Multiple-source heterotrophy fueled by aged organic carbon in an urbanized estuary

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Abstract

The lower Hudson River is a highly urbanized estuary that receives large inputs of treated wastewater. To determine how organic matter from wastewater influences carbon cycling in this type of system, we measured chlorophyll *a*, pCO_2, dissolved organic carbon (DOC), δ^{13}C-DOC, and Δ^{14}C-DOC along the salinity gradient and at wastewater treatment plants. Isotopic mixing curves indicate a net removal of DOC that is ^{13}C enriched and ^{14}C depleted. The amount of DOC removed was consistent with CO_2 evasion from the estuary. During two transects at average to low flow, the lower Hudson River Estuary was a heterotrophic system with CO_2 evasion balanced by the utilization of aged DOC derived from wastewater and marine phytoplankton that enter the estuary at the seaward end-member. DOC removals were largest during a period of high river flow, when isotopic mixing curves also suggest large contributions from labile terrestrial OC sources. Overall, our results suggest that net heterotrophy in the lower Hudson River Estuary is fueled by aged labile DOC derived from a combination of sources, which are influenced by seasonal phytoplankton blooms, hydrological conditions, and the nature of wastewater inputs.

Keywords

Dissolved organic carbon; Wastewater; Sewage; Heterotrophy; ^{13}C; ^{14}C; Isotope mixing curves; Carbon dioxide; Chlorophyll; Carbon cycle; USA; Hudson River Estuary

1. Introduction

Estuaries are complex aquatic systems that link the terrestrial and ocean carbon cycles and can influence the amount and character of dissolved organic carbon (DOC) delivered to the coastal ocean. Within and across estuaries, DOC has variable composition, concentration, isotopic signature, and lability. DOC character is largely determined by its sources and the processes that transform it within rivers and estuaries (McCallister et al. 2006; Moran et al. 1999; Raymond et al. 2004).

Several major processes alter DOC as it travels through an estuary, including autotrophic additions (Cole and Caraco 2006; Peterson et al. 1994; Raymond and Bauer 2001a), heterotrophic removals (Howarth et al. 1996; Maranger et al. 2005; Taylor et al. 2003; Van den Meersche et al. 2009), photochemistry (Mopper and Kieber 2002), flocculation (Sholkovitz 1978), and interactions with suspended particles (Hedges and Keil 1999; Servais and Garnier 2006). Taken together, these processes have major implications for land-ocean carbon and nutrient fluxes and the global carbon cycle.

There are gaps in our understanding of how DOC is transformed within estuaries, especially in saline reaches surrounded by megacities. In highly urbanized estuaries, human wastewater can be a significant freshwater input that drives two competing processes with respect to an estuary's net metabolism and carbon cycling. In the first, nitrogen and phosphorus from wastewater can increase primary productivity leading to autochthonous organic carbon production (Howarth et al. 2006; Muylaert et al. 2000). Conversely, labile organic matter from wastewater can promote heterotrophic removal of organic carbon and subsequent production and release of CO_2. Although most estuaries are generally supersaturated in CO_2 and release a large amount of carbon into the atmosphere (Borges et al. 2006; Cai and Wang 1998; Frankignoulle et

al. 1998), these fluxes can vary with water residence time and organic matter inputs. The effect of wastewater inputs on organic carbon dynamics and the autotrophic-heterotrophic balance in urbanized estuaries remains unclear and context dependent (Abril et al. 2002; Alvarez-Salgado and Miller 1998; Muylaert et al. 2005).

The Hudson River Estuary is a highly urbanized system that receives effluent from more than 100 wastewater treatment plants (WWTPs) and is home to ~ 8.5 million inhabitants. Previous studies of carbon dynamics in the saline, "lower" estuary are limited and have focused on primary and bacterial production (Howarth et al. 2006; Taylor et al. 2003). In contrast, work on the tidal freshwater section of the estuary has been more intense and indicates a strongly heterotrophic system driven by inputs of labile organic matter from aged terrestrial sources (Cole and Caraco 2001; del Giorgio and Pace 2008; Howarth et al. 1992; McCallister et al. 2004; Raymond and Bauer 2001c). These and other studies highlight the importance of internal DOC processing in estuaries (see Peterson et al. 1994).

The goal of the present study was to evaluate the effect of wastewater inputs on net carbon dynamics in a mega-city estuary by measuring pCO_2, chlorophyll *a*, DOC, δ^{13}C-DOC, and Δ^{14}C-DOC across a salinity gradient in the lower Hudson River Estuary.

2. Methods

2.1 Study site

The Hudson River Estuary is tidal from New York City to Troy, NY (river kilometer 240); the location of the salt front depends on river flow and tidal strength. Mean annual discharge at the southern tip of Manhattan is 545 $m^3 s^{-1}$ and varies between typical summertime low flows of 200 $m^3 s^{-1}$ and spring/fall freshets of 2000 $m^3 s^{-1}$. In 2000, wastewater input into the

entire estuary was ~ 70 m^3 s^{-1} (80% of which derives from the New York City metropolitan area), representing a large percentage of the total summer flow of the Hudson (Brosnan et al. 2006).

2.2 Sampling

Estuarine surface water was sampled along the Hudson River, from West Point to the Statue of Liberty, during three sampling cruises: 8 June 2005, 19 April 2006, and 18 July 2006 (Fig. 1). On these days, Hudson River discharge was 307 m^3 s^{-1}, 402 m^3 s^{-1}, and 785 m^3 s^{-1} respectively (Fig. 2; estimated as 167% of the discharge from the Green Island, NY river gauge (Howarth et al. 1996)). In August 2006, wastewater effluent was sampled from six representative WWTPs that were spread along the lower Hudson River Estuary (Fig. 1) and had average discharges ranging from 4.6 to 254 million gallons per day (MGD; Table 1).

During each estuarine transect, pCO_2, salinity, and chlorophyll *a* were measured continuously with an on-board flow-through system. Discrete water samples were also collected using ^{14}C-clean techniques at 6 - 7 sites in acid-washed (1% HCl) polycarbonate bottles and immediately sub-sampled for subsequent laboratory measurements of DOC, $\delta^{13}C$-DOC, $\Delta^{14}C$-DOC, and chlorophyll *a*. All samples were transported to the lab on ice and then frozen (DOC) or refrigerated (chlorophyll *a*). WWTP effluent samples were collected at the outflow access points with dip bottles or dedicated sampling spigots (WWTP 3) and handled in the same way as the estuarine samples.

2.2.1 Flow-through system (pCO_2, salinity, chlorophyll a*)*

The on-board flow-through system was configured as follows: a YSI 600XLM Sonde probe that measured salinity was connected in series to a chlorophyll *a* probe ("WET Labs WETStar Chlorophyll Fluorometer") and a CO_2 shower head equilibrator (Raymond and

Hopkinson 2003). Each probe was attached to a data logger that took readings and global positioning system (GPS) coordinates every second.

2.2.2 Chlorophyll a *calibration*

Laboratory measurements of chlorophyll *a* were used to calibrate the flow-through chlorophyll *a* probe. Refrigerated sub-samples were passed through baked GFF filters under low light conditions within 24 hours of returning to the lab. Each filter was then wrapped in baked foil and frozen. At a later date, chlorophyll *a* concentrations were measured fluorometrically after a 24-hour acetone extraction. Reported concentrations have been corrected for chlorophyll *a* degradation products (Environmental Protection Agency Method 445.0).

2.2.3 DOC, δ^{13}C-DOC, Δ^{14}C-DOC

In the field, DOC sub-samples were passed through a 1.0 μm combusted QMA filter and collected in 125 mL acid-washed polycarbonate bottles. Upon returning to the lab, DOC samples were acidified to pH ~ 2.5 with 1 mL of 60% phosphoric acid and frozen. The entire 125 mL sample was placed in a quartz reaction tube and converted to CO_2 by high-energy UV irradiation (2400 W; see Williams and Gordon 1970) followed by purification and collection on a gas extraction line (Bauer et al. 1992; Raymond and Bauer 2001a). DOC concentrations and recoveries were determined using a calibrated Baratron absolute pressure gauge to measure CO_2 pressure. The sample was then isolated in a glass ampoule to await δ^{13}C and Δ^{14}C analysis.

2.3 Analytical methods

δ^{13}C-DOC measurements were made at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) and the National Science Foundation-University of Arizona Accelerator Mass Spectrometry Facility (NSF-Arizona AMS) by mass spectral analysis of CO_2 gas previously purified on a gas extraction line at Yale University. Natural ^{14}C sampling and

analysis required meticulous attention to detail to avoid contamination (e.g., all bottles, filtration apparatuses, forceps, etc. were appropriately cleaned, acidified, or baked). Δ^{14}C-DOC measurements took place at NOSAMS (June and April transects) and NSF-Arizona AMS (July transect and WWTPs). At each facility, purified CO_2 from each sample was catalytically converted to graphite (McNichol et al. 1992; Vogel et al. 1987) and pressed into a target that was run on an accelerator mass spectrometer. Total measurement uncertainty for the Δ^{14}C analyses of these samples is ~ 5 - 7‰. Radiocarbon ages are reported according to Stuiver and Polach (1977) and Stuiver (1980).

2.4 Isotope Mixing Curves

Isotope mixing models are valuable and sensitive tools for assessing elemental cycling in estuaries. Specifically, a dual isotope mixing approach using both ^{14}C and ^{13}C has been successful at resolving the source and age of organic matter in aquatic systems (McCallister et al. 2004; Raymond and Bauer 2001b; Spiker 1980). Isotope mixing curves use salinity as a conservative tracer and require that both the total DOC concentration and isotopic composition (*I*) of the riverine (*riv*) and high-salinity/marine (*mar*) end-members are known. The conservative isotope value (δ^{13}C or Δ^{14}C) for a sample at a known salinity (I_s) is then calculated as:

$$I_s = \frac{(f_{riv} I_{riv} DOC_{riv} + (1 - f_{riv}) I_{mar} DOC_{mar})}{DOC_{mix}} \quad (1)$$

where the riverine fraction, f_{riv}, is calculated from salinity, and DOC_{mix} is the amount of DOC expected from conservative mixing of the freshwater and marine end-members. A conservative mixing curve is drawn by evaluating I_s across the relevant salinity range (see Fig. 3). If actual isotope values differ from the conservative mixing curve, one concludes that processes apart from simple end-member mixing are at work (see Cifuentes and Eldridge 1998). As an example,

consider an estuary where measured Δ^{14}C-DOC values lie below (are depleted relative to) the conservative curve. This could be interpreted as a net addition of ^{14}C depleted DOC and/or removal of ^{14}C enriched DOC.

The two end-member mixing model described above assumes no major inputs of fresh water along the estuarine study site. In our case, this is a good assumption as tributary inputs are minor and most WWTP effluent is discharged outside of our study area. The mixing model also assumes that the composition of each end-member is invariant on timescales of estuarine mixing.

2.5 Estimates of net DOC removal

Measured DOC concentrations were plotted as a function of salinity and fit with simple quadratic equations to estimate net DOC removals along each transect using the method of Kaul and Froelich (1984). The internal flux (input or removal) of DOC is defined as

$$Internal\ flux = Q(C_s - C_o) \quad (2)$$

where Q is freshwater flow, C_o is the concentration at zero salinity, and C_s is the concentration where the tangent of the quadratic equation at the saline end-member intercepts the y-axis (at zero salinity). Raymond and Bauer (2001a) describe this method in detail.

2.6 Gas exchange estimates

Rough estimates of CO_2 exchange between water and atmosphere were calculated using average pCO_2 values for each transect and a gas exchange coefficient (k) of 5 ± 1 cm h^{-1}. This particular k was chosen because it lies in the middle of the predicted range (3 - 7 cm h^{-1}) for estuaries and rivers such as the Hudson (Raymond and Cole 2001) and also agrees well with field and empirical studies of gas exchange in the freshwater tidal Hudson River at average wind speeds and tidal velocities (Clark et al. 1994; Raymond and Cole 2001; Ho et al. 2002; Zappa et al. 2007).

3. Results

3.1 DOC mixing curves

Results for all three transects indicate that DOC concentrations were highest at the freshwater end member and decreased non-conservatively towards the saline end member, indicating net removal of DOC (Fig. 3a). Using Eq. 2 and the method of Kaul and Froelich (1984), we estimate internal DOC removal fluxes of 22 mmol C m^{-2} d^{-1}, 35 mmol C m^{-2} d^{-1}, and 72 mmol C m^{-2} d^{-1} for the June 2005, April 2006, and July 2006 transects respectively. The δ^{13}C-DOC and Δ^{14}C-DOC isotope mixing curves also exhibit non-conservative behavior with depleted δ^{13}C-DOC values (Fig. 3b) and enriched Δ^{14}C-DOC values (Fig. 3c) relative to the conservative mixing curves.

3.2 Wastewater

WWTP effluent had similar characteristics despite differences in location, source water, and outflow (Fig. 1; Table 1). On average, effluent DOC had a concentration of 954 ± 306 μM (SD), a δ^{13}C value of -26.6 ± 1.1‰ (SD), and a Δ^{14}C value of -163.5 ± 24.4‰ (SD) (Griffith et al. 2009). The latter corresponds to an average wastewater ^{14}C-DOC age of 1379 years BP (Table 1). Using a conservative estimate of wastewater discharge into the lower Hudson (60 m^3 s^{-1}) we estimate that the total DOC input from wastewater is 33 mmol C m^{-2} d^{-1}. Most WWTPs are required to measure biological oxygen demand (BOD; a proxy for labile carbon) in the effluent on a regular basis. For the six WWTPs we sampled, average BOD (5 day; 20 ^{o}C) varied by 18 - 37% between June 2005 and July 2006 (EPA Envirofacts database; http://oaspub.epa.gov/enviro/ef_home2.water). It is noteworthy that most of the wastewater

entering our study area is actually discharged from WWTPs on the East River and in New York Harbor then pushed northwards into the estuary by tides.

3.3 pCO_2 and chlorophyll a

pCO_2 and chlorophyll *a* data for each transect are presented in Figs. 4a and 4b respectively. Comparisons of pCO_2 and chlorophyll *a* averages and ranges are shown in Table 2. Mean pCO_2 values of 1000 µatm, 670 µatm, and 2059 µatm (for June, April, and July transects respectively) are all above atmospheric saturation (380 µatm). Therefore, in all three cruises the estuary was a net source of CO_2 to the atmosphere. Generally, pCO_2 was highest near the north end of Haverstraw Bay (river km 65), decreased to a local minima at the south end of Haverstraw Bay (river km 35), then increased along the length of Manhattan before declining sharply in NY Harbor (Fig. 4a). The June 2005 transect was the only one to exhibit a reach of sub-atmospheric pCO_2 values (<380 µatm). Using a gas exchange coefficient of 5 ± 1 cm h^{-1} (Clark et al. 1994; Raymond and Cole 2001; Ho et al. 2002; Zappa et al. 2007) and mean pCO_2 values for each transect, we estimate CO_2 effluxes of 23 ± 5 mmol C m^{-2} d^{-1}, 11 ± 2 mmol C m^{-2} d^{-1}, and 61 ± 12 mmol C m^{-2} d^{-1} (June, April, and July respectively).

A relationship between the chlorophyll *a* probe and laboratory-based fluorometric chlorophyll *a* measurements (Fig. 5) allowed us to convert probe voltages into chlorophyll *a* concentrations at high temporal resolution. In the April 2006 transect, chlorophyll *a* concentrations were highest at the freshwater and saline extremes (Fig. 4b). Chlorophyll *a* concentrations were significantly higher throughout the July 2006 transect and displayed a sharp peak at the south end of Haverstraw Bay (river km 40; Fig. 4b). In the June 2005 transect, chlorophyll *a* is low in Haverstraw Bay but displays several sharp downstream peaks (river km

10 - 35). Chlorophyll *a* data from the June 2005 transect were measured with the same probe and corrected using the relationship derived from the April and July 2006 transects (Fig. 5).

4. Discussion

4.1 pCO_2 and chlorophyll a

Our study suggests that the saline Hudson River Estuary is less supersaturated in CO_2 than many other large rivers and estuaries around the world (Cole and Caraco 2001; Frankignoulle 1998; Raymond et al. 2000), a surprising result given large sewage treatment plant loadings. Still, our pCO_2 data suggest that the lower Hudson is a net heterotrophic system (Fig. 4a). This finding is consistent with several previous studies (Howarth et al. 2006; Taylor et al. 2003) but estimates of the magnitude vary. Our estimate of metabolism is consistent with long-term studies in the tidal, freshwater Hudson (Cole and Caraco 2001; Raymond et al. 1997), but differ markedly from those of Taylor et al. (2003). Based on bottle incubations, the latter reported average dissolved inorganic carbon (DIC) production rates of 330 mmol C m^{-2} d^{-1} in the saline Hudson. In our study, pCO_2 was between 300 μatm and 2434 μatm. Using a gas exchange coefficient of 5 cm h^{-1} (Clark et al. 1994; Raymond and Cole 2001; Ho et al. 2002; Zappa et al. 2007), we calculate a maximum air-water flux of ~ 90 mmol C m^{-2} d^{-1}, or about ¼ of the flux reported by Taylor et al. (2003). This discrepancy could be due to problems with scaling bottle incubations, a lack of seasonal pCO_2 data, or a large export of carbonate alkalinity from the Hudson.

It is likely that several processes determine the spatial patterns observed in our pCO_2 data (Fig. 4a). Respiration drives pCO_2 increases but is counteracted by primary production and gas exchange in supersaturated estuaries (Borges et al. 2006). Carbon dioxide concentrations may

also change along salinity gradients due to mixing of buffered seawater with freshwater. Turbidity maxima may be sites of in situ DOC production (Alvarez-Salgado and Miller 1998) and enhanced organic matter oxidation by bacteria attached to suspended particles (Servais and Garnier 2006; Uncles et al. 2000). In the Hudson, pCO_2 maxima were observed near turbidity maxima (near the top of Haverstraw Bay and at the George Washington Bridge) where bacterial respiration may be high and primary production is limited by a low photic zone to mixed layer depth ratio (Cole et al. 1992).

Generally, chlorophyll *a* and pCO_2 trends were inversely related (Figs. 4a and 4b). Yet the large localized chlorophyll *a* peak in July 2006 was not accompanied by significantly lower pCO_2 values. If excess chlorophyll is produced in the shoals and exported to the main stem of the Hudson, these results suggest a possible decoupling of the effect of shoal primary production on main stem CO_2 and O_2 dynamics. The observation that chlorophyll *a* concentrations and freshwater discharge were both at their highest during the July 2006 transect suggests an alternative explanation. Given that pCO_2 levels were also high during this transect, it seems likely that elevated chlorophyll *a* concentrations were due in part to primary production in the tidal freshwater Hudson followed by downstream transport into our study site.

4.2 DOC dynamics

Studies have shown that DOC behavior varies widely between estuaries (Abril et al. 2002; Middelburg and Herman 2007; Peterson et al. 1994). For example, conservative mixing of DOC has been documented in the Humber (Alvarez-Salgado and Miller 1998), Rhine, Thames, Elbe, Douro (Abril et al. 2002; Middelburg and Herman 2007), and Columbia (Prahl and Coble 1994). Net additions of DOC characterize the York (Raymond and Bauer 2001a), Betsiboka (Ralison et al. 2008), Gironde, Sado, Loire, and Ems (Abril et al. 2002; Middelburg and Herman

2007), while net removals have been observed in the highly urbanized Scheldt (Abril et al. 2002). Recent studies in the Mississippi, Tyne, Tweed, and Pawcatuck River estuaries also suggest that DOC dynamics vary with the season and hydrological regime (McKenna 2004; Spencer et al. 2007; Wang et al. 2004).

DOC mixing curves for the lower Hudson Estuary indicate a net removal of DOC (22, 35, and 72 mmol C m^{-2} d^{-1}; Fig. 3a). One reason to expect net removals is the low potential for autochthonous production in the lower Hudson. Phytoplankton and tidal marshes are important sources of DOC (Baines and Pace 1991; Peterson et al. 1994; Raymond and Hopkinson 2003), yet the lower estuary has very small spatial coverage of marshes and phytoplankton are generally still light limited (Cole and Caraco 2006). These observations are consistent with a net removal of DOC. The net DOC removal rates are similar to pCO_2 efflux estimates (23 ± 5, 11 ± 2, and 61 ± 12 mmol C m^{-2} d^{-1}; Fig. 4a) despite uncertainties associated with quadratic curve fitting and the use of average pCO_2 and gas transfer velocity values. The general agreement between the DOC removals and pCO_2 efflux estimates, coupled with low primary production, are consistent with a net heterotrophic system in which evasion is balanced partly by DOC consumption by bacteria.

Potential sources of allochthonous labile DOC that could drive net DOC removals and CO_2 evasion include riverine DOC, wastewater DOC, saltmarsh DOC from the Raritan, Passaic, and Hackensack drainages, and DOC from primary production in NY harbor and the coastal ocean. A recent study concluded that most of the labile riverine OC delivered to the Hudson River Estuary is utilized near Troy (river km 240), leaving behind generally refractory DOC that is then transported conservatively through the tidal freshwater Hudson (del Giorgio and Pace 2008). The amount of wastewater DOC that enters the saline Hudson (mostly released outside of our study area and subsequently pushed in by tides) is on the same order as estimates of DOC

297 removal and pCO_2 efflux. The amount of marine OC advected into the lower Hudson is difficult
298 to quantify but remains a potentially large source of labile OC. Below we use dual isotope
299 mixing curves to further constrain possible sources of labile DOC.

300 ***4.3 δ^{13}C-DOC and Δ^{14}C-DOC dynamics***

301 In the lower Hudson, δ^{13}C-DOC values were depleted and Δ^{14}C-DOC values were
302 enriched relative to their respective conservative mixing curves (Figs. 3b and 3c). Together with
303 evidence of net DOC removal (from both mixing curves and pCO_2 levels), the isotope data
304 generally suggest removal of a DOC pool that is ^{13}C enriched and ^{14}C depleted. When the
305 method of Kaul and Froelich (1984) was applied using quadratic fits to ^{12}C, ^{13}C, and ^{14}C
306 concentrations individually ($R^2 = 0.97$), it was possible to quantify the net isotopic signature of
307 the DOC pool that was removed in the lower Hudson during the June transect. This labile DOC
308 pool was enriched in ^{13}C ($\delta^{13}C = -20‰$) and depleted in ^{14}C ($\Delta^{14}C = -232‰$).

309 Three major sources of labile DOC having enriched ^{13}C and depleted ^{14}C signatures are
310 autochthonous estuarine DOC, allochthonous marine DOC, and wastewater DOC. Our
311 chlorophyll *a* data and previous estimates of near zero net ecosystem production (Baines and
312 Pace 1991; Swaney et al. 1999) suggest that the amount of DOC derived from autochthonous
313 production within the lower Hudson will be small. Wastewater ($\delta^{13}C = -26.6‰$ and $\Delta^{14}C = -$
314 163.5‰ (Table 1)) and marine phytoplankton ($\delta^{13}C \sim -20‰$ and $\Delta^{14}C \sim -50‰$) are both large
315 potential sources of labile DOM that are enriched in ^{13}C and depleted in ^{14}C relative to our
316 mixing curves (Figs. 3b and 3c). However, the δ^{13}C value of the removed DOC pool in June (-
317 20‰) points to a marine or C_4-derived OC source (Currin et al. 1995) while the Δ^{14}C value (-
318 232‰) is much closer to the wastewater OC source. In fact, the pool of removed DOC is even
319 more depleted in ^{14}C (i.e., older) than both wastewater and marine OC, suggesting significant

contributions from a component of highly aged DOC. One possible source is particle bound terrestrial OC that has been mobilized or desorbed within the estuary (Alvarez-Salgado and Miller 1998; McCallister et al. 2004). Another possibility is wastewater OC derived from petrochemicals ($\Delta^{14}C$ = -1000‰), such as surfactants, pharmaceuticals, and personal care products (Griffith et al. 2009). Removing even small amounts of highly aged DOC could help explain the $\Delta^{14}C$ value of the removed DOC pool (-232‰). Furthermore, although the ^{13}C signature of removed DOC (-20‰) is not consistent with the removal of only C_3/petroleum (~ -28‰), wastewater organic matter also has a large contribution of C_4 (~ -12‰) OC from crops (such as corn and sugar cane) that are potentially labile and highly ^{13}C-enriched.

In general, OC from wastewater and fresh marine phytoplankton is easily biodegraded (Baines and Pace 1991; Servais et al. 1995). Thus, the contribution of each source to net DOC removal should depend on the relative magnitude of their inputs. In the saline Hudson we hypothesize that allochthonous wastewater DOC is utilized by bacteria throughout the year, supplemented by labile DOC from seasonal phytoplankton blooms in the New York Bight (see Malone 1977). Thus, an interesting feature of the Hudson River Estuary is that both of these labile sources of DOC are brought into the estuary from the seaward end-member through tidal mixing, as opposed to many other estuaries where the major source of labile DOC is from the riverine end-member. Thus, at times the saline Hudson River Estuary operates as an "upside-down estuary," fueled by labile DOC from the seaward end-member.

Our July 2006 transect also suggests that removal of labile riverine DOC may increase during periods of high river discharge. Both discharge (USGS; Fig. 2) and net DOC removal were the largest for the July transect. The corresponding mixing curves suggest removal of ^{14}C-depleted OC, but show mostly conservative ^{13}C behavior (Figs. 3b and 3c). We attribute this to

343 an increased utilization of pre-aged and ^{13}C-depleted riverine OC, which balances a concurrent
344 removal of ^{13}C-enriched OC from wastewater and marine phytoplankton. Other sources of
345 uncertainty include groundwater DOC flux and the effect of combined sewage overflows (CSOs)
346 and raw sewage releases, which also become more frequent during rainy periods and probably
347 contain highly labile DOC.

348 Using a multiple-isotope approach on bacterial nucleic acids in the York and Hudson
349 River estuaries, McCallister et al. (2004) concluded that bacteria are utilizing "old" (^{14}C
350 depleted) carbon derived from petroleum hydrocarbons or terrigenous sources that became labile
351 after dissociation from mineral particles or photolysis and degradation of humic compounds.
352 Recently, Hood et al. (2009) found that the lability of DOM in glacial watersheds was positively
353 correlated with the ^{14}C age of DOC. The present study suggests that "old" DOC derived from
354 wastewater and marine phytoplankton are important sources of labile carbon to the saline
355 Hudson River Estuary during periods of low to average river flow. Together, these studies
356 suggest that "old" does not necessarily mean "refractory."
357

358 **5. Conclusion**

359 Although the saline Hudson River Estuary is a net heterotrophic system that receives
360 large wastewater inputs, we find lower pCO_2 levels than previously reported for several
361 urbanized European estuaries (Frankignoulle 1998). The reasons for this discrepancy are unclear,
362 but may have to do with differences in estuary geomorphology, bacterial assemblages, or
363 wastewater organic carbon character and concentration. Isotope mixing curves point towards the
364 utilization of aged DOC derived from wastewater and/or marine phytoplankton, with potential
365 contributions from labile riverine OC during high river discharge.
366

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542 **TABLE 1.** DOC concentration and isotope data for six WWTPs associated with the lower

543 Hudson River Estuary (Griffith et al. 2009). The location of each outfall is shown in Fig. 1.

WWTP	Date sampled	Flow (2005 AVG) (MGD)	DOC (μM)	δ^{13}C DOC (‰)	Δ^{14}C DOC (‰)	DOC age (years BP)
WWTP 1	1 August 2006	9.5	nd	nd	nd	nd
WWTP 2	1 August 2006	23	1108	-25.4	nd	nd
WWTP 3	1 August 2006	254	1368	-26.8	-162.5	1364
WWTP 4	14 August 2006	5.3	848	-27.3	-197.3	1707
WWTP 5	14 August 2006	4.6	898	-27.9	-154.5	1290
WWTP 6	14 August 2006	105	548	-25.5	-139.6	1153
average		nd	954	-26.6	-163.5	1379
std deviation		nd	306	1.1	24.4	236

544 nd = not determined
545 MGD = million gallons per day
546 AVG = average
547 WWTP = wastewater treatment plant
548

549 **TABLE 2.** Summary of pCO_2 and chlorophyll *a* data during three longitudinal transects in the

550 lower Hudson River Estuary (see Figs. 4a and 4b).

Transect	pCO_2 average (µatm)	pCO_2 range (µatm)	chl *a* average (µg L^{-1})	chl *a* range (µg L^{-1})
8 June 2005	1000	300 - 1609	nd	nd
19 April 2006	670	587 - 773	4.5	3 - 13
18 July 2006	2059	1028 - 2434	24	16 - 83

551 nd = not determined
552

553

554 **TABLE 3.** Summary of DOC concentration and isotope data during three longitudinal transects

555 in the lower Hudson River Estuary (see Figs. 3a - 3c).

Transect	Station	Latitude (decimal degrees)	Longitude (decimal degrees)	Salinity	DOC Concentration (μM)	δ^{13}C DOC (‰)	Δ^{14}C DOC (‰)
8 June 2005	1	41.427	-73.986	0	232	-27.2	13
8 June 2005	2	41.277	-73.960	1.4	214	-28.9	31
8 June 2005	3	41.163	-73.905	3.8	176	-29.2	46
8 June 2005	4	41.023	-73.889	5.1	137	-29.7	59
8 June 2005	5	40.813	-73.975	9.2	135	-29.0	nd
8 June 2005	6	40.749	-74.017	12.5	114	-28.8	-59
19 April 2006	1	41.373	-73.956	0.2	216	-27.7	43
19 April 2006	2	41.282	-73.954	0.8	183	-29.7	101
19 April 2006	3	41.197	-73.929	2.7	155	-30.2	89
19 April 2006	4	41.041	-73.886	4.5	117	-29.4	95
19 April 2006	5	40.928	-73.912	7.3	123	-29.5	105
19 April 2006	6	40.830	-73.962	9.3	139	-28.9	91
19 April 2006	7	40.687	-74.041	18.8	103	-25.0	-10
18 July 2006	1	41.385	-73.953	0.2	400	-29.0	57
18 July 2006	2	41.268	-73.971	0.3	274	-26.7	83
18 July 2006	3	41.163	-73.914	0.8	297	-29.0	64
18 July 2006	4	41.038	-73.885	2.4	276	-28.9	70
18 July 2006	5	40.837	-73.957	5.0	242	-29.3	46
18 July 2006	6	40.687	-74.039	14.5	149	-28.6	18

556 nd = not determined
557

558

559 **Figure Legends**

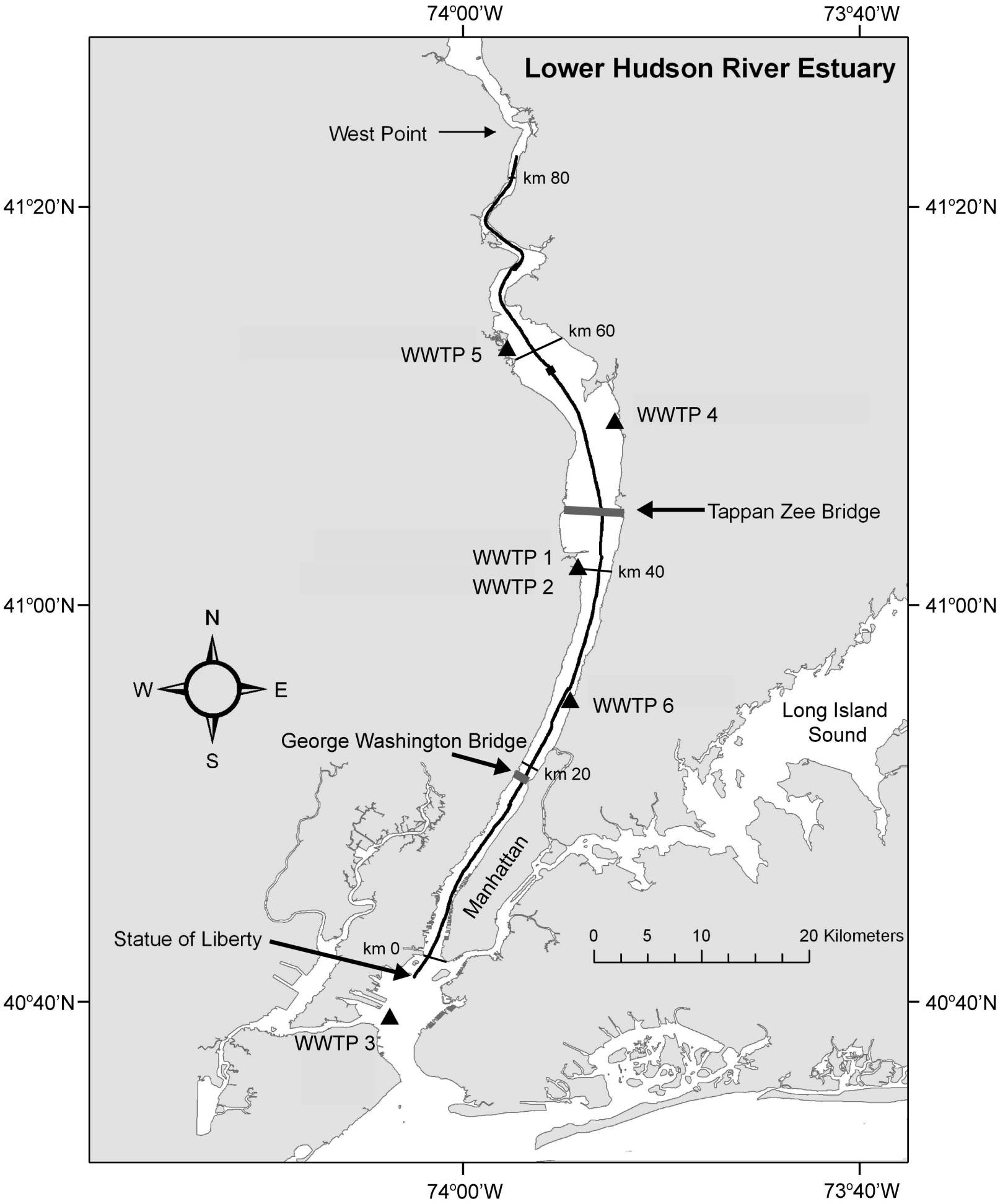
560 **Fig. 1.** Map of the lower Hudson River Estuary. The black curve is a representative transect (19
561 April 2006) and triangles indicate the location of sampled wastewater treatment plant outfalls.

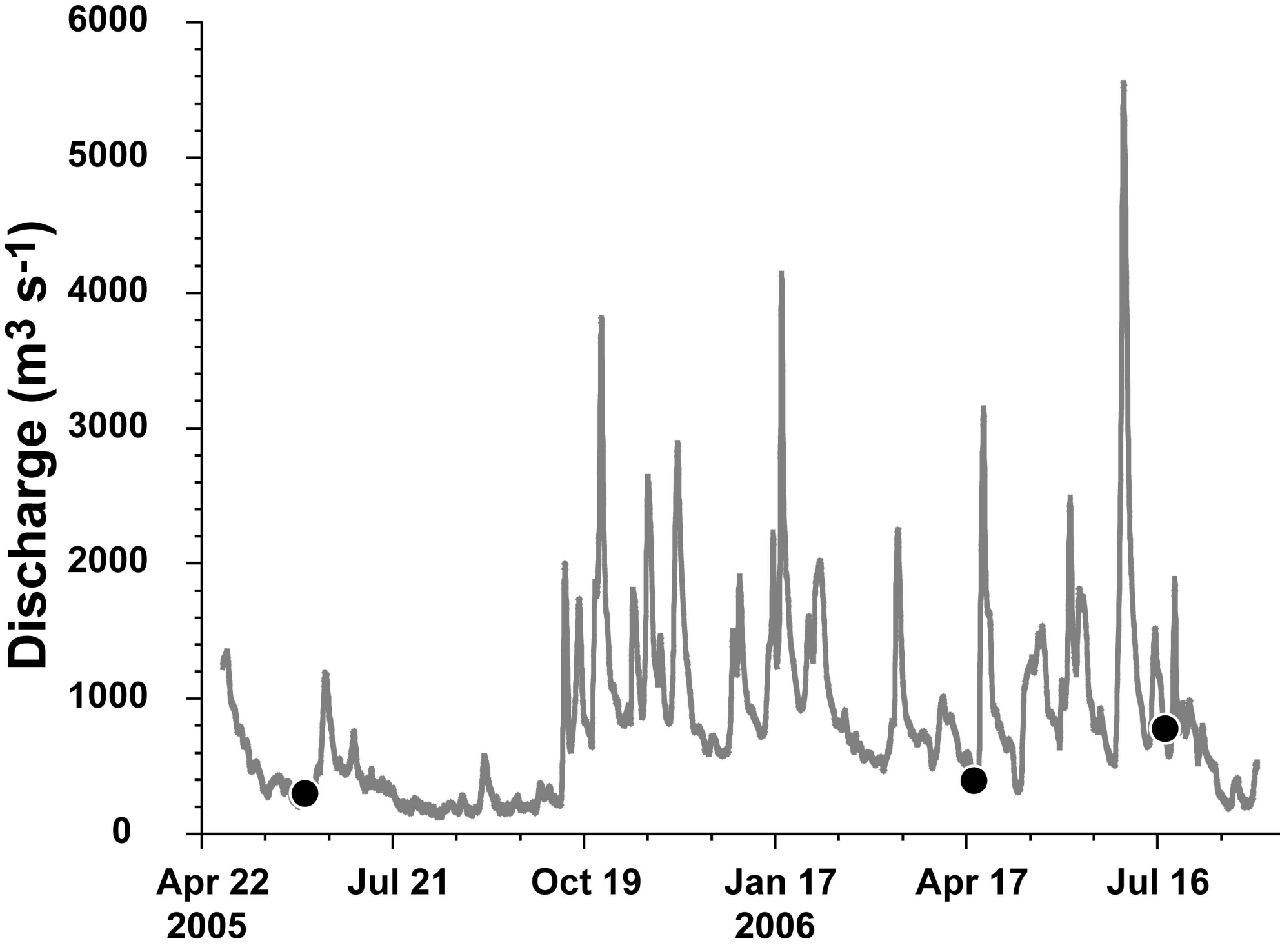
562 **Fig. 2.** Discharge of the Hudson River at the southern tip of Manhattan (river km 0) was
563 estimated as 167% of the discharge from the Green Island, NY river gauge (Howarth et al. 1996;
564 USGS). Our three transects (8 June 2005, 19 April 2006, and 18 July 2006) are shown as circles.

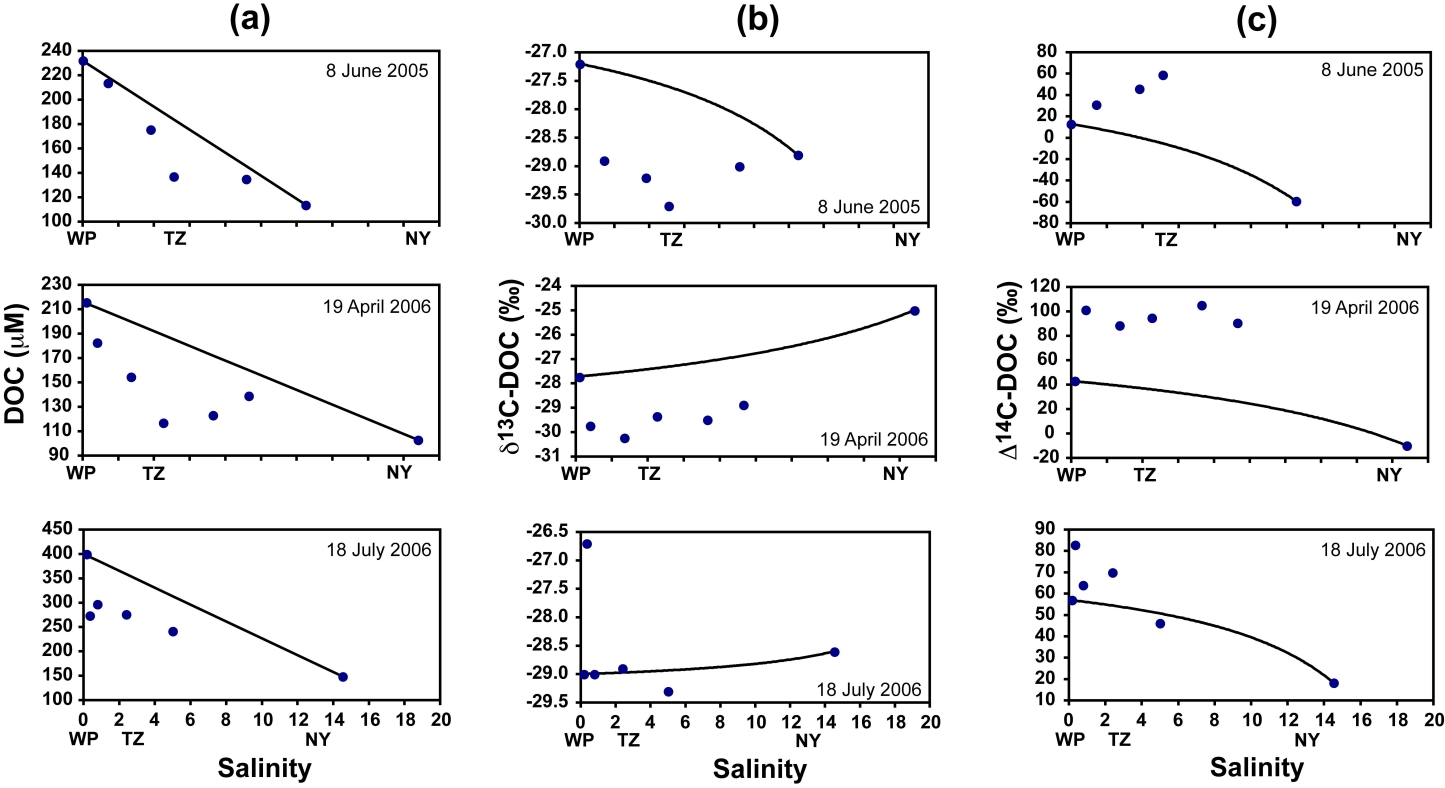
565 **Fig. 3. a)** DOC, **b)** δ^{13}C-DOC, and **c)** Δ^{14}C-DOC mixing curves for three transects in the Hudson
566 River Estuary where circles are measured values and solid lines represent conservative mixing
567 scenarios. Landmarks are abbreviated NY (river km 0, New York City), TZ (Tappan Zee
568 Bridge), and WP (West Point).

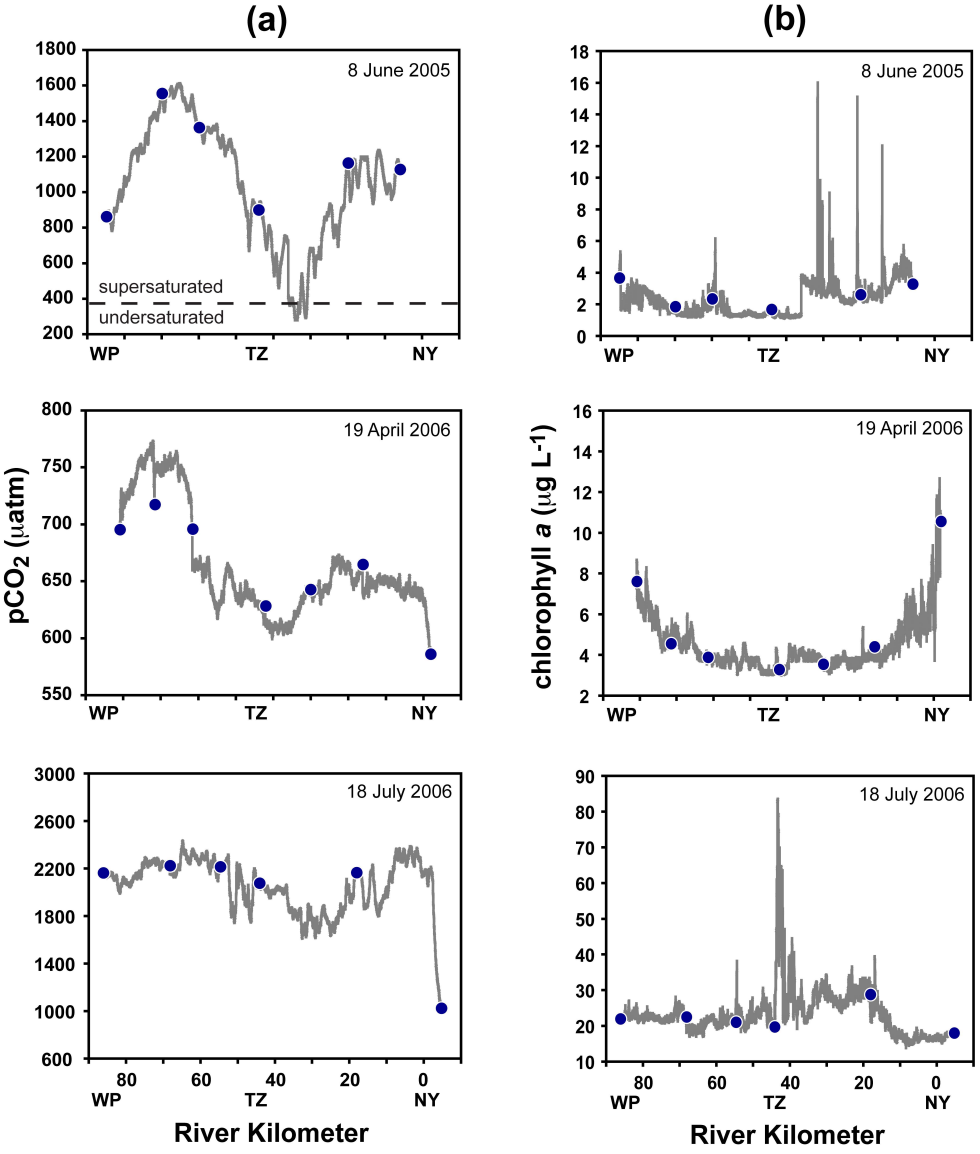
569 **Fig. 4. a)** pCO_2 and **b)** chlorophyll *a* as a function of river kilometer (km 0 is at the southern tip
570 of Manhattan; see Fig. 1). Landmarks are abbreviated NY (river km 0, New York City), TZ
571 (Tappan Zee Bridge), and WP (West Point). The dashed line at 380 μatm represents saturation
572 with atmospheric CO_2, and circles indicate the location of discrete sampling sites.

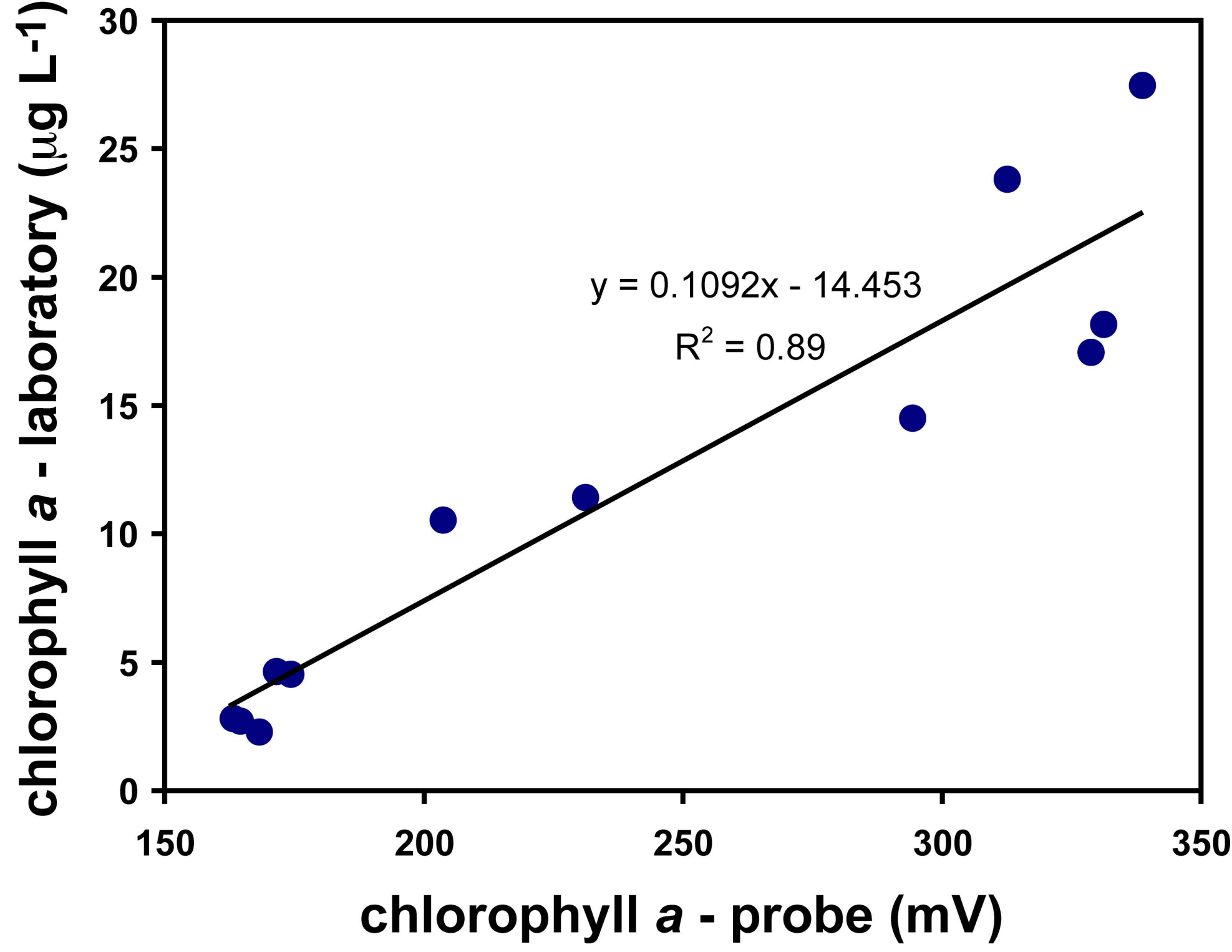
573 **Fig. 5.** Comparing chlorophyll *a* concentrations as measured by a fluorometer in the laboratory
574 and a flow-through probe while underway. The best linear fit was used to convert high spatial
575 resolution probe voltages to the chlorophyll *a* concentrations reported in Fig. 4b.


Lower Hudson River Estuary
74°00'W
73°40'W
41°20'N
41°00'N
40°40'N
West Point
km 80
km 60
WWTP 5
WWTP 4
Tappan Zee Bridge
WWTP 1
WWTP 2
km 40
WWTP 6
George Washington Bridge
km 20
Long Island Sound
Manhattan
Statue of Liberty
km 0
WWTP 3
N
W
E
S
0 5 10 20 Kilometers


6000
5000
4000
3000
2000
1000
0
Discharge (m3 s-1)
Apr 22
2005
Jul 21
Oct 19
Jan 17
2006
Apr 17
Jul 16


(a)
(b)
(c)
DOC (μM)
δ13C-DOC (‰)
Δ14C-DOC (‰)
Salinity
8 June 2005
19 April 2006
18 July 2006
WP
TZ
NY


(a)
(b)
8 June 2005
supersaturated
undersaturated
19 April 2006
18 July 2006
pCO2 (µatm)
chlorophyll a (µg L-1)
WP
TZ
NY
River Kilometer


chlorophyll a - laboratory (µg L-1)
30
25
20
15
10
5
0
y = 0.1092x - 14.453
R2 = 0.89
150
200
250
300
350
chlorophyll a - probe (mV)