PHARMACEUTICAL TRANSPORT AND TRANSFORMATION IN *TRAPA NATANS* BEDS

A Final Report of the Tibor T. Polgar Fellowship Program

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Plum, C.I., S.K. Hamilton, R. Henry, D.T. McCarthy, E.J. Rosi and M.R. Grace. 2021. Pharmaceutical Transport and Transformation in *Trapa natans* Beds. Section VI: 1-52 pp. *In* D.J. Yozzo, S.H. Fernald, and H. Andreyko (eds.), Final reports of the Tibor T. Polgar Fellowship Program, 2019. Hudson River Foundation.

ABSTRACT

Pharmaceutical and pesticide pollution is ubiquitous in the Hudson River due to anthropogenic inputs including agricultural runoff, wastewater treatment and leaky sewage infrastructure. Understanding the transport and transformation of pharmaceuticals and pesticides in the river system is fundamental to gaining insight into how these contaminants affect aquatic biota and, conversely, how the biota may retain or remove these contaminants. An invasive, floating aquatic plant known as water chestnut or *Trapa* (*Trapa natans*) is abundant along the freshwater reaches. *Trapa* beds are microbially active environments that are key contributors to removal of anthropogenic nitrate, a process essential to maintaining a healthy functioning ecosystem. This project aimed to test the hypothesis that *Trapa* beds are hotspots of pharmaceutical accumulation or degradation as well as other changes in water quality including nitrate removal. Concentrations of pharmaceuticals and pesticides, as well as the conservative tracer sucralose, and several traditional water quality metrics, were measured in water flowing into and out of *Trapa* beds at two locations. Preliminary results indicate that the beds alter water quality, most notably by reducing dissolved oxygen concentrations, particularly at low tide. Rates of respiration and denitrification were quantified in open water as well as water, biofilms, and the sediment-water interface within the beds. Respiration and denitrification was measured at elevated levels within the *Trapa* beds compared to the adjacent water column. These biological processes explain the decreased nitrate and dissolved oxygen concentrations observed, sometimes resulting in anoxia, within the *Trapa* beds. Concentrations of a number of pharmaceuticals and pesticides were detectable outside and within the beds. The full analysis of how pharmaceuticals and pesticides interact with *Trapa* beds awaits completion of laboratory measurements of contaminants.

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INTRODUCTION

Hudson River Estuary

The Hudson River Estuary is a singularly important waterbody for New York and the broader region, providing cultural, economic, and environmental values and services (Levinton and Waldman 2006). The freshwater, tidal part of the estuary extends from Troy to Newburgh and is 900 m wide and 8.3 m deep, on average. Strong tidal currents reverse direction every 6 hours and generally keep the water column in the main channel well mixed both vertically and laterally, and cause the water level to vary on the order of about a meter.

Despite a long history of industrial and municipal pollution, the Hudson River today is relatively clean, and serves as a source of drinking water for local populations. Nevertheless, excessive loading of nutrients from both point- and non-point sources, as well as the presence of various trace contaminants in the river system including pharmaceuticals, polychlorinated biphenyls (PCBs) and polyfluoroalkyl substances (PFAS), continue to be a source of concern. The latter two contaminants are legacies of past industrial activity whereas pharmaceuticals come from current wastewater inputs, and their use and inputs to the river may be increasing. A diversity of pharmaceuticals and personal care products have been found in the Hudson River system (Cantwell et al. 2017; Carpenter and Helbing 2018). The ecological effects of trace contaminants including pharmaceuticals, as well as their transport and fate in the Hudson River system, are not well understood.

***Trapa natans* beds contribute to denitrification in the Hudson River**

Aquatic vegetation is essential for maintaining water quality and ecosystem health and can improve water quality through the removal of nutrients and contaminants. Dominance by invasive plants may not compromise those ecosystem services in spite of the other negative impacts (Schlaepfer et al. 2011), and can even enhance services like nutrient and contaminant retention if the invasives are more productive than native species (e.g. Martina et al. 2014).

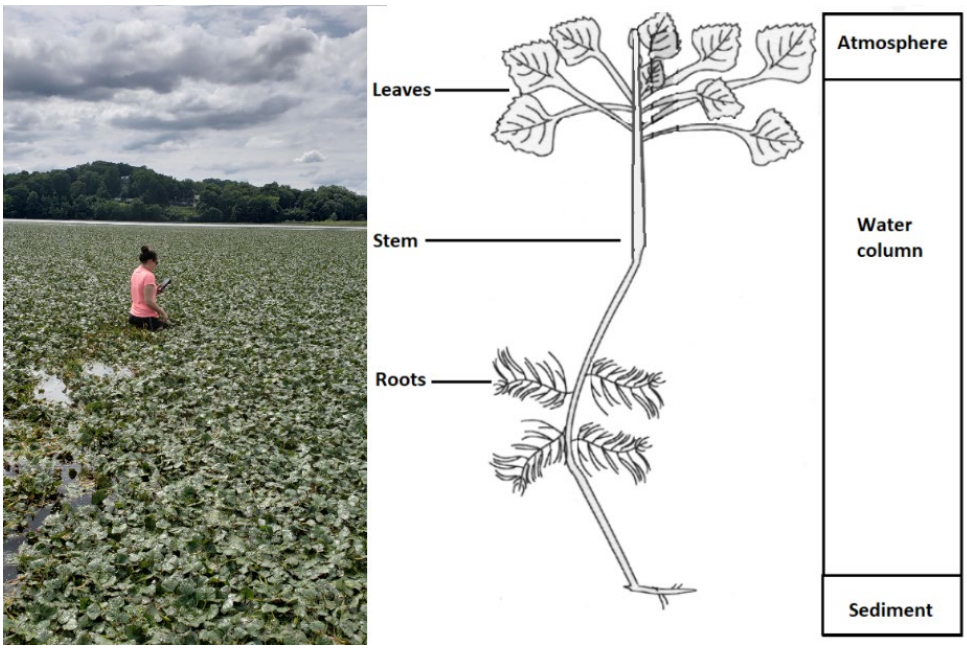

Figure 1: ***Trapa* bed (left) and *Trapa* plant diagram showing components and vertical situation within the bed (right) adapted from Caraco et al. 2006.**

In the Hudson River between Beacon and Troy, many of the shallow backwaters along the main channel are densely vegetated with the invasive floating macrophyte *Trapa natans*, commonly known as water chestnut (Figure 1). These beds are likely to

function as biogeochemical hotspots in the river system. In particular, *Trapa* beds are recognized to play an integral part of the Hudson River's ecology due to their considerable capacity for denitrification, a microbe-mediated process that converts bioavailable nitrate to inert nitrogen gas (N_2). Denitrification in aquatic systems is vital as high nitrogen loads can lead to eutrophication and detrimental algal bloom events which can have implications for public health, economic returns, fishing and aquatic recreation (Backer 2012). Previous work has estimated that *Trapa* beds remove ~25% of nitrogen loads from the Hudson River annually, despite only being active for three months of the year (Tall et al. 2011). The high capacity for denitrification in *Trapa* beds compared to native submersed aquatic vegetation or open water areas is due to *Trapa's* floating leaves, which favor anoxic conditions by limiting light availability and air-water gas exchange (i.e. reaeration), combined with the tidal system which continuously removes and replaces oxygen- and nutrient-depleted water.

Pharmaceutical pollution

Pharmaceuticals, an emerging class of environmental contaminants, have been detected in freshwater systems worldwide, usually in the ng/L to µg/L range (Hughes et al. 2012; Kolpin et al. 2002; Murdoch 2015; Scott et al. 2014; Watkinson et al. 2009). In a recent study, 16 pharmaceuticals were detected at up to 72 sites along a 250-km transect of the Hudson River (Cantwell et al. 2017). The Hudson River catchment contains 114 wastewater treatment plants (WWTPs) that continuously release treated effluent containing pharmaceuticals (Carpenter and Helbling 2018; NYCDEP 2019). Some

pharmaceutical concentrations in close proximity to a WWTP discharge point at the mouth of Rondout Creek, which drains into the Hudson River at Kingston, have been detected in the mg/L range (Carpenter and Helbling 2018). Furthermore, many combined sewer and stormwater overflows discharge directly into the Hudson River and its tributaries, including Rondout Creek. Increasing incidences of extreme rainfalls and stormwater runoff, as well as flooding of WWTPs and other sewage infrastructure due to sea level rise caused by climate change, can result in uncontrollable releases of large amounts of pharmaceutical-containing untreated effluent (Azevedo de Almeida and Mostafavi 2016; Flood and Cahoon 2011). This is of concern as pharmaceuticals have the demonstrated ability to act on non-target organisms, including environmental microbes (Rosi-Marshall et al. 2013), potentially disrupting fundamental ecosystem services such as nutrient removal via denitrification.

Environmental interactions and the ultimate fate of pharmaceuticals are poorly understood. Pharmaceutical pollution includes a broad range of chemical compounds exhibiting a variety of properties that could cause disruption of important ecological processes mediated by microbes and algae (Rosi-Marshall et al. 2013; Richmond et al. 2017). For example, some pharmaceuticals remain in the water column while others accumulate in sediments or biofilms such as those on underwater plant surfaces (Ferrer et al. 2004; Scott et al. 2014). Accumulation may be problematic due to toxicity exhibited by increased concentration in microsites of accumulation, and the increased potential for movement into the food web via particle-feeding aquatic invertebrates and fishes (Richmond et al. 2018). Furthermore, degradation of some pharmaceuticals yields toxic by-products (Neuwoehner et al. 2010; Santos et al. 2010), and these may have a greater

capacity to interfere with microbial communities involved in processes such as denitrification. Pharmaceutical transport, transformation, and the short- and long-term impacts of pharmaceutical pollution must be investigated in order to understand how these contaminants affect aquatic biota and the ecosystem services they provide, and conversely, how the aquatic biota may retain or remove these contaminants.

While it is thought that *Trapa* beds are integral to nitrogen removal throughout the Hudson River, their relationship with pharmaceutical pollution is unknown. Tides cyclically force the movement of large volumes of water containing nutrients and pharmaceuticals into and out of the beds. Pharmaceutical retention or removal may be influenced by the chemical properties of the pharmaceutical compounds, and by interactions with the *Trapa* roots, particulate matter, microbes on sediment surfaces, and biofilms on underwater plant stems and roots. Pharmaceuticals are expected to be present within *Trapa* beds, but whether they show net decreases on concentrations and how they affect microbial processes within the beds are currently unknown. If pharmaceuticals are removed in *Trapa* beds, these beds may help mitigate the impacts of pharmaceutical pollution arising from stormwater and combined sewer overflows and the associated public health risks.

There is some evidence that pharmaceuticals at currently measured environmental concentrations can act negatively on important microbial processes. For example, diphenhydramine (an antihistamine), caffeine (a stimulant), cimetidine (a histamine H2 receptor antagonist) and ciprofloxacin (an antibiotic) have each been demonstrated to suppress biofilm respiration (Rosi-Marshall et al. 2013; Robson et al. 2020). Further, the antibiotic amoxicillin has been shown to decrease denitrification rates (Costanzo et al.

2005). In the Hudson River Estuary, the effects of pharmaceuticals are of particular interest in relation to denitrification, the microbial process that enhances water quality and mitigates eutrophication, thereby improving the health of the estuary and coastal waters into which it discharges.

Project aims and hypotheses

This study uses a multidisciplinary design to investigate the interactions among pharmaceutical pollution, microbial communities, denitrification rates, and *Trapa* beds and the implications for water quality in the Hudson River (Figure 2).

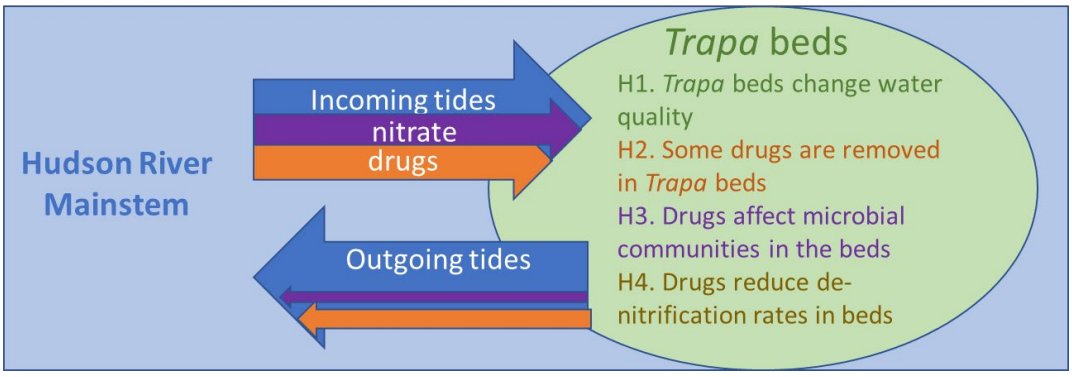

Figure 2: Conceptual framework for this study including the four major hypotheses under investigation.

This study will address four main research questions that relate directly to the hypotheses in Figure 2:

1. **How does water quality change when main stem river water enters the *Trapa* beds?**

As noted above, the dense beds of *Trapa* are predicted to be biogeochemical hotspots compared to the open waters. The plant's photosynthesis takes place largely

above water, and the aquatic metabolism within the beds is expected to be heterotrophic overall. In other words, microbial and root respiration within the beds will result in net consumption of dissolved oxygen and promote anaerobic microbial processes including denitrification. Anaerobic processes increase with lower dissolved oxygen even if the oxygen in the water column is only modestly depleted because anaerobic microsites in biofilms and the sediment-water interface become more important with lower oxygen concentrations.

Spatial and temporal variation is expected within the *Trapa* bed, with the degree of changes in water quality dependent on water residence time with the bed and on the depth of the water column. Therefore, the largest changes are expected at low tide, in the centers of the beds or in water exiting the centers, and especially when water depths are shallow (the underwater parts of *Trapa* expand and contract vertically with changes in depth).

2. Do *Trapa* beds remove pharmaceuticals in the Hudson River?

The tidal dynamics of the Hudson River drive the continuous exchange of water and nutrients between open waters and the *Trapa* beds. This study compared the concentrations of pharmaceuticals and their degradation products in water flowing into and out of *Trapa* beds over tidal cycles by sampling along transects from open water into the beds at various points in the tidal cycle and across the *Trapa* growing season (Figure 2). In addition to pharmaceuticals, sucralose (artificial sweetener) was measured as a conservative tracer to estimate net changes in reactive pharmaceutical concentrations in the face of uncertain mixing and dilution of waters within the beds. The utility of sucralose as a conservative tracer of wastewater effluent due to its resistance to

degradation has been shown by Soh et al. (2011) and Cantwell et al. (2017), the latter study conducted in the Hudson River.

Decreases in the ratios of a particular pharmaceutical to sucralose between inflows and outflows will reveal the net retention or removal of the pharmaceutical during residence of water within the beds. If degradation products increase in concentration relative to sucralose, their potential effects on the biota will also be considered.

As the *Trapa* beds are a microbially rich and biologically active hotspot, it is hypothesized that they will remove pharmaceuticals through adsorption and/or degradation. Pharmaceuticals with a lower solubility in water (i.e. greater hydrophobicity) are expected to have stronger particle interactions and partition with organic matter, allowing bioaccumulation in sediments or biofilms or uptake by *Trapa* roots, resulting in a decreased concentration in the water column. Accordingly, it is expected that lower concentrations of pharmaceuticals, and perhaps higher concentrations of degradation products, will be present in water well within or exiting the bed (i.e., on the outgoing tide). A gradient of pharmaceutical exposure from the main channel into the beds may then alter microbial response and perhaps resistance within the *Trapa* bed. Investigating whether *Trapa* beds remove pharmaceuticals will lay the groundwork for future research to understand how pharmaceuticals affect aquatic biota, and in particular, whether they may affect the rate of microbial denitrification.

This research will provide insights into the role of *Trapa* beds in pharmaceutical effects and fate in the Hudson River system and will provide the basis for investigating the role of pharmaceuticals influencing microbial community structure (question 3

below) and denitrification rates (question 4). This study has been designed and carried out with sufficient sampling to address the four key hypotheses described in Figure 2; however, this report will focus on water quality with some brief discussion of pharmaceuticals. Preliminary rates of denitrification are also presented here. Further results pertaining to the remaining hypotheses await completion of additional laboratory analyses. Nevertheless, the remaining questions to be further addressed are briefly described here:

3. Do pharmaceuticals in *Trapa* beds influence microbial communities?

Characterization of the microbial community composition is necessary to determine whether the presence of pharmaceuticals affects diversity and function. Microbial diversity is essential for an ecosystem's ability to be resilient in the face of environmental stresses and change.

The *Trapa* beds within the Hudson River are a hydrodynamically variable environment, with water levels and water quality varying rapidly in space and time over a tidal cycle. For example, a 60% increase in dissolved oxygen concentrations in less than 20 minutes on the turning tide was measured. Accordingly, conditions for microbes was expected to change quickly. Suspended microbial communities from the main channel enter *Trapa* beds through tidal water movement, but such transient communities would not be adapted to the periodically hypoxic and nutrient-rich conditions in the beds, in contrast to those found in sediment and biofilms within the beds that must be able to survive and function in the highly variable environment. In addition, characterization of the endogenous microbial community will allow further investigation into the effects of pharmaceuticals on individual denitrifying species. In a separate study, this

characterization is being achieved by DNA extraction from water, biofilm and plant material for 16S rRNA gene sequencing and whole genome metagenomics.

It is predicted that greater exposure and thus greater microbial resistance to pharmaceuticals will be observed on the outer edges of the *Trapa* bed compared to the interior. Additionally, a greater diversity of denitrifying microbes is expected in the interior of the *Trapa* bed where dissolved oxygen, labile carbon, and nitrate concentrations are all subject to greater variability.

4. Do pharmaceuticals in *Trapa* beds influence the microbe-mediated biogeochemical process of denitrification?

The dose-response relationship of denitrifying bacteria exposed to commonly detected pharmaceuticals has been investigated. Water incubations were dosed with a cocktail of commonly detected pharmaceuticals at environmentally relevant concentrations (Table ***1***) in order to measure any effects on denitrification rates. Additionally, comparison of two sites with different influence of wastewater will allow assessment across an impact gradient of pharmaceutical pollution.

Table 1: List of pharmaceuticals of interest in this study

Drug	Class	Final concentration ng/L
Acetaminophen	Pain killer	350
Atenolol	Beta blocker	1,000
Caffeine	Stimulant	2,500
Cimetidine	H2 antagonist	250
Ciprofloxacin HCl	Antibiotic	200
Diphenhydramin HCl	Antihistamine	1,000
Ibuprofen NaCl	Anti-inflammatory	3,000
Metformin	Antidiabetic	100,000
Ranitidine HCl	H2 antagonist	1,000

Respiration was measured simultaneously with denitrification, and microbial communities are being extracted for genomic analysis to explore microbial interactions with pharmaceuticals.

As in the case of microbial community effects, it is predicted that pharmaceuticals will have greater impact on respiration and denitrification on the outer edges of the *Trapa* bed because of the gradient of pharmaceutical exposure and thus microbial response. It is possible that some denitrifying species are susceptible, but that denitrification is maintained by resistant species. Alternately, competitors that assimilate nitrate may be susceptible, resulting in more nitrate availability for growth of denitrifying species.

METHODS

Experimental Overview

A variety of variables were sampled from each location up to 27 times between 18 June 2019 and 25 September 2019, over various points in the tidal cycle on multiple dates to account for short-term and seasonal variation. Not all variables were measured on every trip. Parameters from the first and final sampling trips, collected on 4 June and 23 October respectively, were not included as no *Trapa* was present.

Site Descriptions

Two areas containing *Trapa* beds were chosen for investigation in this study, and are denoted as Kingston and Norrie Point (Figure 3). These sites were selected because they differ in nearby land use and receive different amounts of wastewater effluent, and both have long-term nutrient monitoring data.

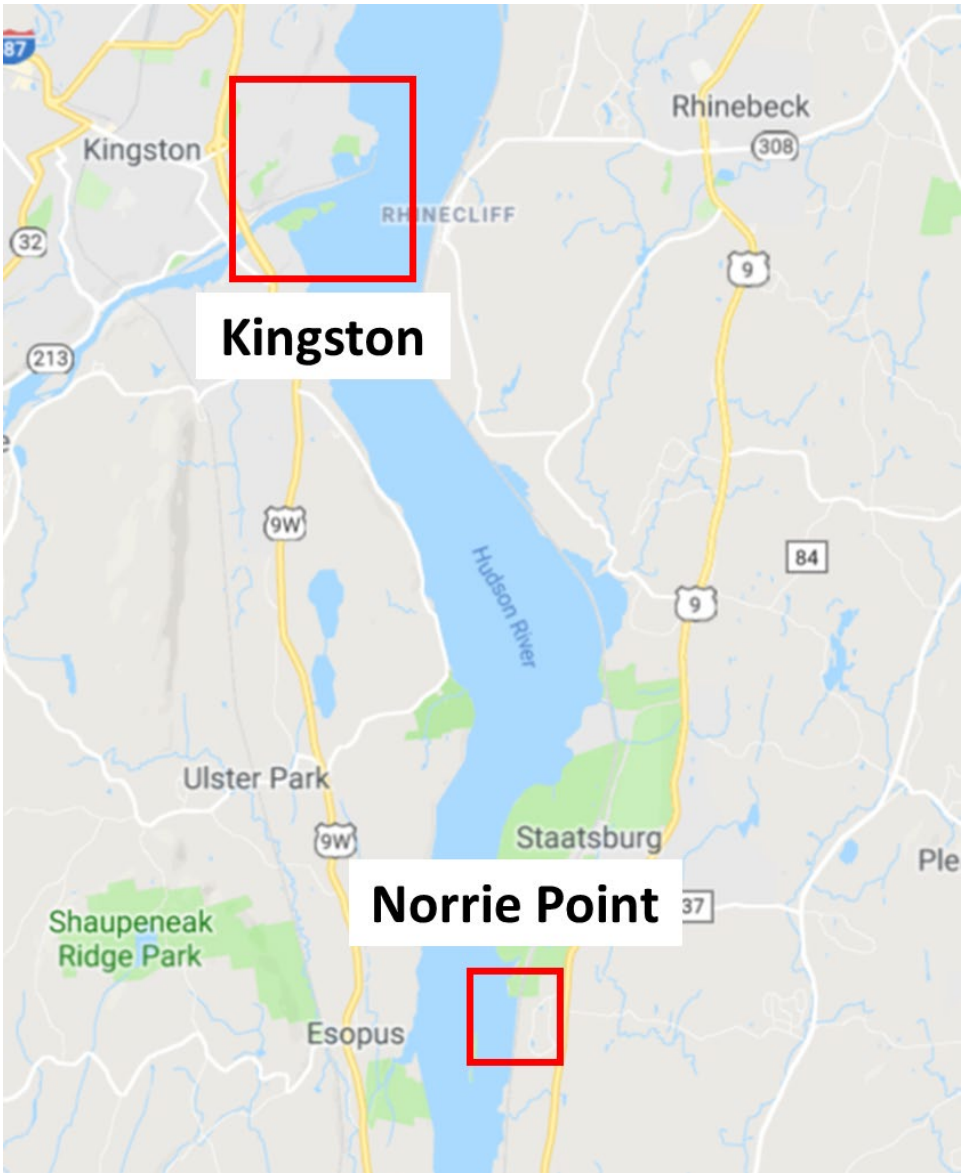

Figure 3: Kingston and Norrie Point sampling locations on the Hudson River

The Kingston *Trapa* bed lies south of the Rondout Creek. This bed receives Kingston WWTP effluent released into the vicinity of the mouth of Rondout Creek. Three locations along a transect into the center of the *Trapa* bed were sampled (Figure 4):

- KBA (41°55'10.7"N 73°58'01.9”W) is a site adjacent to the *Trapa* bed in which water flows from the Rondout Creek to the Hudson River,
- KBB (41°55'09.6"N 73°58'05.3”W) is located 80 m west of KBA and lies 45 m into the *Trapa* bed, and
- KBC (41°55'08.5"N 73°58'08.0”W) is the furthest transect point and is situated 110 m into the *Trapa* bed and 150 m east of KBA.

Two river monitoring sites with long-term data were also sampled:

- Rondout Creek (RC, 41°55'04.7"N 73°58'55.7"W) in front of Kingston Public Dock, which is the site of a combined sewer and stormwater outfall. Water quality data collected by RiverKeeper are available for this location (described in section 3.2).
- Kingston Hudson River (KHR, 41°56'12.1"N 73°57'38.2”W), which is located in the main stem of the Hudson River was included in routine sampling. Long term data for this site are available through the Long Term Research in Environmental Biology (LTREB) project led by Cary scientists and funded by the National Science Foundation.

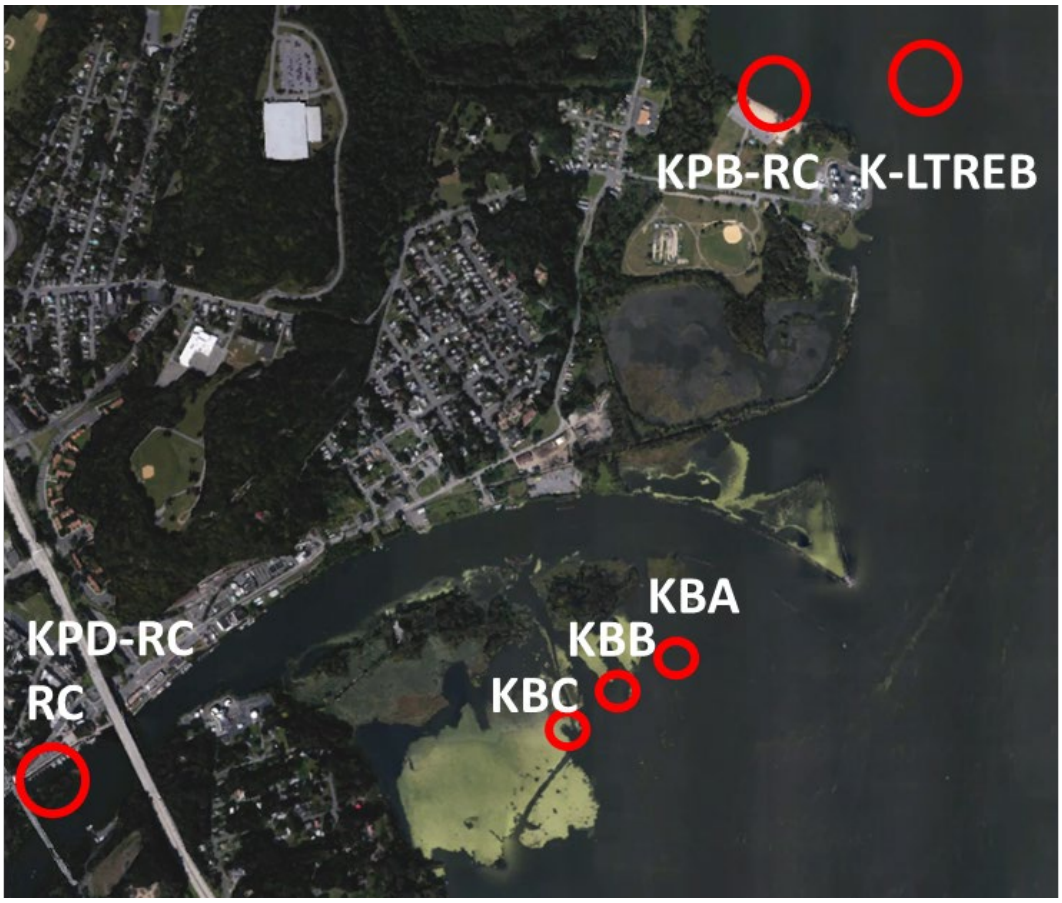

Figure 4: **Map of Kingston *Trapa* bed (bright green, annual variability) identifying the three sampling sites along a transect into the bed as well as the Rondout Creek and Hudson River sampling sites. The WWTP effluent is discharged along the north bank of Rondout Creek.**

Norrie Point was chosen for sampling as it is well characterised from previous studies, easily accessible and routinely monitored by the Hudson River Environmental Conditions Observing System (HRECOS) station maintained by the Hudson River National Estuary Research Reserve (HRNERR). A site within the bed (NB, 41°49'54.3"N 73°56'29.9"W) and another in the open water of the Hudson River (NHR, 41°49'54.7"N 73°56'33.6”W) were sampled. NHR is the site of the HRECOS monitoring station, which is further described in section 3.2 below.

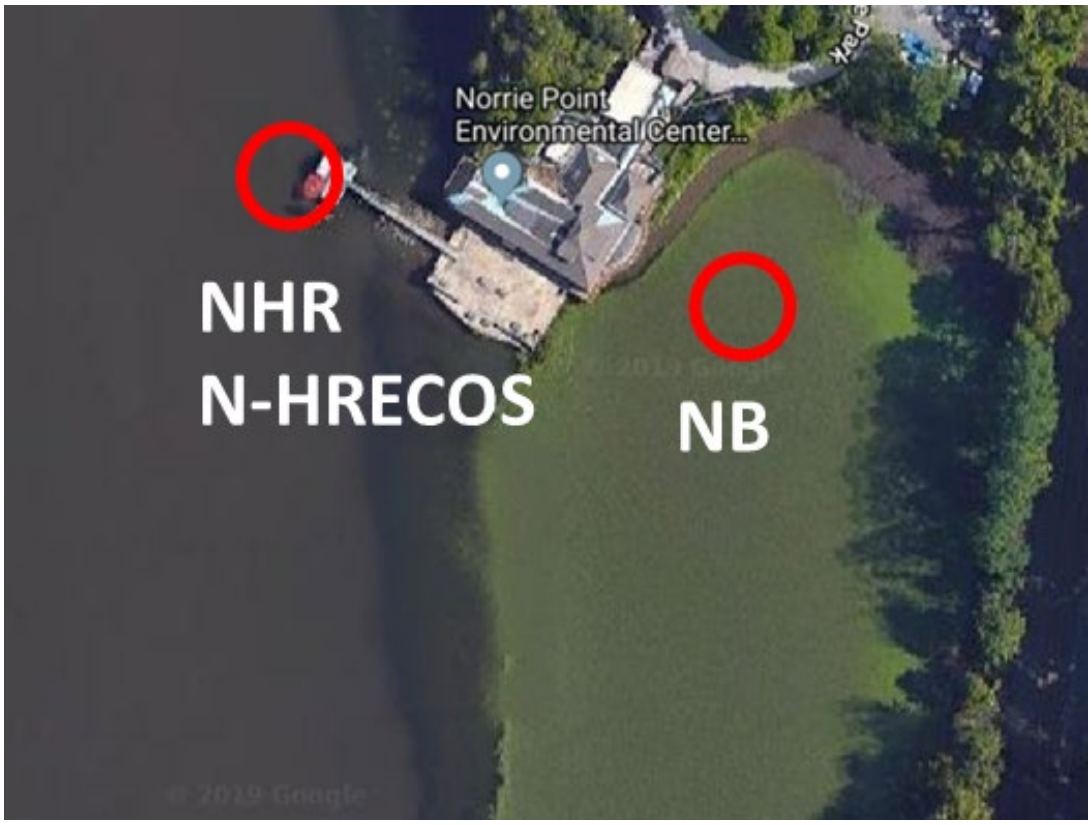

Figure 5: Map of Norrie Point *Trapa* bed (bright green) identifying the sampling sites within and exterior to the bed.

Water quality

Traditional water quality metrics

Temperature and dissolved oxygen (mg/L) were measured in situ using a pre-calibrated ProODO probe (YSI). Electric conductivity (EC) was measured *in situ* using an EC300 probe (YSI).

Depth-integrated samples of the water column were collected using a pump and transported to the laboratory on ice. Fluorescence (Relative Fluorescence Units, RFU) was measured for each sample using a Trilogy fluorometer (Turner designs, model #

7200-000), then converted to Nephelometric Turbidity Units (NTU) using a calibration curve constructed from serial dilutions of YSI 6073G Turbidity Standard (126 NTU).

In addition, long-term water quality monitoring data for the main river were obtained from the following sources:

- The New York State Department of Environmental Conservation (NYSDEC) Hudson River National Estuarine Research Reserve (HRNERR) monitoring programs including the meteorologic and hydrologic station at Norrie Point operated as part of the Hudson River Environmental Conditions Observing System (HRECOS). Long-term data include measurements at 15-minute intervals of temperature, wind, precipitation, pH, turbidity, chlorophyll fluorescence, dissolved oxygen etc. Data are available at https://ny.water.usgs.gov/maps/hrecos/ .
- The Long Term Research in Environmental Biology (LTREB) site at Kingston, which Cary scientists have monitored bi-weekly since 2000. Long-term monitoring of water chemistry, nutrient concentrations and phytoplankton and zooplankton have contributed to assessing the ecological effects of invasive zebra mussels (*Dreissena polymorpha*) (Strayer et al. 2006; Strayer et al. 2008; Fernald et al. 2007; Findlay et al. 2006; Caraco et al. 2000; Strayer et al. 2014).
- The non-profit environmental organization RiverKeeper makes monthly observations at 74 sites between the Mohawk River and Gowanus Canal. RiverKeeper provides counts of *Enterococcus*, a bacterium that is an EPA-approved indicator of faecal contamination, as well as measurements of water

temperature, turbidity, oxygen etc. This information is useful for beach-goers or others who interact with the river recreationally and is available at https://www.riverkeeper.org/.

Nutrient concentrations

Depth-integrated samples of the water column were collected using a pump and transported to the laboratory on ice. Samples for the analysis of ammonium, filterable reactive phosphorus, nitrate + nitrite (APN), and non-purgeable organic carbon (NPOC) were filtered using a 0.2-µm syringe membrane filter (Sartorius). Samples for total phosphorus and total nitrogen (TP and TN) remained unfiltered. DOC samples were preserved with 300 µL of 2 M H_2SO_4 and TP, TN and APN samples were preserved with 500 µL of 2 M H_2SO_4 and all were stored at room temperature until analysis. NPOC was analysed on a Total Organic Carbon Analyzer (Shimadzu, model #TOC-VCSH).

Diel variability in dissolved oxygen

To provide an indication of diel changes in dissolved oxygen, which are indicative of the balance between aquatic photosynthesis and respiration, miniDOT oxygen loggers (PME) were deployed from 27 August to 5 September 2019. The miniDOTs were attached to the inside of the roof of a lobster cage filled with bricks and set in the *Trapa* bed such that the loggers were situated approximately 30 cm above the bottom. Temperature and oxygen measurements were recorded every 5 minutes. These data were compared to the HRECOS oxygen data at the open water off Norrie Point, for the same dates, which were logged every 15 minutes.

Tide information was obtained from Tides & Currents Pro (Nobeltec version 3.5.107) using Kingston data based on New York, The Battery (NOAA; 41°55'06N, 073°59'00W, Station ID 1337) and Norrie Point based on New York, The Battery (NOAA; 41°47'N 073°57'W, Station ID:1335).

Pharmaceuticals

Depth-integrated samples of the water column were collected using a pump and transported to the laboratory on ice in clean 1-L Nalgene bottles. Samples were prefiltered by gravity using 1 µm filter paper, then the pharmaceuticals were pre-concentrated on Oasis HLB 6cc 200mg extraction cartridges (preconditioned with 5 mL of HPLC grade methanol then 5 mL of de-ionised water (Brodin et al. 2013) at a flow rate of 5 mL/min. Cartridges were then refrigerated in the dark to prevent UV degradation. Samples were transported to Monash University, Melbourne, Australia and analysed on a triple quadrupole liquid chromatograph mass spectrometer (SPE-triple quad-LCMS-MS**,** Agilent) with a 1 ng/L limit of detection. Pharmaceuticals analysed include atenolol, atropine, caffeine, carbamazepine, cetrizine, ciprofloxacin, diclofenac, diphenylhydramine, enrofloxacin, flucanozole, flunixin, fluoxetine, gemfibrozil, imidacloprid, meloxicam, metformin, oxytocin, pimobendan, ranitidine, sertraline, sulfadoxine, sulfamethaxozole, telmisartan, triclosan, trimethoprim and xylazine. In addition, herbicides that were measured include 2,4-dichlorophenoxyacetic acid (a.k.a. 2,4-D), atrazine, DCMU, dicamba, MCPA and triclopyr.

Denitrification and respiration

Water incubations to indicate rates

Depth-integrated samples of the water column were collected in a bucket using a pump with its outlet submerged to avoid reaeration. 12.5 mL glass vials with septa (Exetainers) were filled and capped underwater to exclude air bubbles. Immediately, biological activity in a set of triplicate samples was terminated by adding 0.2 mL $ZnCl_2$ (50% w/v) using a purge needle to eliminate over-pressurization. This time was recorded as T0. All remaining samples were incubated in the dark to preclude photosynthetic activity, at ambient temperature (in a cooler filled with site water). At various time intervals, triplicate control and dosed samples were terminated and stored inverted in the dark until the time of analysis.

This was repeated at each site before and after low tide. Later, slurries of plant biofilm material and sediment were incubated under the same protocol; however, only the results for water collected on the outgoing (ebb) tide, which best reflects conditions within the beds, are discussed in this report.

Membrane Inlet Mass Spectrometry (MIMS) Analysis

Terminated water samples were analyzed for dissolved N_2:Ar and O_2:Ar ratios at 20°C on a MIMS. Sample temperatures were maintained at 20 °C. Atmospheric pressure

was recorded at the time of each sample. A recirculating distilled water bath at 20°C was used as a standard for air-equilibrated dissolved gas concentrations and measured every 12 samples to correct for any instrumental drift (Reisinger et al. 2016). MIMS readings were averaged over a 30-second time period for each sample and converted to mg/L. The "Linest" function in Excel was used to perform a regression of N_2 and O_2 over time, the slope of which was taken as the rate (denitrification as μg N_2/L-hr produced and respiration as μg O_2/L-hr consumed, respectively).

RESULTS

Water Quality

Average *in situ* water quality variables (Table 2), except dissolved oxygen, show little variation among the sampling sites at Kingston. Temperature was in a similar range (24 ± 3 °C) at all Kingston sites, except for those measured by RiverKeeper (KPD-RK and RPB-RK) which were higher. Conductivity was highest at RC (360 μS/cm) and decreased from 316 to 296 μS/cm along the transect into the *Trapa* bed; however, these differences are not statistically significant. Conductivity was lowest at the K-LTREB site (254 uS/cm). Turbidity was similar and low (mean ranging from 10 to 19 NTU) at all Kingston sites.

Dissolved oxygen differed between the open channel and the *Trapa* beds. On average, % oxygen saturation was >100% at all open channel sites. The oxygen measured next to the *Trapa* bed was similar to that measured in Rondout Creek (97 ± 7% and 98 ±

7%, respectively). Oxygen was lower and more variable within the *Trapa* bed (KBB= 81 ± 14% and KBC=92 ± 15%). The minimum oxygen concentration measured in the bed (47% DO; Appendix A) is considerably lower than the minimum dissolved oxygen measured in Rondout Creek by RiverKeeper or in the main river by LTREB (86% and 95% saturation, respectively).

Temperature and conductivity were similar between sites within the *Trapa* bed and in the nearby open channel. Turbidity was lower and less variable in the river compared to in the *Trapa* bed. Temperature was higher in the Norrie Point main channel (NC-RK 30 ± 2 °C) compared with N-HRECOS and measurements taken in this study (25 ± 2 °C).

Temperature and turbidity were similar between Kingston and Norrie Point (Table 2). Conductivity was slightly lower at Norrie Point than Kingston, but similar to K-LTREB. Both Kingston and Norrie Point showed lower % oxygen saturation in the *Trapa* bed compared to the nearby open channel; however, within the beds Norrie Point was found to have a lower average oxygen concentrations (69%) than at Kingston (81% and 92%).

Ammonium, nitrate, phosphate, total nitrogen and total phosphorus concentrations measured at three sites within the Kingston *Trapa* bed were lower than concentrations measured in Rondout Creek. Nitrate concentrations decreased leading further into the bed, supporting the denitrification capacity of *Trapa* beds. Despite variation in tidal stages, results showed less variability than expected. All nutrients had lower concentrations at the Norrie Point sites compared to Kingston, perhaps reflecting more local sources in the vicinity of Kingston, including the nearby WWTP.

Table 2: **Mean water quality and nutrient variables ± S.D. for six sites measured in this study (Kingston - KBA, KBB, KBC & RC; Norrie Point - NHR & NB) and five routinely monitored sites (KLTREB = Kingston LTREB site, KPD-RK= RiverKeeper Kingston Public Dock, PBO-RK=RiverKeeper Kingston Public Beach, N-HRECOS= HRECOS monitor at Norrie Point & NC-RK= RiverKeeper Norrie Mid Channel) for samples collected between 4 June and 25 September 2019 . Summary statistics for each site are presented in Appendix I. Asterisks (*) denoted measurement unit of formazin nephelometric units (FNU).**

	Kingston							Norrie Point			
Variable	**KBA**	**KBB**	**KBC**	**RC**	**K-LTREB**	**KPD-RK**	**KPB-RK**	**NHR**	**NB**	**N-HRECOS**	**NC-RK**
Sampling dates	10 trips 18 Jun - 24 Sep	10 trips 18 Jun - 24 Sep	9 trips 18 Jun - 9 Sep	7 trips 23 Jul - 24 Sep	19 Jun - 24 Sep	11 Jul - 12 Sep	11 Jul - 12 Sep	9 trips 26 Jun - 25 Sep	9 trips 26 Jun - 25 Sep	19 Jun - 25 Sep	11 Jul - 12 Sep
Number of samplings	27	27	25	10	8	3	3	14	14	All data	3
Temperature (˚C)	24 ± 2	24 ± 2	25 ± 2	25 ± 2	24 ± 3	31 ± 3	30 ± 3	25 ± 2	25 ± 2	25 ± 2	30 ± 2
Electrical Conductivity (µS/cm, 25°C)	316 ± 66	310 ± 75	296 ± 52	360 ± 68	258 ± 36	n/a	n/a	272 ± 20	273 ± 20	262 ± 38	n/a
Turbidity (NTU)	19 ± 17	17 ± 25	10 ± 10	10 ± 4	16 ± 15	9 ± 9	9 ± 3	13 ± 3	20 ± 20	6 ± 4*	7 ± 3
Dissolved oxygen (mg/L)	8.2 ± 0.7	7 ± 1	8± 1	8.0 ± 0.7	8.5 ± 0.6	n/a	n/a	8 ± 1	6 ± 4	7.6 ± 0.8	n/a
% Oxygen saturation	98 ± 7	81 ± 14	92 ± 15	97 ± 7	101 ± 5	101 ± 18	100 ± 7	100 ± 14	69 ± 43	92 ± 8	93 ± 7
NPOC (mg-C/L)	4.6 ± 0.7	4.3 ± 1.0	4.3 ± 0.6	6 ± 1	3.5 ± 0.2	n/a	n/a	4.0 ± 0.3	4.0 ± 0.2	n/a	n/a
NH_4^+ (mg-N/L)	0.10 ± 0.7	0.07 ± 0.1	0.06 ± 0.03	0.19 ± 0.1	0.04 ± 0.03	n/a	n/a	0.03 ± 0.02	0.04 ± 0.02	n/a	n/a
NO_3^- (mg-N/L)	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	0.34 ± 0.07	n/a	n/a	0.3 ± 0.1	0.2 ± 0.1	n/a	n/a
PO_4^{3-} (mg-P/L)	0.03 ± 0.03	0.03 ± 0.02	0.02 ± 0.01	0.07 ± 0.03	0.02 ± 0.01	n/a	n/a	0.02 ± 0.01	0.02 ± 0.01	n/a	n/a
TN (mg-N/L)	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.7	1.0 ± 0.2	0.8 ± 0.1	n/a	n/a	0.64 ± 0.05	0.6 ± 0.1	n/a	n/a
TP (mg-P/L)	0.08 ± 0.03	0.07 ± 0.05	0.06 ± 0.02	0.11 ± 0.2	0.05 ± 0.005	n/a	n/a	0.05 ± 0.01	0.07 ± 0.04	n/a	n/a

Diel variability in dissolved oxygen

Continuous measurements of dissolved oxygen over nearly two weeks showed concentrations to be lower overall in the *Trapa* bed (24-hour mean, 44% oxygen saturation) than in the open channel (average 89%), and the 24-hour means are lower than the daytime samples reported above (69% for the *Trapa* bed and 92% for the open channel, Table 2).

Over the diel cycle, dissolved oxygen was much more variable in the *Trapa* bed compared to the open channel, ranging from 9% to 89 % saturation within the bed while the open channel ranged from 76% to 104% saturation. Norrie Point data are shown in Figure 6.

On four occasions during low tide the miniDOT oxygen logger was exposed to air, so the minimum % oxygen saturation reported here is conservative and is likely to have decreased further had it been measurable at the lowest tides. This is supported by field measurements as indicated in Appendix B where the lowest concentration measured at site NB was 5% oxygen saturation.

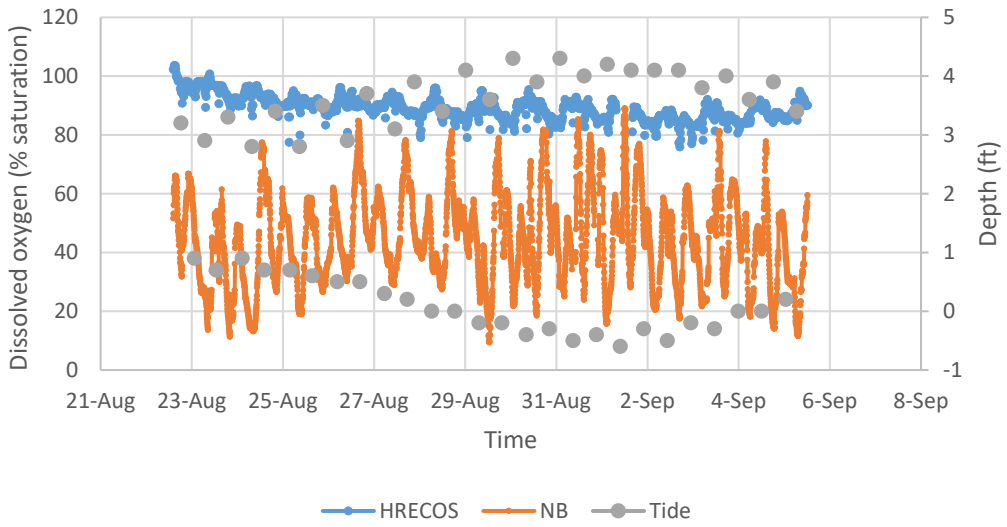

Figure 6: **Comparison of % oxygen saturation for the open water of the Hudson River and within the *Trapa* bed at Norrie Point from 22 August to 5 September 2019. NB denotes Norrie Point data collected in this study and HRECOS refers to the Norrie Point monitoring station. Maximum and minimum water depths for each tidal cycle are also shown**

Temperatures within the open channel and *Trapa* bed were similar (Figure 7). The mean water temperature in the bed (25.0 ± 0.9°C) was similar to that in the river (25.4 ± 0.6°C). The mean water temperatures within this two-week period are close to the mean temperature across the entire sampling period (25 ± 2°C); however, higher temperature fluctuations were found within the bed, with a range of 4.7°C compared to the river with a range of 2.4 °C.

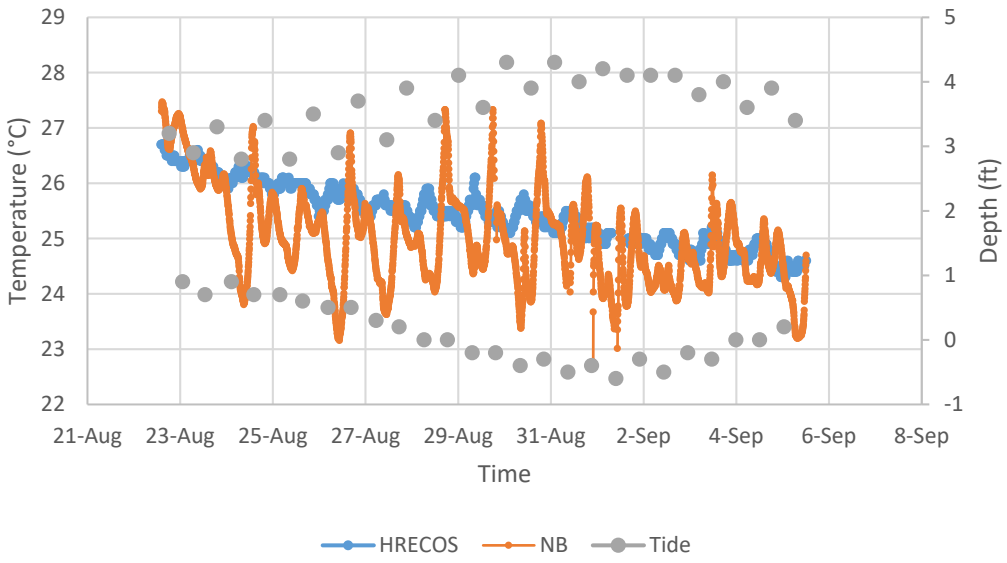

Figure 7: **Temperature for Hudson River and *Trapa* bed at Norrie Point from 22 August to 5 September 2019 where NB denotes data collected in this study and HRECOS refers to long term Norrie Point data. Temperature for HRECOS and NB is on the left y-axis and depth above mean tide is on the right y-axis.**

Pharmaceuticals

At the time of this report, 41 of 153 water samples have been analysed for pharmaceutical concentrations, and only preliminary data are available because some additional post-processing is needed to ensure accuracy. Out of 33 compounds analysed, 23 were detected, as outlined in Table 3. Eight compounds (2,4-dichlorophenoxyacetic acid, atenolol, atrazine, caffeine, cetirizine, diphenhydramine, metformin and sulfamethoxazole) were detected in all samples collected from both Kingston and Norrie Point. For samples collected from Norrie Point, 12 compounds were detected in all samples. Five herbicides (all except atrazine) and one antihistamine (diphenhydramine) detected at Norrie Point had concentrations >10% than those detected at Kingston. 12

compounds had concentrations >10% higher at Kingston than at Norrie Point. Concentrations detected are being correlated with tidal cycles to determine any changes during residence of water in the *Trapa* bed. The eight compounds analysed but not detected at any site were atropine, diclofenac, enrofloxacin, flunixin, imidacloprid, oxytocin, pimobendan, sertraline, xylazine and meloxicam.

Table 3: Pharmaceutical and herbicide detection frequency for 23 compounds measured in site water from three sites near Kingston and two sites in Norrie Point by Monash University via SPE-triple quad-LCMS-MS. Asterisks (*) denote mean concentration >10% higher at Norrie Point. Daggers (†) denote concentrations were >10% higher at Kingston.

PPCP	Class	Kingston frequency detected (of 31 samplings)	Norrie Point frequency detected (of 10 samplings)
2,4-D	Herbicide*	31	10
Atenolol	Beta blocker†	31	10
Atrazine	Herbicide	31	10
Caffeine	Stimulant†	31	10
Carbamazepine	Anticonvulsant†	30	10
Cetirizine	Antihistamine†	31	10
Ciprofloxacin	Antibiotic†	7	5
DCMU	Herbicide*	26	10
Dicamba	Herbicide*	15	9
Diclofenac	Anti-inflammatory†	18	8
Diphenhydramine	Antihistamine*	31	10
Fluconazole	Antifungal†	28	10
Fluoxetine	Antidepressant	4	0
Gemfibrozil	Cholesterol reduction†	14	4
MCPA	Herbicide*	6	9
Metformin	Anti-diabetic†	31	10
Ranitidine	Antihistamine	3	0
Sulfadoxine	Antimalarial†	9	4
Sulfamethoxazole	Antibiotic†	31	10
Telmisartan	Antihypertensive†	28	10
Triclopyr	Herbicide*	14	8
Triclosan	Antibacterial	5	2
Trimethoprim	Antibiotic†	21	5

Denitrification and Respiration

Denitrification and respiration rates in the *Trapa* bed and open channel at both Kingston and Norrie Point were measured on three dates throughout the sampling period. Both sites had very low denitrification (Figure 8) and respiration (Figure 9) rates for the first two samplings, not detectably different from zero, as indicated by error bars crossing over zero on the y-axis.

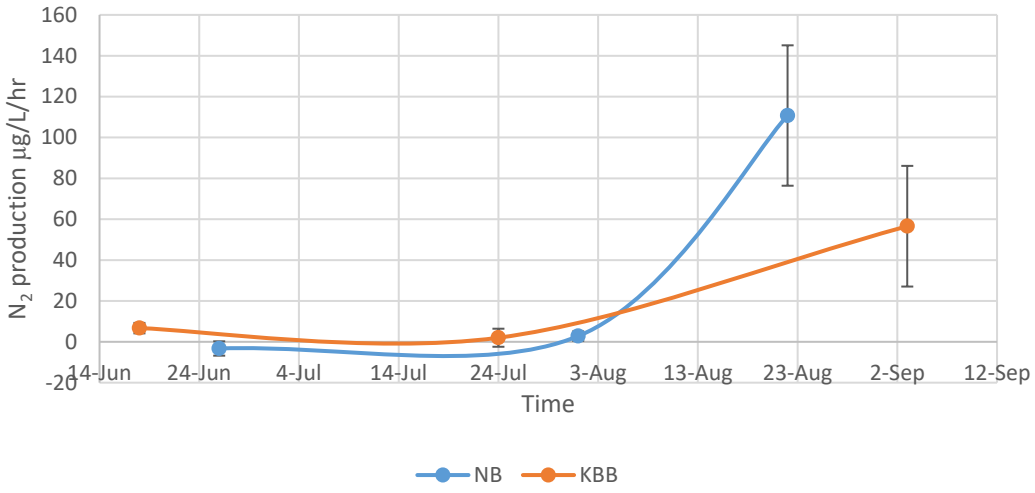

Figure 8: Comparison of denitrification rates over time in the Norrie Point and Kingston *Trapa* beds on the incoming tide for samples collected between 26 June and 3 September 2019. Error bars depict uncertainty in the estimate of the slope of the change over time.

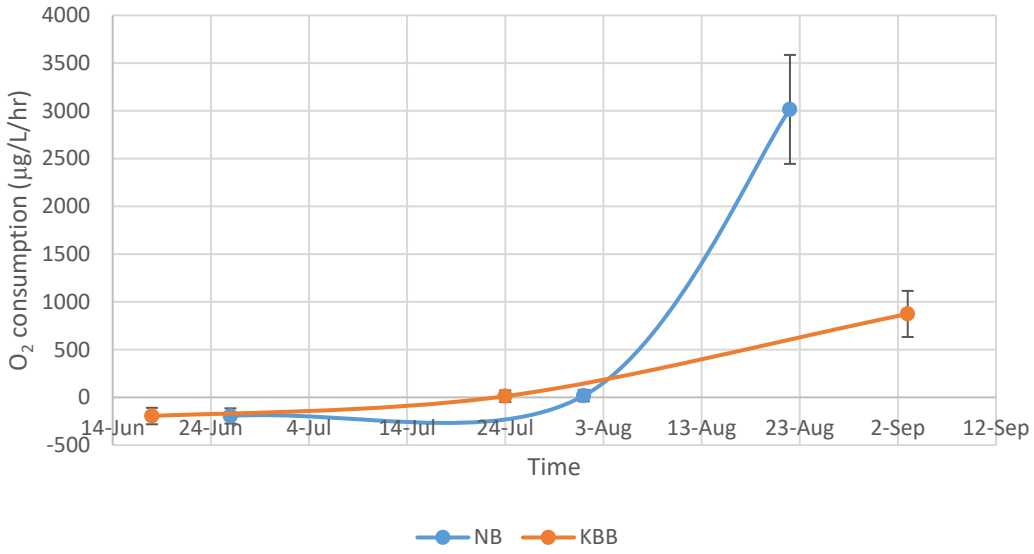

Figure 9: **Comparison of respiration rates over time in the Norrie Point and Kingston *Trapa* beds only for samples collected on the incoming tide for samples collected between 26 June and 3 September 2019. Rates were obtained using the Linest function and error bars depict uncertainty in the estimate of the slope of the change over time.**

DISCUSSION

Findings from this study highlight changes in water quality and pharmaceutical concentrations between the *Trapa* beds and the open water of the Hudson River. The documented changes over time scales ranging from diel cycles to the seasonal cycle of growth and senescence of *Trapa*. In addition, the study also reveals differences between the Kingston and Norrie Point sites. Differences between the *Trapa* bed and the open channel are evident in traditional metrics of water quality, particularly dissolved oxygen, but also by pharmaceutical concentrations and rates of respiration and denitrification. Furthermore, dissolved oxygen and denitrification show evidence for change over the seasonal cycle. This high spatiotemporal variability highlights the need to account for the phenological stage of *Trapa* as well as tidal cycles, time of day, complex hydrodynamics,

and spatial variability to fully comprehend how these beds affect ecosystem processes and are affected by emerging contaminants like pharmaceuticals.

Comparison with data from long term monitoring programs

Water quality data collected in this study are broadly consistent with data from other sampling at Kingston, K-LTREB and KBA, indicating that the open channel next to the *Trapa* bed is well mixed and that these long-term stations are reasonably representative of water quality and nutrient concentrations in open water adjacent to the *Trapa* beds sampled. NHR and HRECOS temperature and oxygen data are in particularly good agreement with measurements in this study. Higher temperatures and dissolved oxygen concentrations in the RiverKeeper data are likely explained by their relatively infrequent sampling, occurring during daylight hours and not accounting for tidal variations.

Variability over a tidal cycle

Continuous measurements with miniDOTs show variability in dissolved oxygen in the *Trapa* beds over a tidal cycle. As hypothesized, dissolved oxygen in the *Trapa* bed was always lower than that in the open waters of the river, indicating an excess of respiration over photosynthesis in the beds; however, during the period of 23 August to 8 September 2019, the dissolved oxygen in the bed was highest and most similar to the dissolved oxygen concentrations in the Hudson River during the daytime high tide, when depth of water in the beds exceeded 3.6 feet. High tides decrease density of plant mass,

allowing for greater water movement and mixing of water within the *Trapa* bed, allowing more reaeration and diluting the effects of biological oxygen production or consumption within the bed on oxygen concentrations. Nevertheless, even at the end of the incoming tide, water that has flooded into the bed would have mixed with low-oxygen water that was retained over low tide, resulting in a lower concentration of dissolved oxygen compared to the main channel.

Dissolved oxygen in the *Trapa* beds also shows diel patterns with higher concentrations in the daytime, reflecting the photosynthetic production of oxygen, but overall the beds showed net oxygen consumption. Although there is the potential for photosynthesis by underwater parts of the *Trapa* as well as by algae and duckweed within the beds, photosynthesis is likely subject to light limitation in most of the water column. Root respiration by the *Trapa* as well as high labile carbon availability from senescing plant parts likely explain the overall excess of respiration over photosynthesis.

Compared to the open water of the river, dissolved oxygen in the *Trapa* bed was more variable, ranging from 9-89% saturation, compared to the river water's range of 76-104%. It is clear that fishes and invertebrates in *Trapa* beds can experience periodic hypoxic stress given that dissolved oxygen concentrations of < 20-30% saturation are potentially stressful to aquatic biota (Rao et al. 2014). Previous studies show that *Trapa* beds are a hot spot for biodiversity (Kornijów et al. 2010) supporting diverse invertebrate communities. During this study, a diversity of fauna was observed both in the water column and inhabiting the submerged and emergent portions of the *Trapa* plants, including fish and invertebrates, showing that despite periodic low dissolved oxygen the

Trapa bed is vital for supporting aquatic biota (Kornijów et al. 2010; Findlay et al. 1989; Yozzo and Odum 1993).

Dissolved oxygen concentrations within the *Trapa* bed only exceeded 100% saturation for a 90-min period on 22 August (approximately halfway through the incoming tide) and only 1 instance (one data point) on 23 August (approximately halfway through the outgoing tide).

While biogeochemical processes in the *Trapa* bed may influence the water in the main channel of the Hudson River, it appears that physical processes are effective in re-oxygenating water from the *Trapa* bed and mixing it with main channel water, which rapidly increases dissolved oxygen concentrations. Thus, hypoxia would be an acute stress that may make it more readily tolerable or avoidable by fauna within the beds.

The overall mean dissolved oxygen concentrations over the two-week miniDOT sampling period (27 August to 5 September 2019) were lower than averages for the entire sampling period (18 June to 25 September 2019). Towards the end of summer, *Trapa* beds were lower in oxygen, a period corresponding with higher denitrification rates, as expected since anoxic conditions favour denitrification. This may also be a period of enhanced microbial activity as *Trapa* plants mature and begin to senesce.

Mean water temperatures in the open water of the river over the course of the sampling period were similar at both sites (NHRECOS = 25.4 ± 0.6 °C, NB = 25.0 ± 0.9 °C). Temperature was more variable in the *Trapa* bed compared to the open water. The open channel of the river is deeper and has a greater thermal mass and is thus not strongly influenced by variability in air temperature. The shallower depth and more protected

waters within the bed resulted in greater diel temperature fluctuation compared to the open waters of the river. Temperature changes are driven more by air temperature changes.

Although turbidity was similar at all sites, some tidally driven variability was observed. Suspended particulate matter in the *Trapa* bed increased on the incoming tide when particulates that had settled on the benthos and plant material were disturbed by increased flow. These particulates quickly settled out, but this observation shows the effectiveness of mixing by tidal water incursions within the bed. On the outgoing tide, a decrease in water volume resulted in an increased density of plant matter within the water column, resulting in restricted flow that further trapped suspended material.

Available forms of nitrogen and phosphorus were above critical limiting levels for aquatic primary production in all sampling sites (Table 2) (Wurtsbaugh at al. 2019; Guildford and Hecky 2000), consistent with previous studies of Hudson River environments (e.g., Lampman et al. 1999) and suggesting that light and mixing are more limiting factors for algal and submersed plant growth even in the more stagnant interstitial waters of the *Trapa* beds. All nutrient concentrations were higher in RC compared to *Trapa* bed sites. However, nutrient concentrations were similar within the three sites in the Kingston bed: KBA, KBB and KBC.

Change over the season

Lower concentrations of dissolved oxygen were recorded at low tide as the season progressed. The development of more extreme oxygen depletion towards September (e.g.

5% saturation measured on 5 September 2019) is expected due to increasing plant growth as the summer progressed, which limits 95% of light availability to the *Trapa* bed due to leaf cover (Tall et al. 2011). In addition, increased growth would encourage greater microbial activity within the *Trapa* beds as biofilms develop on plant surfaces and higher plant densities trap incoming nutrients and particulates, and the plants begin to senesce soon after seed production.

Lower dissolved oxygen stimulates denitrification, which takes place under anoxic conditions that would become more prevalent in microsites like biofilms as the water column oxygen concentration decreases. Linear correlation between denitrification and dissolved oxygen has previously been reported (Tall et al. 2011). Only *Trapa* bed samples collected on the ebb tide are presented in this report. Denitrification and respiration rates were negligible at Norrie Point in 26 June (when there was not a lot of plant biomass or biofilm) but measurable in early August and increased significantly on 22 August.

Site comparison between Kingston and Norrie Point

Dissolved oxygen concentrations and conductivity differed between the sites. Norrie Point had lower dissolved oxygen saturation (69 ± 43%) compared to Kingston (81 ± 14%). This may be due to flow patterns and plant density; the bed at Norrie Point is more protected while Kingston has adjacent channels flowing to and from Rondout Creek. Additionally, the Kingston bed was somewhat sparser even though it was larger. Differences in water quality between sites may further be influenced by sediment type; the sediments beneath the Norrie Point bed are softer and siltier than those at the

Kingston bed. Release of large volumes of sediment gas bubbles (presumably mostly composed of methane) when walking through soft sediment was consistently observed at Norrie Point, but not at Kingston, supporting the observation of higher rates of microbial processes at Norrie Point. For example, respiration measured at Norrie Point (3015 ± 570 □g O_2 consumed/L/hr) was higher than measured at Kingston (873 ± 241 □g O_2 consumed/L/hr) (Figure 9). Lower dissolved oxygen may be indicative of more plant activity (as respiration by *Trapa* roots would contribute to oxygen depletion) and/or higher microbial activity resulting in increased respiration in the water column. Conductivity was lower at Norrie Point, which may be indicative of lower wastewater influence (de Sousa et al. 2014).

Pharmaceutical types and concentrations varied between sites. Finding pharmaceuticals at concentrations above the detection limit of 1 ng/L is not surprising given that pharmaceuticals are known to be present throughout the 250 km of the Hudson River Estuary (Carpenter and Helbling 2018; Cantwell et al. 2017). Additionally, the WWTP outlet into Rondout Creek is a known pharmaceutical input into the Hudson River (Carpenter and Helbling 2018).

Pharmaceutical analysis reflects variation in input sources and land-use. Generally, Kingston had higher concentrations of pharmaceuticals and Norrie Point had higher concentrations of pesticides. The mean concentration of all herbicides was higher at Norrie Point than at Kingston while the mean concentration of all pharmaceuticals, except diphenhydramine, was higher at Kingston (when comparing average concentrations at all sites including in and out of the bed; further analysis needed in order to compare sites). This is not surprising given the differing land use in the vicinity. Norrie

Point is surrounded by maintained parkland while Kingston is more urbanized and has a known wastewater and combined stormwater/sewer overflow outfall. Further quality analysis of the analytical results is required before determining if the mean differences are statistically significant.

The sampling design of this study was such that it incorporated an array of dates and tidal periods to compare concentrations in the river with those in the *Trapa* beds and explore the net effect of the beds on pharmaceutical concentrations over a tidal cycle; however, at the time of this report only 41 of 153 samples have been analysed. Further analysis accounting for tidal interactions is required to fully understand how the beds affect pharmaceutical concentrations. In addition to the pharmaceutical samples described above, Polar Organic Chemical Integrative Samplers (POCIS) were deployed at Norrie Point for the same period as the miniDOTs. The POCIS data, which are not yet available, will show any change in pharmaceutical concentrations across four sites at Norrie Point over the two week deployment. By showing the net effect of the *Trapa* bed, this method will account for complex hydrodynamics. Differences in caffeine and carbamazepine across sites show that these relatively conservative compounds may be useful for comparisons within a site but not between sites. Analysis of sucralose, a better conservative tracer of wastewater due to low degradability (Cantwell et al. 2017; Soh et al. 2011; Torres et al. 2011), in the water samples will assist in understanding changes in pharmaceutical concentrations over a tidal cycle. This, together with the pending microbiological assays, will help to answer further questions such as

- Do pharmaceutical concentrations decrease in *Trapa* bed over tidal cycle?
- Can *Trapa* be used for pharmaceutical remediation?

- How do dissolved oxygen and pharmaceuticals interact to affect microbial activity and biogeochemical processes in *Trapa* beds?

Preliminary data show that carbamazepine was stable within the *Trapa* bed, suggesting that the fractional wastewater influence was similar; however, water samples collected on 18 June show that diphenhydramine (an antihistamine) decreased in concentration during water residence within the *Trapa* bed at both KBB and KBC sites. While the mode of action is unknown, it is interesting as diphenhydramine has been shown to negatively affect environmental bacteria by decreasing biofilm respiration (Rosi-Marshall et al. 2013). The decrease in concentration of diphenhydramine indicates potential pharmaceutical removal by *Trapa* beds, although this conclusion is preliminary since further post-processing of the pharmaceutical measurements is required.

CONCLUSIONS AND RECOMMENDATIONS

The preliminary results reported here show that *Trapa* beds function as biogeochemical hotspots in the river system. During residence of river water within the beds, dissolved oxygen tended to be reduced in concentration, sometimes reaching levels that would be stressful to the aquatic biota, particularly at low tides; however this hypoxia is rather ephemeral because the water was soon exchanged as the tide rose. Water temperatures fluctuated more than in the deeper, open waters. Rates of microbial respiration and denitrification were elevated compared to the adjacent open water. Nutrient measurements show reductions in nitrate concentrations leading into the bed, which were lower than the adjacent Rondout Creek.

Pharmaceuticals and pesticides in water outside and within the *Trapa* beds were detected. Some of these compounds appear to have been reduced in concentration during residence of water in the beds (e.g. diphenhydramine), whereas others seem unaffected (carbamazepine); however, fewer than half the samples have been analyzed, and those analyses need more post-processing to ensure accuracy. In the future, measurements of sucralose, which is perhaps the best conservative tracer of wastewater in this system, will be analysed.

Preliminary results suggest that concentrations of pesticides were higher at Norrie Point whereas pharmaceuticals were higher at the site near Kingston. The proximity of the Kingston site to wastewater treatment plant effluent may explain this difference.

Forthcoming results will provide insight for further investigation of the influence of *Trapa* on biogeochemical processing and interaction with emerging contaminants; however, it is recommended that modes of interactions, such as plant uptake, adsorption to plant surfaces or breakdown by microbes, is investigated for all pharmaceuticals that are shown to have a net decrease during residence within the *Trapa* bed. Further, mesocosm experiments may be useful in investigating pharmaceutical dose-response relationships and/or acute vs long-term exposure on microbial community diversity and function.

ACKNOWLEDGEMENTS

I appreciate the guidance and mentorship of the Hudson River Foundation throughout this project. Thank you to the Cary Institute for hosting me and providing the invaluable experience of working on the Hudson River. I express sincere gratitude to David Fischer, Heather Malcolm, Stephanie Robson and Chris Mitchell for encouragement as well as practical and logistical support. I acknowledge and thank my supervisors for their direction and assistance as well as those involved in the RiverKeeper, LTREB and HRECOS long term monitoring initiatives.

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APPENDICES

APPENDIX A –

Kingston Summary data

	KBA Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	27	24	2	28	24	20
Conductivity (uS/cm)	18	316	66	491	299	259
Turbidity (NTU)	27	19	17	66	12	6
Dissolved oxygen (mg /L)	27	8.2	0.7	9.3	8.5	6.5
% Oxygen saturation	27	97.8	7.3	108.7	100.9	78.6
NPOC (mg-C/L)	21	4.6	0.7	6.1	4.4	3.6
NH_4^+ (mg-N/L)	22	0.10	0.07	0.29	0.08	0.02
NO_3^- (mg-N/L)	27	0.4	0.1	0.8	0.4	0.1
PO_4^{3-} (mg-P/L)	27	0.03	0.03	0.10	0.02	0.01
TN (mg-N/L)	13	0.8	0.1	1.0	0.8	0.6
TP (mg-P/L)	13	0.08	0.03	0.15	0.07	0.04

	KBB Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	27	24	2	28	24	21
Conductivity (uS/cm)	18	310	75	485	291	244
Turbidity (NTU)	27	17	25	90	7	1
Dissolved oxygen (mg /L)	27	7	1	9	7	4
% Oxygen saturation	27	81	14	109	85	47
NPOC (mg-C/L)	21	4.5	0.7	6	4	4
NH_4^+ (mg-N/L)	23	0.07	0.1	0.22	0.06	0.02
NO_3^- (mg-N/L)	27	0.3	0.1	0.5	0.3	0.1
PO_4^{3-} (mg-P/L)	27	0.03	0.02	0.09	0.02	0.01
TN (mg-N/L)	13	0.7	0.1	1.0	0.7	0.6
TP (mg-P/L)	13	0.07	0.05	0.15	0.05	0.02

	KBC Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	25	25	2	28	25	21
Conductivity (uS/cm)	16	296	52	427	295	244
Turbidity (NTU)	25	10	10	43	7	2
Dissolved oxygen (mg /L)	25	8	1	9	8	5
% Oxygen saturation	25	92	15	115	92	56
NPOC (mg-C/L)	21	4.3	0.6	6	4	4
NH_4^+ (mg-N/L)	18	0.06	0.03	0.14	0.05	0.02
NO_3^- (mg-N/L)	25	0.3	0.1	0.5	0.3	0.1
PO_4^{3-} (mg-P/L)	25	0.02	0.01	0.05	0.02	0.01
TN (mg-N/L)	13	0.9	0.7	3.3	0.7	0.5
TP (mg-P/L)	13	0.06	0.02	0.09	0.05	0.04

	RC Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	10	25	2	27	25	22
Conductivity (uS/cm)	10	360	68	492	337	311
Turbidity (NTU)	10	10	4	17	9	6
Dissolved oxygen (mg /L)	10	8.0	0.7	9.3	7.9	6.9
% Oxygen saturation	10	97	7	106	96	86
NPOC (mg-C/L)	7	6	1	9	5	5
NH_4^+ (mg-N/L)	10	0.19	0.1	0.33	0.19	0.08
NO_3^- (mg-N/L)	10	0.5	0.3	1.3	0.4	0.3
PO_4^{3-} (mg-P/L)	10	0.07	0.03	0.12	0.06	0.04
TN (mg-N/L)	6	1.0	0.2	1.2	1.0	0.6
TP (mg-P/L)	6	0.11	0.02	0.15	0.11	0.07

	K-LTREB Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	8	24	3	29	24	21
Conductivity (uS/cm)	8	258	36	293	269	212
Turbidity (NTU)	8	16	15	50	11	6
Dissolved oxygen (mg /L)	8	8.5	0.6	9.5	8.4	7.6
% Oxygen saturation	8	101	5	108	100	95
NPOC (mg-C/L)	8	3.5	0.2	3.8	3.5	3.2
NH_4^+ (mg-N/L)	8	0.04	0.03	0.10	0.04	0.01
NO_3^- (mg-N/L)	8	0.34	0.07	0.46	0.36	0.25
PO_4^{3-} (mg-P/L)	8	0.02	0.01	0.03	0.02	0.01
TN (mg-N/L)	8	0.8	0.1	0.9	0.8	0.6
TP (mg-P/L)	8	0.05	0.005	0.06	0.05	0.05

	KPD-RK Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	3	31	3	33	31	28
Conductivity (uS/cm)						
Turbidity (NTU)	3	9	9	19	5	3
Dissolved oxygen (mg /L)						
% Oxygen saturation	3	101	18	122	96	87
NPOC (mg-C/L)						

	KPB-RK Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	3	30	3	32	32	27
Conductivity (uS/cm)						
Turbidity (NTU)	3	9	3	13	8	7
Dissolved oxygen (mg /L)						
% Oxygen saturation	3	100	7	108	101	93
NPOC (mg-C/L)						

Norrie Point Summary data

	NB Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	14	25	2	28	25	21
Conductivity (uS/cm)	9	273	20	311	264	252
Turbidity (NTU)	15	20	20	72	12	0
Dissolved oxygen (mg /L)	13	6	4	12	7	0.5
% Oxygen saturation	13	69	43	143	84	5
NPOC (mg-C/L)	11	4.0	0.2	4.4	3.7	3.7
NH_4^+ (mg-N/L)	11	0.04	0.02	0.08	0.04	0.02
NO_3^- (mg-N/L)	15	0.2	0.1	0.4	0.3	0.1
PO_4^{3-} (mg-P/L)	15	0.02	0.01	0.03	0.02	0.00
TN (mg-N/L)	11	0.6	0.1	0.9	0.6	0.5
TP (mg-P/L)	11	0.07	0.04	0.16	0.05	0.04

	NHR Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	14	25	2	27	25	21
Conductivity (uS/cm)	9	272	20	298	277	247
Turbidity (NTU)	15	14	3	20	14	10
Dissolved oxygen (mg /L)	14	8	1	11	8	7
% Oxygen saturation	14	100	14	127	94	84
NPOC (mg-C/L)	11	4.0	0.3	4.6	3.6	3.6
NH_4^+ (mg-N/L)	11	0.03	0.02	0.09	0.03	0.02
NO_3^- (mg-N/L)	15	0.3	0.1	0.4	0.3	0.2
PO_4^{3-} (mg-P/L)	15	0.02	0.01	0.02	0.02	0.01
TN (mg-N/L)	11	0.64	0.05	0.69	0.65	0.56
TP (mg-P/L)	11	0.05	0.01	0.07	0.05	0.03

	N-HRECOS Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	All data	25	2	29	26	21
Conductivity (uS/cm)	recorded	262	38	320	250	170
Turbidity (NTU)	every 15 mins	7	4	180	6	0
Dissolved oxygen (mg /L)	from 19 Jun	7.6	0.8	11.3	7.5	5.5
% Oxygen saturation	to 25 Sep	92	8	132	90	69
NPOC (mg-C/L)	2019					

	NC-RK Summary					
	n	Mean	StDev	Max	Median	Min
Temperature (˚C)	3	30	2	32	31	28
Conductivity (uS/cm)						
Turbidity (NTU)	3	7	3	10	7	5
Dissolved oxygen (mg /L)						
% Oxygen saturation	3	93	7	100	93	85
NPOC (mg-C/L)						