FIELD AND LABORATORY INVESTIGATIONS ON THE EFFECTS OF SALINITY ON DECOMPOSITION DYNAMICS AMONG THE HUDSON RIVER'S FRESHWATER TIDAL WETLANDS

A Final Report of the Tibor T. Polgar Fellowship Program

Craig T. Connolly

Polgar Fellow

Ecology, Evolutionary, and Organismal Biology Program
College of the Holy Cross
Worcester, MA 01610

Project Advisors:

Stuart E.G. Findlay
Senior Scientist
Cary Institute for Ecosystem Studies
Millbrook, NY12545

William V. Sobczak
Associate Professor of Biology and Environmental Studies
College of the Holy Cross
Worcester, MA 01610

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ABSTRACT

Sea level rise due to climate change will expose Hudson River tidal marshes to chronic shifts in salinity, thus altering habitat conditions and biogeochemical processes. Increased salt intrusion may affect macroinvertebrates and microbial colonies important in the decomposition of the invasive plant species, *Phragmites australis.* It was hypothesized that litter breakdown varies along the Hudson River salinity gradient, and salinity intrusion will negatively affect macroinvertebrate and micro-organisms. To study the role of salinity in dictating decomposition dynamics, leaf packets were deployed along the Hudson River for measurements of microbial respiration, fungal biomass, and mass loss. The tolerance of a freshwater isopod (*Ligidium sp.*) and microbial colonies to varying salt concentrations found along the Hudson River was examined. Salinity negatively affected isopod survivorship and microbial activity in controlled laboratory treatments. However, the effect of a varying salinity regime on field measurements is unclear. This study provides a model of a river undergoing continuous sea level rise and changing decomposition dynamics.

Key words: decomposition, salinity, respiration, litter, fungi, microbial colonies, Hudson River, *Phragmites australis*

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INTRODUCTION

Rising temperatures causing thermal expansion of oceans and melting of continental sheet ice have made global sea level rise a current and future concern (Larsen et al. 2010). Global sea level rise has increased through the 20th century and is projected to accelerate, adding 190 cm by 2100 (Vermeer and Rahmstorf 2009). Immediate impacts include increased coastal flooding and salt intrusion of surface waters (Nicholls and Cazenave 2010). Wetlands are highly valued ecosystems (Costanza et al. 1998), with services linked to plant biomass, sediment and nutrient retention, and high rates of above- and below- ground net production (Findlay et al. 1990; Findlay et al. 2002B). Tidal wetlands are detritus-based systems with large quantities of organic matter available for decomposition (Mendelssohn et al. 1999; Quintino et al. 2009). As high impact areas, sea level rise threatens the long-term persistence and functioning of coastal wetlands (Titus 1988; Morris et al. 2002; Nicholls and Cazenave 2010; Larsen et al. 2010). Tidal wetlands show spatial patterns of salinity variations from the daily scale of tides to the annual scale of climate patterns, making them prone to increasing salt intrusion (Quintino et al. 2009). Decomposition of plant material supplies organic matter for the metabolism of rivers and wetlands, nutrient cycling for sustaining food chains and primary production, and supports a rich biodiversity of macroinvertebrates, fish, and wildlife (Jordan et al. 1989; Gessner and Chauvet 1994; Baldy et al. 1995; Mendelssohn et al. 1999; Komínková et al. 2000; Quintino et al. 2009). Litter breakdown involves tissue softening and consumption by fungi, microbial colonies, and detritus feeding macroinvertebrates (Webster and Benfield 1986; Gessner and Chauvet 1994; Baldy et al.

1995; Komínková et al. 2000; Graça 2001; Hieber and Gessner 2002; Van Ryckegem et al. 2007).

Phragmites australis (common reed) is an invasive species in tidal wetlands. *Phragmites* stands are productive systems that enhance wetland biomass, modify community structure, and promote resource availability (Gessner 2001; Morris et al. 2002; Findlay et al. 2002B; Quintino et al. 2009). *Phragmites* is tolerant of brackish conditions and is expected to spread as the brackish zone of the Hudson River Estuary moves north (Baldwin and Mendelssohn 1998; Chambers et al. 2003).

Several studies have examined how salinity and macro-and micro-organisms control rates of decomposition of *Phragmites* along a salinity gradient (Reice and Herbst 1982; Hemminga et al. 1991; Mendelssohn et al. 1999; Piscart et al. 2005; Quintino et al. 2009). In response to salinization and the extensive invasion of *Phragmites*, it is important to identify factors controlling organic matter decomposition in tidal freshwater wetlands for future mitigation and wetland restoration projects (Mendelssohn et al. 1999).

This study examined the effect of sea level rise on decomposition within a major river with a salinity gradient. Literature has indicated that mass loss from field measurements (Jordan et al. 1989; Findlay et al. 1990; Hemminga et al. 1991; Windham 2001; Findlay et al. 2002B; Hieber and Gessner 2002; Quintino et al. 2009), respiration by microbial colonies (Findlay et al. 1990; Komínková et al. 2000), ergosterol content as an indicator for fungal biomass (Baldy et al. 1995; Komínková et al. 2000; Gessner 2001; Gessner and Newell 2002; Findlay et al. 2002A; Hieber and Gessner 2002), and salt treatments to macroinvertebrates and microbial colonies (Blasius and Merritt 2002; Baumann and Marschner 2011) are appropriate measures to predict the contribution of

consumer organisms to decomposition, and the effect of salinity on macroinvertebrate and microbial activity. This study presents a novel approach to use well established field and laboratory techniques to measure decomposition within emergent, tidal wetlands along a river spanning a full estuarine salinity gradient.

METHODS

Site Description

The Hudson River Estuary extends 250 km (154 miles) from the Federal Lock and Dam at Troy, New York to The Battery, at the southern tip of Manhattan Island (Yozzo et al. 2005). Mean tidal amplitude ranges from 0.8 m (West Point) to 1.4 m (Troy). The estuary is tidal freshwater (< 0.1 ppt) from Troy south to Poughkeepsie (River Mile "RM" 75) (Yozzo et al. 2005). Depending on freshwater discharge rates, the salt front migrates between the Tappan Zee Bridge (RM 30) and Newburgh (RM 60) throughout the year (Limburg et al. 1986; Yozzo et al. 2005). Intertidal wetlands occur throughout the estuary and tidal freshwater wetlands are found from Albany south to Manitou Marsh (Yozzo et al. 2005). Oligohaline and mesohaline marshes occur along an increasing salinity gradient from Manitou Marsh south to New York City (Yozzo et al. 2005). This study was conducted at the Piermont, Iona, Manitou, Con Hook, and Constitution Marshes from early June to late August 2012 (Fig. 1a, b). Piermont Marsh is a tidal mesohaline marsh (5-15 ppt) located along the west shoreline of the Hudson River about 26 km south of Iona Island (Yozzo and Osgood 2012). Piermont Marsh is covered predominately by *Phragmites* (65% in 1991); native vegetation is restricted to the interior of the marsh (Yozzo and Osgood 2012). Iona Marsh is an oligohaline intertidal marsh and upland habitat, also predominately covered by *Phragmites* (70%

Phragmites; 30% narrowleaf cattail, *Typha angustifolia*) as well as other minor vegetation: broadleaf emergent plants [e.g., pickerelweed (*Pontedaria cordata*) and arrow-arum (*Peltandra virginica*)] (Yozzo and Osgood 2012).

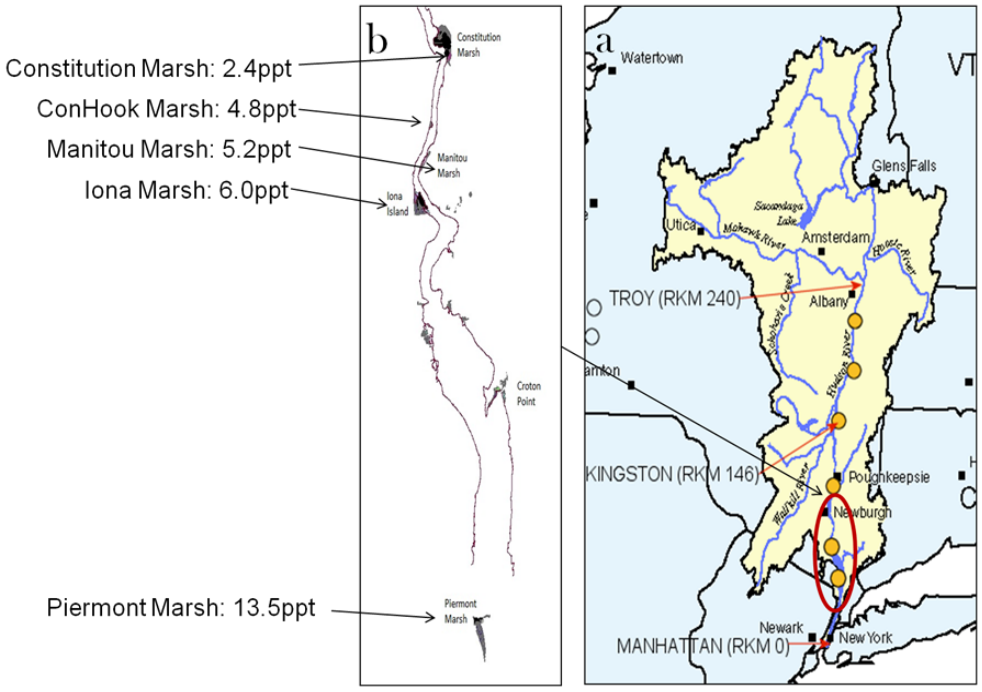

Figure 1. A map of the Hudson River Watershed (a) and Estuary (b). Salinity weekly average for Piermont, Iona, Manitou, Con Hook, and Constitution Marshes were measured during mid-August.

Manitou Marsh is a freshwater to slightly brackish enclosed tidal marsh (0-7 ppt) located on the eastern shore of the Hudson River (Limburg et al. 1986). Manitou Marsh is partially isolated from the Hudson River by a railroad and natural ledge and contains *Phragmites*, narrowleaf cattail, purple loosestrife (*Lythrum salicaria*) and spotted touch-me-not (*Impatiens capensis*). Con Hook Marsh is ~1 km distance north of Manitou Marsh and likely has similar salinity ranges. Constitution Marsh is a freshwater to slightly brackish, enclosed tidal marsh (0-5 ppt) located on the eastern shore of the Hudson River (Limburg et al. 1986). Constitution Marsh is dominated by narrowleaf cattail, but arrow-

arum, pickerelweed, broadleaf arrowhead (*Sagittaria latifolia*), rice cutgrass (*Leersia oryzoides)*, and wild rice (*Zizania sp.*) are also common, while *Phragmites* stands are minor and controlled.

Salinity Range

Salinity data for Piermont, NY and Hastings, NY were analyzed using long-term USGS records (archived conductivity data provided by Gary Wall, USGS, Troy, NY). Daily averages were calculated from October 2005-November 2010 for Hastings and November 2010-September 2011 for Piermont due to differing lengths of records. A histogram of the frequency of salinities (ppt) containing bins: 0, 5, 10, 15, 20 was used to assess the range of salinities from October 2005-September 2011. The frequency of daily averages clearly reached 15 ppt (13% days from 2005-2010, Hastings; 1.8% days from 2010-2011, Piermont) for experimental purposes. During the experimental period, salinity data were collected with YSI Sondes logging at 15 min intervals at all five sites, and averages were calculated during 9 -14 days from 11 July-3 August. Additional salinity measurements were made from grab samples collected during field work at each site in mid-July and early-August.

Macroinvertebrate and Microbial Tolerance to Salinity

A laboratory experiment was conducted to examine macroinvertebrate tolerance to salinity. A simple feeding experiment was performed, giving a common freshwater isopod (*Ligidium sp.*) 1 cm leaf discs of *Phragmites.* Mass loss measurements of leaf discs were made 16 days later. In addition, *Ligidium sp.* were collected at Constitution Marsh and were exposed to salt concentrations similar to conditions spanning the Hudson River Estuary (Blasius and Merritt 2002; Yozzo and Osgood 2012). Daily salinity

averages calculated from October 2005 – September 2011 for Hastings and Piermont were used to select salinity concentrations (ppt): 0, 5, 10, 15, and 20. Salt solutions were made using Instant Ocean and freshwater from the Hudson River and monitored using a YSI salinity meter. Isopods were held in aerated tanks until exposed to salt treatments. Each experimental unit was replicated 4x per treatment and contained four isopods, 20 ml of water varying in salinity, and some detritus. Isopods were exposed to salt treatments for 96 hrs with observations of behavior and mortality recorded every 24 hrs. A similar controlled, laboratory experiment was conducted to assess the effect of salinity on microbial respiration. *Phragmites* leaves were incubated at Constitution Marsh for four weeks. Upon retrieval, 1 cm diameter leaf discs were placed into cups with water from Constitution Marsh and salt amendments (ppt) to comprise: 0, 5, 10, 15, and 20. Leaf discs sat in salt treated water for 96 hrs, while water was changed every 24-48 hrs. After 96 hrs, 10 leaf discs were placed into 60 ml BOD bottles to measure respiration (mg DO ml^{-1} hr^{-1}) over a 12-13 hr interval.

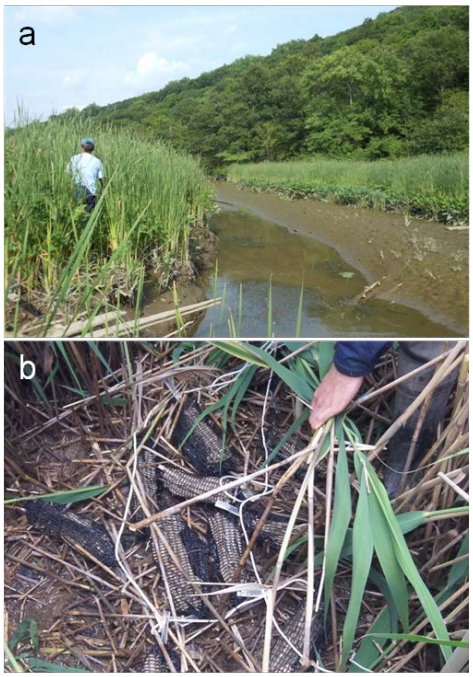

Figure 2. Research approach (a) Typical *Phragmites australis* stand at Manitou Marsh, (b) leaf packet design.

In situ Mass Loss

To study the effect of a salinity gradient on mass loss, leaf litter packets containing 5 g dry weight of *Phragmites* leaves were deployed for approximately four and eight weeks at the five marsh sites (Fig. 2 a, b). Leaf packets were deployed 4-7 June. The first round of packets

was collected 11-19 July, and the second round was collected 31 July- 3 August. Leaf packets consisted of a 1 cm x 1 cm mesh size to allow macroinvertebrate colonization. Twenty-three leaf packets were deployed at each site and were held together by a nylon rope and PVC piping staked into the marsh sediment. Leaf packets were transported to the field individually in paper bags in order to collect mass loss during handling. Three leaf packets at each site were removed immediately to estimate mass loss during field transfer. Leaf packets were placed in varying densities of *Phragmites* and narrowleaf cattail during low tide and in small tributaries off the main channel of the Hudson River, where disturbance would be minimal. Attempts were made to place packets at the same elevation so they were inundated equally.

Upon retrieval, leaf litter was cleaned of sediment and a portion of each leaf packet was removed for leaf discs and small segments for respiration and ergosterol measurements. Leaf litter was dried at 70^{o}C for 24 hrs and combusted in a muffle furnace at 450^{o}C for four hours for ash free dry mass (AFDM) determination. Mass loss during handling and field transfer, and mass removed for respiration discs and ergosterol segments were converted to an AFDM corresponding to the percent loss AFDM of original leaf matter from packets. These values were added back to the AFDM of litter from packets to obtain a final percent AFDM after four and eight weeks. The average mass lost due to handling and field transfer, and mass removed for respiration and ergosterol measurements were 2.7% and 12.7% respectively of the average mass of leaf litter from packets.

Microbial Respiration

To estimate rates of respiration (mg DO ml^{-1} hr^{-1}), dissolved oxygen remaining in

BOD bottles containing leaf litter associated microbes over a 12-13 hr interval was measured. Respiration was measured on leaf litter collected after four and eight weeks. Prior to measurements, leaf litter was cleaned using Hudson River water from the corresponding site to minimize variation between laboratory and field conditions and prevent shock to microbial colonies. Leaf discs were cut using a core (1 cm diameter) immediately following retrieval of packets and placed in BOD bottles filled with corresponding Hudson River water. Hudson River water was used at room temperature to prevent supersaturation of DO. BOD bottles were measured for initial DO concentration and a final DO concentration after a 12-13 hr interval.

Fungal Biomass

To estimate fungal biomass, ergosterol content (μg erg/mg DM) was measured (Gessner and Newell 2002; Findlay et al. 2002B). Upon retrieval, sediment was cleaned with Hudson River water from corresponding sites and 10 leaf segments (2 cm long) were made from random leaves. Leaf segments were stored in 20 ml methanol and placed in a freezer (-20^{o}C). For the first round of leaf packets, an additional 10 leaf segments per leaf packet were made for AFDM determination. Samples were extracted for two hours at 65^{o}C and then cooled in an ice bath. A 5 ml saponification solution containing 4% KOH was used, followed by additional warming and cooling. A series of 10 ml and 5 ml pentane solutions were added and mixed. Pentane containing ergosterol was extracted and evaporated to dryness. Dissolved ergosterol residue was put in 1 ml methanol, sonicated, and then filtered using acrodisks in 2 ml HPLC vials. Samples were stored in the freezer until HPLC analysis. The UV detector was set to 282 nm, while methanol as a mobile phase was set to 1.0-1.5 ml/min. Ergosterol standards were

included during the HPLC analysis. Retention time of ergosterol was dependent on flow rate, temperature, and column properties, and ranged from 4-5 min. A 20 µl injection of samples was used for analysis of amount of ergosterol.

Statistical Analysis

Statistica software was used for statistical analysis. ANOVA was used to compare cumulative mortality of *Ligidium sp.* and microbial respiration across salinity treatments. ANOVA was also used to compare field microbial respiration, ergosterol content, and decomposition across sites. Model significance required p-value ≤ 0.05. Tukey's post-hoc test was used to delineate significance among treatments and report data as mean ±SE. The data do not meet the ANOVA assumption of normality when log transformed due to small sample sizes.

RESULTS

Average salinities (ppt) for Piermont, Iona, Manitou, Con Hook, and Constitution Marshes were 13.5, 6.0, 5.2, 4.8, and 2.4, respectively. Spot measurements for these marshes after four and eight weeks were in the range of average salinities (ppt) calculated from 11 July- 3 August: 7.5 and 9.7, 3.8 and 3.0, 2.6 and 2.4, 2.9 and 2.1, and 1.9 and 1.6, respectively. Results show *Ligidium sp.* fed on leaf litter causing roughly a 2%/day reduction in mass of leaf discs. Cumulative mortality over the 96 hr salinity exposure showed significant differences among treatments (Fig. 3, ANOVA $p< 0.001$). No mortality occurred in 0 ppt, indicating that mortality was due to salt treatments and lab conditions provided suitable conditions. Immediate negative salinity effects were found with significant differences from exposure to 15 ppt and 20 ppt (2.75± 0.71, $p <0.05$, LSD test; 4.0± 0, $p < 0.05$, LSD test). Observations of isopod behavior exhibited less

responsiveness to prodding and lethargy when exposed to salinity compared to the control. This behavior intensified in 10 ppt, 15 ppt, and 20 ppt treatments with increased exposure. Microbial respiration rates (mg DO ml^{-1} hr^{-1}) significantly differed among treatments (Fig. 4, ANOVA $p < 0.001$). Freshwater-colonized microbes had decreased respiration at all salinities greater than 2 ppt (25%, $p < 0.05$, LSD test).

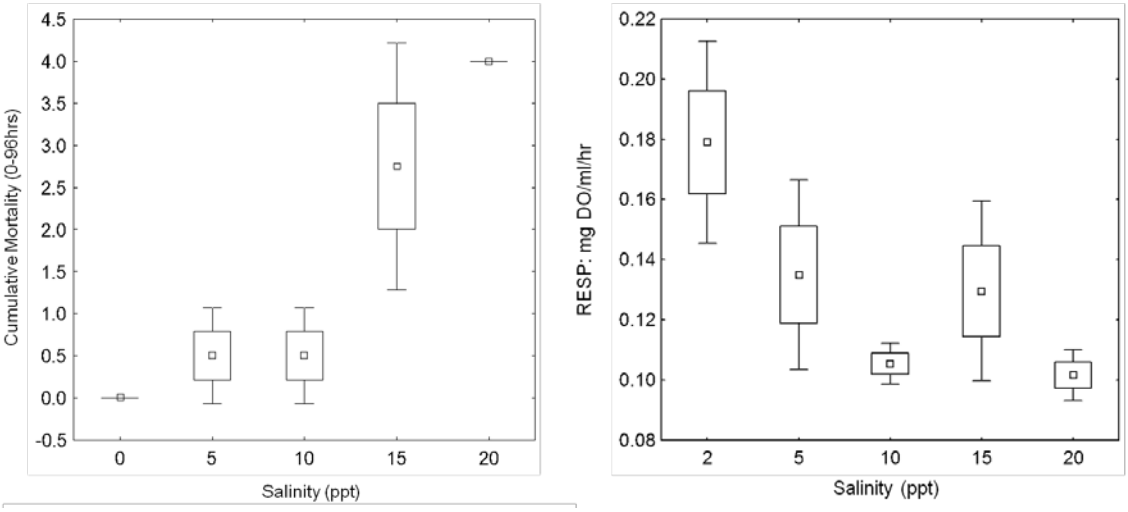

Figure 4. Microbial respiration across salinity treatments after 96hrs.

In situ mass loss experiments showed field measurements of percent mass loss AFDM (g) after eight weeks were significantly different among sites (Fig. 5, ANOVA $p<0.0001$); however, there was no clear relationship between salinity and percent mass loss. Percent mass loss was highest at Iona Marsh (62.95± 1.46%) and lowest at Con Hook Marsh (37.61± 0.92%). Field respiration rates (mg DO $ml^{-1}hr^{-1}$) after four and eight weeks were combined since there was no effect of time in the field. Respiration was significantly different among sites (Fig. 6, ANOVA $p<0.001$), but there was no clear relationship between salinity and *in situ* microbial respiration. Microbial respiration was highest at Piermont Marsh (0.40± 0.02 mg DO $ml^{-1}hr^{-1}$) and lowest at Con Hook Marsh

(0.23± 0.01 mg DO $ml^{-1}hr^{-1}$). Fungal biomass (reported as ergosterol) was significantly different among sites (Fig. 7, ANOVA *p<0.001*), but there was no clear relationship between salinity and ergosterol content. Ergosterol content was highest at Con Hook Marsh (0.17 ± 0.02 μg erg/mg DM) and lowest at Iona Marsh (0.06± 0.005 μg erg/mg DM).

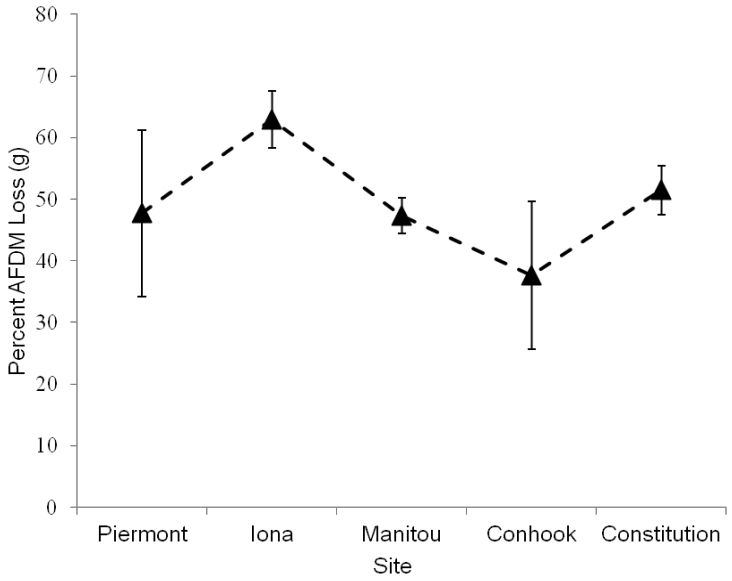

Figure 5. Percent ash free dry mass (AFDM) loss after 8 weeks across the Hudson River salinity gradient.

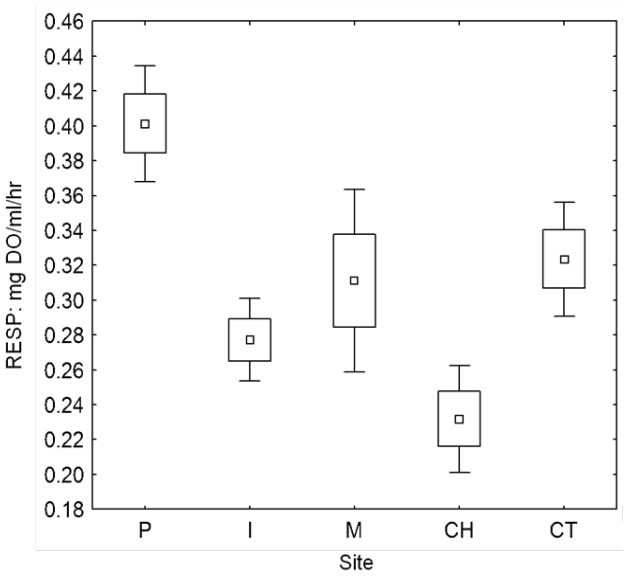

Figure 6. Field microbial respiration across the Hudson River salinity gradient combined 4 and 8 week measurements: Piermont (P), Iona (I), Manitou (M), Con Hook (CH), and Constitution (CT).

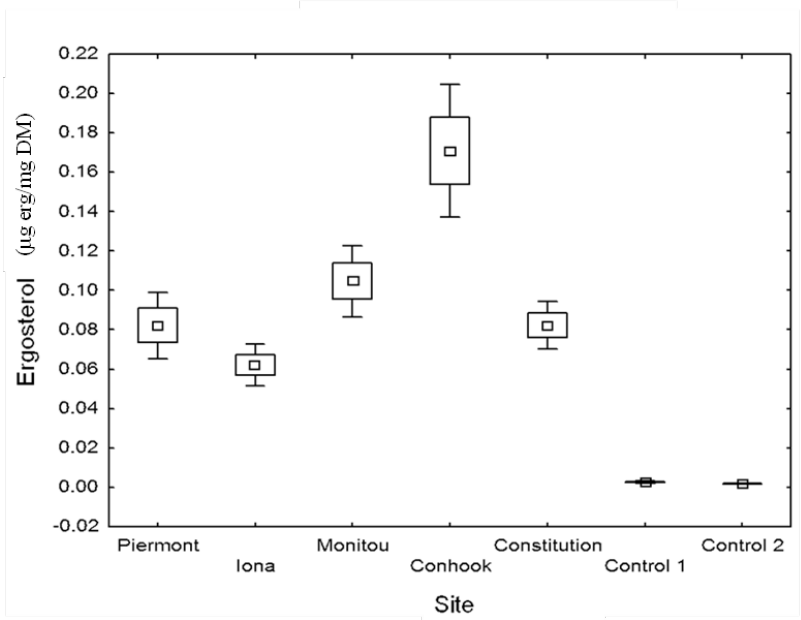

Figure 7. Field ergosterol content across the Hudson River salinity gradient 4 and 8 week measurements combined.

DISCUSSION

The impacts of sea level rise from climate change are a current and future concern for the structural integrity and community composition of wetlands (Titus 1988; Costanza et al. 1998; Morris et al. 2002; Nicholls and Cazenave 2010; Larsen et al. 2010). Litter decomposition has been widely studied in inland wetlands, providing ecosystem services that drive nutrient cycling, and primary and secondary productivity (Webster and Benfield 1986; Jordan et al. 1989; Hemminga et al. 1991; Gessner 2001; Windham 2001; Findlay et al. 2002B). The dominant wetland macrophyte, *Phragmites australis* (common reed) has received considerable attention due to its extensive invasiveness and ability to drive wetland detrital processes (Findlay et al. 2002B). Several studies have examined the role of salinity in dictating decomposition dynamics of *Phragmites* associated with litter mass loss, microbial respiration, and fungal abundances (Reice and Herbst 1982; Hemminga et al. 1991; Mendelssohn et al. 1999; Blasius and Merritt 2002; Piscart et al. 2005; Roache et al. 2006; Quintino et al. 2009; Baumann and Marschner 2011). Only

one study to date has considered the effect of sea level rise and salt intrusion on decomposition dynamics of *Phragmites* across a full salinity gradient (Quintino et al. 2009).

Salinity changes at 15 -20 ppt negatively affected the freshwater isopod, *Ligidium sp.*. Isopods held at 5 -10 ppt exhibited less responsiveness to prodding and a gradual mortality compared to freshwater conditions. This behavior intensified at 15 -20 ppt and with increasing time. Isopods exposed to 10 -20 ppt exhibited immediate mortality, indicating intolerance to rapid, large changes in salinity. Macroinvertebrates contribute to shredding and breakdown of leaf litter (Brinson et al. 1981; Webster and Benfield 1986; Graça 2001; Blasius and Merritt 2002; Collins et al. 2007). *Ligidium sp.* is a common macroinvertebrate at Constitution Marsh that shreds and decomposes *Phragmites*. Increasing salinity at sites near freshwater conditions would be expected to decrease decomposition of *Phragmites* by *Ligidium sp.*. Shifts in salinity might change the biodiversity of common shredding macroinvertebrates of the Hudson River, leading to further decreases in decomposition rates (Yozzo and Osgood 2012). Similar patterns were seen of increasing mortality of *Gammarus* (Amphipoda) from exposure to high road salt concentrations in a laboratory study on the effect of road salts (NaCl) on macroinvertebrate communities in Lake Michigan (Blasius and Merritt 2002).

Exposing microbial colonies on *Phragmites* to acute salinity increases showed a strong negative effect on microbial respiration. Respiration decreased from changes as low as 5 ppt. This trend intensified at 10 -15 ppt, indicating intolerance to chronic changes in salinity. Microbial assimilation of detritus is critical for softening of leaf tissue and contributes to the decomposition of leaf matter (Brinson et al. 1981; Webster and

Benfield 1986; Gessner and Chauvet 1994; Baldy et al. 1995; Hieber and Gessner 2002). Thus, increasing salinity would be expected to decrease the decomposition of *Phragmites* by microbial colonies adapted to freshwater conditions. Similar decreases in microbial respiration with increasing soil salinity were observed in a study of drying and rewetting (Baumann and Marschner, 2011). Evidence from laboratory experiments also showed reduced microbial activity on a gradient of increasing salinity in a freshwater wetland near Gippsland Lakes, eastern Victoria, Australia (Roache et al. 2006).

Natural variation in salinity among Hudson River marshes did not reveal clear patterns in field respiration, fungal biomass, and mass loss from decomposition. Recent studies on *Phragmites* decomposition along a full salinity gradient (34.6 ppt at the mouth-0 ppt, at the head) of the Mira Channel, Ria de Averio in Western Portugal, showed clearer relationships (Quintino et al. 2009). It was estimated that a 51%, 71%, 70%, and 71% mass loss occurred in 5 mm litter bags containing 3 g *Phragmites* after 60 days in salinities (ppt) of approximately 34.6, 16.2, 2.4, and 0.0, respectively.

Studies show an inverse relationship between water or soil salinity and microbial activity. Decreasing micro-organism activity with increasing salinity was seen in a terrestrial ecosystem from shifts in species diversity (van Bruggen and Semenov 2000). Other studies in terrestrial ecosystems also indicated decreasing microbial biomass (Muhammad et al. 2006; Wichern et al. 2006), and activity (Rietz and Haynes 2003; Sardinha et al. 2003) with increasing salinity. Rising salinity along a gradient has been shown to reduce denitrification activity and the diversity of nitrogen cycling communities, suggesting nitrogen removal capacity will reduce as freshwater marshes become more saline (Larsen et al. 2010). Evidence from laboratory studies showed a

decrease in leaf mass decay for three plant species along an increasing salinity gradient (Roache et al. 2006). Discrepancy between laboratory and field results could be due to varying conditions at field sites compared to a controlled lab setting. These conditions include tides of changing salinity, and sediment on leaf matter which alters respiration rates and the AFDM. A "shock" from instant exposure to salt treatments may contribute to differences between field and laboratory results. The effect that salinity has on fungal communities is poorly understood, but could contribute to variations in fungal dynamics.

In years and decades to come, this pressing issue will become increasingly important as chronic changes in salinity will continue to alter and drive wetland processes. Consequences include adaptation, changes in productivity and community diversity, and diminishing of buffering and restorative services of wetlands. Future studies examining decomposition of *Phragmites* across a salinity regime are required to enhance the knowledge of wetland responses to salt intrusion from sea level rise. Increasing salinization in tidal wetlands is a current issue for preventing the expansion of *Phragmites australis,* and for establishing restoration goals. Climate change projections indicate continuous sea level rise; hence, this study provides a useful approach and initial benchmark for future studies examining projected salinity increases in tidal wetlands along the Hudson River and other freshwater-tidal rivers.

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