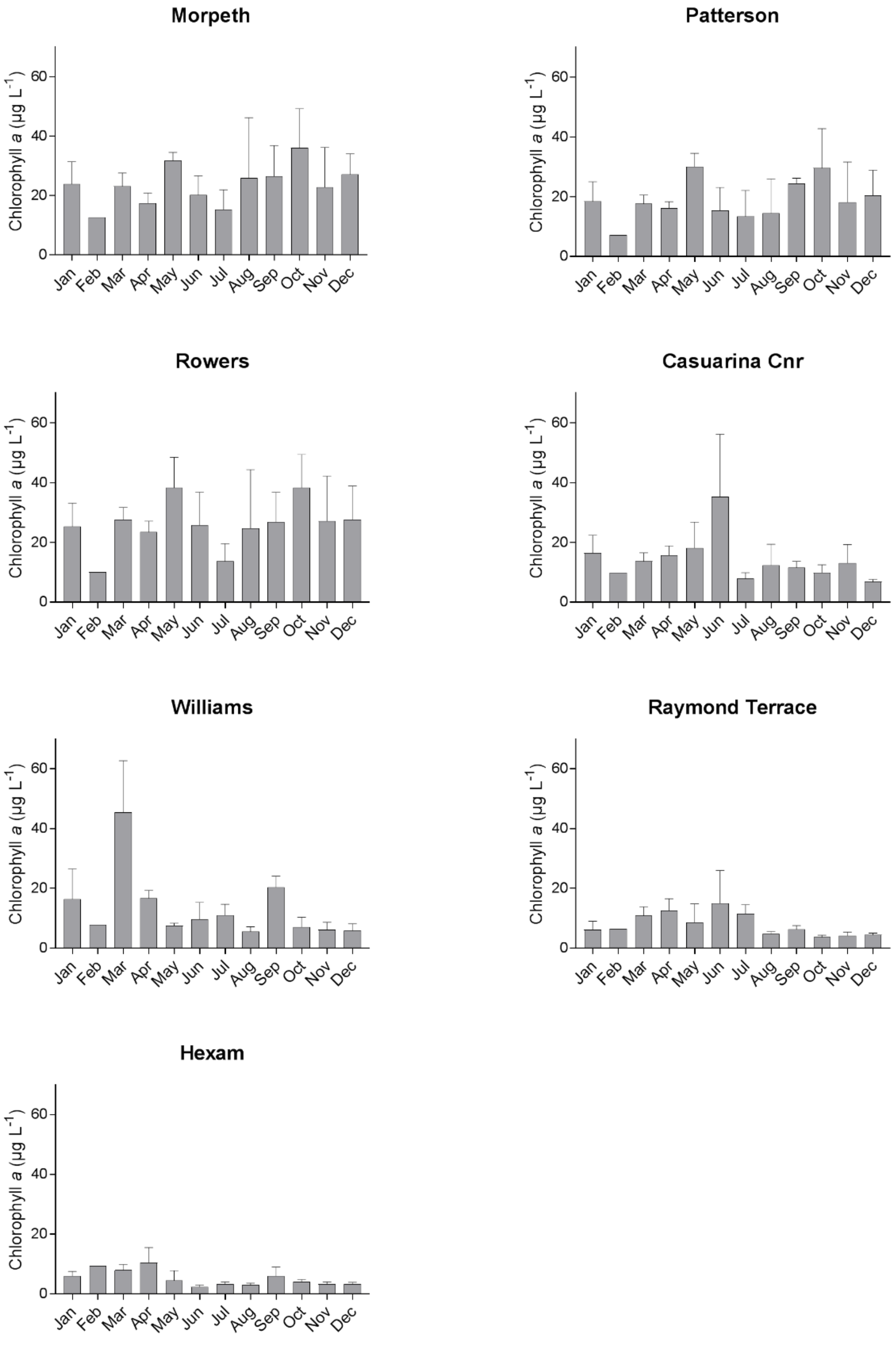
	Nitrogen				**Phosphorus**		
	DON (%)	**NOx (%)**	**NH4 (%)**	**PTN (%)**	**SUP (%)**	**SRP (%)**	**PTP (%)**
Morpeth	47.2	20.3	4.0	28.5	18.4	28.9	52.6
	± 2.4	± 2.9	± 0.9	± 3.5	± 2.5	± 3.8	±4.3
Paterson	58.2	11.5	4.8	25.5	20.3	34.9	44.8
	± 3.2	± 2.2	± 1.0	±2.9	± 4.4	± 4.5	±7.6
Rowers	48.5	20.7	3.6	27.2	16.1	35.5	48.3
	± 2.7	± 3.5	± 0.9	±3.4	± 2.4	± 4.4	±4.1
Casuarina Cnr	52.8	22.3	5.0	19.9	8.1	56.8	35.0
	± 2.3	± 2.7	± 0.8	±3.0	± 2.3	± 6.1	±5.4
Williams	62.8	10.5	4.7	22.0	15.5	45.5	39.0
	± 4.0	± 2.5	± 0.9	±3.8	± 2.7	± 4.8	±5.2
Raymond Terrace	51.7	21.2	7.8	19.4	6.8	60.1	33.2
	± 3.0	± 3.2	± 1.1	±3.6	± 2.6	± 5.1	±5.1
Hexam	51.5	20.6	9.5	18.4	13.4	63.3	23.3
	± 3.0	± 2.5	± 2.8	±2.8	± 3.4	± 4.9	±4.0



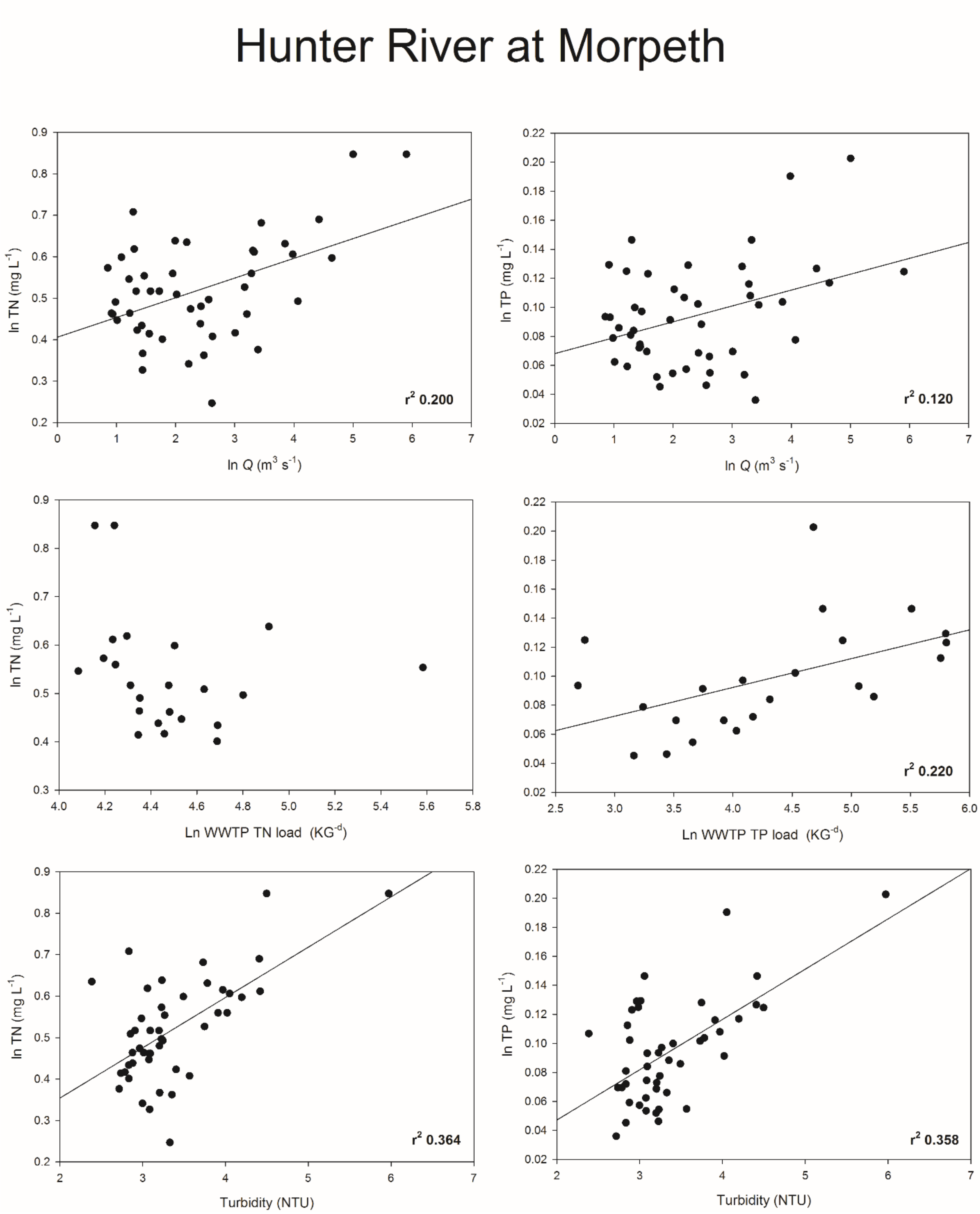
Appendix A Figure 2: Monthly mean total nitrogen concentrations, 2010 – 2014. Error bars are standard error.



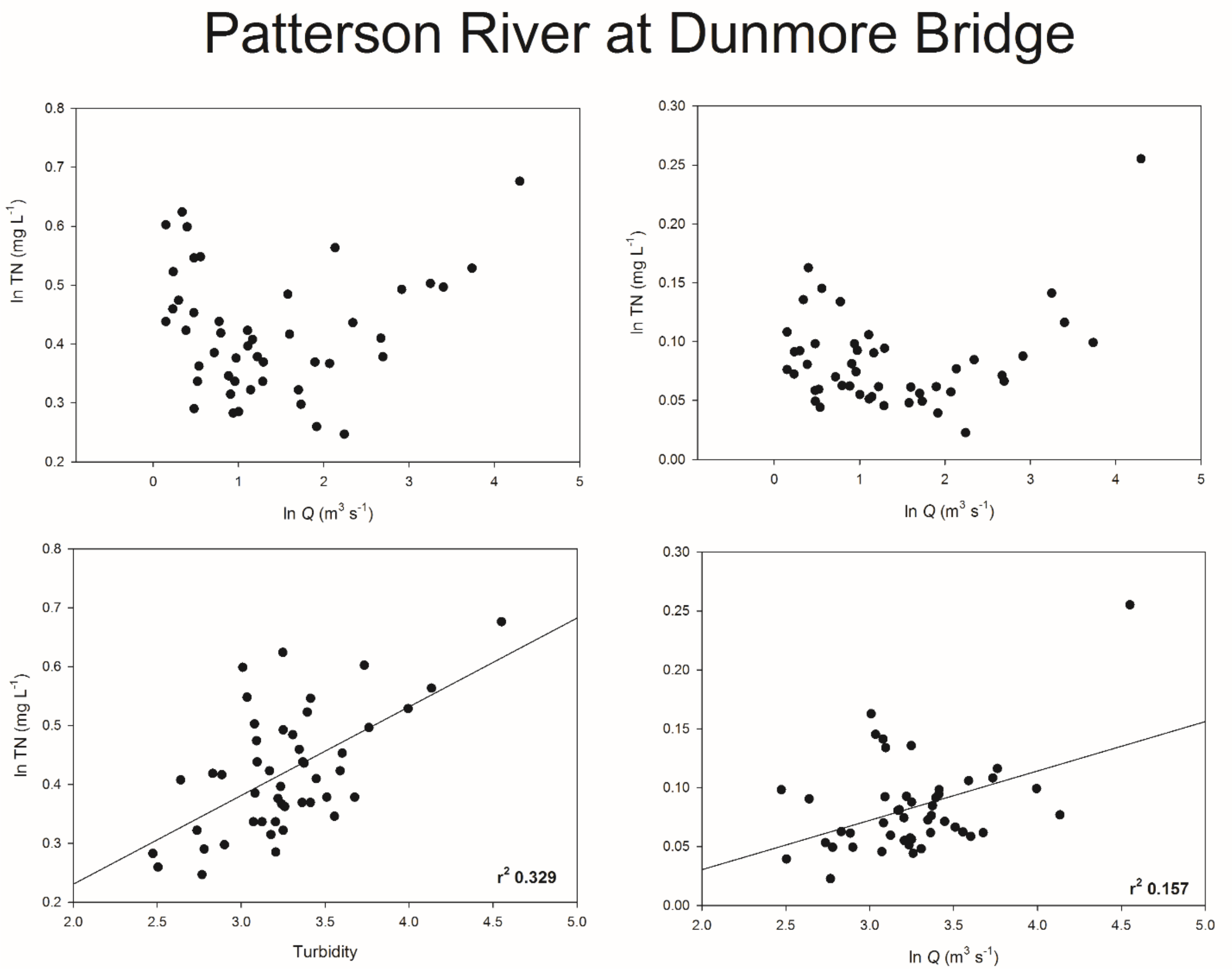
Appendix A Figure 3: Monthly mean total phosphorus concentrations, 2010 – 2014. Error bars are standard error.



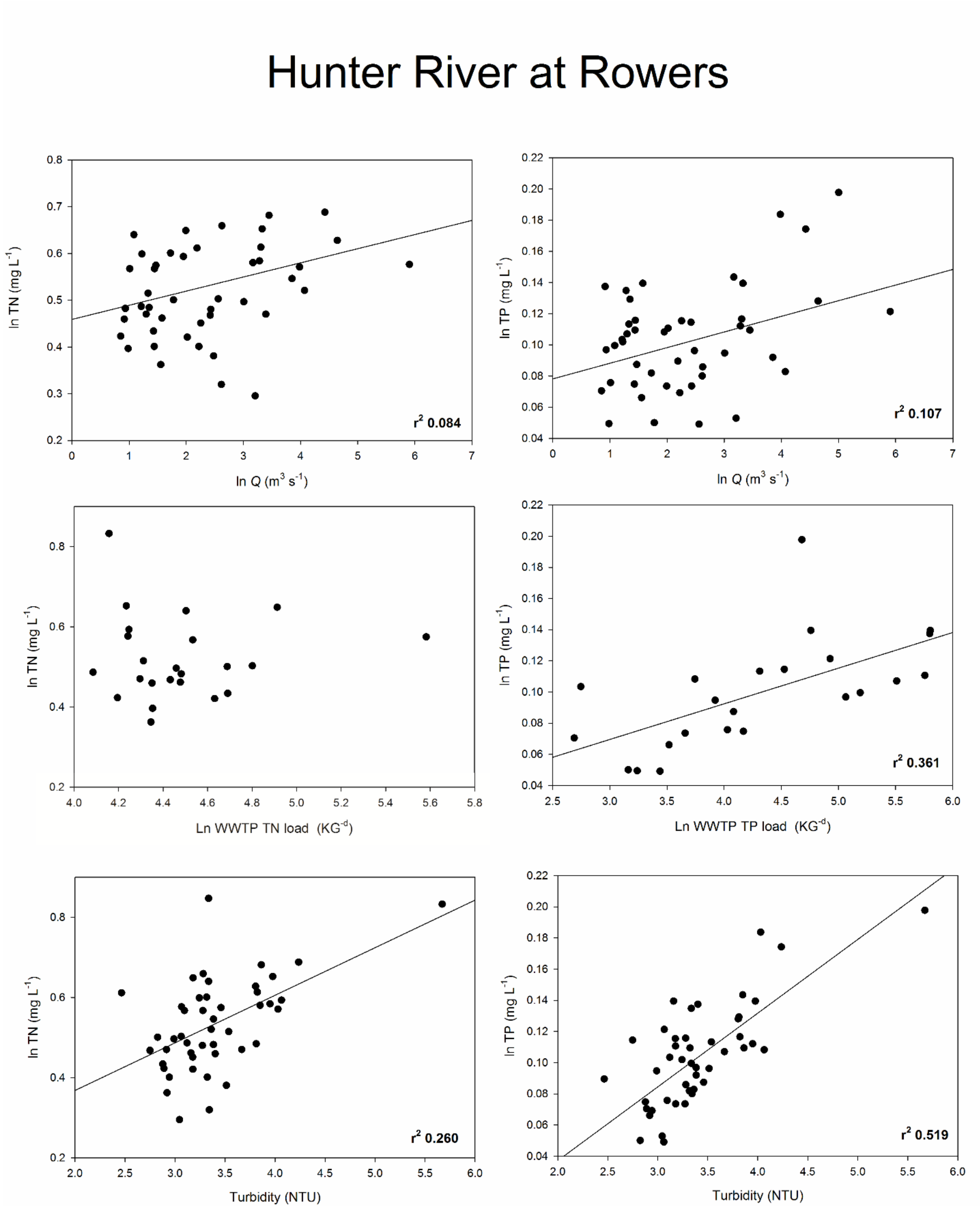
Appendix A Figure 4: Monthly mean chlorophyll *a* concentrations, 2010 – 2014. Error bars are standard error.



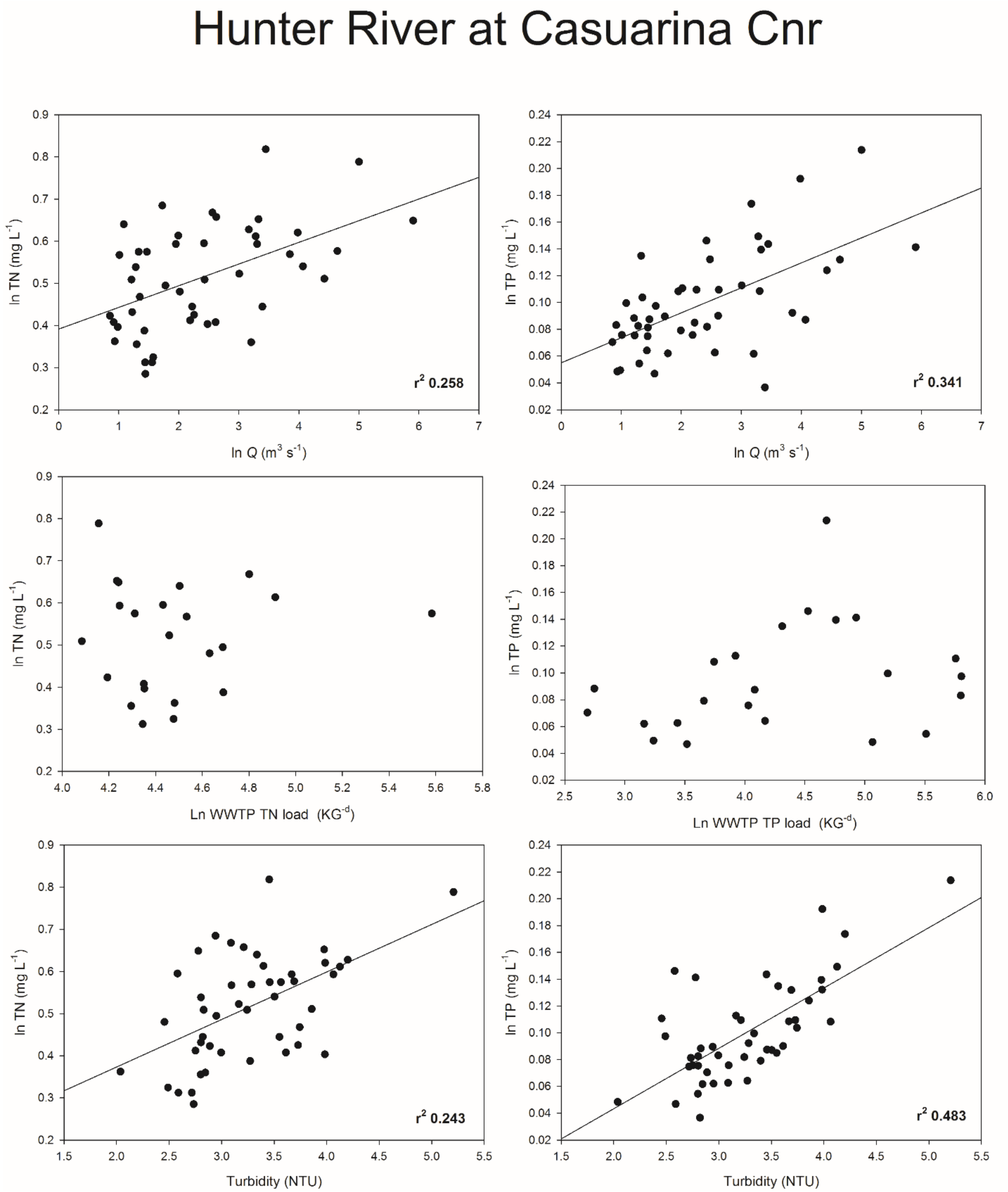
Appendix A Figure 5: Relationships between total nitrogen and total phosphorus, and discharge, WWTP nutrient loads to the estuary, and turbidity for the Hunter River at Morpeth, 2010-2014. WWTP loads are those for the Morpeth outfall.



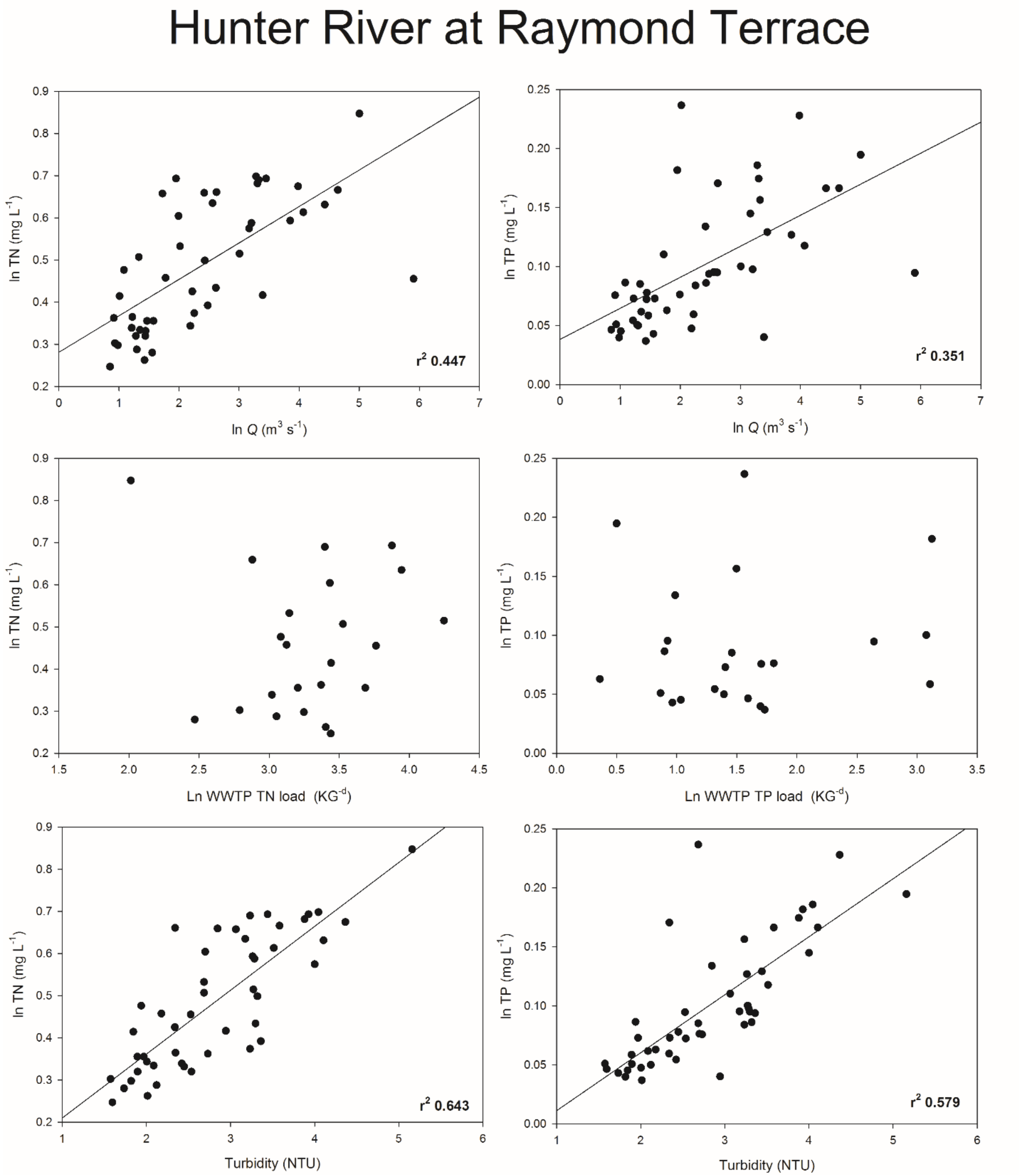
Appendix A Figure 6: Relationships between total nitrogen and total phosphorus, and discharge and turbidity for the Paterson River at Dunmore Bridge, 2010-2014. .



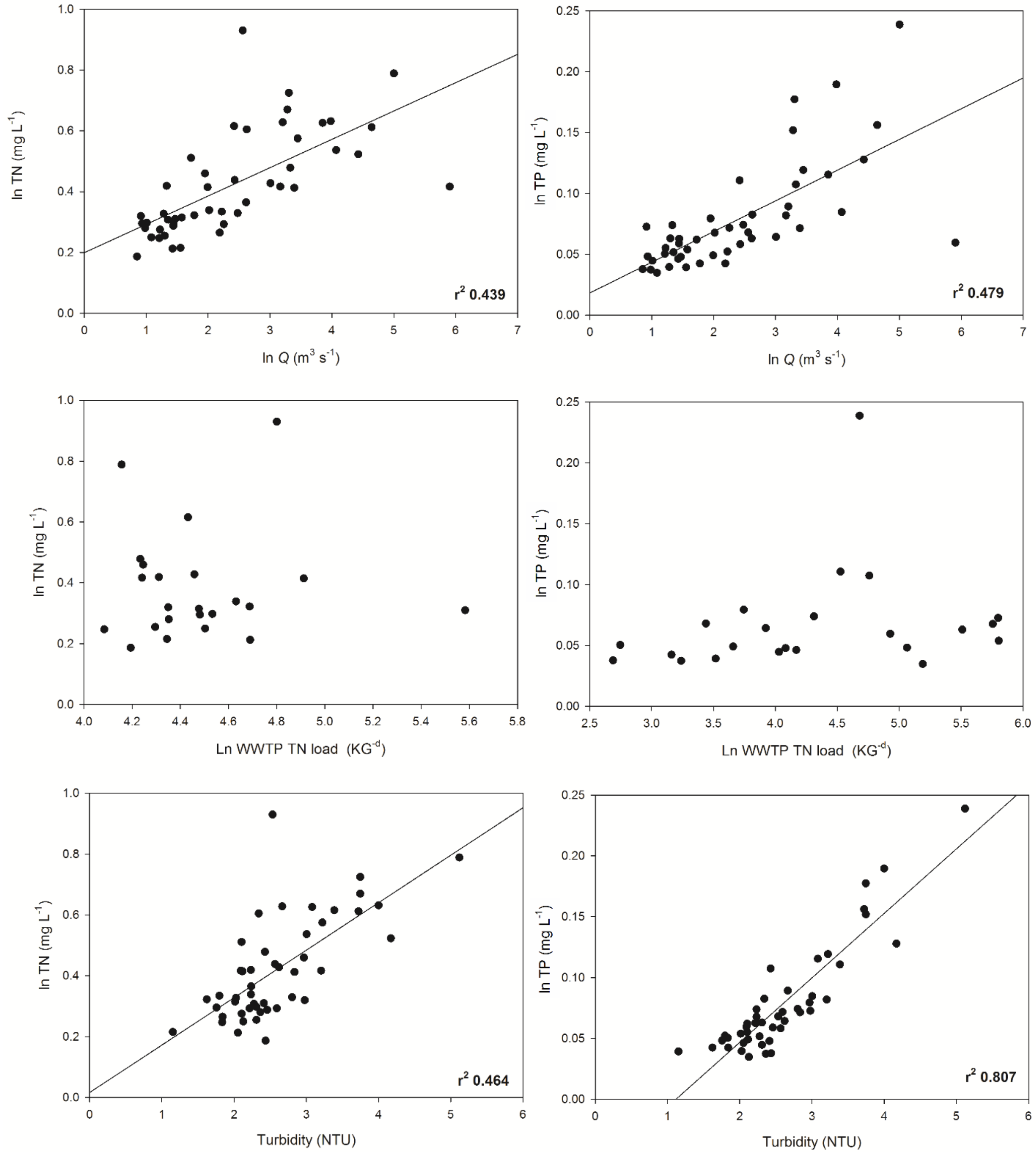
Appendix A Figure 7. Relationships between total nitrogen and total phosphorus, and discharge, WWTP nutrient loads to the estuary, and turbidity for the Hunter River at Rowers, 2010-2014. WWTP loads are those for the Morpeth outfall.



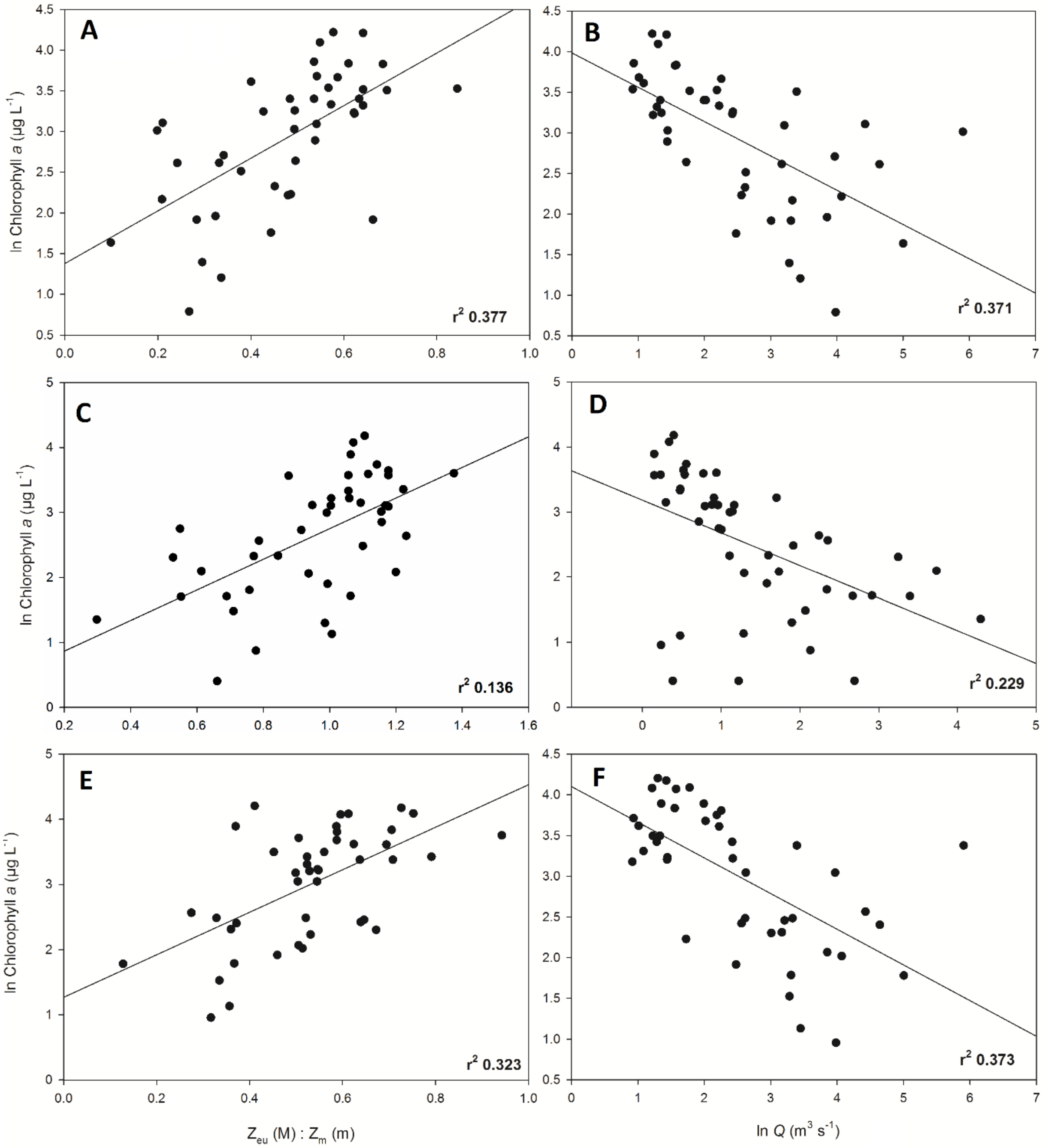
Appendix A Figure 8: Relationships between total nitrogen and total phosphorus, and discharge, WWTP nutrient loads to the estuary, and turbidity for the Hunter River at Casuarina cnr, 2010-2014. WWTP loads are those for the Morpeth outfall.



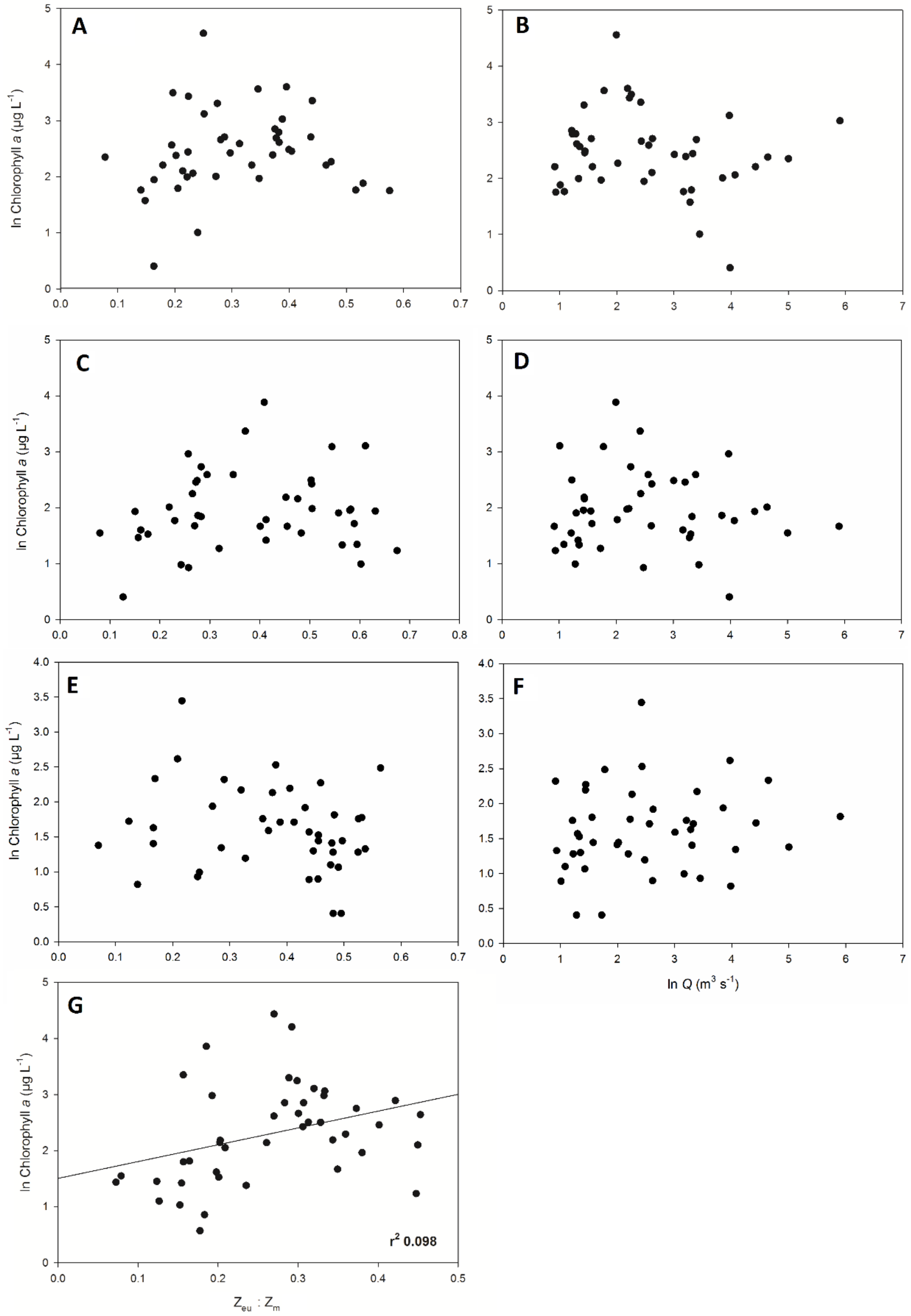
Appendix A Figure 9: Relationships between total nitrogen and total phosphorus, and discharge, WWTP nutrient loads to the estuary, and turbidity for the Hunter River at Raymond Terrace, 2010-2014. WWTP loads are those for the Raymond Terrace WTTP discharge to Windeyers Creek.


Hunter River at Hexam

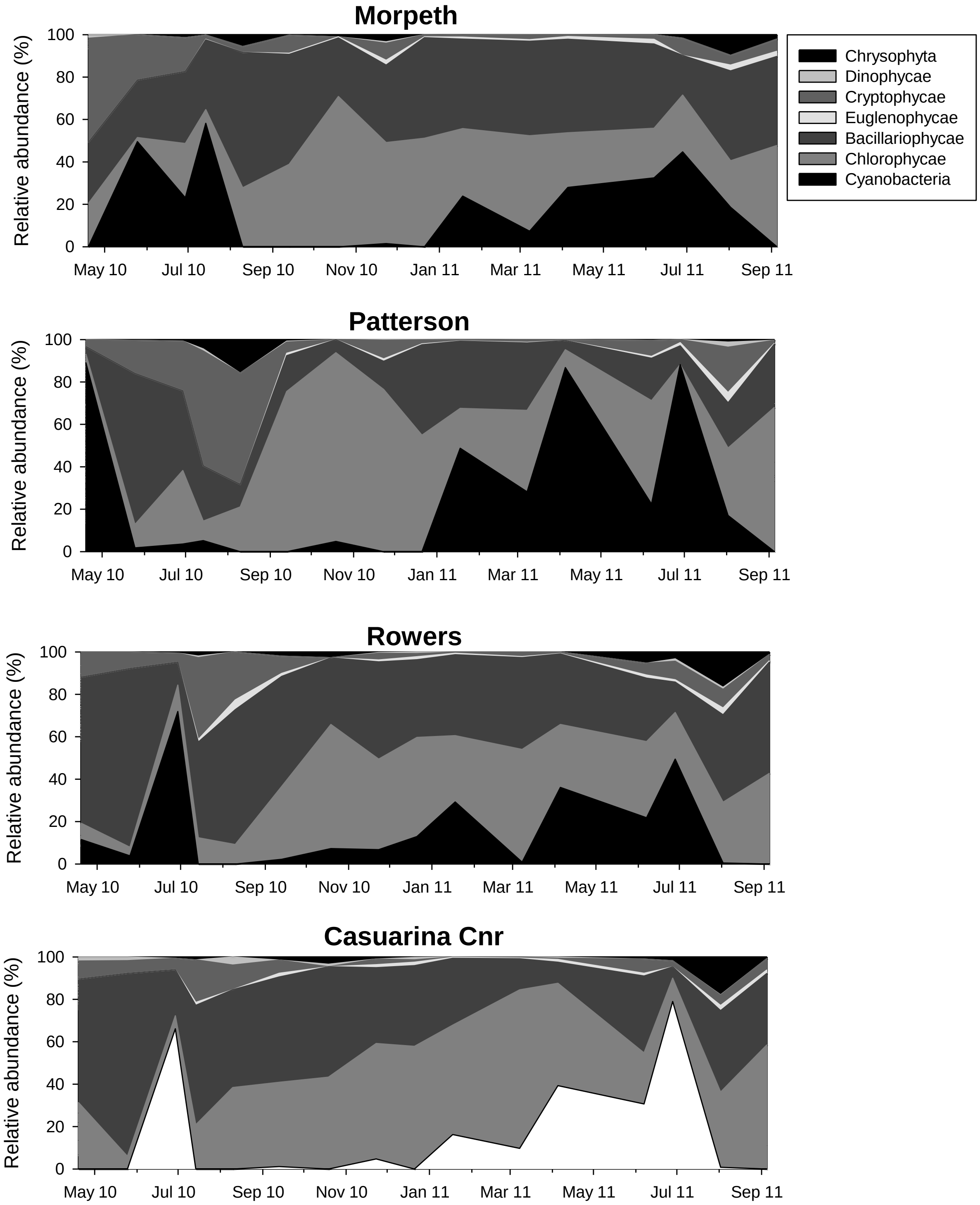
Appendix A Figure 10. Relationships between total nitrogen and total phosphorus, and discharge, WWTP nutrient loads to the estuary, and turbidity for the Hunter River at Hexam, 2010-2014. WWTP loads are those for the Raymond Terrace WTTP discharge to Windeyers Creek.



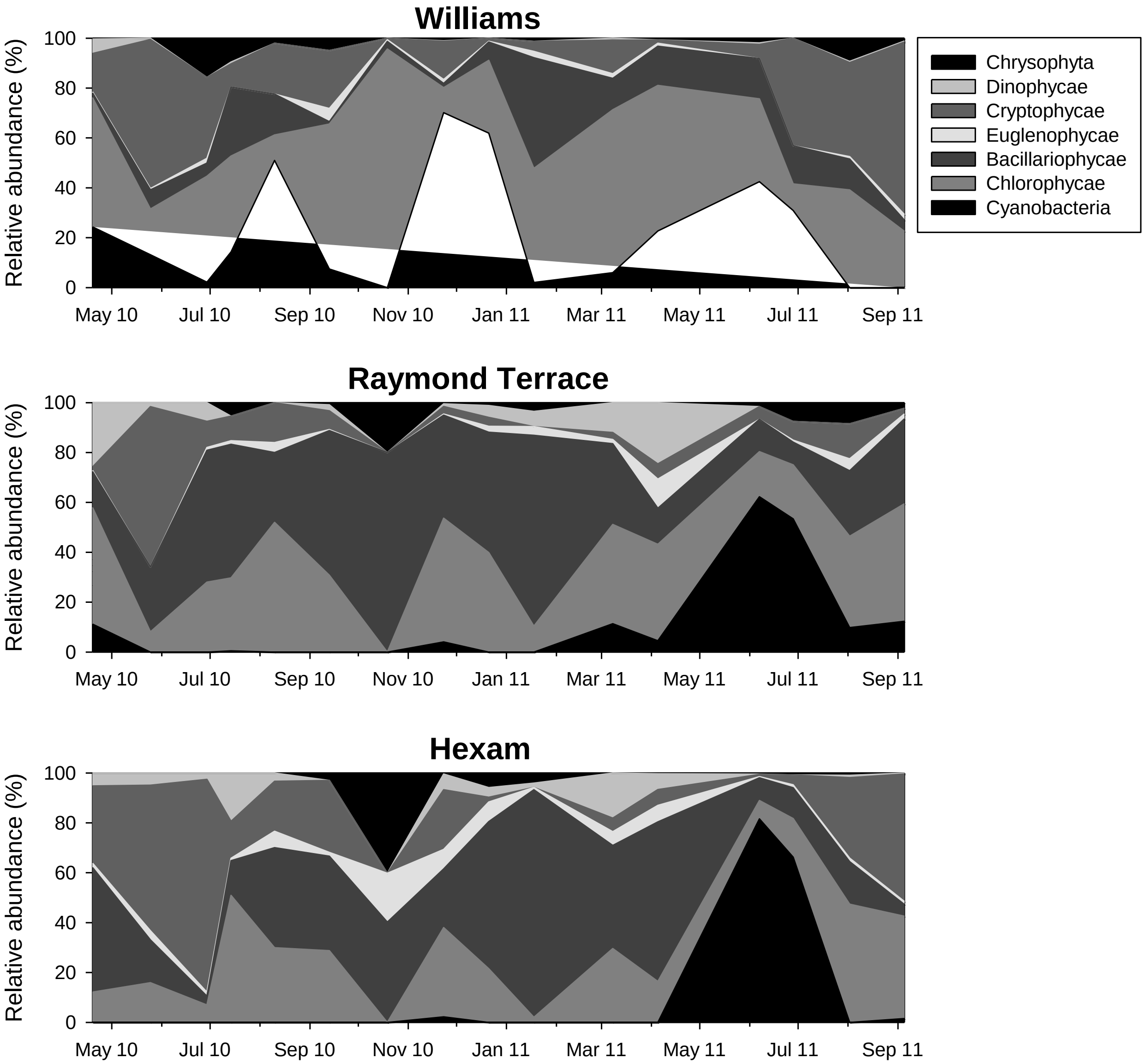
Appendix A Figure 11. Relationship between chlorophyll *a*, and Discharge and Zeu:Zm for 2010-2014 at Hunter River at Morpeth (A,B) Paterson River at Dunmore Bridge (C, D) and the Hunter River at Rowers (E, F)



Appendix A Figure 12. Relationship between chlorophyll *a*, and Discharge and Zeu:Zm for 2010-2014 at Hunter River at Casuarina Cnr (A,B) Hunter River at Raymond Terrace (C, D), Hunter River at Hexam (E, F), and the Williams River at Seaham Weir (G).



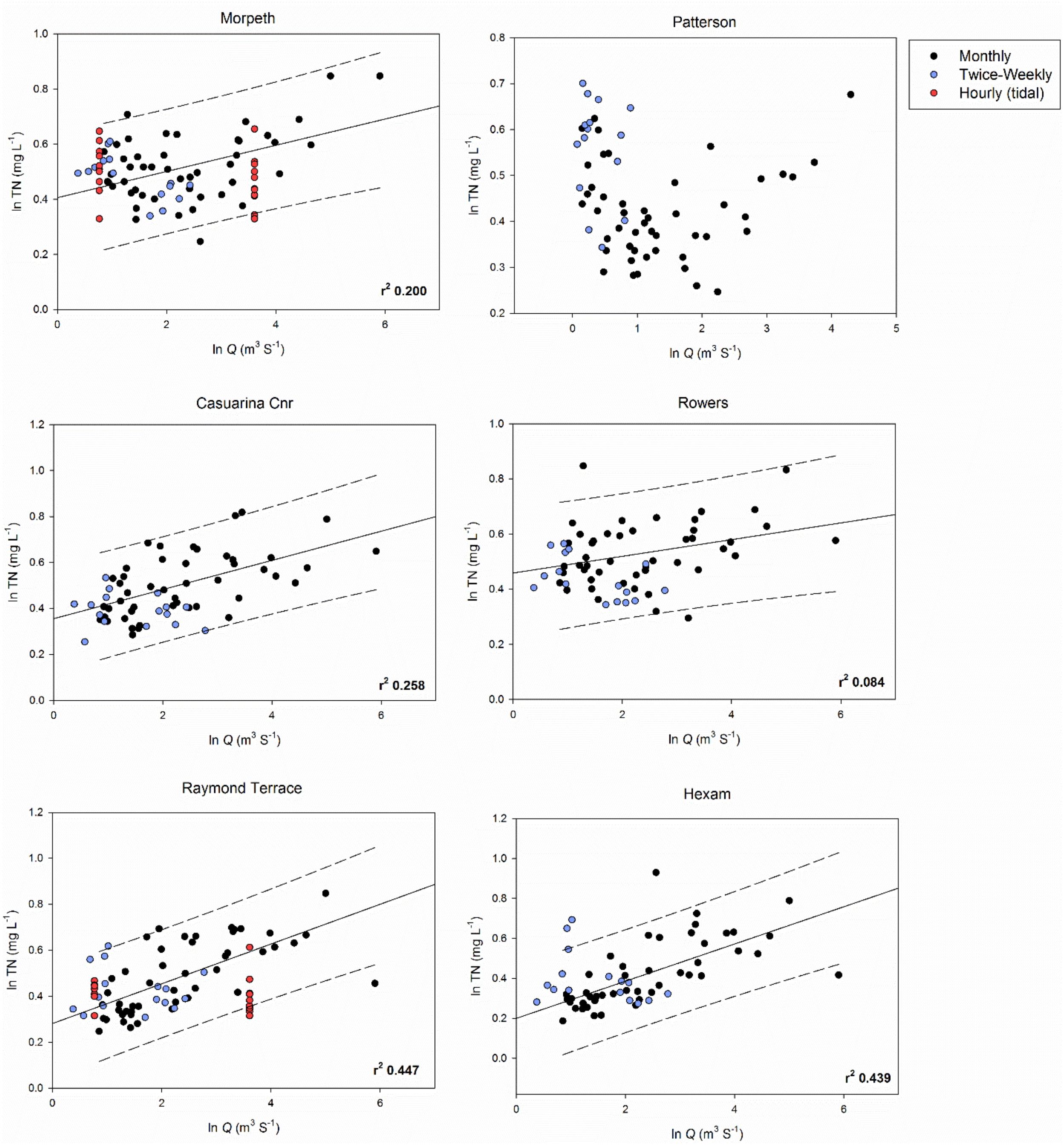
Appendix A Figure 13. Relative phytoplankton abundance (families) for 2010 – 2011 the Hunter River at Morpeth, Paterson River at Dunmore Bridge, Hunter River at Rowers, and the Hunter River at Casuarina Cnr.



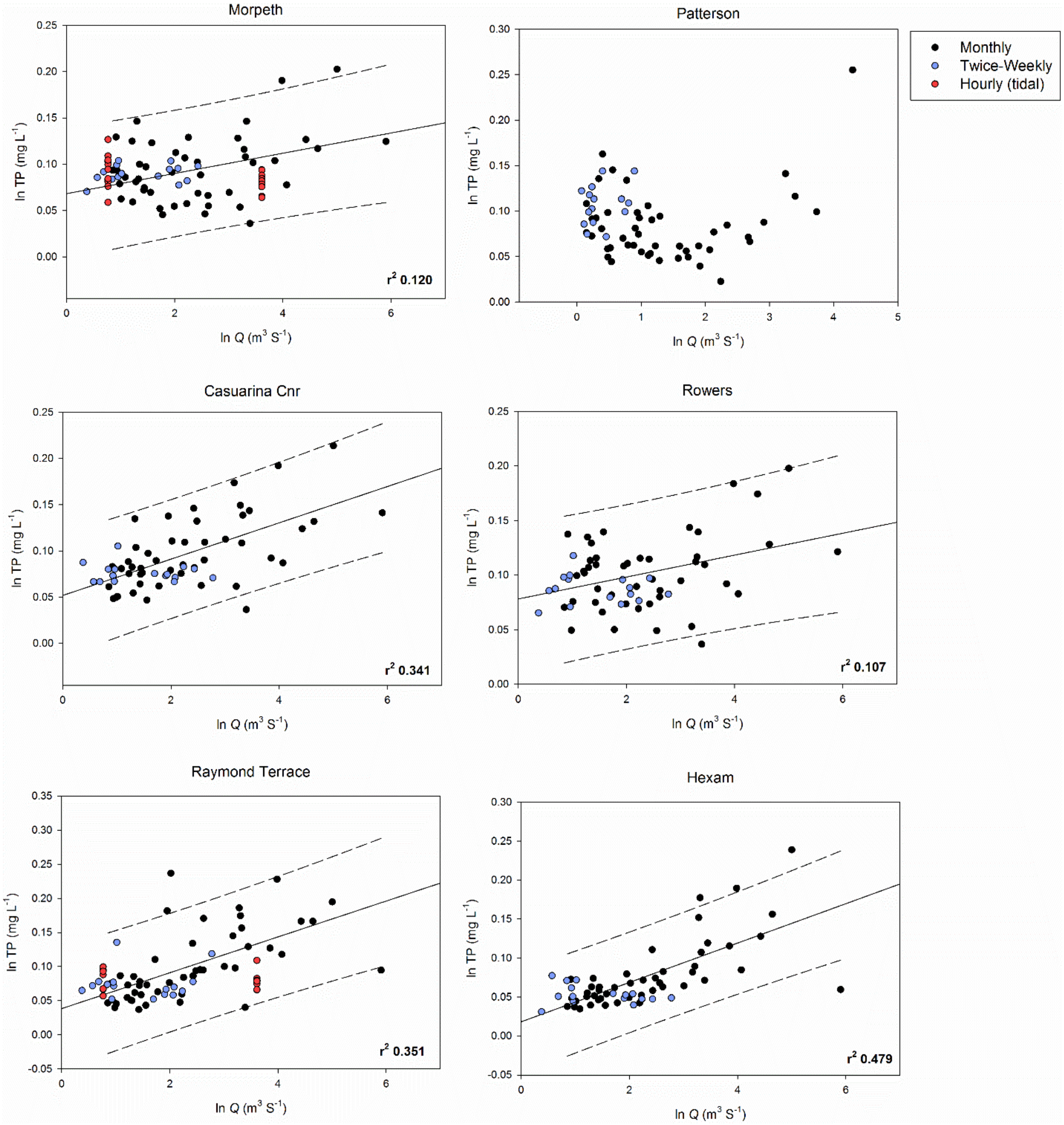
Appendix A Figure 14. Relative phytoplankton abundance (families) for 2010 – 2011 the Williams River at Seaham Weir, Hunter River at Raymond Terrace, and Hunter River at Hexam.

Appendix A Table 2. Permanova results testing the difference between monthly and twice-weekly data. A sub-set of data was selected from the monthly monitoring set for dates falling between September to April when discharge fell within the range present during summer sampling (13-14 sampling occasions). Data included turbidity, chlorophyll *a*, and all nutrient data (excluding DON, DTP, DTN).

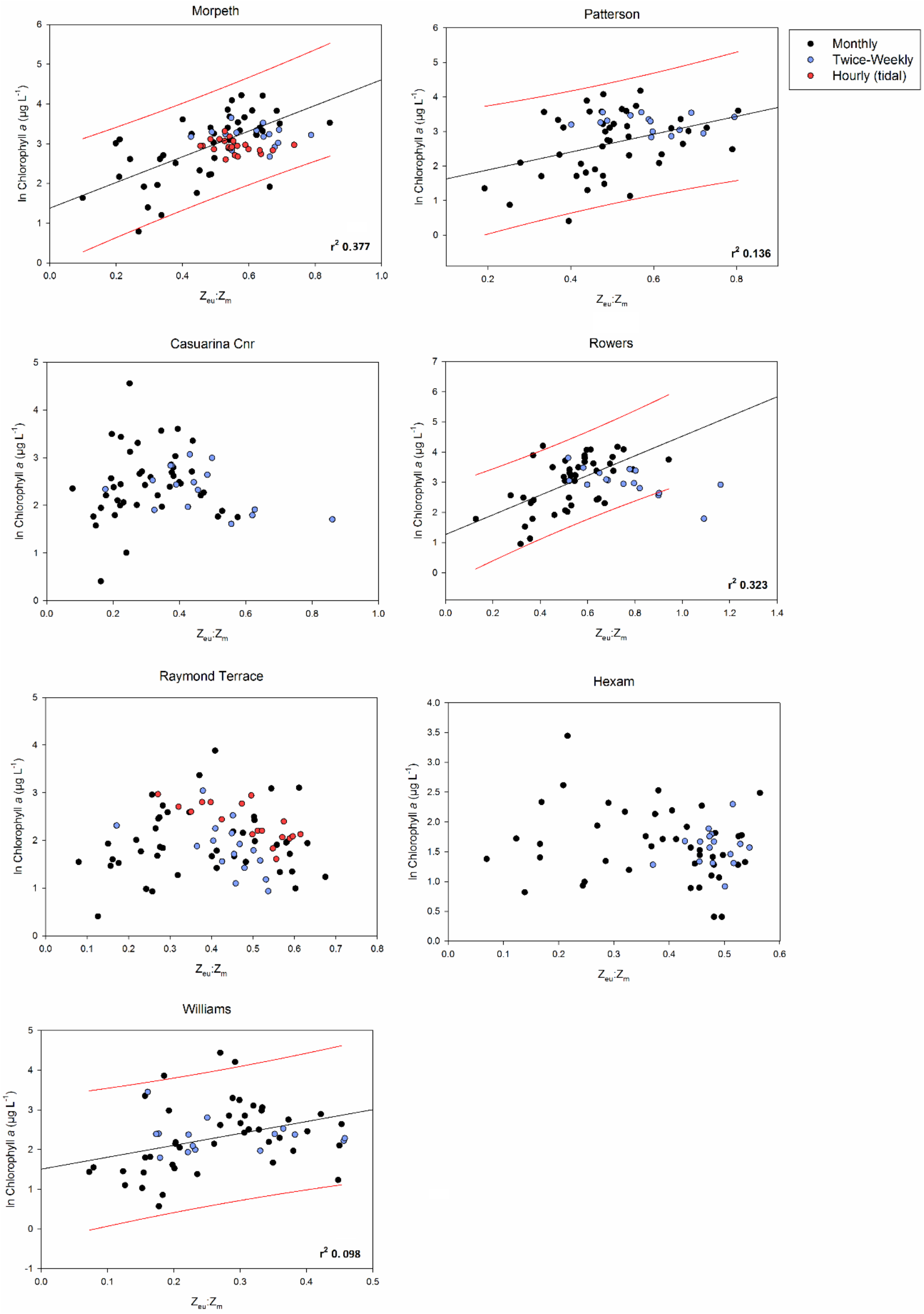
	Pseudo f	**df**	**P**	**Perms**
Hunter River at Morpeth	2.0075	1	0.063	998
Paterson River	2.2432	1	0.051	998
Hunter River at Rower	4.8194	1	**0.001**	999
Hunter River at Casuarina Cnr	2.7417	1	**0.024**	998
Williams River at Seaham Weir	2.0411	1	0.076	998
Hunter River at Raymond Terrace	1.4371	1	0.188	999
Hunter River at Hexam	1.6146	1	0.144	999



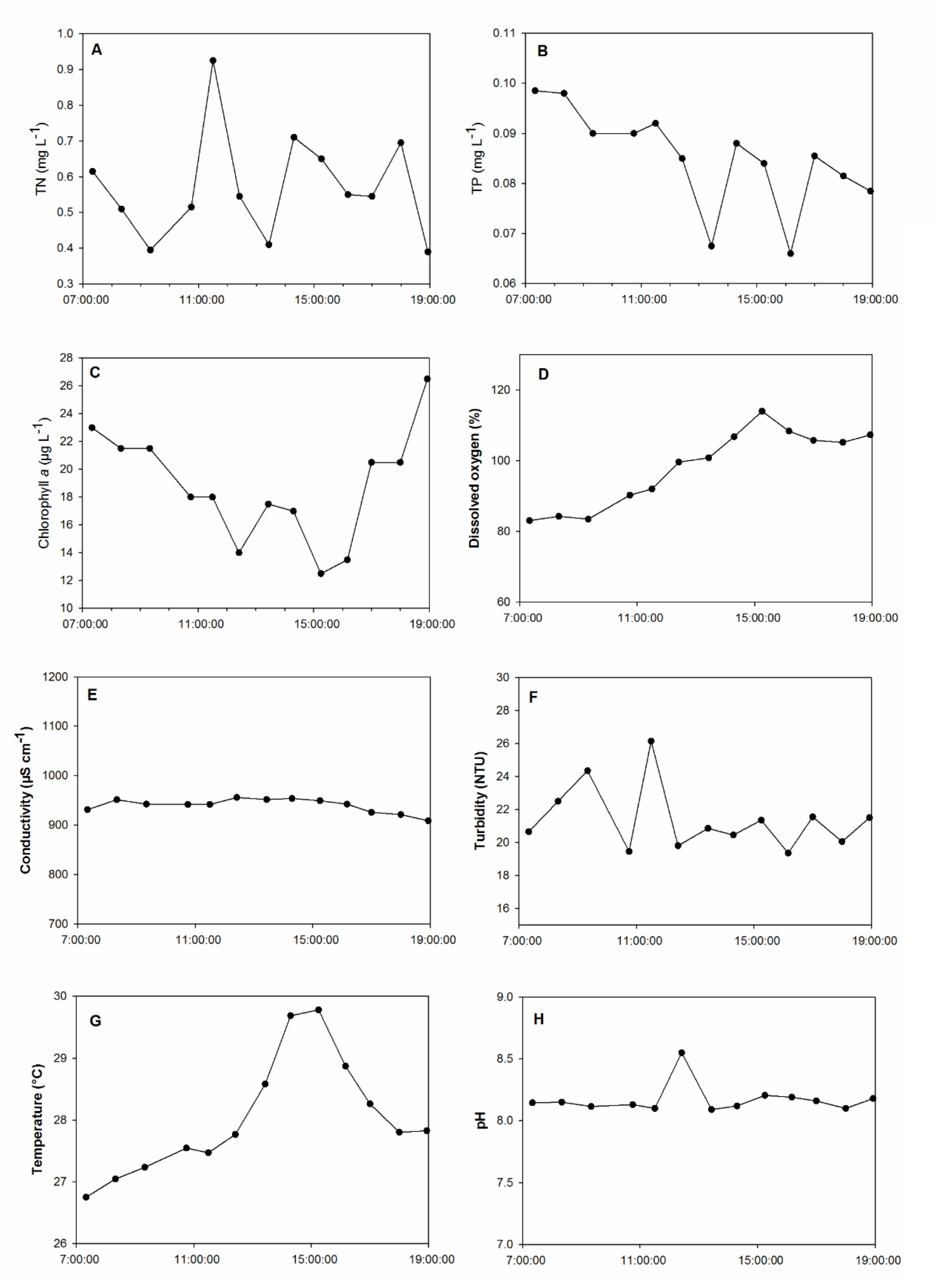
Appendix A Figure 15. Relationships for total nitrogen vs discharge. Where a significant regression was present for the 2010 – 2014 data (solid line) 95% prediction intervals were calculated (dashed lines.). Black circle = monthly data, blue circle = twice weekly data, and red circles = hourly data.



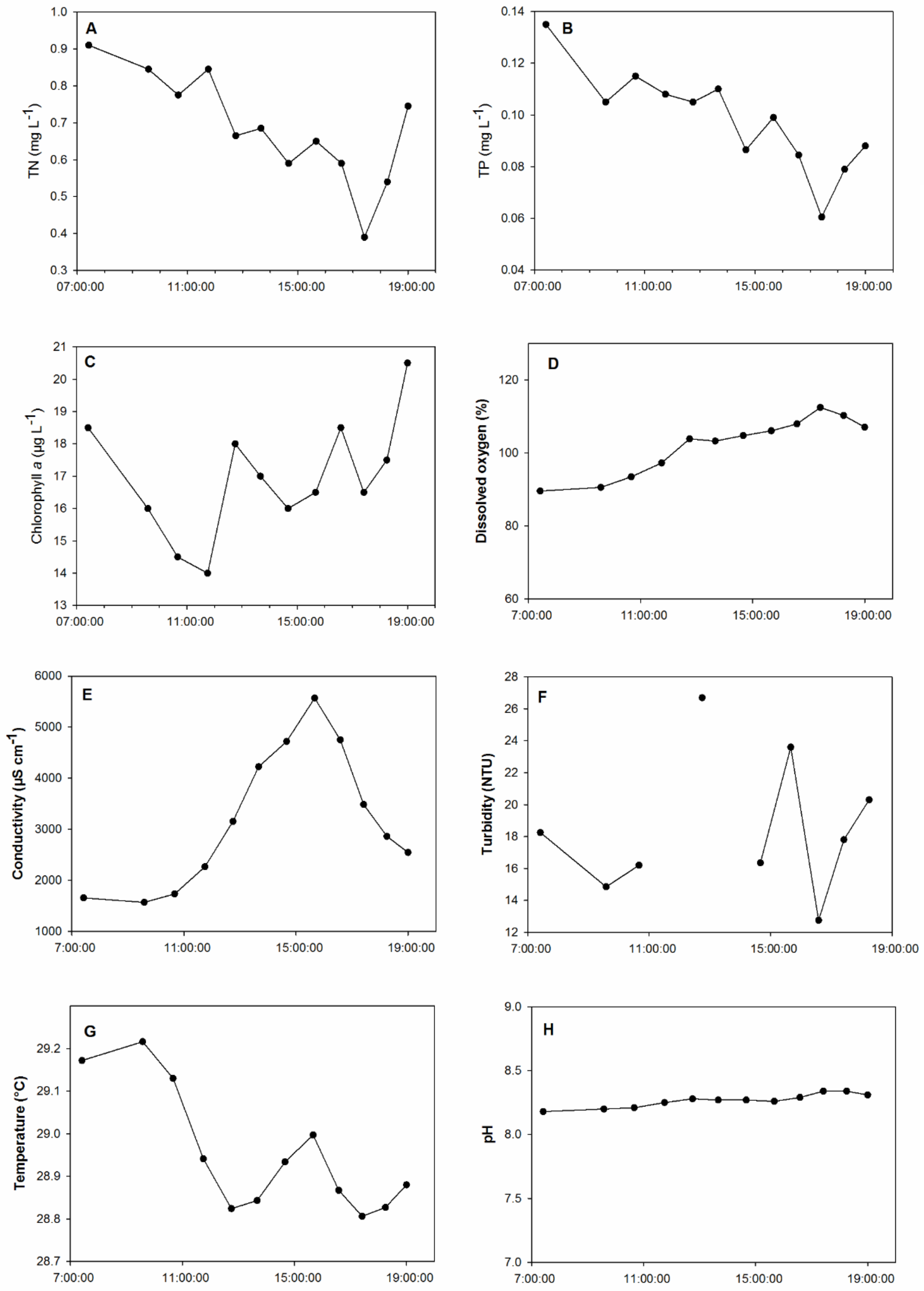
Appendix A Figure 16. Relationships for total phosphorus vs discharge. Where a significant regression was present for the 2010 – 2014 data (solid line) 95% prediction intervals were calculated (dashed lines.). Black circle = monthly data, blue circle = twice weekly data, and red circles = hourly data.



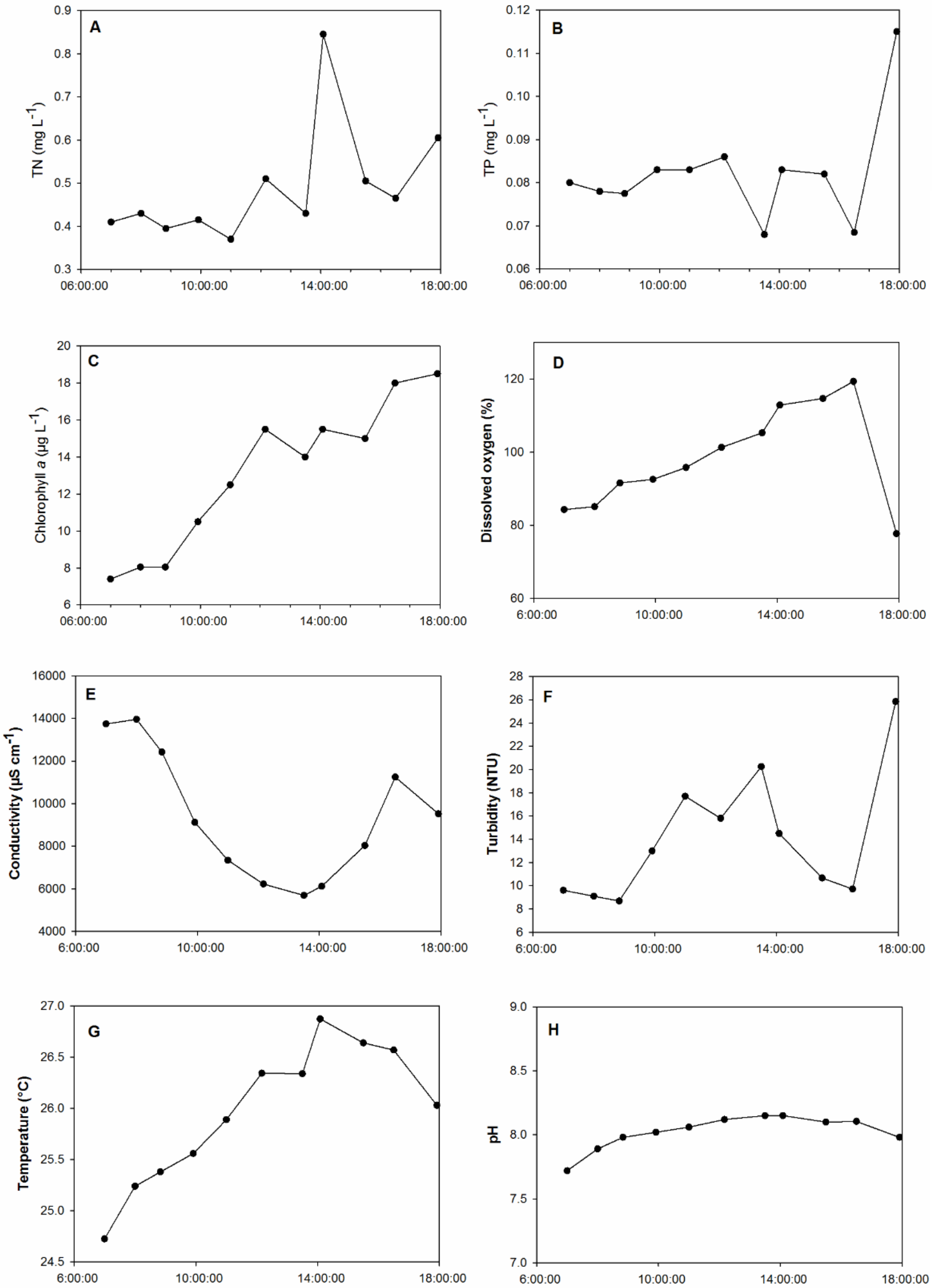
Appendix A Figure 17. Relationships for chlorophyll *a* vs Z_{eu}:Z_m. Where a significant regression was present for the 2010 – 2014 data (solid line) 95% prediction intervals were calculated (red lines.). Black circle = monthly data, blue circle = twice weekly data, and red circles = hourly data.



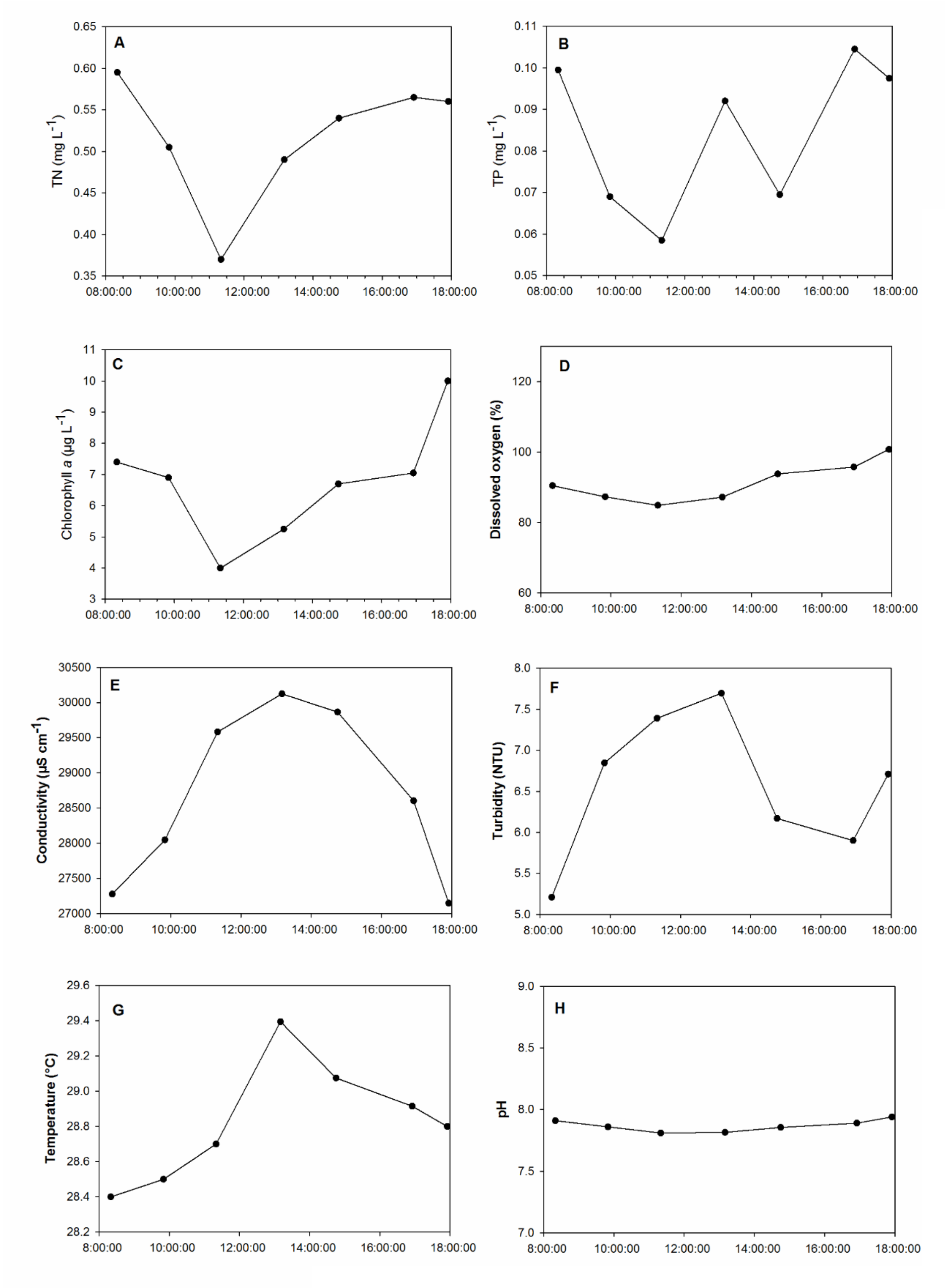
Appendix A Figure 18. Hourly sampling across the tidal cycle for the Hunter River at Morpeth 22 November 2016. A) TN, B) TP, C) Chlorophyll *a*, D) dissolved oxygen, E) Conductivity, F) turbidity, G) temperature, H) pH.



Appendix A Figure 19. Hourly sampling across the tidal cycle for the Hunter River at Morpeth 14 February 2017. A) TN, B) TP, C) Chlorophyll *a*, D) dissolved oxygen, E) Conductivity, F) turbidity, G) temperature, H) pH.



Appendix A Figure 20. Hourly sampling across the tidal cycle for the Hunter River at Raymond Terrace 22 November 2016. A) TN, B) TP, C) Chlorophyll *a*, D) dissolved oxygen, E) Conductivity, F) turbidity, G) temperature, H) pH. NB: during the last hour of sampling there was a large storm.

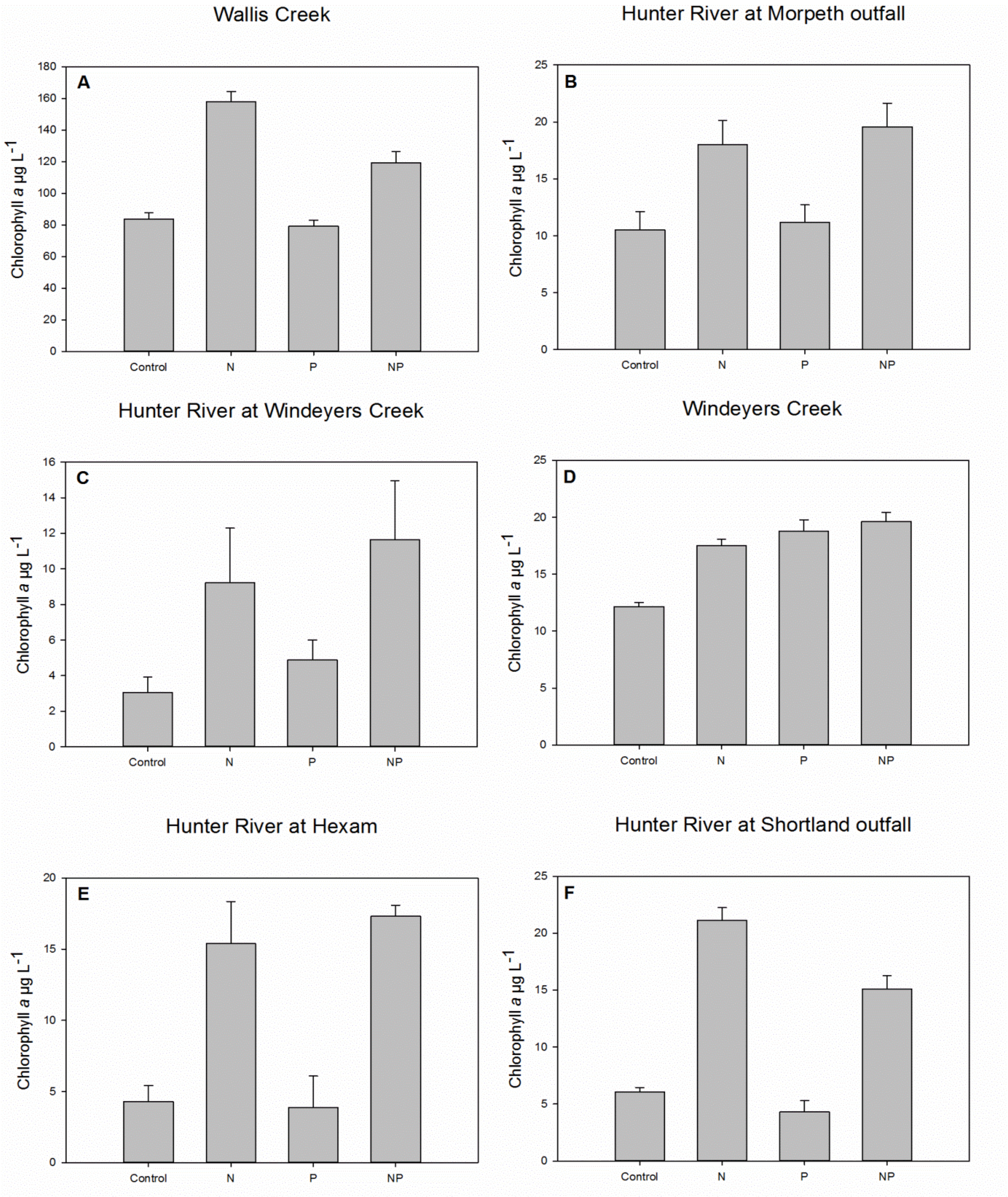


Appendix A Figure 21. Hourly sampling across the tidal cycle for the Hunter River at Raymond Terrace 14 February 2017. A) TN, B) TP, C) Chlorophyll *a*, D) dissolved oxygen, E) Conductivity, F) turbidity, G) temperature, H) pH.

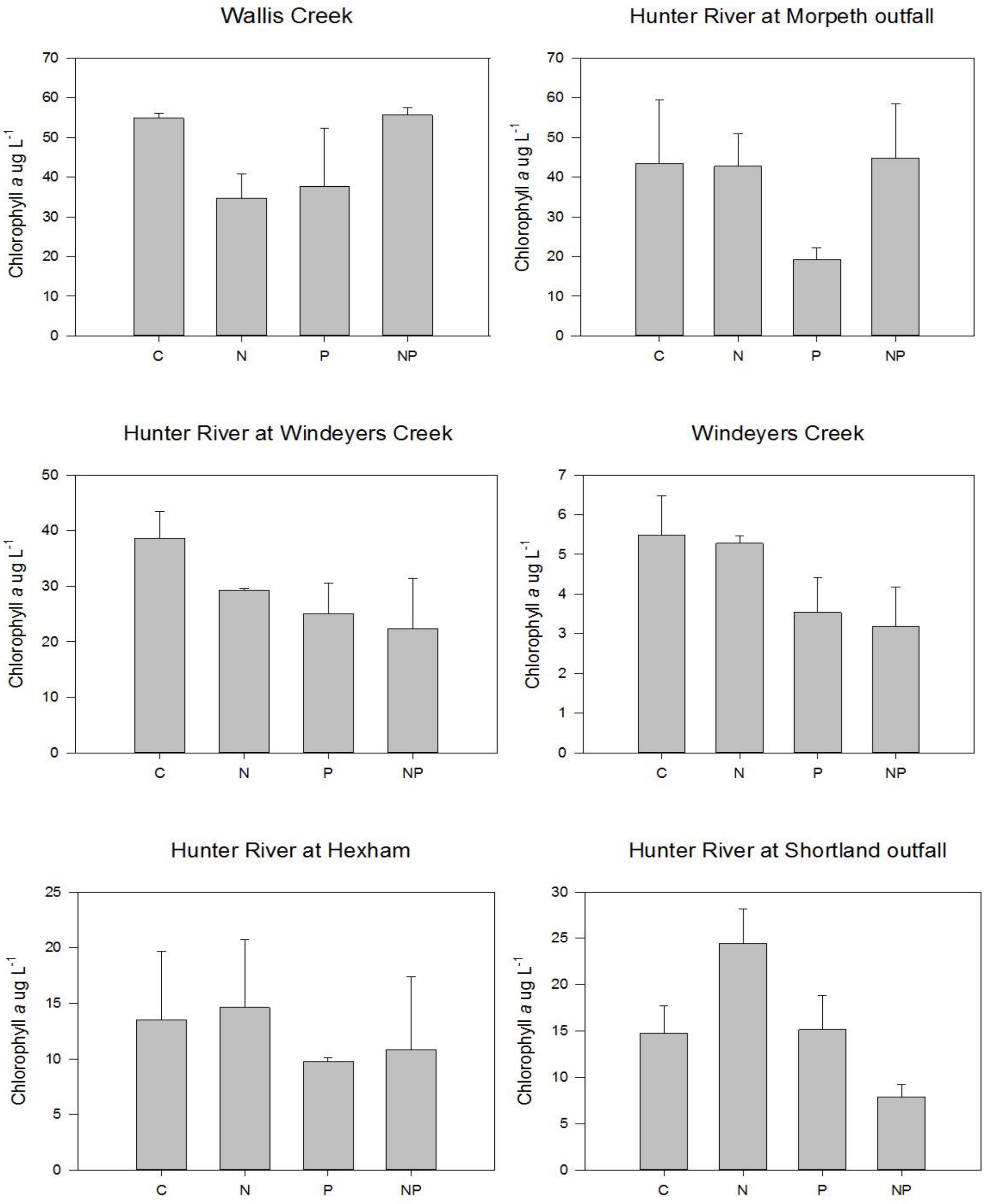
Appendix B Nutrient amendment experiment results

Appendix B Table 1. Permanova results comparing difference between treatments for the nutrient amendment experiments. Where significant differences were present (P-values listed in the bold), pair-wise permanova test was used to determine which treatments were different from the control. *N treatment a significantly higher than NP treatment but not control.

	Summer				Autumn				Winter				Spring			
	Pseud f	**df**	**P**	**Perms**	**Pseud f**	**df**	**P**	**Perms**	**Pseud f**	**df**	**P**	**Perms**	**Pseud f**	**df**	**P**	**Perms**
Hunter River at Morpeth Outfall	5.7383	3	**0.025**	968	1.2577	3	0.358	964	0.9981	3	0.445	968	2.0482	3	0.205	959
Hunter River at Windeyers Creek	2.1486	3	0.198	971	1.5158	3	0.306	967	2.0535	3	0.166	966	2.4327	3	0.121	968
Hunter River at Hexam	7.4015	3	**0.01**	963	0.20513	3	0.89	969	171.44	3	**0.002**	968	4.4513	3	**0.05**	942
Hunter River at Shortland Outfall	24.334	3	**0.001**	945	5.3045	3	**0.026***	964	8.8458	3	**0.006**	969	1.0572	3	0.438	961
Wallis Creek	28.559	3	**0.002**	968	1.9824	3	0.168	937	4.3393	3	0.051	972	14.075	3	**0.002**	961
Windeyers Creek	19.344	3	**0.001**	964	1.9621	3	0.183	969	0.9267	3	0.462	952	0.6084	3	0.641	969



Appendix B Figure 1. Chlorophyll *a* results for summer nutrient amendment experiments. Error bars are standard error.



Wallis Creek

Hunter River at Morpeth outfall

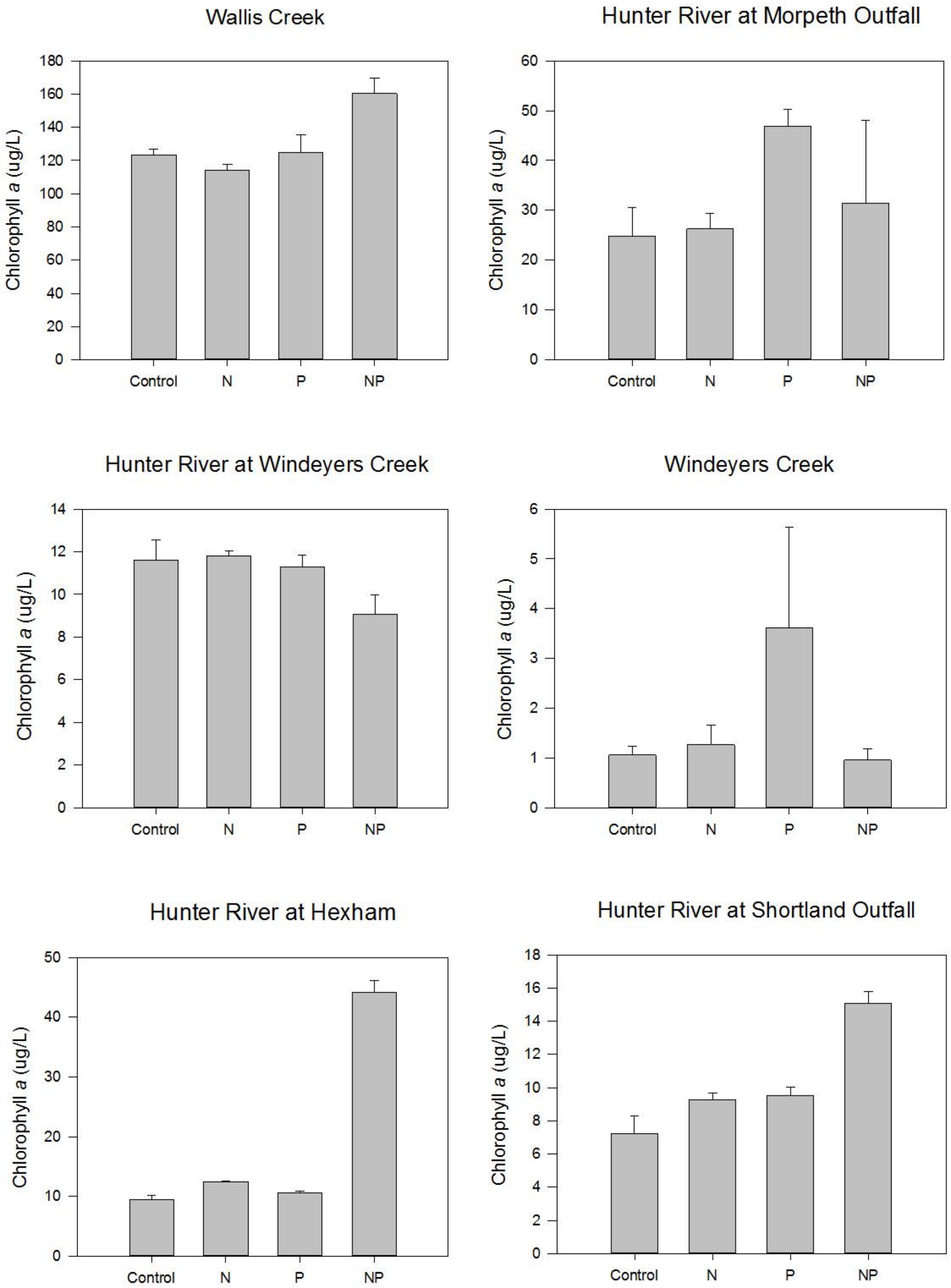
Hunter River at Windeyers Creek

Windeyers Creek

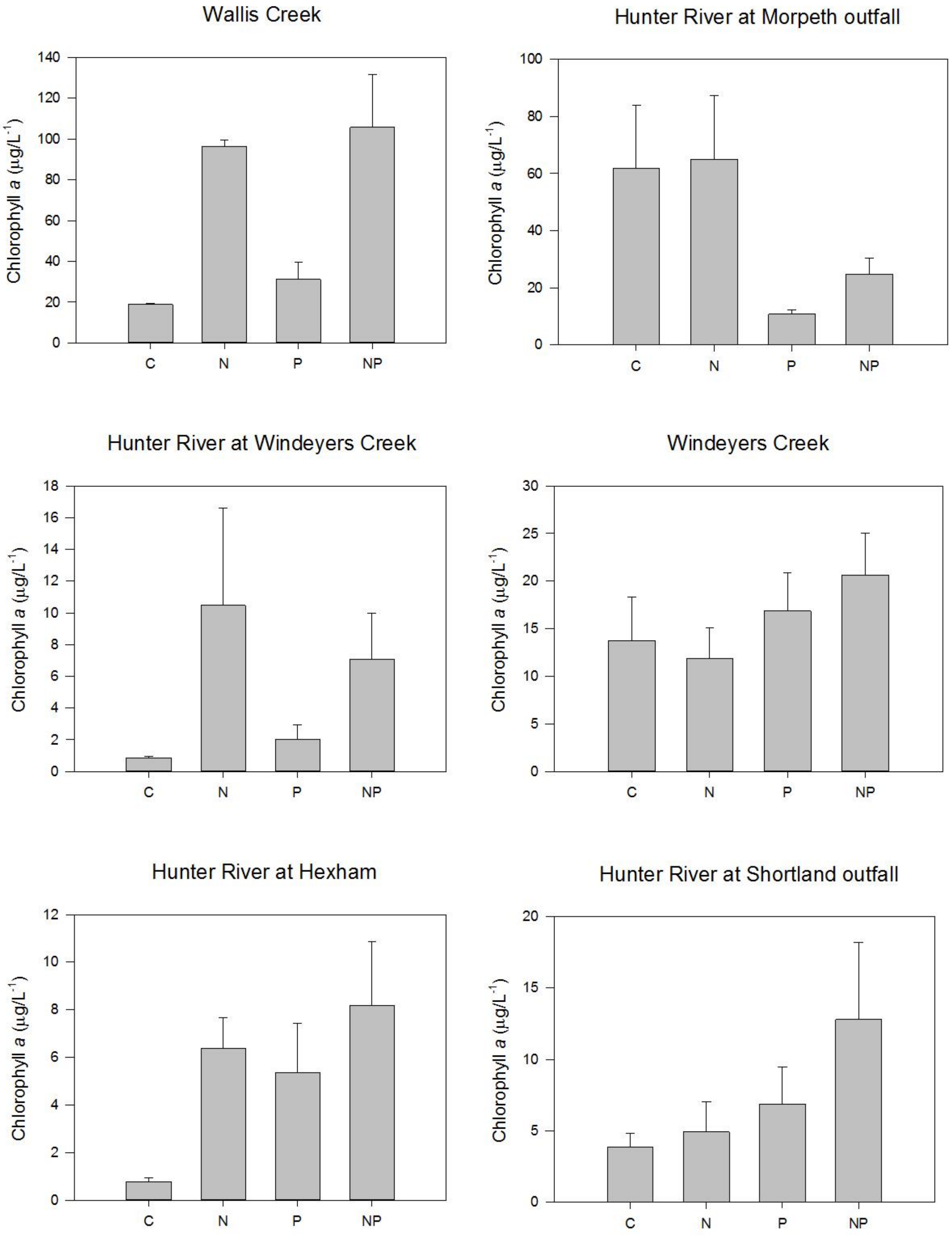
Hunter River at Hexham

Hunter River at Shortland outfall

Appendix B Figure 2. Chlorophyll *a* results for the autumn nutrient amendment experiments. Error bars are standard error.



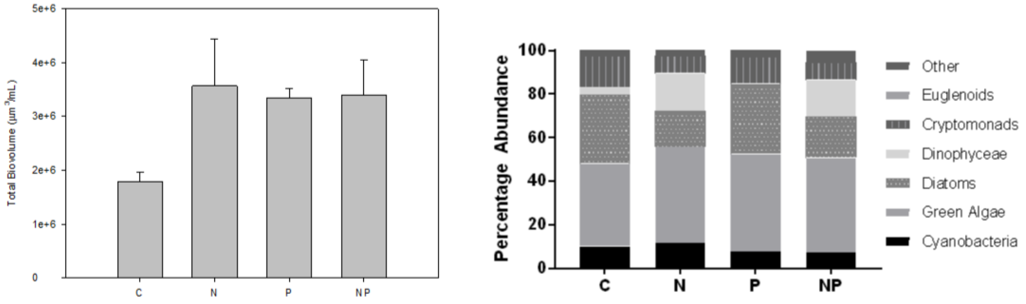
Appendix B Figure 3. Chlorophyll *a* results for the winter nutrient amendment experiments. Error bars are standard error.



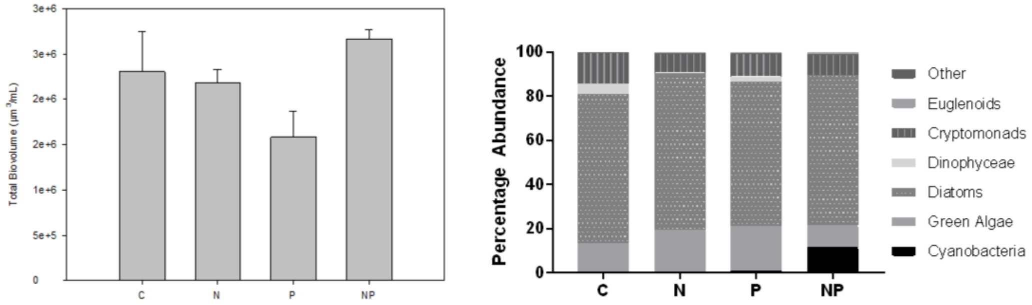
Appendix B Figure 4. Chlorophyll *a* for the spring nutrient amendment experiments. Error bars are standard error.

Hunter River at Morpeth Outfall

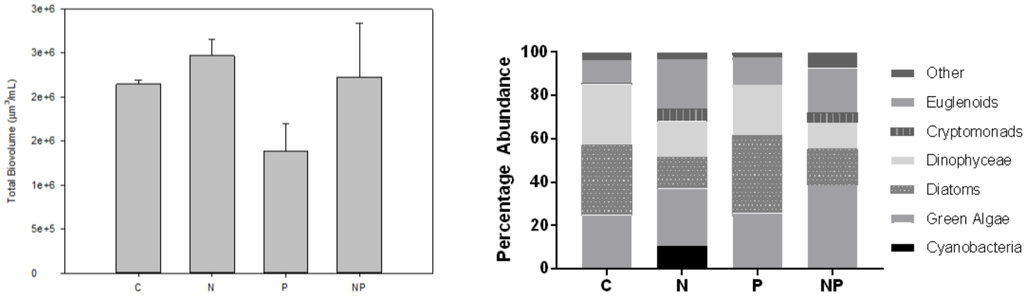
Summer



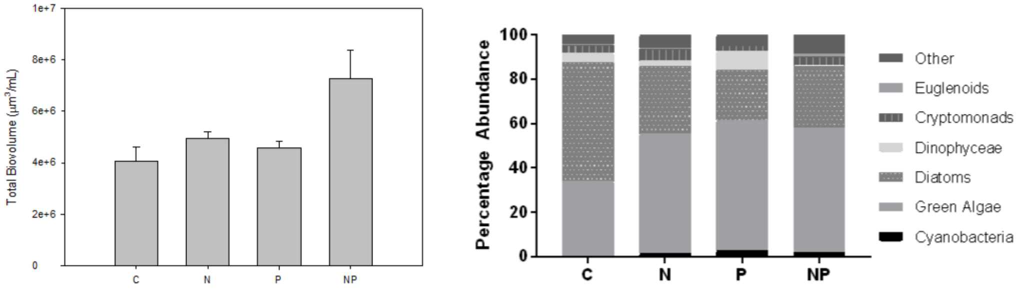
Autumn



Winter



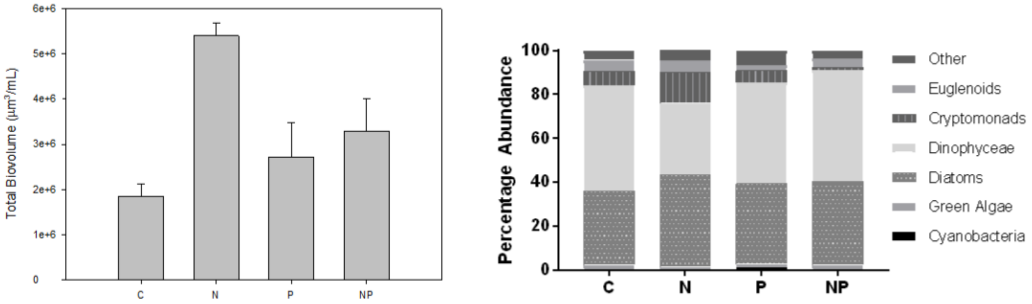
Spring



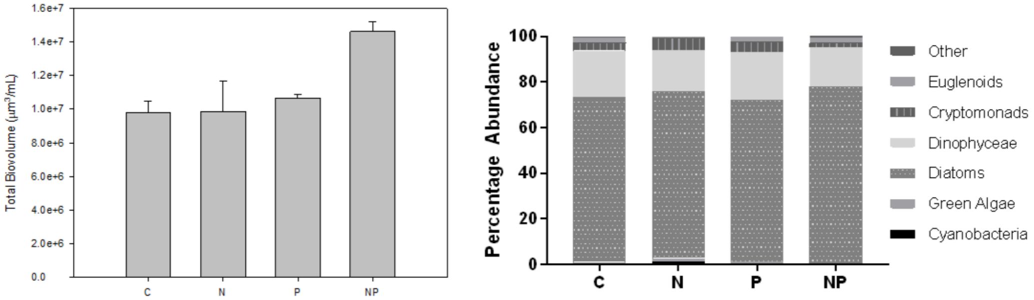
Appendix B Figure 5. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for the Hunter River at Morpeth. Error bars are standard error.

Hunter River at Windeyers Creek

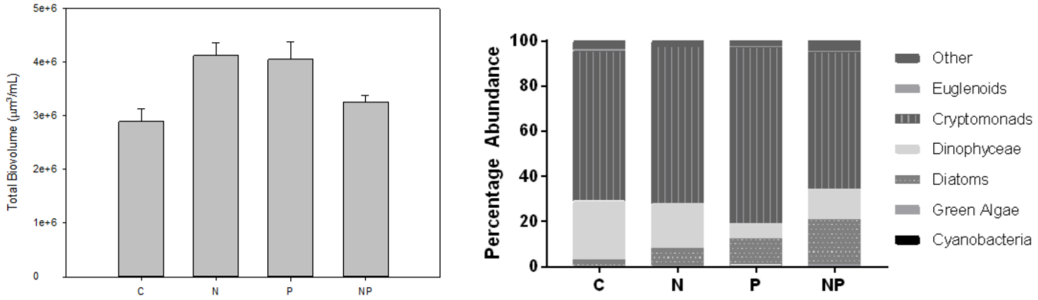
Summer



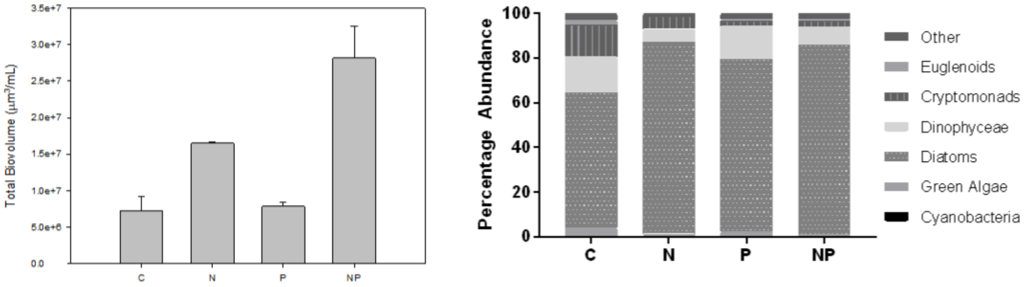
Autumn



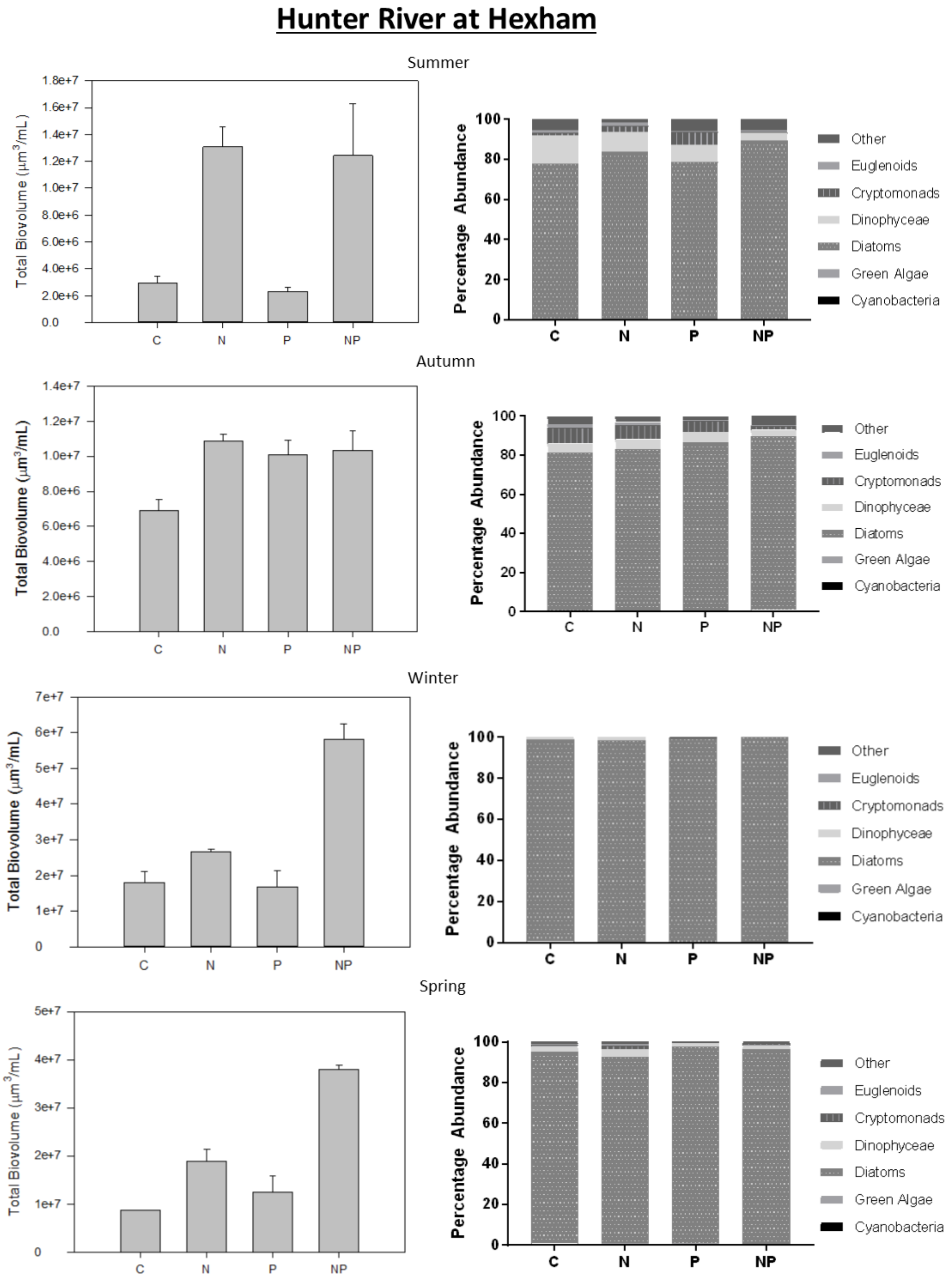
Winter



Spring

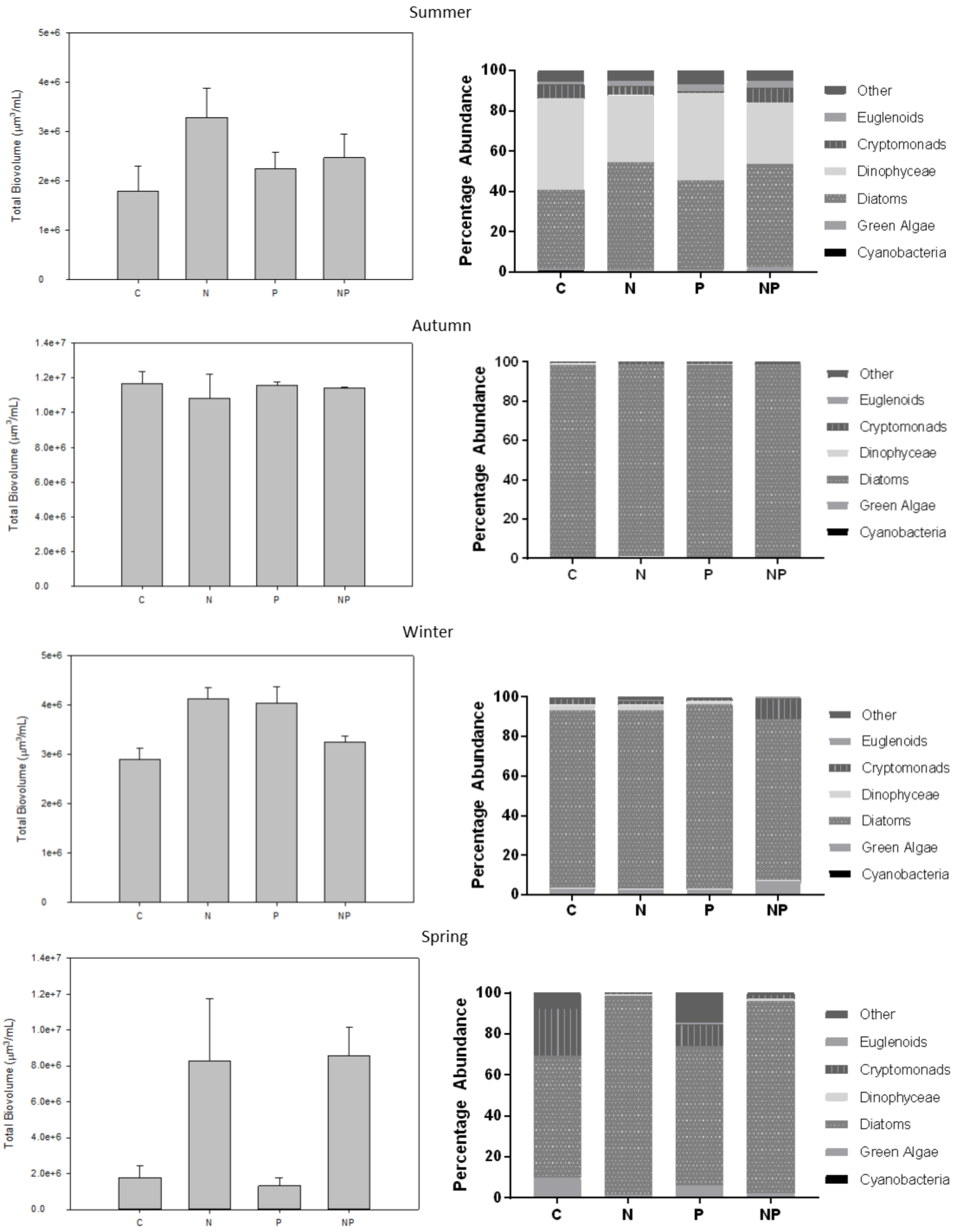


Appendix B Figure 6. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for the Hunter River at Windeyers Creek. Error bars are standard error.



Appendix B Figure 7. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for the Hunter River at Hexam. Error bars are standard error.

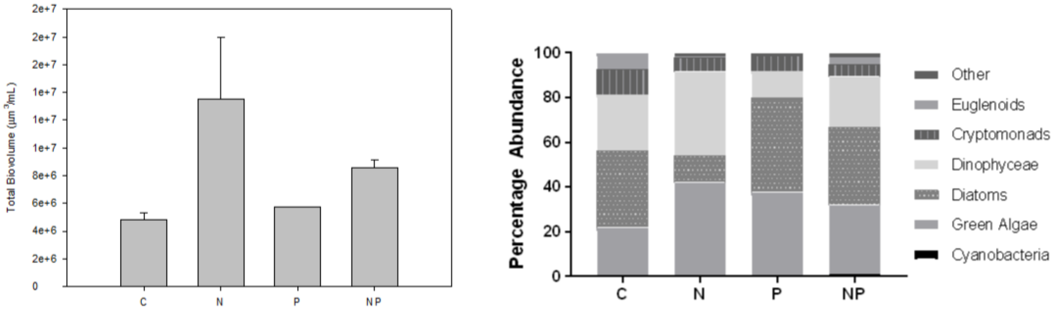
Hunter River at Shortland Outfall



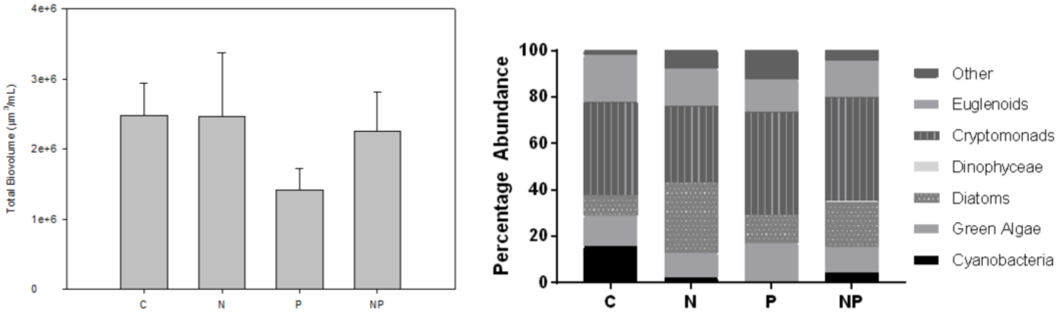
Appendix B Figure 8. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for the Hunter River at Shortland. Error bars are standard error.

Wallis Creek

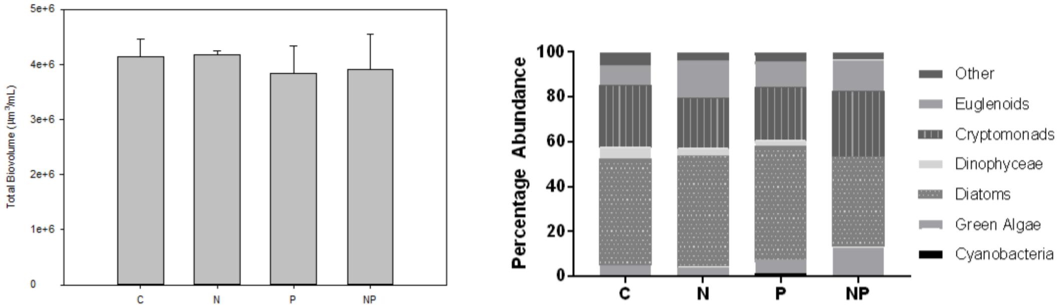
Summer



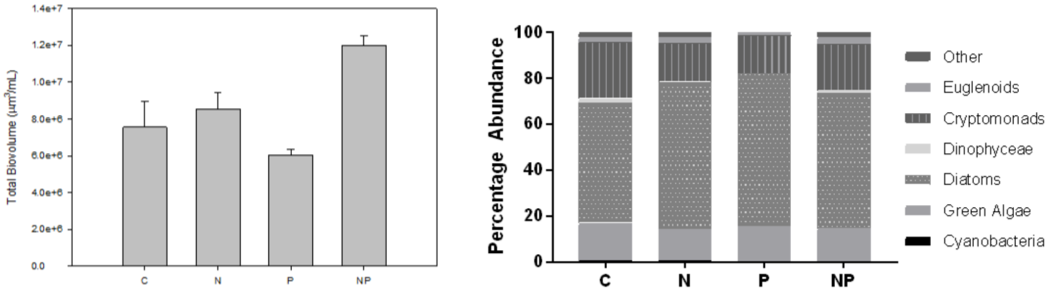
Autumn



Winter

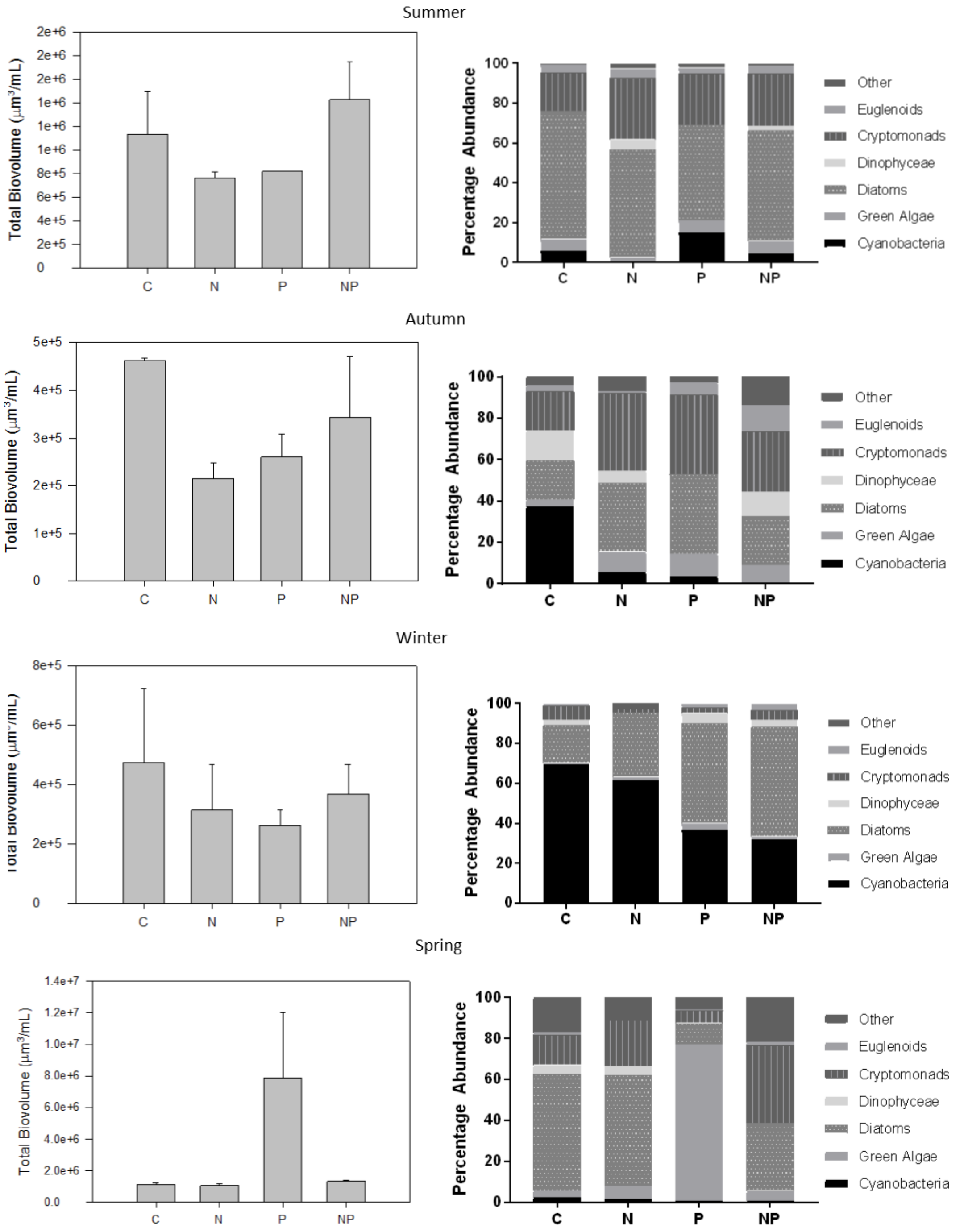


Spring



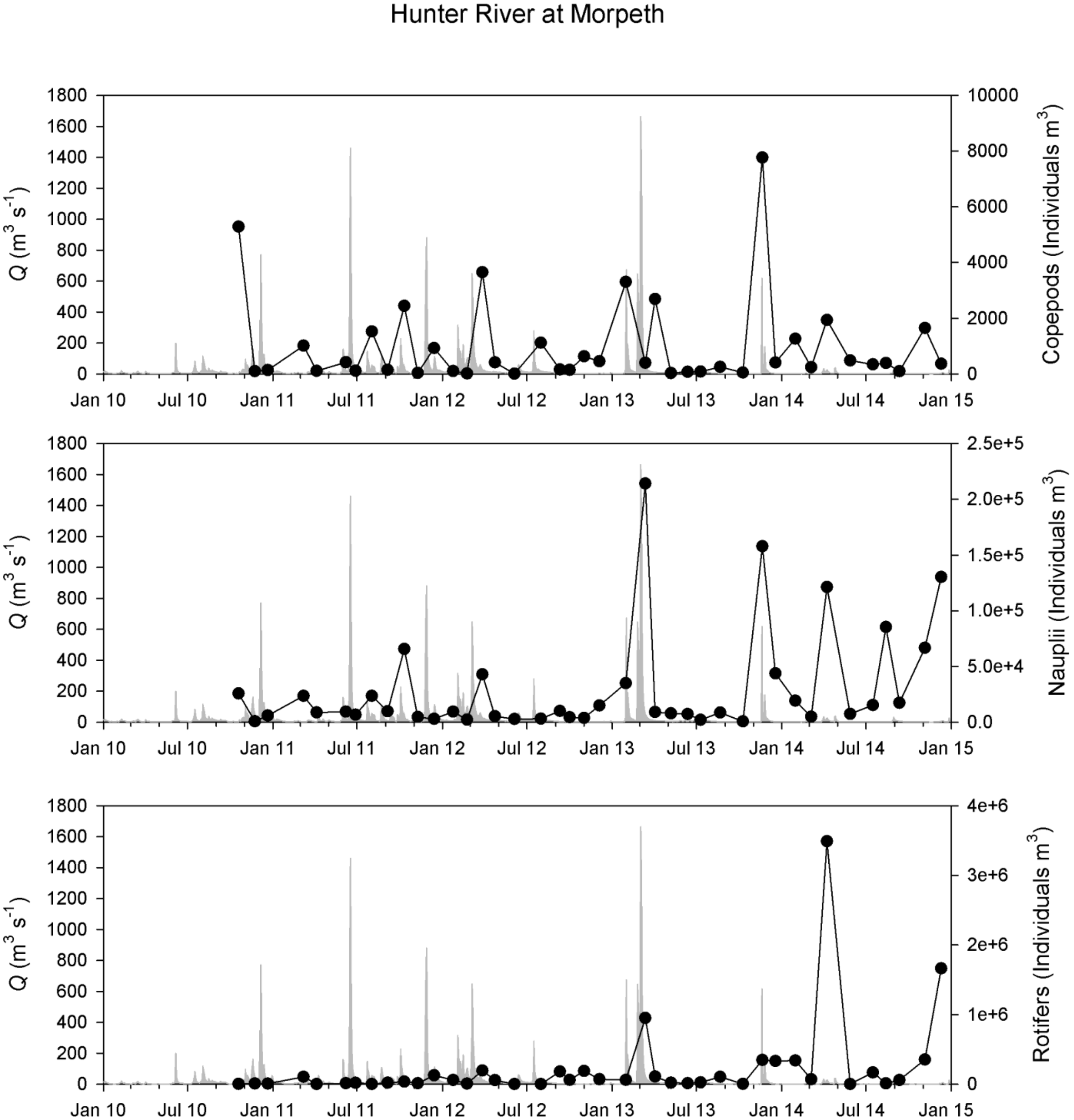
Appendix B Figure 9. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for Wallis Creek. Error bars are standard error.

Windeyers Creek

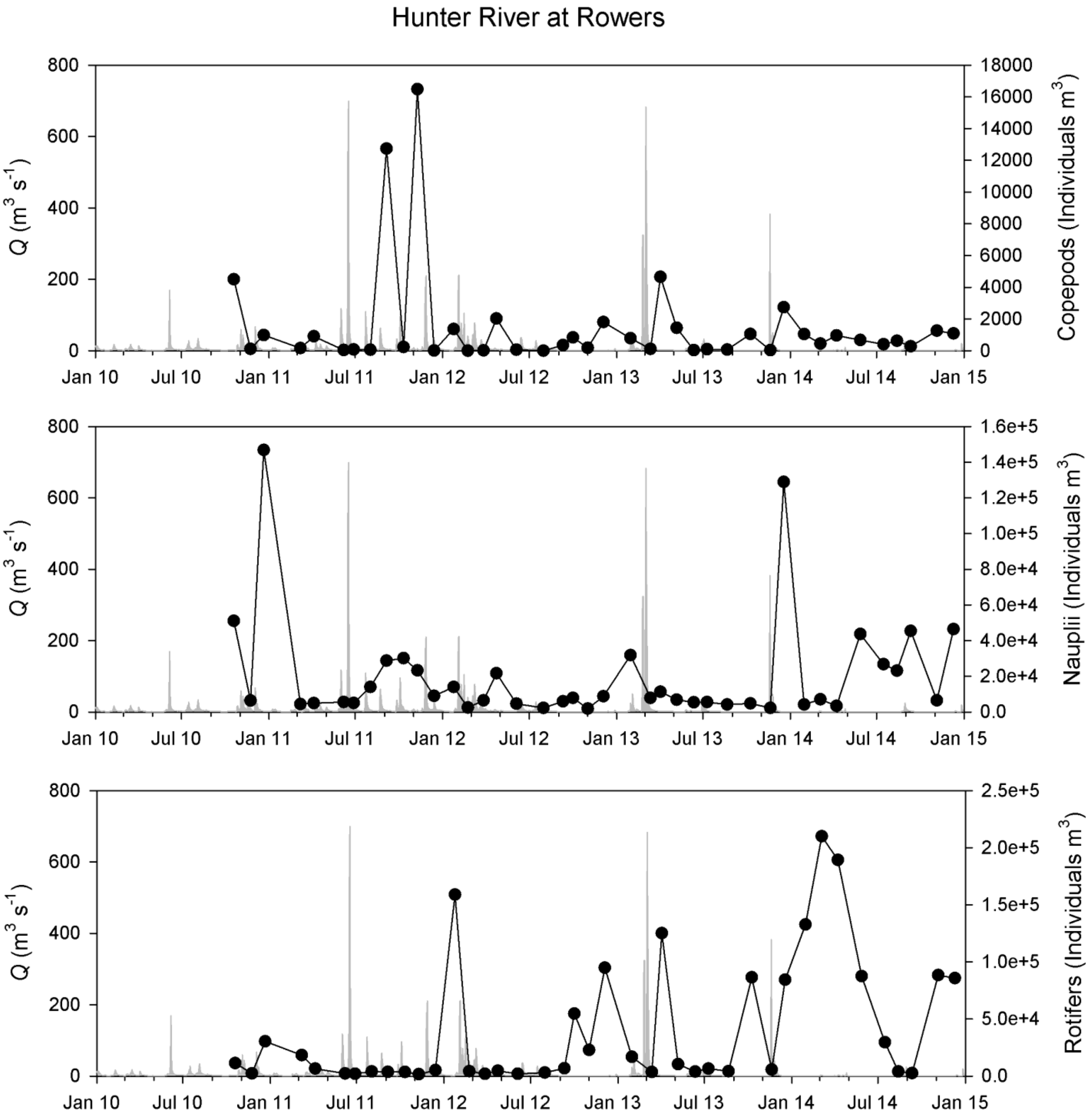


Appendix B Figure 10. Phytoplankton biomass and relative abundance during the seasonal nutrient amendment experiments for Windeyers Creek. Error bars are standard error.

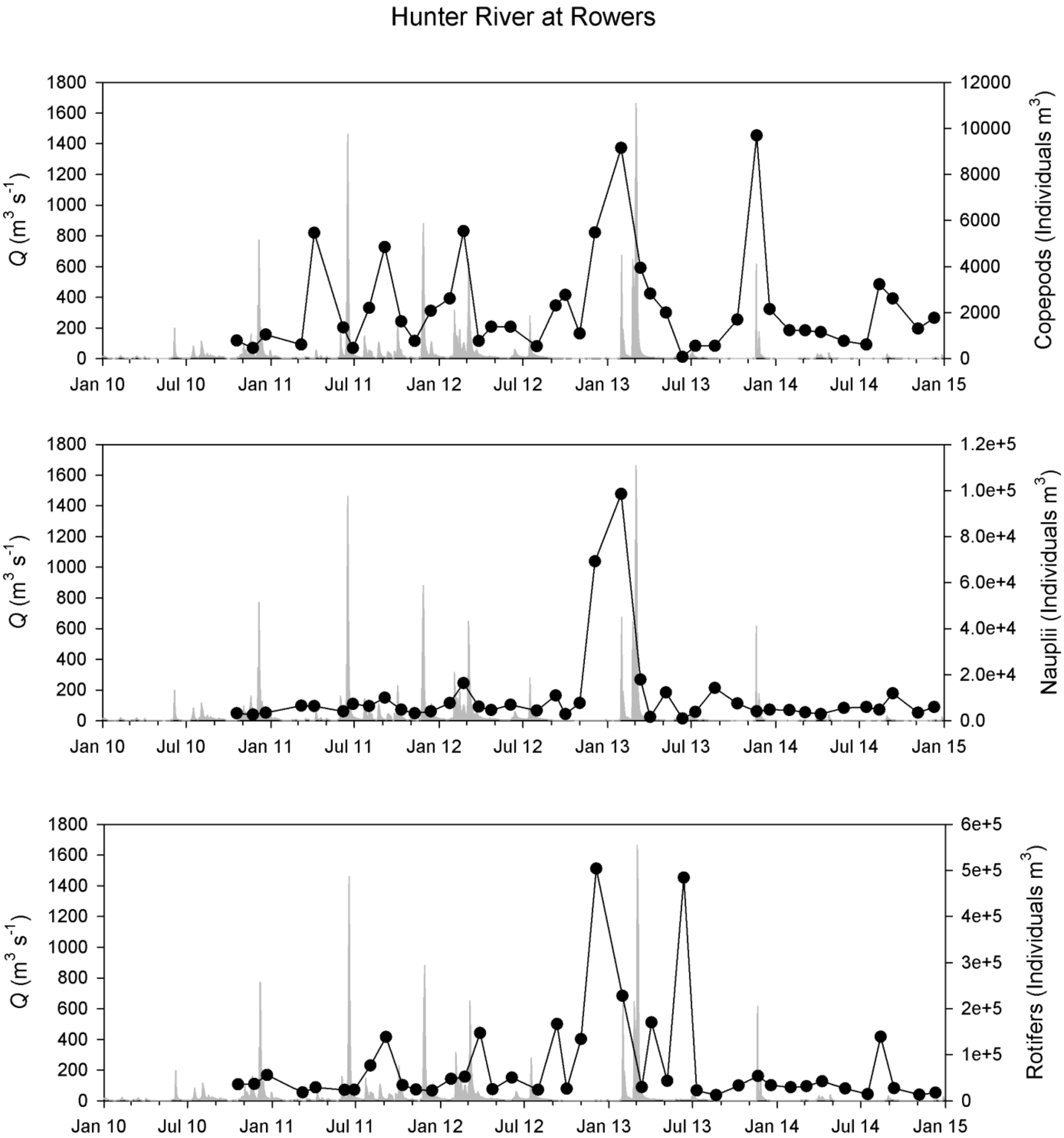
Appendix C Zooplankton results



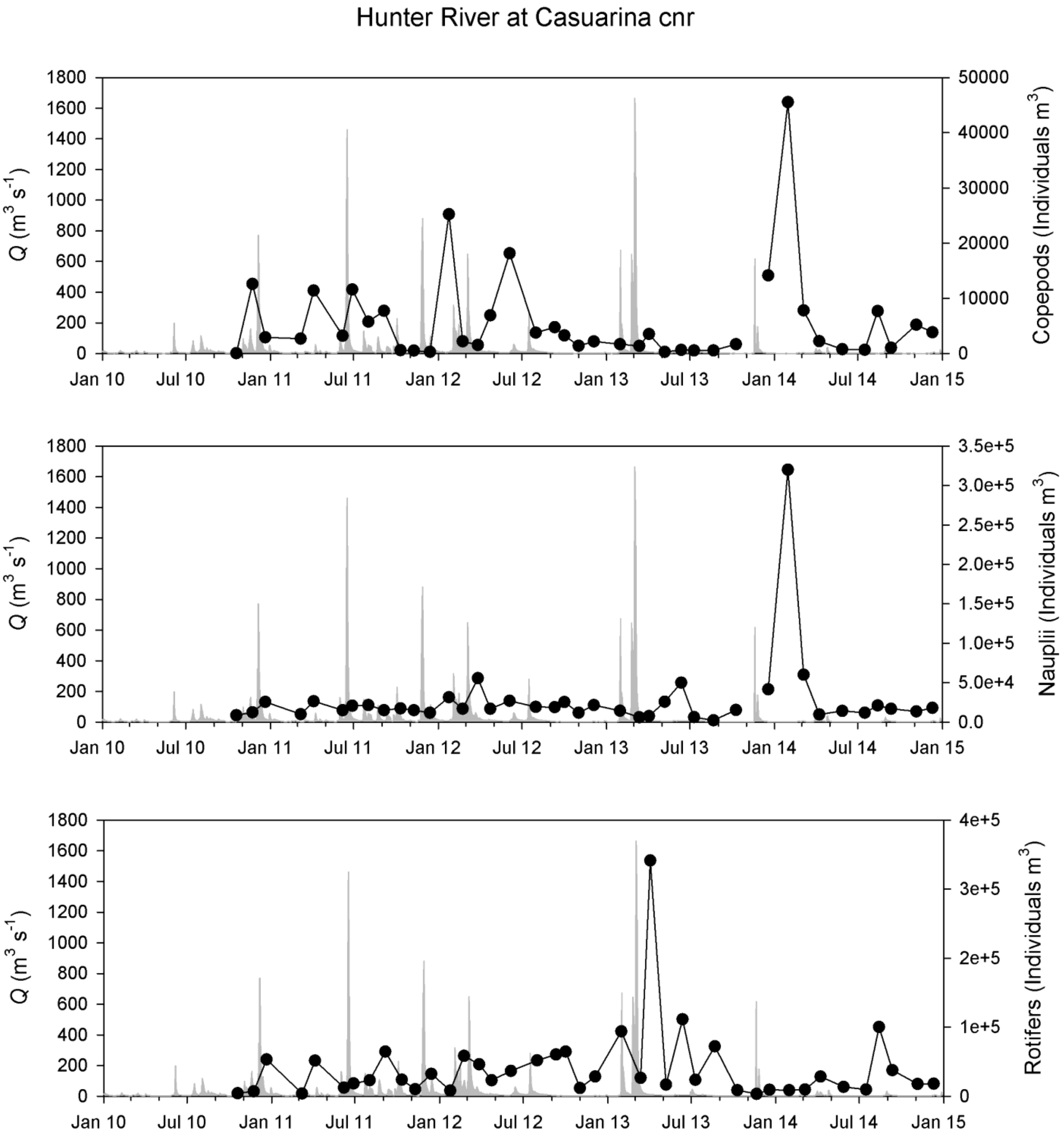
Appendix C Figure 1. Major zooplankton group abundance for the Hunter River at Morpeth. Discharge values are combined data for gauging stations on the Hunter River at Greta and Paterson River at Gostwyck.



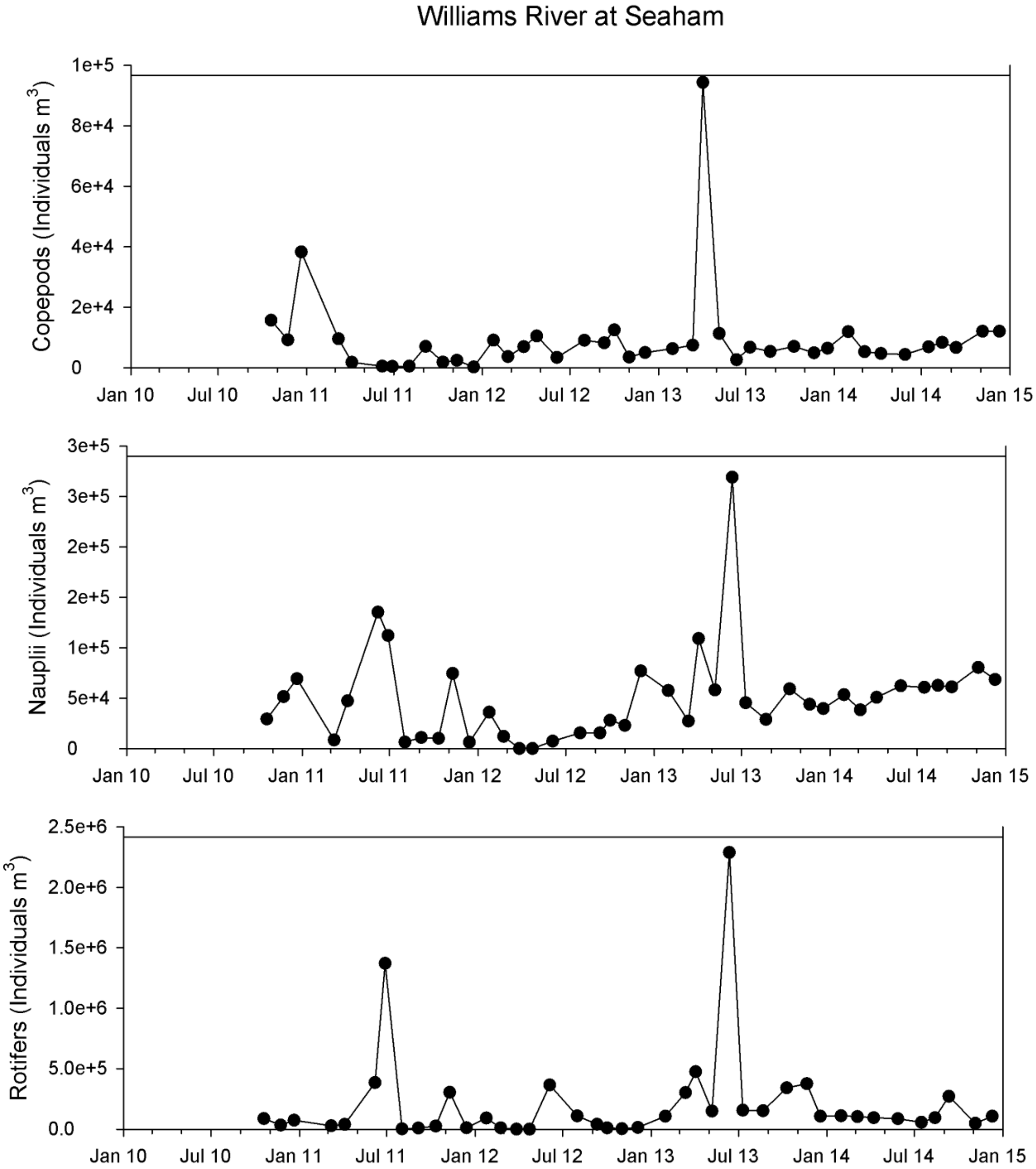
Appendix C Figure 2. Major zooplankton group abundance for the Paterson River at Dunmore Bridge. Discharge values are combined data for the gauging station on the Paterson River at Gostwyck.



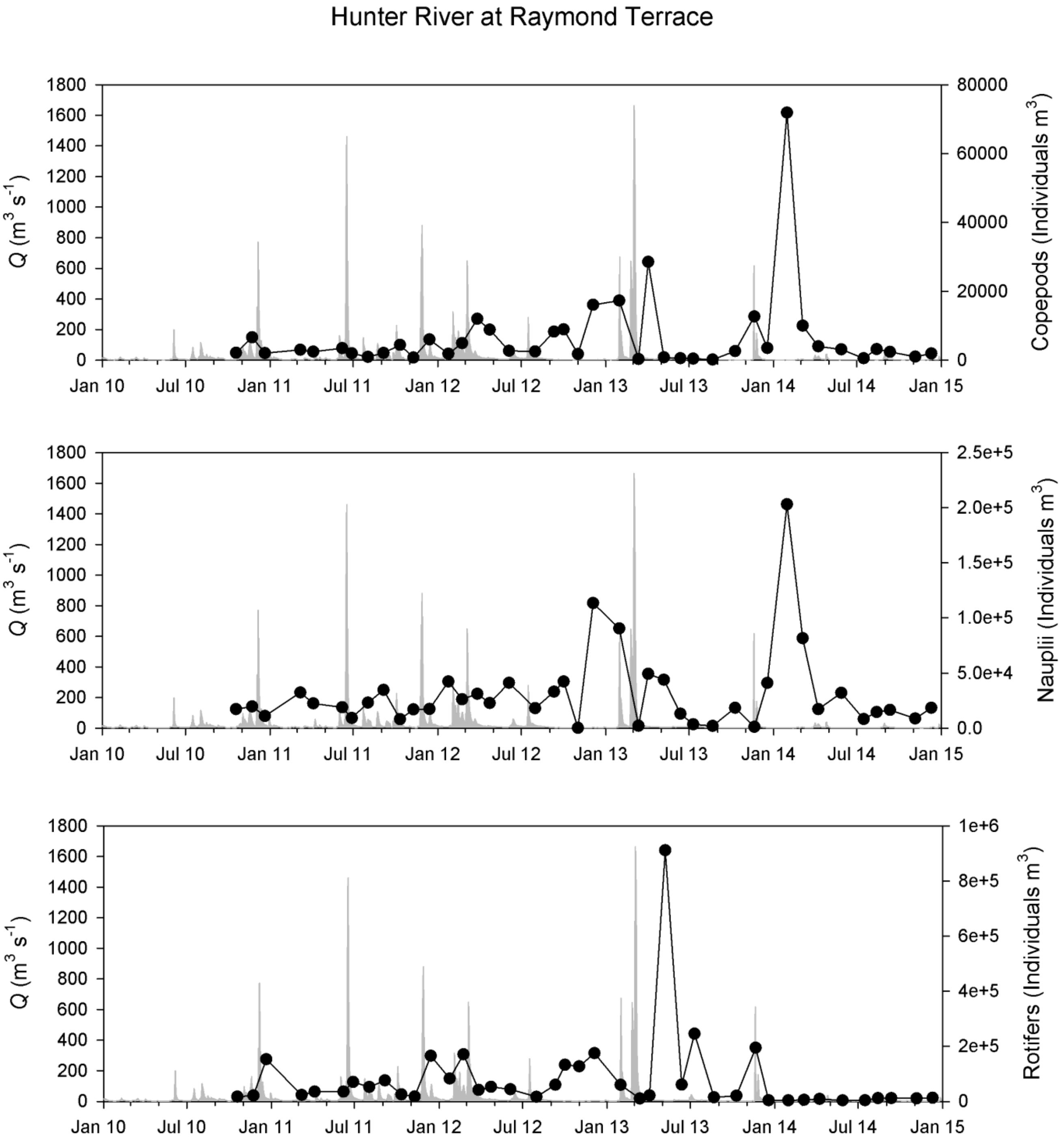
Appendix C Figure 3. Major zooplankton group abundance for the Hunter River at Rowers Club. Discharge values are combined data for gauging stations on the Hunter River at Greta and Paterson River at Gostwyck.


Hunter River at Casuarina cnr

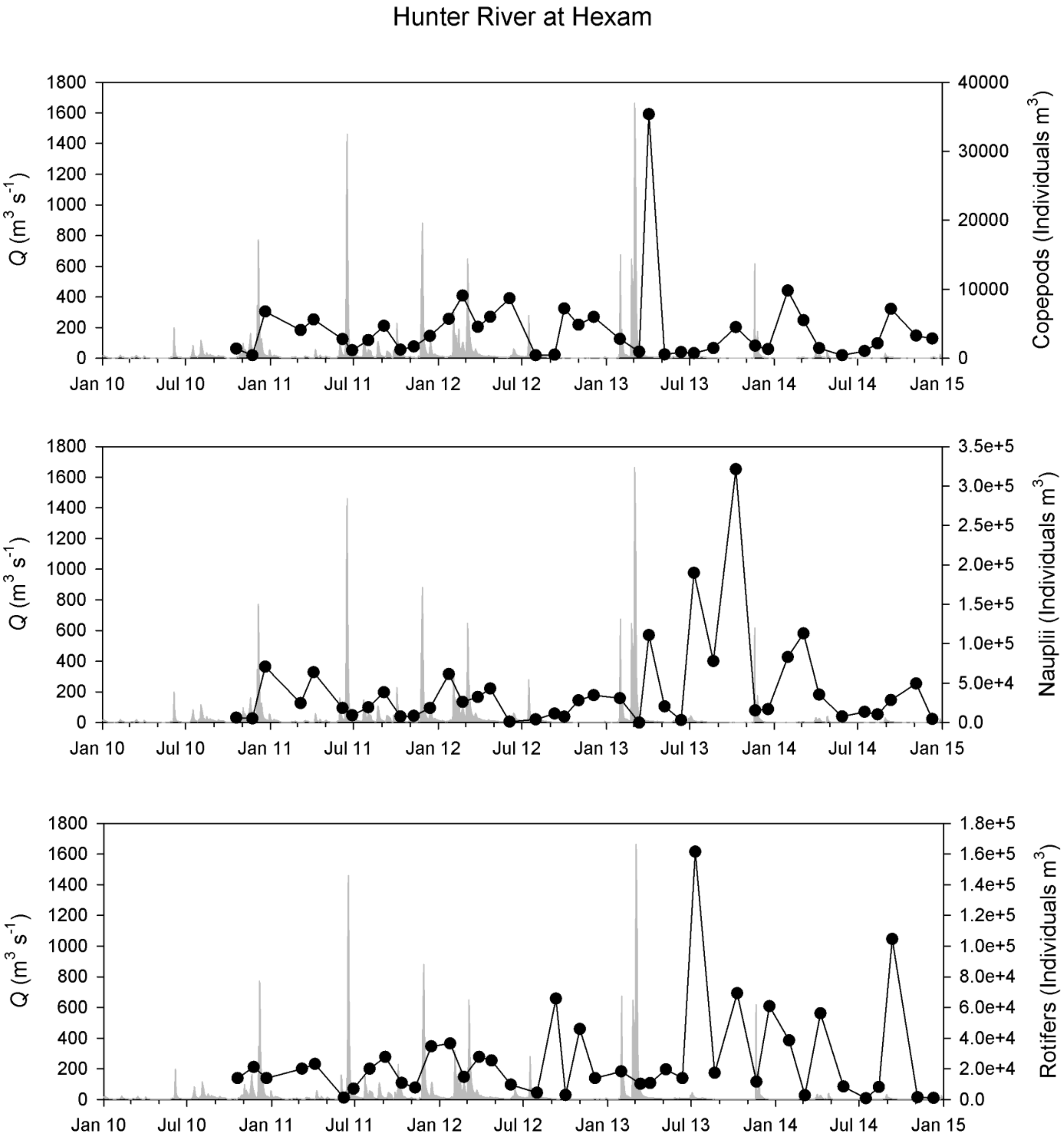
Appendix C Figure 4. Major zooplankton group abundance for the Hunter River at Casuarina Cnr. Discharge values are combined data for gauging stations on the Hunter River at Greta and Paterson River at Gostwyck.



Appendix C Figure 5. Major zooplankton group abundance for the Williams River at Seaham.



Appendix C Figure 6. Major zooplankton group abundance for the Hunter River at Raymond Terrace. Discharge values are combined data for gauging stations on the Hunter River at Greta and Paterson River at Gostwyck.



Appendix C Figure 7. Major zooplankton group abundance for the Hunter River at Hexam. Discharge values are combined data for gauging stations on the Hunter River at Greta and Paterson River at Gostwyck.