The Differential Impact of Eutrophication Along Three Prince Edward Island Estuaries on the Biotic and Physiochemical Characteristics of Sediment

Jerrica Cormier
Department of Biology
University of Prince Edward Island
Charlottetown, PE, Canada

A thesis submitted in partial fulfilment of the requirements of the Honours Program in the Department of Biology

This Thesis is

_____

Accepted

Dean of Science
University of Prince Edward Island

May 2018

ABSTRACT

Estuaries are a diverse habitat characterized by the transition from fresh to salt water and are influenced by the activities on adjacent land. The excess nutrients that enter the estuary resulting from agriculture (a prominent business on Prince Edward Island (PEI)) result in eutrophication – the overgrowth of plants. The occurrence of eutrophication can greatly impact both the faunal and floral communities, as well as the physiochemical properties of the sediment. For this study, sediment samples were collected from three PEI estuaries (Wheatley, Wilmot, and Souris) every two-three weeks throughout the Summer of 2016. For each sample carbon content was determined and DNA was extracted. Additionally, sediment from the Trout-Stanley estuary was collected and the production of ammonium was monitored under both oxic and hypoxic conditions. The objectives of this experiment were to determine the physiochemical and biotic differences along estuary gradients in relation to eutrophication, as well as to compare the patterns of ammonium production under oxic and hypoxic conditions. Riverine estuary regions were more seriously influenced by eutrophication compared to lower regions, and ammonium production was highest under hypoxic conditions compared to oxic conditions. Additionally, both organic and inorganic carbon, as well as DNA concentrations correlated positively.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor, Dr. Mike van den Heuvel, for taking me on as an honours student, sharing is knowledge, and giving me this opportunity to advance my research and laboratory skills in biology.

I would also like to thank Dr. Larry Hale for agreeing to be the second member of my supervisory committee. He was supportive and very approachable.

PhD candidate Kyle Knysh also deserves acknowledgement for taking the time to answer my never-ending questions, for showing me how to perform lab techniques as needed, and for lending a helping hand. Overall Kyle gave me guidance and direction throughout my project, and was always willing to read over and edit my work before submission.

Additionally, I would like to thank the numerous people who assisted me throughout my honours experience including Christina, for ordering supplies when I ran out and answering questions, Carissa, for lending a helping hand, and Travis, for taking the time to walk me through the DNA extraction technique in the lab. Lastly, I would like to thank my friend Courtney for moral support throughout my honours experience, as well as my family for always stressing the importance of education, for supporting me in everything I do, and for always encouraging me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The shores of estuaries tend to be highly populated by humans, making them vulnerable to negative anthropogenic influences (Lotze et al. 2006). Agricultural practices are one anthropogenic influence that significantly alter land structure relative to natural vegetation growth, impacting surrounding aquatic ecosystems in a variety of ways. For example, in Prince Edward Island, approximately 42% of land is used for agriculture (PEI Department of Agriculture and Fisheries, 2015). With Prince Edward Island's high proportion of agricultural land and small watersheds, the potential for agricultural impacts on those estuaries is high. Agricultural practices have been shown to be correlated with a phenomenon known as eutrophication (Kolenbrander 1971; Carpenter 2005; Andersen et al. 2014; Tromboni and Dodds 2017). Eutrophication by agricultural land use is caused by an excess of nutrients entering the estuary, primarily as a result of fertilizer usage and manure, first leading to an overgrowth of plants such as macro-algae, and often leading to a lack of dissolved oxygen in affected estuaries known as hypoxia, or, in extreme cases, anoxia where excess organic material increases biochemical oxygen demand (Bugden et al. 2014). Nutrient increases in estuaries and the resulting oxygen depletion change the physiochemical and biotic characteristics within the estuary, and of particular importance to this study: within sediment. Eutrophication often leads to an accumulation of organic material in sediments when living organisms die and settle to the bottom. This can be useful for examining biodiversity since deposition rates and species diversity can be

tracked by collecting and analyzing samples of water and sediment by molecular means (i.e. eDNA analysis). The ability to re-release nutrients back into the water, further adding to eutrophication, can be determined through sediment incubation experiments.

It is possible to analyze species diversity because estuarine sediment contains a historical record of organisms that live in both the water column, and the sediment itself. Recently, environmental DNA (eDNA) techniques have been used in place of traditional means of biodiversity monitoring. DNA can be extracted from materials such as sediment and sequenced in order to classify the species that may be present, or have been present in the past (Bohan et al. 2017). Additional parameters gathered from sediment such as organic carbon within sediment can also provide information on the amount of overall primary production in an estuary (Downing et al. 2008).

Microorganisms within the sediment play a large role in nutrient cycling. Remineralization occurs when bacteria break down the organic matter in sediment and release inorganic nutrients ammonium and phosphate. Sulphate reducing bacteria in marine systems are known to dominate under hypoxic conditions and are thought to perform key steps in the remineralization of phosphate and ammonium (Sinkko et al. 2013). The production of ammonium can be a significant addition to the overall load of nutrients to an estuary which would further accelerate eutrophication, making it an influential factor worth studying. Plants can use ammonium as a source of nitrogen for growth, leading to high oxygen production during photosynthesis, low oxygen during respiration and eventually, anaerobic decomposition as plants die off.

The aim of this research was to compare and contrast the impact of eutrophication along estuaries on the biotic and physiochemical characteristics of sediment found in

Prince Edward Island (PEI), Canada. It was predicted that areas receiving higher nutrient input (i.e. riverine areas) would reflect this occurrence by having increased levels of organic carbon in the sediment (Hopkinson et al. 1999). Due to the higher levels of organic matter predicted in the riverine regions of estuaries, it was predicted that DNA concentrations would also be higher in those areas. Inorganic carbon on the other hand was predicted to be slightly higher at the mid-estuary region, where shelled organisms (and therefore carbonate) are more abundant. In general, communities were expected to change along the salinity gradient. Finally, ammonium production measured using sediment from the Trout-Stanley river, PEI was predicted to be higher under hypoxic conditions as opposed to oxic conditions based on the efficiency of anaerobic microbes (particularly sulfate-reducing bacteria).

LITERATURE REVIEW

Nutrient Enrichment

Nutrient enrichment refers to the anthropogenic elevation of plant nutrients beyond their natural levels. Plants require macronutrients such as C, N, O, S, and P, at high concentrations to grow, and also require micronutrients such as Ca, Mg, Si in lesser quantities. In aquatic systems, N and P are generally considered the most limiting nutrients for plant growth given that C availability (derived from the atmosphere) is held constant. This led to the development of the Redfield ratio, a formula consisting of the C, N, and P levels typically found in the organic matter of marine phytoplankton (Anderson 1995). Redfield's ratio is 106:16:1 (C:N:P), although there has been some debate about the simplicity of this ratio. While decades of limnologic study suggest that P is often the limiting element in lentic systems (Schindler 1977), in marine systems, N is usually considered the limiting element (Moore et al. 2013).

Nitrogen, while abundant in its elemental form in the atmosphere, is a limiting agent for most organismal development (Howarth and Marino 2006). Only 2% of nitrogen present in soil is readily available for organisms. Thus, it has become common practice for farmers to use artificial nitrogen fertilizers on their crops to increase productivity (Bianchi 2007). Phosphorus has been found to have similar effects on waterways, and one of the main passages for elements to enter the waterway is through runoff of agricultural lands that have been applied with manures and fertilizers (Carpenter

2005). The creation of farmland through deforestation and the successive planting of crops has had detrimental effects on land structure as the removal of deeply rooted trees being replaced with temporary crops has allowed soil to become unstable, increasing runoff (Zheng 2006). PEI typically has a high abundance of row crops (particularly potatoes) subjecting the island to heavy chemical fertilizer usage (Jiang et al. 2011). Although runoff from farmland can bring nutrients into the estuary, it is not the only available pathway; groundwater discharge is the dominant means of transportation of nitrogen from field to estuary (Johannes 1980; Bugden et al. 2014; Jiang et al. 2015). Interestingly, Ünlü et al. (1999) found that the occurrence of nitrogen leaching (closely dependent upon soil hydraulic properties) primarily as nitrate, increased significantly with increased fertilization rates, but that nitrogen absorption decreased. While these fertilizers aid in the production of healthy, fully developed crops for consumers, they can end up in groundwater, particularly in the form of the nitrate ion that is water soluble, and can result in very troublesome issues concerning watershed communities and water quality (Jiang and Somers 2008).

Eutrophication

Enrichment of nutrients in aquatic systems can lead to eutrophication, an event that decreases the overall water quality. While eutrophication itself refers to the overgrowth of plants, Bugden et al. (2014) elaborated on the consequences associated with eutrophication in estuaries which include: toxic or nuisance phytoplankton blooms, excessive macro-algal growth, decreased submerged vegetation, higher levels of organic matter in the sediment, hypoxia/anoxia, changes in community structure, possible fish or

shellfish kills, limited recreational water use and diminished aesthetic appeal. Consequently, overall biomass found in the estuary is increased, and can cause issues through the production of cyanobacterial toxins, decreased oxygen availability, and even habitat destruction as phytoplankton and/or macro-algae block sunlight from lower level vegetation (Anderson et al. 2002). Diminished oxygen levels can be very dangerous for aquatic life and in serious cases large scale fish kills may occur. An additional characteristic of eutrophication is the occurrence of harmful algal blooms (HABs) (Paerl 1997). HABs (sometimes referred to as green or red tides depending on the dominant species involved) are events in which phytoplankton grow rapidly, resulting in the harm of other organisms either through the toxins they release or via indirect influences (i.e. foams, and oxygen depletion) (Anderson et al. 2002).

Phytoplankton blooms can also contribute to increased sedimentation (Krümmel et al. 2003; Carstensen et al. 2007; Janetski et al. 2012 as cited by Shi et al. 2017). Evidence from Grimm et al. (1997) suggests that phytoplankton actually engage in a process they defined as "self-sedimentation". Self-sedimentation is a term used to describe the ability of phytoplankton to excrete dissolved polymers which react with water molecules forming sticky, gel-like substances referred to as transparent exopolymer particles (TEPs). This phenomenon typically occurs when blooms stop proliferating, and increases the aggregative abilities of phytoplankton allowing them to sink rapidly, making them less likely to be consumed by other organisms (Grimm et al. 1977). Ultimately, this process permits more organic material to settle in the estuary.

In the dynamic environment of an estuary, estuarine characteristics can greatly influence how nutrient enrichment manifests as eutrophication. For example, tidal

amplitudes are very influential in eutrophication of estuaries as they determine water residence times; micro-tidal estuaries are more strongly affected by hypoxia than macro-tidal estuaries (Coffin et al. 2017). In areas with small tidal amplitudes such as on the north and west coasts of PEI (of importance to this study: The Wheatley estuary), flushing in watersheds is minimal, increasing the chance for hypoxic conditions as new oxygen is not being transported into the estuary to meet the biochemical oxygen demands brought on by plant decomposition (Coffin et al. 2017). Additionally, smaller tidal fluctuations are better associated with increased sedimentation (Abril et al. 1999), potentially increasing organic matter on the benthos. PEI estuaries such as Souris and Wilmot are on the eastern and southern sides of PEI respectively, and therefore experience more relief from eutrophication through more dramatic tidal dynamics (Coffin et al. 2018).

Sediment chemical characteristics

The chemical characteristics of sediment are influenced by sources of deposition. Sediment derived from land on PEI is generally sand and fine sand (Alberto et al. 2016), with low organic matter content (MacDougall et al. 1988). Sources of organic material in estuarine sediment are generally related to biological processes within the estuary, e.g. deposition of phytoplankton, macro-algae and organic matter from aquaculture; however, external sources (such as leaves and terrestrial/marsh vegetation) can also be influential and should not be disregarded. Thus organic carbon in sediment is indicative, in part, of the level of productivity within that estuary (Cranford et al. 2009). Eutrophication will lead to increased deposition of organic matter and should therefore amount to higher

levels of organic carbon within the sediment (Bugden et al. 2014). Organic carbon has also been shown to correlate with abiotic factors. For instance, Coffin et al. (2018) report findings indicating that organic matter is negatively correlated with salinity and nitrate levels.

Anoxia due to eutrophication leads to an increase in anaerobic bacterial decomposition of deposited organic matter. This anaerobic endogenous decay is more efficient than under aerobic conditions and can lead to a return of nutrients into the estuary (Middelburg and Levin 2009). Bacteria that degrade organic matter in aquatic systems reduce sulfate to create hydrogen sulfide (Morse et al. 1987), a toxic compound to many organisms (Cranford et al. 2017). Sulfidic sediments are expected to occur in hypoxic conditions such as those mentioned above and Cranford et al. (2009) found results indicating that mussel farms were directly related to increased hypoxic and sulfidic sediments. These sulfate-reducing bacteria also increase water alkalinity, and aid in carbonate precipitation (Baumgartner et al. 2006). Sulfate-reducing bacteria are also thought to be a major bacterial group responsible for remineralization of organic matter with the resultant release of ammonium and phosphate (Sinkko et al. 2013).

Inorganic carbon measures provide information on recent deposited shellfish abundance in the estuary as it is a major component of their shells (McConnaughey et al. 1997 as cited by McConnaughey and Gillikin 2008). Generally, there will be more carbonate deposition resulting from the farming of bivalves and other shelled organisms in saltwater areas compared to freshwater areas. The biomass of algae and shellfish, on the other hand, contribute to organic matter content in sediments when they decompose and settle into the benthic zone of watersheds (Cranford et al. 2009).

Human activity is also a contributing factor to carbon within rivers and oceans. Humans have caused an escalation in atmospheric carbon dioxide levels which can be slightly mediated through oceanic absorption of this greenhouse gas (Feely et al. 2010). Unfortunately, this increase in oceanic carbon dioxide has resulted in a decrease in pH, as well as a reduction in carbonate saturation (Feely et al. 2010). When carbonate levels fall below saturation (i.e. they begin to precipitate), the ocean will begin to break down materials such as shells to absorb more carbonate, ultimately inhibiting calcification for some marine species (Feely et al. 2010). Interestingly, Borges and Gypens (2010) determined that eutrophication affects the carbon cycle in such a way that it can resist the effects of ocean acidification. Considering these findings, higher amounts of carbonate (in other words inorganic carbon) in the sediment of estuaries subject to more intense or frequent eutrophication would be expected.

Biotic Community Differences and eDNA

Species patterns along estuary salinity gradients are expected to change based on the salinity tolerance of each species. Typically, species abundance is highest in euhaline and oligohaline salinities as well as tidal freshwater areas, and is found to be lowest in mesohaline salinities (Whitfield et al. 2012). Eutrophication is another factor that can affect what species inhabit estuaries. Some species, such as the four-spined stickleback and the mummichog can tolerate, and even benefit from the macro-algal abundance characteristic of eutrophic estuaries (Schein et al. 2012; Finley et al. 2013; Bremner et al. 2015), while other species, for example the three-spined stickleback, are negatively influenced by these conditions (Schein et al. 2012; Bremner et al. 2015). A study

performed by Hughes and Thomas (1971) looked at species along the salinity gradient of Bedeque Bay, PEI, and found that both plant and animal species differed from the upper to the lower estuary (with higher species diversity towards the ocean). It is therefore predicted that there will be more species diversity along the salinity gradient (towards the lower estuary). Similarly, more marine species would be expected in the lower estuarine areas.

Of particular interest however, are the species found in the benthic sediment zone that cannot be easily monitored or identified, such as changes in micro-algal communities within estuaries (Underwood et al. 1998). Phytoplankton blooms or HABs may arise from an array of varying water quality changes, and can occur in both fresh and salt water (Paerl 1988). Differences in the bacterial communities along estuary gradients have been reported by Bouvier and Giorgio (2002). Additionally, Bird and Kalff (1984) found that in both fresh and salt waters bacterial numbers were strongly and positively correlated with chlorophyll levels. This evidence suggests that eutrophic waters will therefore have higher bacterial abundance than oligotrophic or mesotrophic waters. The ensuing depletion of oxygen through bacterial decomposition can, in turn, decrease overall species abundance if oxygen levels become too low for species survival (Cranford et al. 2017). Biomonitoring can reveal more about these species patterns between and within estuaries which can provide information on which depositing bacteria and archaea are causing anoxia.

Next-generation sequencing (NGS) is a quickly growing family of molecular biology techniques (Medinger et al. 2010). These methods of massively parallel DNA sequencing make it possible to quickly sequence entire genomes or transcriptomes as well

as to comprehend the diversity of organisms that inhabit any environment (Medinger et al. 2010). Fragments of DNA from species living in an aquatic ecosystem may be deposited in the sediment; DNA can then be extracted from sediment specific marker genes, amplified by polymerase chain reaction (PCR), and subsequently sequenced using NGS (Gabor et al. 2003). EDNA will primarily provide information on what species are contributing to the sediment. Tessler et al. (2017) were able to use eDNA techniques to study bacterioplankton using the 16S rRNA gene (a gene representative of bacteria). Similarly, Pawlowski et al. (2015) were able to use eDNA to study metazoans using the 18S rRNA gene (a gene representative of metazoans). These studies are examples of what is now possible to discover using eDNA and how widely applicable it can be.

One of the most important reasons for adopting this technique is the minimal invasiveness (i.e. to metazoans) required to collect adequate samples (Goldberg et al. 2016). The technique also allows more definitive identity of known organisms which may typically be difficult to examine and identify (particularly micro-organisms) and, using this technique increases chances of detecting rare species in an environment (Goldberg et al. 2016). Another benefit of using DNA sequencing techniques for the detection of aquatic organisms include reducing the time it takes to identify large quantities of organisms (Chariton et al. 2010).

Unlike alternative methods, next generation sequencing does not require the use of bacterial cloning in the process of genetic amplification; instead, PCR can be used to amplify specific fragments of DNA (Mardis 2008). Once strands are amplified, DNA sequencing can commence. In the case of deposited sediments, the temporal variability of species composition is integrated into the sediment as a historical record of all species

present, rather than at one particular time point. This is potentially important in estuaries where species composition may vary dramatically over the year. Although there are many advantages to using eDNA there are still some issues that researchers must be made aware of when using this technique (Roussel et al. 2015). The main disadvantage of eDNA compared to traditional biomonitoring techniques is that the relationship between a species collected DNA abundance within an environment, and its absolute density in the environment is not well validated, however, determining relative abundance through eDNA may be useful (Bohan et al. 2017).

METHODS

Sampling sites and data collection

Three PEI estuaries: Souris, Wheatley, and Wilmot (Fig. 1) were sampled in this study throughout the Summer of 2016 (Table 1). These three estuaries were meant to provide the range of variables of space and time for the depositional and benthic processes of agriculturally influenced estuaries. Triplicate sediment samples were taken at each of three locations: riverine, upper, and mid estuary (defined by their salinity ranges shown in Table 2 and their GPS coordinates shown in Table 3), using Mini-Ponar benthic grab. Benthic grab samples were collected and stored in glass jars covered with aluminum (both of which were solvent-rinsed with acetone). For additional protection from contaminants the glass jars were sealed in plastic zip-lock bags and frozen at -20°C.

Environmental DNA analysis

DNA from thawed benthic grab samples, was extracted using a power soil extraction kit (as per MO BIO Laboratories, Inc. instructions). Approximately 5 µl of the extracted DNA was then pipetted onto a micro volume spectrophotometer (NanoVue Plus) which was used to determine the concentration of eDNA in each sample.

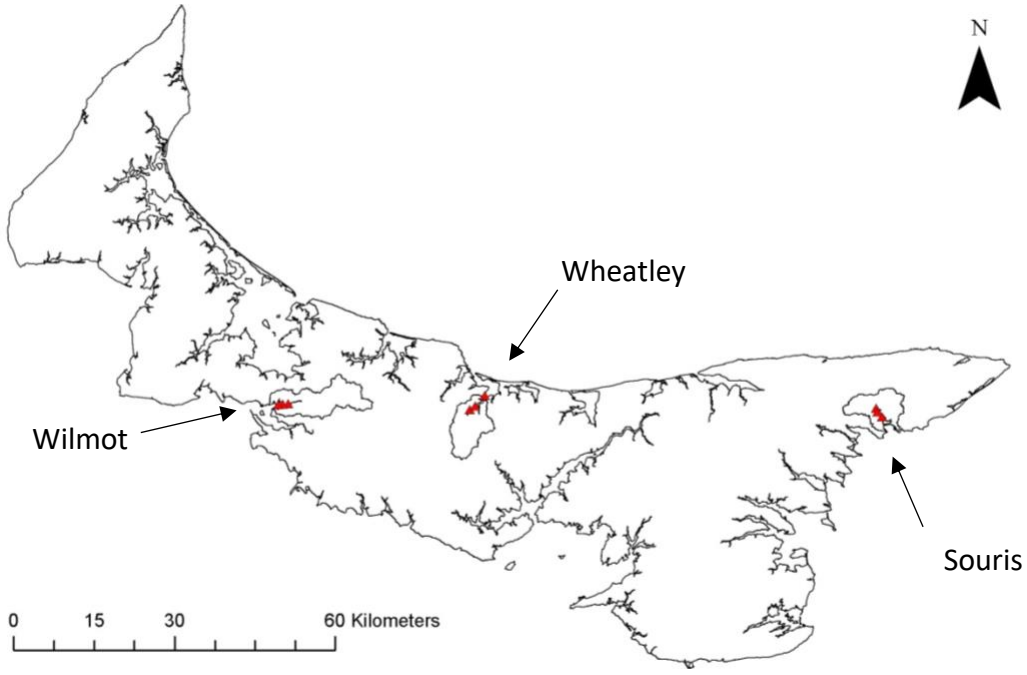


Figure 1. A map of Prince Edward Island, Canada, highlighting Wheatley (north shore), Wilmot (south shore) and Souris (east shore) estuaries and their three respective sample sites (riverine, upper, and mid-estuary).

Table 1. Salinity (PSU) levels at riverine, upper, and mid sites of the Souris, Wheatley and Wilmot estuaries.

Estuary	Site	Salinity (PSU)
Souris	Riverine	10-28
	Upper	21-29
	Mid	25-30
Wheatley	Riverine	3-20
	Upper	15-27
	Mid	24-29
Wilmot	Riverine	7-21
	Upper	9-24
	Mid	19-27

Table 2. Sample collection dates for Souris, Wheatley, and Wilmot estuaries, Prince Edward Island, Canada.

Estuary	Sampling Period	Date
Souris	1	15-Jun-16
	2	06-Jul-16
	3	20-Jul-16
	4	11-Aug-16
	5	06-Sep-16
Wheatley	1	11-Jun-16
	2	04-Jul-16
	3	18-Jul-16
	4	02-Aug-16
	5	30-Aug-16
Wilmot	1	21-Jun-16
	2	08-Jul-16
	3	24-Jul-16
	4	15-Aug-16
	5	08-Sep-16

Table 3. GPS coordinates for each sampling site (riverine, upper and mid-estuary) from Souris, Wheatley, and Wilmot estuaries, Prince Edward Island, Canada.

Estuary	Site	Latitude	Longitude
	Riverine	46.383125	-62.295442
Souris	Upper	46.376770	-62.292400
	Mid	46.368000	-62.282120
	Riverine	46.382400	-63.282740
Wheatley	Upper	46.388783	-63.269489
	Mid	46.406537	-63.247455
	Riverine	46.390580	-63.724300
Wilmot	Upper	46.389930	-63.738170
	Mid	46.389113	-63.748847

Organic and inorganic carbon content

The loss on ignition technique (LOI) as described by Heiri et al. (1999) was used in this study to assess organic and inorganic carbon content of the collected sediment samples. Initially, the sediment was oven-dried in a PRECISION Economy Incubator at 60°C for approximately 48 hours. Organic matter was then combusted in an Isotemp® Programmable Muffle Furnace, by heating to 550°C for four hours. The following equation was then used to calculate the percent mass of organic carbon present in each sample:

$$LOI_{550} = \frac{(DW_{60} - DW_{550})}{DW_{60}} \times 100 \quad (1)$$

where DW_{60} is the dry weight before combustion, and DW_{550} is the weight after the first combustion (at 550 °C). A second combustion to determine inorganic carbon was then performed, this time at a temperature of 950°C for two hours. The following equation was then used to calculate the percent mass of inorganic carbon present in each sample:

$$LOI_{950} = \frac{(DW_{550} - DW_{950})}{DW_{60}} \times 100 \quad (2)$$

where DW_{950} is the weight after the second combustion (at 950 °C).

Nutrient remineralization in oxygenated and deoxygenated sediments

For this experiment approximately 18 liters of sediment were collected from the upper estuary section of the Trout-Stanley river, PEI, Canada (46.419522, -63.439300), on February 15th, 2018, using a post hole digger, and an ice auger to drill through the ice. Artificial seawater was then made as per Kester et al. (1967), although only NaCl, KCl, $CaCl_2$*$2H_20$, $MgSO_4$*$7H_20$, MgCl*$6H_20$, and $NaHCO_3$ salts were included to avoid

major nutrients. The seawater was diluted with distilled water until a salinity of 25 PSU was reached.

Approximately 500 ml of the collected sediment and 500 ml of artificial seawater were placed into each of 12 PVC pipes sealed at one end. Six of the 12 pipes were then bubbled with compressed air, while the other six were given only N_2 gas. Water samples (100 ml) from each tube were taken at 1, 3, 6, 9, 12, 24, 48, 72, 96, 120, and 144 hours after the start of the experiment. Following each collection, water was replaced (100 ml) with clean, artificial seawater.

Half of the samples were put in the freezer for later phosphate and nitrate analysis; the other half were analyzed for ammonium levels using a fluorometric method outlined by Holmes et al. (1999), but modified for a 96-well microplate fluorometer (FLx800 Microplate Fluorescence Reader by Bio-Tek, Instruments, INC). This method works well over a range of water salinities as well as a range of ammonium concentrations.

The stable working reagent was a commercially available orthophthaldialdehyde solution in a borate buffer (Fluoraldehyde™) with added sodium sulfite. 180 µl of this reagent and 20 µl of sample were mixed and incubated in the dark for 15 minutes. For this procedure a standard made from dissolved ammonium chloride was also used. According to Holmes et al. 1999, excitation wavelengths lower than 380 nm and emission wavelengths greater than 400 nm were functional for the analysis of ammonium samples. Filter range was M360/460. The product of this test revealed the concentration of ammonium from a quantified sample of water, determined by the strength of light emitted.

Other measures such as temperature, oxygen, pH, and salinity were also taken regularly. Water temperature, dissolved oxygen, conductivity and salinity were measured with a YSI 550 multimeter, while pH was taken with a VWR benchtop pH meter. Additionally, one oxic and one hypoxic pipe had their dissolved oxygen levels and water temperature measured every 15 minutes with an Onset HOBO® Dissolved Oxygen logger.

Statistical analysis

All sediment analyses were completed in R version 3.4.4 (R Core Team 2018). Correlations between variables were done with a spearman rank correlation in the R packages dplyr (Wickham et al. 2017) and Hmisc (Harrell et al. 2018). Exploratory analysis of sediment carbon treated sites as separate factors. A one-way ANCOVA was used to compare sites over the summer with time being treated as a continuous variable. This linear model was done using the package car (Fox and Weisberg 2011). Post hoc comparisons of sites were done with a Tukey's test, in the package Multcomp (Hothorn et al. 2008). Normality was examined using normal probability plots. Organic carbon content was natural log transformed in the model.

Incubator ammonium concentration was converted to the mass of N evolved for every time point. In order to account for sample removal, the mass of N at each time point was corrected for the cumulative N previously removed (volume x concentration for each previous time point). The evolution of N was modeled using a first-order rate equation of the form:

$$N = C \times (1 - e^{(-k \times t)}) \quad (3)$$

where N is the cumulative mass of nitrogen released from sediment, C is the asymptotic constant representing the maximum mass of nitrogen evolved at infinite time, and t is the incubation time. Curves were fit using nonlinear estimation in STATISTICA (v.13) software.

RESULTS

Organic carbon content

Wheatley estuary was found to have the most organic carbon content overall (Fig. 2). More specifically, the riverine section of both the Souris and Wheatley estuaries had the highest organic carbon levels along the estuary (Fig. 3a and 3b respectively; Table 4). Wilmot estuary showed a different trend with the highest organic carbon content at the upper-estuary site (Fig. 3c; Table 4). The mid-estuary region of each estuary had consistently low (the lowest in Wheatley and Wilmot) amount of organic carbon (Fig. 3a, 3b, and 3c; Table 4). The effect of time was not a statistically significant factor on organic carbon content.

Inorganic carbon content

Wheatley estuary had the highest concentrations of inorganic carbon overall for the three estuaries (Fig. 4). Within each estuary the concentrations varied throughout the estuary as Souris' riverine site had much higher concentrations than either upper or mid-estuary (Fig. 5a), whereas the highest inorganic concentrations found within Wheatley and Wilmot both occurred at the upper-estuary site (Fig. 5b and 5c respectively).

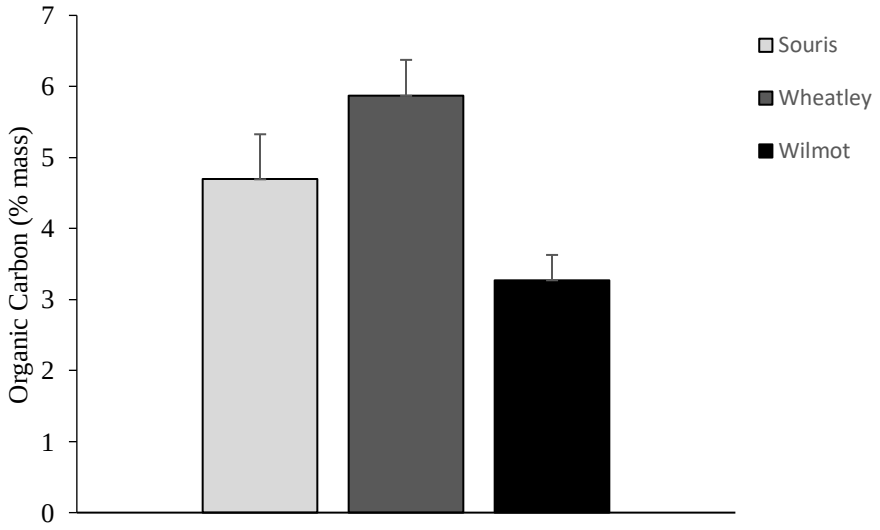
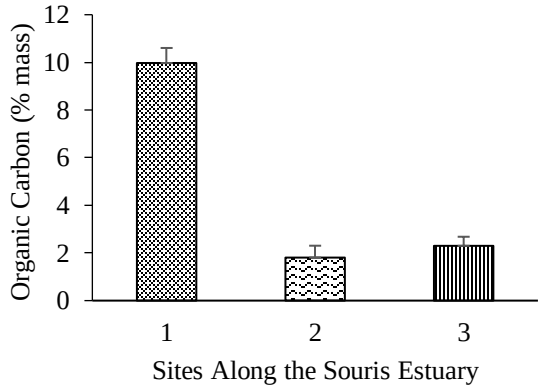
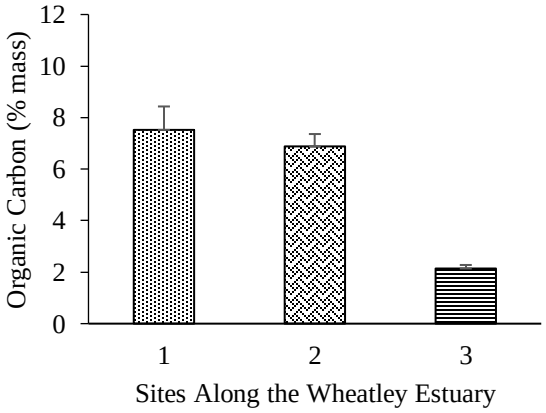


Figure 2. Mean percent mass of organic carbon content measured in each of three PEI estuaries (Souris, Wheatley, and Wilmot). Bars represent standard error.

a)



b)



c)

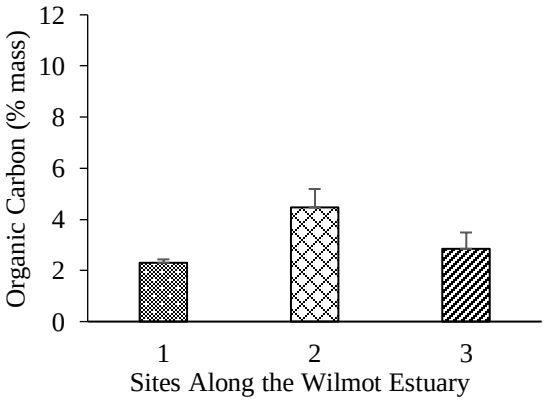


Figure 3. Mean percent mass of organic carbon content along the salinity gradient of Souris (a), Wheatley (b), and Wilmot (c) estuaries. Site 1 represents the riverine section of the estuary, site 2 the upper estuary and site 3 the mid-estuary. Bars represent standard error.

Table 4. Matrix of Tukey contrasts between the organic carbon content of the riverine, upper, and mid-estuary sites of the Souris, Wheatley and Wilmot estuaries.

	Souris Riverine	Souris Upper	Souris Mid	Wheatley Riverine	Wheatley Upper	Wheatley Mid	Wilmot Riverine	Wilmot Upper	Wilmot Mid
Souris Riverine		***	***	-	-	***	***	***	***
Souris Upper			-	***	***	-	-	***	-
Souris Mid				***	***	-	-	**	-
Wheatley Riverine					-	***	***	*	***
Wheatley Upper						***	***	*	***
Wheatley Mid							-	.	-
Wilmot Riverine								-	-
Wilmot Upper									-
Wilmot Mid									

Asterisks and period represent significance. 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 1 ‘-‘

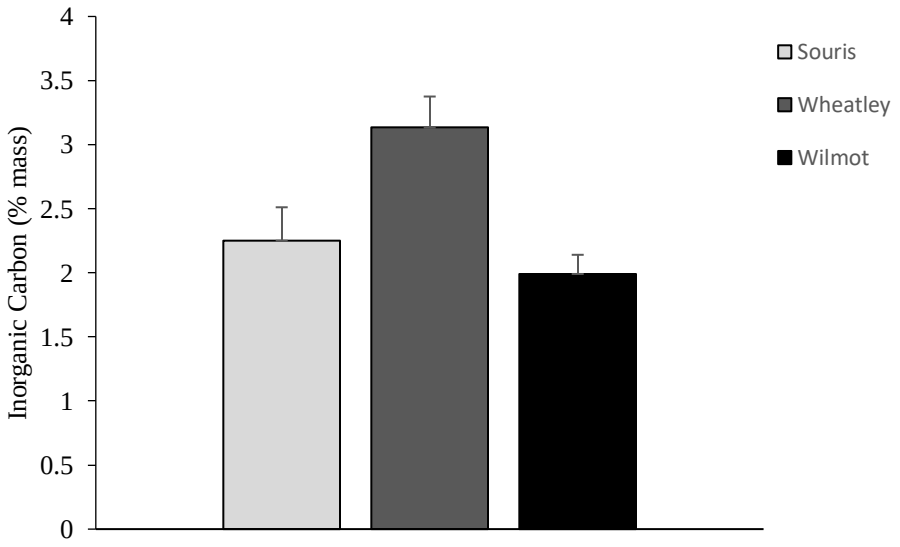
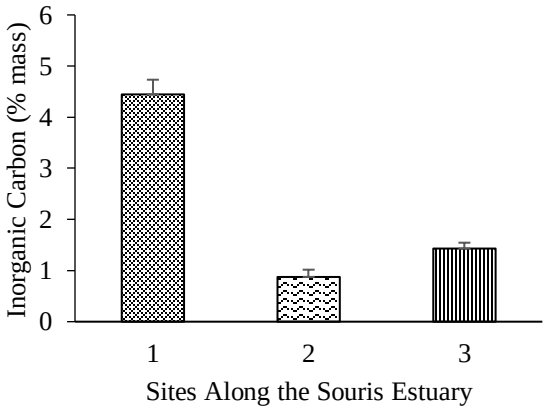
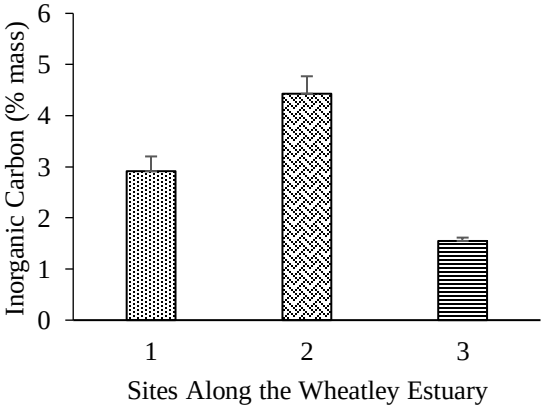


Figure 4. Mean percent mass of inorganic carbon content measured in each of three PEI estuaries (Souris, Wheatley, and Wilmot). Bars represent standard error.

a)



b)



c)

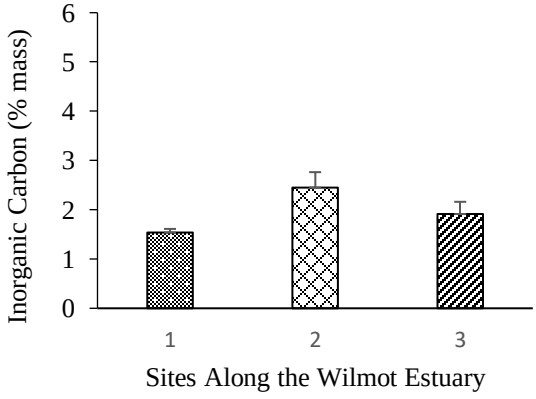


Figure 5. Mean percent mass of inorganic carbon content along the salinity gradient of Souris (a), Wheatley (b), and Wilmot (c) estuaries. Site 1 represents the riverine section of the estuary, site 2 the upper estuary and site 3 the mid-estuary. Bars represent standard error.

DNA concentration

DNA was highest overall in the Wheatley estuary (Fig. 6). The riverine site of both the Souris and Wheatley estuaries had the highest levels of DNA compared to upper and mid-estuary (Fig. 7a and 7b respectively). Contrastingly, Wilmot's upper estuary had the highest amount of DNA within the estuary (Fig. 7c).

Relations between carbon content and DNA concentrations

A strong positive correlation was found between organic carbon content and inorganic carbon content ($N=130$, $rho=0.76$, $p<0.001$; Fig.8). Inorganic carbon content was also positively correlated with DNA concentration ($N=130$, $rho=0.54$, $p<0.001$; Fig.8). Finally, organic carbon content was also found to correlated positively with DNA concentrations ($N= 130$, $rho= 0.40$, $p<0.001$; Fig.8).

Ammonium production

The highest overall ammonium production in sediment reactor experiments occurred under hypoxic conditions as opposed to oxic conditions (Figure 9). Temperature and oxygen remained relatively constant over time. Mean temperature in the incubations was 14.07 ± 0.02 °C, and 14.04 ± 0.02 °C for the oxic and hypoxic reactors, respectively (Appendix I). Mean dissolved oxygen was measured to be 8.27 ± 0.36 mg/L and 3.21 ± 0.52 mg/L in the oxic and hypoxic reactors, respectively (Table 5). Ammonium concentrations increased similarly within both conditions but diverged over time as the rate of increase became faster in the hypoxic samples and concentrations became consistently higher (Figure 9). First order rate equations showed that the predicted production of nitrogen at infinite time was 3.96 g for oxic conditions (corresponding to

504 g/m^2) and 6.29 g for hypoxic conditions (corresponding the 801 g/m^2). Between the two conditions, pH and salinity also experienced minor changes. Salinity increased under both conditions although the overall concentration was higher and the rate of increase was faster under hypoxic conditions (Appendix II). pH remained fairly stable under the oxic conditions as well, but increased slightly over time within the hypoxic condition (Appendix III).

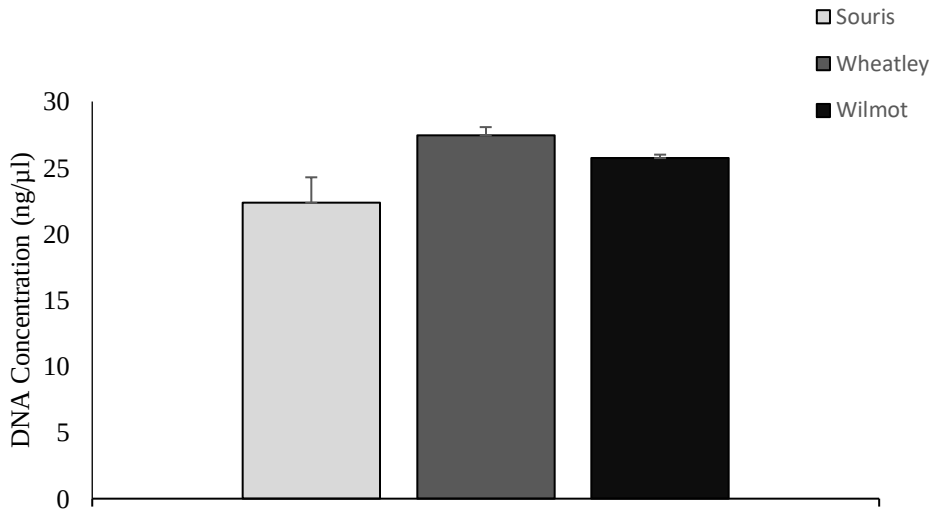
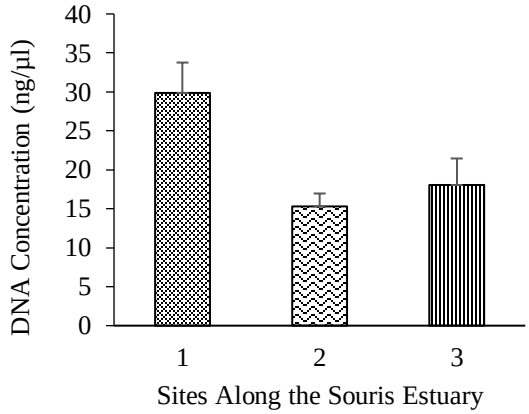
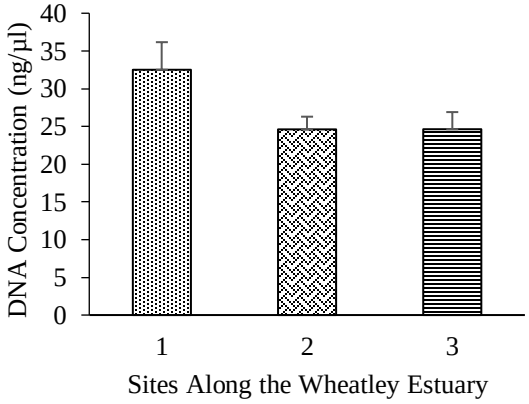


Figure 6. DNA concentration (ng/µl) measured in each of three PEI estuaries (Souris, Wheatley, and Wilmot). Bars represent standard error.

a)



b)



c)

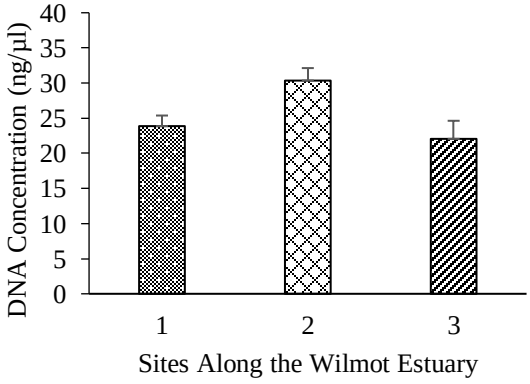
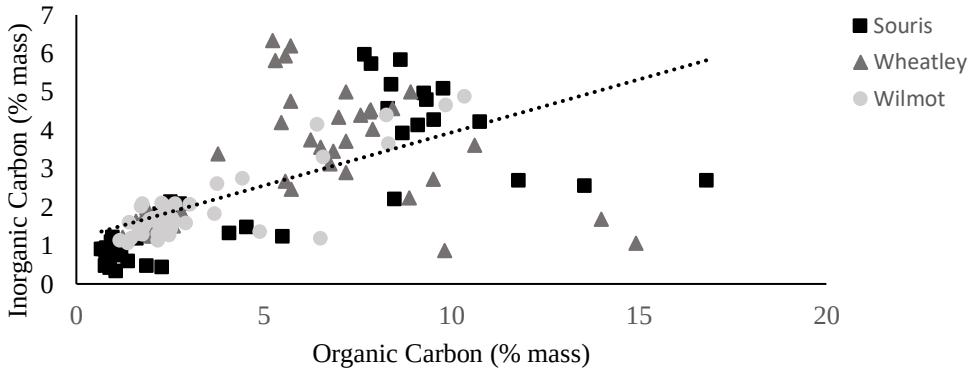
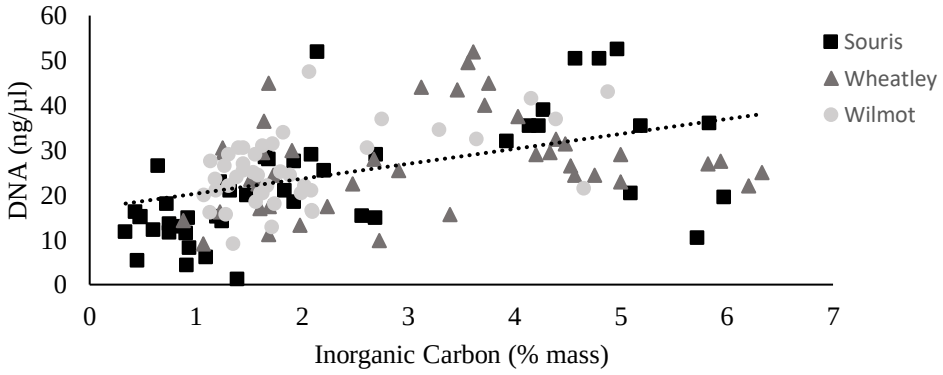


Figure 7. DNA concentration (ng/µl) along the salinity gradient of Souris (a), Wheatley (b), and Wilmot (c) estuaries. Site 1 represents the riverine section of the estuary, site 2 the upper estuary and site 3 the mid-estuary. Bars represent standard error.

a)



b)



c)

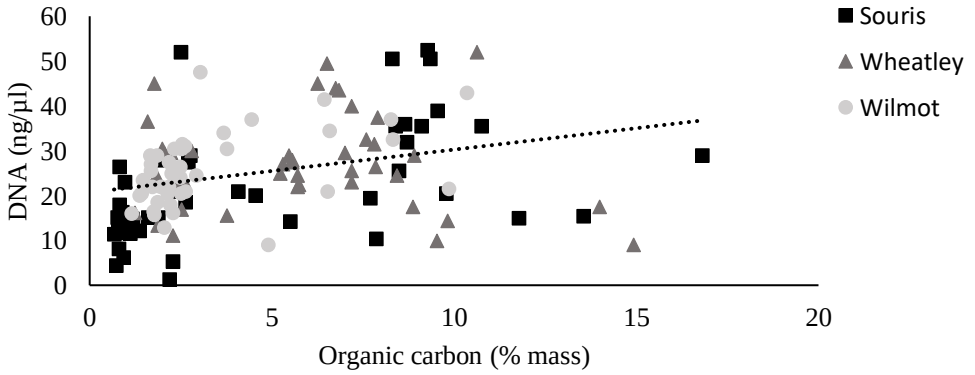


Figure 8. Scatterplots showing the positive correlations between inorganic carbon and organic carbon (a), DNA and inorganic carbon (b), and DNA and organic carbon (c).

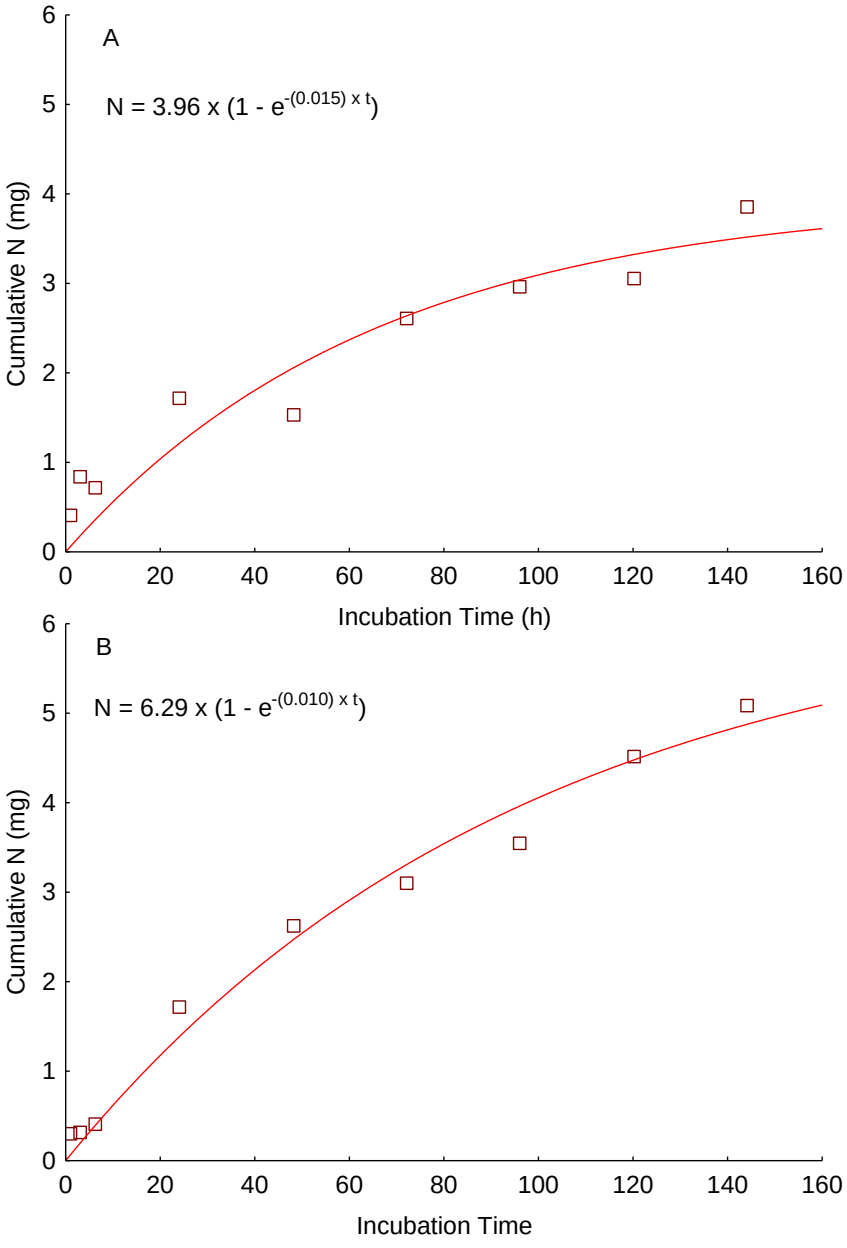


Figure 9. Nitrogen production in A) oxic reactors and B) hypoxic reactors measured over a period of 144 hours.

Table 5. Average oxygen (mg/L) levels throughout the reactor experiment for hypoxic and oxic conditions, measured over a period of 144 hours.

Average Oxygen (mg/L)		
Time after start of experiment (hours)	Hypoxic conditions (mg/L ± std. error)	Oxic conditions (mg/L ± std. error)
1	3.21 ± 0.17	8.23 ± 0.07
3	2.98 ± 0.15	8.28 ± 0.16
6	3.26 ± 0.09	8.02 ± 0.04
24	3.03 ± 0.24	8.62 ± 0.06
48	4.08 ± 0.22	8.80 ± 0.04
72	3.24 ± 0.13	8.56 ± 0.06
96	3.47 ± 0.13	8.02 ± 0.04
120	2.94 ± 0.09	8.20 ± 0.06
144	2.69 ± 0.11	7.74 ± 0.09

DISCUSSION

This study found that organic carbon was generally highest in the uppermost parts of the estuaries examined. Furthermore, DNA content generally followed the trends in organic matter. Sediment organic carbon also changed between June and September, though not consistently at each estuary. Reactor experiments with estuary sediment demonstrated that significant amounts of nitrogen are released from sediment within a 144 h period. Hypoxia in the overlying water, accelerated the rate and increased the ultimate mass of N produced.

In addition to nutrient loading itself, one of the most probable explanations for the differing levels of organic matter deposition seen along the estuary gradients as well as between estuaries is differences in tidal dynamics. Nitrogen loading was previously determined to be 49, 149, and 427 kg/ha/yr of N for the Souris, Wheatley and Wilmot estuaries, respectively (Coffin et al. submitted). As such, nitrogen alone does not at all explain the higher level of organic matter accumulation in the Souris and Wheatley estuaries. However, taking into account the tidal dynamics around PEI may help explain this lack of agreement. Coffin et al. (submitted) found that the overall water residence times were 1.85, 1.74, and 0.73 d, for Souris, Wheatley and Wilmot estuaries, respectively. Thus, the two-fold longer residence time, with subsequently lower flushing in the Wheatley and Souris estuaries can account for some of the variance in organic matter found here as the effective concentration of nutrients may be less, and more

organic matter or nutrients are exported from the estuary with greater flushing.

Another explanation for the high organic carbon content of the Souris and Wheatley estuaries found in the uppermost regions is their proximity to agricultural land, and their narrower structure (compared to lower estuary areas). Nutrients enter the estuary from the river, and plants in the upper estuary, largely macro-algae (*Ulva sp.*) and phytoplankton are able to absorb those nutrients very rapidly, leading to a proliferation of plant growth in this part of the estuary. It has been reported that nitrogen is generally depleted by plants in the water column within the first 400 m of a river entering an estuary (Valiela 1997). A study by Coffin et al. (unpublished) also found hypoxia/anoxia to be more severe in the upper 10% of the Wheatley estuary as compared to half-way down the estuary (by area). Thus, the uppermost estuary, where nutrients first meet marine plants is well established as being the most severe area of eutrophication.

DNA concentrations were also correlated with inorganic carbon and carbon levels as is indicative of the deposition of biological matter. While a relationship with organic carbon was expected, a relationship with inorganic carbon was not. However, these data indicate that organic matter deposited had an inorganic carbon component. Mats of macro-algae can often be covered by a proliferation of snails which may in part explain the inorganic carbon content in those highly eutrophic areas. Coffin et al. (2018) found that snails are predominant in this plant habitat, but become even more dominant when hypoxic or anoxic conditions occur as those organisms are tolerant of the adverse conditions. The proportion of time under 4 mg/L of oxygen (May-November) was 14, 2.5, and 1% for the Wheatley, Souris and Wilmot estuaries, respectively. This may explain the high inorganic carbon mid estuary at Wheatley that tends to incur higher

severity of hypoxia throughout the estuary. This area of the estuary is dominated by thick mats of *Ulva*.

Annual trends in organic matter in PEI estuarine sediment reflect a balance between deposition, and remineralization. Generally, it would have been expected that remineralization would increase through the summer, peaking in august when sediments are most likely to be anoxic. A downward change in organic matter at Souris and Wheatley upper estuary site indicating a reduction in organic matter could potentially have been due to remineralization over the season. The upper estuary is also most likely to incur hypoxia/anoxia, enhancing remineralization. The converse may be true, and the relative rate of organic matter deposition in the uppermost reaches of the Wilmot River are potentially due to the relatively oxic conditions limiting remineralization.

Hypoxia significantly enhanced the internal loading of nitrogen from estuarine sediments. Studies in the Baltic (Sinkko et al.2013) and Chesapeake Bay have also shown the enhanced production of nitrogen from sediments under low oxygen conditions (Buridge 1991). While anaerobic remineralization was thought to be the reason for increased rates of N production, it should be emphasized that the present study did not confirm anaerobic sediments directly, nor was the reduction of sulphate that accompanies this remineralization of carbon measured. The experimental setup only achieved hypoxic overlying water, thus it cannot be presumed that sediment was totally anoxic. However, given relatively low rates of oxygen transfer into sediment, oxygen can decrease with depth from the surface so sediments were likely anoxic at some level below the surface. It is possible that even the oxic reactors had some level of hypoxia/anoxia in sediment. Subsequent experiments could use increased nitrogen bubbling, partially sealed

incubators to reduce overlying oxygen, and a greater height of water to provide near anoxia.

The internal production of nutrients is significance to understanding and predicting estuarine responses to nutrients. Management action to reduce nutrients requires some knowledge of how much to reduce them by in order to prevent severe effects like estuarine anoxia (Bugden et al. 2014). However, at present, we are only able to understand the external loading of nutrients to estuaries in the regions. While models have been developed to predict oxygen from N loading and estuarine flushing (Coffin et al. submitted), external nitrogen was not as strong a predictor as would be expected. This may be due to not accounting for internal loading of nitrogen, which as was seen herein, can be considerable. For example, the Wheatley River has an external loading of 149 kg N/ha/yr (Coffin et al, submitted); the highest value seen for the Trout River sediment was approximately 8000 kg/ha of produced nitrogen. While not all of an estuary is dominated by high organic mud, nor do all estuaries go anoxic, this still imparts the potential relative significant of internal loading of N to the overall nitrogen budget of an estuary.

Each estuary is unique, being influenced by a combination of factors including but not limited to tidal fluctuations, oxygen availability, carbon composition, deposition rates, agriculture, and aquaculture. Therefore, a more comprehensive study combining many biotic and abiotic factors would provide valuable insight that could potentially be used for conservation purposes in the future. Additionally, while it was not possible within the timeframe of the present study, eDNA analysis would provide useful information on the microbial community within estuaries and how it may differ along estuary gradients and between different estuaries. This could also illustrate the dominance

of sulphate reducing bacteria that are critical to remineralization. In order to improve understanding of estuary dynamics and the effects of eutrophication, it would be beneficial to analyze the biogeochemistry of more estuaries located at different locations on PEI – this will be critical to developing models for estuarine impact that let environmental managers make decisions.

LITERATURE CITED

Abril G, Etcheber H, Le Hir P, Bassullet P, Boutier B, Frankignoulle M (1999) Oxic/anoxic oscillations and organic carbon mineralization in an estuarine maximum turbidity zone (the gironde, france). Limnol Oceanogr 44:1304-1315.

Alberto A, St-Hilaire A, Courtenay SC, van den Heuvel MR (2016) Monitoring stream sediment loads in response to agriculture in Prince Edward Island, Canada. Environ Monit Assess 188:415.

Andersen JH, Fossing H, Hansen JW, Manscher OH, Murray C, Petersen DLJ (2014) Nitrogen inputs from agriculture: Towards better assessments of eutrophication status in marine waters. Ambio 43:906-913.

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25:704-726.

Anderson LA (1995) On the hydrogen and oxygen content of marine phytoplankton. Deep Sea Research Part I. Oceanogr Res Pap 42:1675-1680.

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop KM, Visscher PT (2006) Sulfate reducing bacteria in microbial mats: Changing paradigms, new discoveries. Sediment Geol 185:131-145.

Bernhard AE, Donn T, Giblin AE, and Stahl DA (2005) Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. Environ Microbiol 7:1289-1297.

Bianchi TS (2007) *Biochemistry of Estuaries*. Oxford University Press. New York, USA. 687p.

Bird DF and Kalff J (1984) Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can J Fish and Aquat Sci 41:1015-1023.

Bohan DA, Vacher C, Tamaddoni-Nezhad A, Raybould A, Dumbrell AJ, Woodward G (2017) Next-generation global biomonitoring: Large-scale, automated reconstruction of ecological networks. Trends Ecol Evol (Amst) 32:477-487.

Borges A and Gypens N (2010) Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. Limnol Oceanogr 55:346-353.

Bouvier TC and del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47:453-470.

Bremner AM, Methven DA, Munkittrick KR, Frego KA (2015) Spatial and temporal variation in fish assemblages in three small unpolluted estuarine rivers and associated lagoons in Kouchibouguac National Park, southern Gulf of St. Lawrence, Canada. Can Field-Nat 129:121-133.

Bugden G, Jiang Y, van den Heuvel MR, Vandermeulen H, MacQuarrie KTB, Crane CJ and Raymond BG (2014) *Nitrogen Loading Criteria for Estuaries in Prince Edward Island*. Can Tech Rep Fish Aquat Sci 3066: 50 p.

Burdige DJ (1991) The kinetics of organic matter mineralization in anoxic marine sediments. J Mar Res 49:727-61.

Carey CC, Doubek JP, McClure RP, and Hanson PC (2017) Oxygen dynamics control the burial of organic carbon in a eutrophic reservoir. Limnol Oceanogr 50:1.

Carpenter SR (2005) Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. Proc Natl Acad Sci USA 102:10002-10005.

Chariton AA, Court LN, Hartley DM, Colloff MJ, Hardy CM (2010) Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Front Ecol Environ 8:233-238.

Coffin MRS, Courtenay SC, Knysh KM, Pater CC, van den Heuvel MR (2018) Impacts of hypoxia on estuarine macroinvertebrate assemblages across a regional nutrient gradient. Facets 3:23-44.

Coffin MRS, Knysh KM, Theriault EF, Pater CC, Courtenay SC, van den Heuvel MR (2017) Are floating algal mats a refuge from hypoxia for estuarine invertebrates? PeerJ 5:e3080.

Coffin MRS, Pater CC, Courtenay SC, and van den Heuvel MR (Submitted) Monitoring eutrophication across a nutrient gradient at a regional scale: benefits of dissolved oxygen as the response variable. Marine Pollut. Bull.

Corwin DL and Yemoto K (2017) Salinity: Electrical conductivity and total dissolved solids. Methods Soil Anal 2(1).

Cranford PJ, Hargrave BT, Doucette LI (2009) Benthic organic enrichment from suspended mussel (*Mytilus edulis*) culture in Prince Edward Island, Canada. Aquaculture 292:189-196.

Cranford PJ, Brager L, Wong D (2017) A dual indicator approach for monitoring benthic impacts from organic enrichment with test application near Atlantic salmon farms. Marine Poll Bull 124:258-265.

Downing JA, Cole JJ, Middelburg JJ, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA (2008) Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century. Global Biogeochem Cycles 22:GB1018.

Erguder TH, Boon N, Wittebolle L, Marzorati M, Verstraete W (2009) Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiol Rev 33:855-69.

Feely RA, Alin SR, Newton J, Sabine CL, Warner M, Devol A, Krembs C, Maloy C (2010) The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuar Coast and Shelf Sci 88:442-449.

Finley MA, Courtenay SC, Teather KL, Hewitt LM, Holdway DA, Hogan NS, van den Heuvel MR (2013) Evaluating cumulative effects of anthropogenic inputs in Prince Edward Island estuaries using the mummichog (*Fundulus heteroclitus*). Integr Environ Assess Manag 9:496-507.

Fox J, and Weisberg S (2011) An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gabor EM, Vries D, J E, Janssen DB (2003) Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol Ecol 44:153-163.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, et al (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7:1299-1307.

Grimm KA, Lange CB, Gill AS (1997) Self-sedimentation of phytoplankton blooms in the geologic record. Sediment Geol 110:151-161.

Harrell Jr Frank E, with contributions from Dupont C et al. (2018) Hmisc: Harrell Miscellaneous. R package version 4.1-1. https://CRAN.R-project.org/package=Hmisc

Heiri O, Lotter AF, Lemcke G (1999) Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. J Paleolimnol 25:101-110.

Holmes RM, Aminot A, Kérouel R, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can J Fish Aquat Sci 56(10).

Hopkinson CS, Giblin AE, Tucker J, Garritt RH (1999) Benthic metabolism and nutrient cycling along an estuarine salinity gradient. Estuaries 22:863-881.

Hothorn T, Bretz F and Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Howarth RW, and Marino R (2006) Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnol Oceanogr 51:364-376.

Hughes RN and Thomas MLH (1971) The classification and ordination of shallow-water benthic samples from Prince Edward Island, Canada. J Exp Mar Bio Ecol 7:1-39.

Jiang Y and Somers G (2008) Modeling effects of nitrate from non-point sources on groundwater quality in an agricultural watershed in Prince Edward Island, Canada. Hydrogeol J 17:707-724.

Jiang Y, Zebarth B, Love J (2011) Long-term simulations of nitrate leaching from potato production systems in Prince Edward Island, Canada. Nutr Cycl Agroecosyst 91:307-325.

Jiang Y, Nishimura P, van den Heuvel MR, MacQuarrie KTB, Crane CS, Xing Z, Raymond BG, Thompson BL (2015) Modeling land-based nitrogen loads from groundwater-dominated agricultural watersheds to estuaries to inform nutrient reduction planning. J Hydrol 529:213-230.

Johannes RE (1980) The ecological significance of the submarine discharge of groundwater. Mar Ecol Prog Ser 3:365-373.

Kester DR, Duedall IW, Connors DN, Pytkowicz RM (1967) Preparation of artificial seawater1. Limnol Oceanogr 12:176-9.

Kolenbrander GJ (1971) *Contribution of agriculture to eutrophication of surface waters with nitrogen and phosphorus in the Netherlands*. 50p.

Lotze H, S Lenihan H, Bourque B, Bradbury R, Cooke R, C Kay M, M Kidwell S, X Kirby M, H Peterson C, Jackson J (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science (New York, N.Y.) 312:1806-9.

MacDougall JI, Veer C, Wilson F (1988) *Soils of Prince Edward Island*. Contr. No. 141. Land Resource. Research Institute of Agriculture, Ottawa, Ont., Canada. 139 p.

Mardis ER (2008) Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 9:387-402.

McConnaughey TA and Gillikin DP (2008) Carbon isotopes in mollusk shell carbonates. Geo-Mar Lett 28:287-299.

Medinger R, Nolte V, Pandey RV, Jost S, Ottenwälder B, Schlötterer C, Boenigk J (2010) Diversity in a hidden world: Potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. Mol Ecol 19 Suppl 1:32-40.

Middelburg JJ, Levin LA (2009) Coastal hypoxia and sediment biogeochemistry. Biogeosciences 6:1273-1293.

Moore CM, Mills MM, Arrigo KR, Berman-Frank I, Bopp L, Boyd PW, Galbraith ED, Geider RJ, Guieu C, Jaccard SL, et al. (2013) Processes and patterns of oceanic nutrient limitation. Nat Geosci 6:ngeo1765.

Morse JW, Millero FJ, Cornwell JC, Rickard D (1987) The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. Earth-Sci Rev 24:1-42.

Mosier AC, and Francis CA (2008) Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco bay estuary. Environ Microbiol 10:3002-3016.

Paerl HW (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters1. Limnol Oceanogr 33:823-843.

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnol Oceanogr 42:1154-1165.

Palmer SM, Hope D, Billett MF, Dawson JJC, Bryant CL (2001) Sources of organic and inorganic carbon in a headwater stream: Evidence from carbon isotope studies. Biogeochemistry 52:321-38.

Pawlowski J, Esling P, Lejzerowicz F, Visco JA, and Cedhagen T (2015) Next generation sequencing assays for benthic monitoring of the environmental impact associated with salmon farming (pilot study).

PEI Department of Fisheries and Agriculture (2015) Accessed Nov.10/2017. https://www.princeedwardisland.ca/en/information/agriculture-and-fisheries/agriculture-pei

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Roussel J, Paillisson J, Tréguier A, Petit E (2015) The downside of eDNA as a survey tool in water bodies. J Appl Ecol 52:823-826.

Schein A, Courtenay SC, Crane CS, Teather KL, van den Heuvel MR (2012) The role of submerged aquatic vegetation in structuring the nearshore fish community within an estuary of the southern Gulf of St. Lawrence. Estuaries and Coasts 35:799-810.

Schindler DW (1977) Evolution of phosphorus limitation in lakes. Science 195:260.

Shi W, Yu N, Jiang X, Han Z, Wang S, Zhang X, Wei S, Giesy JP, Yu H (2017) Influence of blooms of phytoplankton on concentrations of hydrophobic organic chemicals in sediments and snails in a hyper-eutrophic, freshwater lake. Water Research 113:22-31.

Sinkko H, Lukkari K, Sihvonen LM, Sivonen K, Leivuori M, Rantanen M, Paulin L, Lyra C (2013) Bacteria contribute to sediment nutrient release and reflect progressed eutrophication-driven hypoxia in an organic-rich continental sea. Plos One 8:e67061.

Tessler M, Brugler MR, DeSalle R, Hersch R, Velho LFM, Segovia BT, Lansac-Toha FA, Lemke MJ (2017) A global eDNA comparison of freshwater bacterioplankton assemblages focusing on large-river floodplain lakes of Brazil. Microb Ecol 73:61-74.

Trimmer M, Nicholls JC, Deflandre B (2003) Anaerobic ammonium oxidation measured in sediments along the Thames estuary, United Kingdom. Appl Environ Microbiol 69:6447-54.

Tromboni F and Dodds WK (2017) Relationships between land use and stream nutrient concentrations in a highly urbanized tropical region of Brazil: Thresholds and riparian zones. Environ Manage 60:30-40.

Underwood GJC, Phillips J, Saunders K (1998) Distribution of estuarine benthic diatom species along salinity and nutrient gradients. Eur J Phycol 33:173-183.

Ünlü K, Özeni˙rler G, Yurteri˙ C (1999) Nitrogen fertilizer leaching from cropped and irrigated sandy soil in Central Turkey. Eur J Soil Sci 50:609-620.

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997) Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol Oceanogr 42:1105-18.

Wickham H, Francois R, Henry L and Müller K (2017) dplyr: A Grammar of Data Manipulation. R package version 0.7.4. https://CRAN.R-project.org/package=dplyr

Whitfield AK, Elliott M, Basset A, Blaber SJM, West RJ (2012) Paradigms in estuarine ecology – A review of the Remane diagram with a suggested revised model for estuaries. Estuar Coast Shelf Sci 97:78-90.

Zheng F (2006) Effect of vegetation changes on soil erosion on the Loess Plateau. Pedosphere 16:420-427.

APPENDIX

Table I. Average temperature (°C) for hypoxic and oxic conditions throughout the reactor experiment, measured over a period of 144 hours.

Average Temperature		
Time after start of experiment (hours)	Hypoxic conditions (°C ± std. error)	Oxic conditions (°C ± std. error)
1	14.18 ± 0.04	14.31 ± 0.02
3	14.00 ± 0.03	14.04 ± 0.05
6	13.99 ± 0.04	14.12 ± 0.04
24	13.78 ± 0.03	13.84 ± 0.02
48	14.01 ± 0.05	13.94 ± 0.02
72	14.00 ± 0.03	14.00 ± 0.01
96	14.15 ± 0.03	14.16 ± 0.02
120	14.07 ± 0.03	14.08 ± 0.02
144	14.16 ± 0.03	14.17 ± 0.01

Table II. Average salinity (PSU) levels for hypoxic and oxic conditions throughout the reactor experiment, measured over a period of 144 hours.

Average Salinity		
Time after start of experiment (hours)	Hypoxic conditions (PSU ± std. error)	Oxic conditions (PSU ± std. error)
1	24.55 ± 0.05	24.57 ± 0.07
3	24.62 ± 0.03	24.58 ± 0.08
6	24.70 ± 0.01	24.60 ± 0.05
24	24.86 ± 0.05	24.74 ± 0.07
48	24.97 ± 0.05	24.82 ± 0.06
72	25.08 ± 0.06	24.71 ± 0.14
96	25.12 ± 0.07	24.96 ± 0.05
120	25.27 ± 0.07	25.04 ± 0.05
144	25.32 ± 0.09	25.06 ± 0.07

Table III. Average pH for hypoxic and oxic conditions throughout the reactor experiment, measured over a period of 144 hours.

	Average pH	
Time after start of experiment (hours)	Hypoxic conditions (± std. error)	Oxic conditions (± std. error)
1	8.133 ± 0.014	8.192 ± 0.024
3	8.185 ± 0.025	8.163 ± 0.020
6	8.337 ± 0.020	8.128 ± 0.014
12	8.192 ± 0.093	8.042 ± 0.064
24	8.385 ± 0.009	8.182 ± 0.021
48	8.243 ± 0.014	8.127 ± 0.015
72	8.320 ± 0.041	8.130 ± 0.018
96	8.362 ± 0.036	8.133 ± 0.026
120	8.418 ± 0.030	8.115 ± 0.005
144	8.405 ± 0.039	8.150 ± 0.030