REPORTS OF THE TIBOR T. POLGAR

FELLOWSHIP PROGRAM, 2010

Sarah H. Fernald, David J. Yozzo
and Helena Andreyko

Editors

A Joint Program of
The Hudson River Foundation
and The New York State
Department of Environmental Conservation

March 2012

This volume of the Reports of the Tibor T. Polgar Fellowship Program is dedicated to the memory of Dr. Mark B. Bain.

Mark B. Bain
1955 – 2012

ABSTRACT

Eight studies were conducted within the Hudson River estuary under the auspices of the Tibor T. Polgar Fellowship Program during 2010. Major objectives of these studies included: (1) analyzing the purposes, strengths, and challenges, as well as anticipate future issues, concerning conservation easements along the Hudson through the administration of a survey to easement-holding entities, (2) studying stable carbon and nitrogen isotopes in Hudson River marshes to test how heightened anthropogenic activities such as land-use change and nutrient loading have affected both the biodiversity and sedimentation dynamics of wetlands, (3) examining the spatial and temporal distribution of antibiotic-resistant microbes in the Hudson River in correlation with the sewage indicating bacterium, *Enterococcus*, to test the hypothesis that the occurrence of antibiotic-resistant microbes is positively correlated with sewage loading in wet weather conditions, (4) characterizing the responses of denitrification potential and sediment characteristics in a freshwater tidal marsh in the upper Hudson River to a small-scale removal of *Phragmites australis*, an invasive plant community, (5) understanding the relationship between environmental contaminants and oyster health in the Hudson River Estuary by testing the hypothesis that oysters placed at impacted sites will exhibit a lower overall condition, lower energy reserves, and higher total body burdens of metals (Cd, Cu, Hg) than oysters placed at a reference site, (6) investigating the effect of silver nanoparticles (AgNPs), which have been shown to be toxic and destructive to DNA and metabolic pathways, on aquatic animals, using crayfish (*Orconectes virilis*), a common inhabitant of the Hudson River Watershed, as experimental model, (7) quantifying Hudson River larval fish occurrence within a range of shallow water microhabitats in order to determine the importance of habitat variables for larval fish distribution, and (8) observing stable isotope ratios of carbon (δ13C) and nitrogen (δ15N) in feathers of colonial waterbirds nesting in New York Harbor to examine the foraging ecology of a suite of species, and to identify the key habitats and forage base that were particularly important to each individual population.

TABLE OF CONTENTS

PREFACE

The Hudson River estuary stretches from its tidal limit at the Federal Dam at Troy, New York, to its merger with the New York Bight, south of New York City. Within that reach, the estuary displays a broad transition from tidal freshwater to marine conditions that are reflected in its physical composition and the biota its supports. As such, it presents a major opportunity and challenge to researchers to describe the makeup and workings of a complex and dynamic ecosystem. The Tibor T. Polgar Fellowship Program provides funds for students to study selected aspects of the physical, chemical, biological, and public policy realms of the estuary.

The Polgar Fellowship Program was established in 1985 in memory of Dr. Tibor T. Polgar, former Chairman of the Hudson River Foundation Science Panel. The 2010 program was jointly conducted by the Hudson River Foundation for Science and Environmental Research and the New York State Department of Environmental Conservation and underwritten by the Hudson River Foundation. The fellowship program provides stipends and research funds for research projects within the Hudson estuary and is open to graduate and undergraduate students.

Prior to 1988, Polgar studies were conducted only within the four sites that comprise the Hudson River National Estuarine Research Reserve, a part of the National Estuarine Research Reserve System. The four Hudson River sites, Piermont Marsh, Iona Island, Tivoli Bays, and Stockport Flats exceed 4,000 acres and include a wide variety of habitats spaced over 100 miles of the Hudson estuary. Since 1988, the Polgar Program has supported research carried out at any location within the Hudson estuary.

The work reported in this volume represents the eight research projects conducted by Polgar Fellows during 2010. These studies meet the goals of the Tibor T. Polgar Fellowship Program to generate new information on the nature of the Hudson estuary and to train students in estuarine science.

Sarah H. Fernald
New York State Department of Environmental Conservation

David J. Yozzo
Henningson, Durham & Richardson Architecture and Engineering, P.C.

Helena Andreyko
Hudson River Foundation for Science and Environmental Research

LAND TRUSTS AND CONSERVATION EASEMENTS ALONG THE HUDSON: HOW FEASIBLE IS PERPETUITY?

A Final Report of the Tibor T. Polgar Fellowship Program

Nikki Koenig Nielson

Polgar Fellow

Juris Doctor Program
Albany Law School
Albany, NY 12208

Project Advisor:

Keith H. Hirokawa
Assistant Professor of Law
Albany Law School
Albany, NY 12208

Nielson, N. K. and K. H. Hirokawa. 2012. Land Trusts and Conservation Easements Along the Hudson: How Feasible is Perpetuity? Section I: 1-42 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

Conservation Easements along the Hudson River and within its watershed are a well established property interest. Ownership of these conservation easements, constituting tens of thousands of encumbered acres, rests largely with privately held land trusts. Authorized by New York State statute yet subject to common law property doctrines, conservation easements represent a relatively new and novel interest in land. In order to achieve the explicit and specific intended conservation purposes, land trusts are charged with complex annual monitoring requirements in addition to fulfilling the daily responsibilities and obligations of their organizational mission. There is an increasing proclivity on the part of landowners to donate these property interests in order to benefit from the substantial state and federal tax incentives. Qualification of donated easements for tax purposes also requires that the easement restrict the use of property to meet the conservation purpose in perpetuity. The issue of holding and protecting these interests within the expanse of forever is subject to circumstances of changed conditions, which may serve to modify or terminate the conservation easement. Provisions in the statute acknowledge and identify that forever is a long time, and that on a case-by-case, fact specific basis, there may in fact be a need to terminate the easement interest. The statute's acknowledgement of this reality and flexibility in application may provide the most realistic approach to what is otherwise intended to be perpetual. More challenging to the perpetual nature of conservation easements however, is the organizational capacity of the land trusts that are charged with their enforcement. Because many land trusts in the Hudson Valley are heavily reliant on volunteerism, they are subject to concerns about their ability to exist in the long term to be able to enforce the intended purposes of the

conservation easements they hold. This project seeks to analyze the purposes, strengths, and challenges, as well as anticipate future issues concerning easements along the Hudson through the administration of a survey to easement-holding entities. Survey results illustrate the interdependence between the easements themselves and the entities charged with their enforcement.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

The Hudson River

The Hudson River corridor enjoys a considerable open space inventory. Grand estates, religious retreats, non-profit centers, parkland, and residences characterize significant stretches along the Hudson. When valued in terms of economic development, these properties represent an undeveloped economic asset. Yet keeping the properties undeveloped may also yield substantial conservation benefits, both in the form of what is prevented (impermeable surfaces, water pollution, scarred landscapes and habitat destruction) and what is protected (habitats and species, viewsheds, and water quality).

Conservation easements[1] are proving to be an emerging and effective mechanism for preventing development while preserving land in private ownership. Tens of thousands of acres are currently subject to private conservation restrictions along the Hudson and within its watershed, representing a significant portion of the constituent land trusts' portfolio of properties. The popularity of the conservation easement is likely due both to its use as an economical mechanism for private control over the particular restriction and its status as a property interest. Land trusts[2] act as stewards of the easements that are intended to protect a broad range of conservation interests, including ecosystem services, viewshed and working farmland. Once easement interests are acquired, land trusts are charged with (and entitled to) the duty of monitoring and

---

[1] A conservation easement is a "voluntary agreement between a private landowner and a municipal agency or a qualified not-for-profit corporation to restrict the development, management or use of the land." John R. Nolan, WELL-GROUNDED: USING LOCAL LAND USE AUTHORITY TO ACHIEVE SMART GROWTH 295 (2001).

[2] "[A] land trust is a local or regional not-for-profit organization, private in nature, organized to preserve and protect the natural and man-made environment" *Id*.

enforcing these interests with a substantial level of technical and professional expertise to evaluate the effectiveness of the intended easement purpose. On the other hand, although conservation easements are an established property interest in the Hudson and its watershed, over time the challenge for the continued protection of these interests may be the availability of resources to provide the necessary monitoring and expertise to detect violations and ensure enforcement.

Property and Conservation Easements

Property law has developed as a framework for the enforcement of privileges and rights to use that accompany ownership. This is often characterized as the property owner's "bundle of sticks"[3](Hylton, et al. 2007). Each stick in the bundle represents a separate right, i.e. time, space, use, which can be separated from the others.[4] On property's usurfractory[5] side, use and enjoyment of a property interest may be limited to the extent that it creates a nuisance by interfering with others' use and enjoyment. Limitations on such use have come into effect through common law through the doctrines of trespass, which protects physical boundaries from intrusion and nuisance, and which prohibits unreasonable interference with an owner's possession.[6] A more recent development concerns property use limitations effected under the police power, which

[3] J. GORDON HYLTON, ET AL., PROPERTY LAW AND THE PUBLIC INTEREST: CASES AND MATERIALS 83 (3rd ed. 2007).
[4] *Id*.
[5] "[T]he right of using and enjoying property belonging to another provided the substance of the property remained unimpaired." BLACK'S LAW DICTIONARY 1684 (9th ed. 2010).
[6] Hylton, *supra*, note 3 at 102. Common law property is ever mindful of the maxim, *Sic utere tuo ut alienum non laedus*, meaning that the right to enjoy one's own property is limited to the extent that it interferes with the rights of another.

authorizes the state to determine the types of uses that are protected as property, as well as the types of land uses that threaten the health, safety and welfare of the community.[7]

An easement is a non-possessory interest in real property and is generally granted for a specific use or to prevent a specific use of property.[8] A grantor may execute a positive easement to a neighbor for access to the grantee's property across the grantor's property.[9] In contrast, a negative easement might be granted to protect a view across the granting owner's land by preventing the granting landowner from building over a particular height.[10] Easements need not benefit a specific land: in gross, easements benefit a third party in a way that may not be connected to the grantee's property.[11] Essentially, the required elements of an easement include the (1) intent to bind current (and if expressed, future) owners of the property to the terms of the agreement, (2) restrictions applied to the use of the entire property, and (3) a period of time that conveys permanence.[12]

Although conservation easements are borne from its predecessor forms of easements, they are justifiably thought of as a new form of ownership[13] (Tapick, 2002).

---

[7] *Id*. at 133.
[8] Warren's Weed New York Real Property § 40.03. Easements may be granted to an adjacent landowner (appurtenant) or for the entire property (in gross). Conservation easements are granted in gross.
[9] *Id*.
[10] *Id*.
[11] *Id*. Common law easements in gross are not necessarily assignable to subsequent parties, and the courts are split on whether to enforce such interests when at least one of the original parties to the agreement no longer exists.
[12] *Id.*, *Millbrook Hunt, Inc. v. Smith*, 249 A.D.2d 281, 282—83, 670 N.Y.S.2d 907, 908—09 (2d Dep't 1998) (holding that easements in gross require expressly identifying the intent of the grant is as an easement, a definite, period of time that conveys permanence and notice that the interest exists in order to be enforceable against a subsequent owner).
[13] Jeffrey Tapick, *Threats to the Continued Existence of Conservation Easements,* 27 COLUM J. ENVTL. L. 257, 266—72 (2002) (arguing that conservation easements do not qualify as a negative easement because it is not one of the four recognized areas where such term applies, nor covenants because equitable remedies are not available for enforcement of covenants, only monetary damages that do not support the

The majority of conservation easements involve easement deeds intended to encumber the allowable uses in perpetuity, preventing specific activities[14] (Laitos et al. 2006). These instruments create conservation property interests in the transferee, a third party which is typically a land trust, although other eligible entities include municipal governments and qualified non-profit agencies.[15] As a function of the easement, the right to use property remains substantially that of the traditional and mainstream view of a private owner, with the easement holder gaining the right to restrict types of uses of the property "to preserve the servient[16] land in an undeveloped or natural state"[17] (Bruce and Ely, 2001). The separation of the property interests, and the allocation of different rights (monitoring, maintenance, enforcement, etc.) to multiple parties may find support in the framework of easement law, but the conservation easement also challenges the previous scheme because the entities involved include a third party organization whose priorities and organizational capacity is subject to change. This fluidity often constitutes a challenge to effect the purpose of conservation easements.

---

requirements of conservation, nor servitudes because they do not satisfy the requirement that the interest "touch and concern" the land).

[14] JAN G. LAITOS, ET AL., NATURAL RESOURCES LAW 706–07 (2006) the requirement for conservation purposes does not need to include access, and environmental stewardship is in and of itself a public conservation interest that can be protected; See, Nancy A. McLaughlin, *The Role of Land Trusts in Biodiversity Conservation on Private Lands*, 38 Idaho L. Rev. 453, 460—68 (2002) in JAN G. LAITOS, ET AL., NATURAL RESOURCES LAW at 719, arguing that protection of biodiversity is provided by the existence of a conservation easement because it connects people with nature, providing a comprehensive approach to long term preservation, and "casts a much wider net of land protections than is found in more targeted land conservation programs…." *Id*. at 720.

[15] N.Y. Envtl. Conserv. Law §49-0305(3)(a) (1983) (As not-for-profit organizations, land trusts, municipal governments and other non-profits are authorized to accept an interest in a conservation easement). Occasionally the land trusts acquire fee simple title to a whole or part of the parcel, but the recent trend involves conveyance of a mere easement in which the owner retains the right to continue to use the land, including conveyance by sale.

[16] The servient property is the one "subject to an easement," the property that is in servitude to the owner of the easement. BLACK'S LAW DICTIONARY 1491 (9th ed. 2010).

[17] JON W. BRUCE AND JAMES W. ELY, JR., THE LAW OF EASEMENTS AND LICENSES IN LAND 12.2 (2001).

Project Goals

This project seeks to quantify the easements along the Hudson, including their intended purposes, discuss the strengths and challenges of this new property framework, and identify issues for future research. The project process created and administered a survey to easement-holding entities along the Hudson, the results of which function to illustrate the interdependence between the easements themselves and the entities charged with their enforcement.

LITERATURE REVIEW

The literature review yielded information about legal issues and controversies relating to conservation easements.[18] Property law, which provides the legal framework governing conservation easements, is based on common law that has developed over centuries as a non-linear story of rights to own, transfer, possess, use and exclude others from what is owned[19] (Hylton et al. 2007). American courts have moved towards allowing property to be alienable, disallowing indeterminate restrictions of ownership and use.[20]

---

[18] *Herman v. Comm'r of Internal Revenue*, 98 T.C.M. (CCH) 57931, 2009 Tax Ct. Memo LEXIS 209 *30(2009)(holding that perpetuity in the context of conservation easements requires a "limitation that would survive the sale… to a bona fide purchaser who might not share… subjective intentions.").

[19] J. GORDON HYLTON, ET AL., PROPERTY LAW AND THE PUBLIC INTEREST: CASES AND MATERIALS75 (3rd ed. 2007) (explaining that while there have always been some limitations restricting property use, as imposed the first time the property was transferred from sovereign to private ownership. These restrictions are not clear, and often require adjudication for clarification of the rule.)

[20] *Id*. at 407–09.

Conservation easements are relatively new, having first emerged in the late nineteenth century.[21] However, this form of easement was not routinely used until the availability of the Uniform Conservation Easement Act after 1981[22] (Smith 2010). The New York State Legislature has adopted statutory provisions for conservation easements and definitions of use.[23] The state's adoption of statutory mechanisms to create this expressly acknowledges the availability and legitimacy of easements in the State and establishes standards for interpreting language and implementation of the instruments that purport to create them.

Creation of a Conservation Easement

Recently, there has been an increase in the level of conservation easement donations, which has been correlated to the availability of tax credits[24] (Paden 2010). The tax credit requires that the easement be donated, as opposed to purchased or exacted, and that the

---

[21] Ann Harris Smith, Note, *Conservation Easement Violated: What Next? A Discussion of Remedies*, 20 FORDHAM ENVTL. L. REV. 597, 600–02 (2010), citing Richard R. Powell, Powell on Real Property § 34A.02(1987)(courts were reluctant to address the existence of conservation easements until the 1930s because there was no privity between properties or parties, and the common law did not recognize conservation as a legitimate conservation purpose); see also, Zachary Bray, *Reconciling Development and Natural Beauty: The Promise and Dilemma of Conservation Easements*, 34 HARV. ENVTL. L. REV. 119, 126 (2010).

[22] *Id*. the Uniform Conservation Easement Act, developed by the National Conference of Commissioners on State Laws, is model legislation; Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 305—307 (2004) (the earliest conservation easement statute were Massachusetts in 1956 and California in 1959, with the former allowing non-profits to hold the easements beginning in 1969).

[23] N.Y. Envtl. Conserv. Law §§ 49-0203, 52-0905, 54-0907, 56-0309, 49-0301 (1983); The New York State Department of Agriculture has adopted a standard easement and strongly urges its use for those seeking funds toward easement purchase, http://www.agmkt.state.ny.us/RFPS_archive.html

[24] Peter R. Paden, Presentation at the New York State Bar Association Continuing Legal Education Seminar: Planning, Drafting & Administration of Conservation Easements (June 4, 2010) (NYS has property tax credit given on state income tax for easements that apply in perpetuity, in addition to the federal credit for charitable donation purposes. . Average size of a conservation easement donation is valued at $400k, compared to an average stock donation of $50k and an average large donation of $10k.); *see also*, Zachary Bray, *Reconciling Development and Natural Beauty: The Promise and Dilemma of Conservation Easements*, 34 HARV. ENVTL. L. REV. 119, 137 (2010), Andrew Dana & Michael Ramsey, *Conservation Easements and the Common Law*, 8 STAN. ENVTL. L.J. 2, 38—39 (1989).

donation be granted explicitly in perpetuity.[25] There is no state or Federal statute requiring that the assessed value of the property be automatically reduced once a conservation easement is conveyed. The property owner must demonstrate, as any other owner grieving an assessment, that the easement in and of itself restricts the property value.[26] Because there are many qualified public interests for which the tax credit is available, conservation easements are granted for reasons that are inclusive of conservation purposes other than strictly environmental, e.g. educational, open space, recreational, viewshed[27] (Paden 2010). Land use planning and personal wealth management factor in to the reasons that people choose to make easement donations.[28] The significance of the tax credit and the need to adhere to IRS regulations has resulted in

---

[25] http://www.renstrust.org/information-for-landowners/conservation-easement-tax-credit, (accessed Aug. 11, 2010) ("The New York State Conservation Easement Tax Credit (CETC) offers NY taxpayers whose land is restricted by a conservation easement an annual NY State income tax credit of up to 25% of the school district, county and town real estate taxes paid on the restricted land , up to an annual maximum of $5,000 per taxpayer. Unlike a tax deduction, which is an adjustment to taxable income, a tax credit offsets a taxpayer's lax liability on a dollar-for-dollar basis. The CETC is a REFUNDABLE income tax credit, which means that if a landowner's tax credit exceeds the amount he or she owes in state income taxes, the landowner gets a check for the difference. The CETC is available to individual landowners, estates, trusts, partnerships and certain corporations as long as the land they own is restricted by a perpetual and permanent conservation easement as defined in Article 49 of New York's Environmental Law."); see also, http://www.landtrustalliance.org/community/northeast/ny-tax-credit (accessed Aug. 11, 2010).

[26] *Gibson v. Gleason*, 20 A.D.3d 623, 626—27, 798 N.Y.S.2d 541, 544—45(Sup. Ct. 3rd Dep't 2005) (holding that a conservation easement does not per se reduce property value assessment but if the owner can demonstrate reduced value by evidence of restriction on highest and best use, reduction is available. Further, the court found that a conservation easement prohibiting subdivision requires assessment of the property as a whole, individual parcel); *See also*, *Adirondack Mountain Reserve v. Bd. of Assessors of Town of N. Hudson*, 471 N.Y.S.2d 703, 704, 99 A.D.2d 600, 601 (3d Dep't 1984) (holding that easements granted by land trust to State of New York did not decrease the property value, and that assessor benefits from presumption of validity of evaluation of property value); *Luca v. Lincoln County Assessor*, 2003 WL21252488 *8 (Or. Tax Magistrate Div.) (finding that highest and best use of property restricted by a conservation easement was single-family residential, which was a higher value than open space, because existing single-family residences on the property had a right to be replaced, rebuilt or subdivided).

[27] Peter R. Paden, *Planning Conservation Easements*, in PLANNING, DRAFTING & ADMINISTRATION OF CONSERVATION EASEMENTS 1, 11 (New York State Bar Association Continuing Legal Education, 2010) (The intent of the easement can factor in what entity will hold the easement, as some land trusts have specific areas of focus); Jessica Owley Lippman, *The Emergence of Exacted Conservation Easements*, 84 NEB. L. REV. 1043, 1094 (2006) (arguing that the availability and accessibility of conservation easements provides many public benefits ancillary to improved ecosystem services, such as keeping property on the tax rolls, which benefits schools and local governments.)

[28] Paden, *supra*, note 37 at 6–9 (New York State Bar Association Continuing Legal Education, 2010)

an increase in the level of scrutiny on land trust agencies themselves.[29] The Land Trust Alliance has created a program to provide for accreditation of local land trusts, to ensure compliance with IRS requirements and provide a more transparent process of determining the authenticity and legitimacy of the donation.[30]

An otherwise unlimited conservation easement "runs with the land" (meaning that its restrictions survive transfers of ownership of the underlying land) and can be formed in three ways: by donation, purchase or exaction.[31] The prevailing intention, which is required for a donation to qualify as tax deductible according to Federal Internal Revenue Service code, is that the easement be enforceable in perpetuity[32] (Covington 1996). However, perpetuity, the concept of "forever," can come to an end.[33] This termination of perpetuity essentially constitutes an issue of enforcement distinct from an enforcement action, the latter is brought to enforce a provision of the easement agreement to protect the intended purpose, without questioning or challenging whether the easement itself

---

[29] *Id*.

[30] http://www.landtrustalliance.org/learning/accreditation/accreditation, (accessed Aug. 11, 2010) (The Accreditation program is substantial rigorous, with only 105 land trusts completing the process nationally since 2008. Criteria include responsible governance, ethical considerations, compliance with federal and state regulations, accountability and protection of the public interest. In the Hudson Valley, six Hudson Valley focused organizations including Scenic Hudson, The Hudson Highlands Land Trust, the Dutchess Land Conservancy, Rensselaer Land Trust and Westchester Land Trust, and the nationally focused Open Space Institute have achieved accreditation.)

[31] Peter R. Paden, *supra*, note 27 at 1; Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 294—295 (2004) (discussing the emergence of exacted easements, which are non-voluntary agreements that convey an easement interest as a mitigation effort, in exchange for permission to do something that changes the property). *Smith v. Mendon*, 4 N.Y.3d 1, 822 N.E. 2d 1214 (2004) (A property restricted by zoning that required an Environmental Protection Overlay District for development was further required to grant the municipality a conservation easement as a condition of obtaining a development permit because the restriction did not diminish the value of the property and impose an unfair financial burden)

[32] George M. Covington, *Conservation Easements: A Win/Win for Preservationists and Real Estate Owners*, 84 ILL. B. J. 628, 629 (1996), citing Internal Revenue Code §170(f)(3)(B)(iii) (the easement must be granted to a qualified charitable organization and granted in perpetuity, but the grantee does not have to budget funds towards enforcement in perpetuity); 34A Am. Jur. 2d *Federal Taxation* §143,900.3 (West 2010) (IRS tax credits become part of an estate, but are subject to specific provisions).

[33] Restatement (Third) of Property: Servitudes §7.11 (2000).

should continue to exist, as discussed below. Analysis of enforceability necessarily includes identification of what resources are available to enforce the provisions of the easement.

Termination Under the Common Law

In general, the common-law has developed tools to limit the duration of property interests, and these rules apply to conservation easements. Specifically, easements may be subject to termination despite evidence of an intent to create a perpetual interest within the conveyance instrument. Because easements restrict use in perpetuity, they are disfavored. The "policy against encumbrances" discourages the infinite restrictions on use that a conservation easement represents.[34] An easement can terminate by its own terms, i.e. it was never intended to exist in perpetuity, or a condition for termination is met,[35] changed conditions,[36] condemnation[37] or by merger.

Property and contractual doctrines provide some relief where changed circumstances interfere with the intent of encumbrances. One such doctrine is the Doctrine of Merger, also known as Unity of Title, whereby if a property encumbered with an easement is

---

[34] *Huggins v. Castle Estates, Inc.*, 36 N.Y.2d 427, 431–33, 330 N.E.2d 48, 52–53, 369 N.Y.S.2d 80, 85–87 (1975) (if not unequivocally and explicitly included in the deed, court will not interpret in favor of restriction.); *Turner v. Caesar*, 291 A.D. 2d 650, 651, 737 N.Y.S.2d 426, 428 (3rd Dep't 2002)("The law favors the free and unencumbered use of real property and, to that end, the courts strictly construe restrictive covenants against the party seeking to enforce them."); *Kramer v. Dalton Co.*, 235 Or. App. 494, 502, (Or. Ct. App. 2010) (holding that the covenant restricted residential properties to single-family dwellings located on subdivided lots because of the clear language and location of the restriction in the deed).

[35] JAN G. LAITOS, ET AL., NATURAL RESOURCES LAW 713 (2006) citing Federico Cheever, *Public Good and Private Magic in the Law of Land Trusts and Conservation Easements: A Happy Present and a Troubled Future*, 73 DENV. U. L. REV. 1077, 1087—93 n1 (1996)(the Uniform Conservation Easement Act states that a court retains the power to modify or terminate a conservation easement in accordance with the principles of law or equity, Unif. Conservation Easement Act §3(b), 12 U.L.A. 177 (1996)).

[36] Frederic Cheever, *Public Good and Private Magic in the Law of Land Trusts and Conservation Easements: A Happy Present and a Troubled Future*, 73 DENV. U. L. REV. 1077 (1996).

[37] Richard B. Collins, *Alienation of Conservation Easements*, 73 DENV. U. L. REV. 1103, 1106 (1996).

subsequently purchased or acquired in fee-simple[38] ownership by the owner of the easement, the easement is extinguished[39] (Hylton et al. 2007). If at a later point in time, the owner wants to separately convey an easement, a new one must be created, transferred and recorded.

Conservation easements conveyed to a land trust must serve a public purpose, but such public purposes can be widely disparate and may even be mutually exclusive.[40] The Doctrine of Changed Conditions[41] is one whereby circumstances anticipated or intended at the outset of the conveyance have changed to the point that enforcement of the original agreement is no longer pragmatic or desirable and so a court may choose to extinguish the easement[42] (McLaughlin 2005). Because of the relative novelty of conservation easements, the actual implementation of conservation easements, particularly their

---

[38] "The estate in fee simple is the largest estate known to the law" BLACK'S LAW DICTIONARY 691 (9th ed. 2010).

[39] J. GORDON HYLTON, ET AL., PROPERTY LAW AND THE PUBLIC INTEREST: CASES AND MATERIALS 530 (3rd ed. 2007).

[40] N.Y. Envtl. Conserv. Law §49-303 (1983), authorizes conservation easements as an "Interest in real property, created under and subject to the provisions of this title which limits or restricts development, management or use of such real property for the purpose of preserving or maintaining the scenic, open, historic, archaeological, architectural, or natural condition, character, significance or amenities of the real property in a manner consistent with the public policy."; *County of Colusa v. Cal. Wildlife Conservation Bd.*, 145 Cal. App. 4th 637, 652, 52 Cal. Rptr. 3d 1, 26 (3rd Dist. 2006) (where conservation purposes are adverse to each other, as with an easement that prohibits agricultural use in order to protect wetlands and habitat, and agricultural production is a keystone of the local and state economy, the court allowed modification of the easement to allow agricultural production as an important public interest).

[41] Jeffrey A. Blackie, *Conservation Easements and the Doctrine of Changed Conditions*, 40 HAST. L. J. 1187 (1989); Gerald Korngold, *Privately Held Conservation Servitudes: A Policy Analysis in the Context of in Gross Real Covenants and Easements*, 63 TEX. L. REV. 433, 441—42 (1984) (discussing what constitutes a conservation purpose and how to weigh one conservation purpose against another); *see also*, AMY KASTELY, ET AL., CONTRACTING LAW 659 (4th ed. 2006) (This doctrine is founded in contract law, and specifies that when conditions have changed over time so that the original intent is substantially or entirely no longer in existence, the restriction on the property, here the easement, can be extinguished via petition and court decision).

[42] Nancy A. McLaughlin, *Rethinking the Perpetual Nature of Conservation Easements*, 29 HARV. ENVTL. L. REV. 421, 471—77 (2005) (noting that charitable donations do not require efficiency but must convey a community benefit and when the benefit is such that there is little public benefit offered or an absence of public support for continued enforcement, the court may choose to modify or extinguish the easement under *cy pres*, the doctrine of reform).

enforcement in perpetuity, includes many situations where the law has not been challenged.

Termination Under Statutory Considerations

New York's authorizing statute contemplates that perpetuity is subject to many of the existing common-law doctrines, by identifying mechanisms for termination of a conservation easement.[43] However, the application of these doctrines and how the court chooses to balance donor intent in the context of changed conditions or other common law doctrines for modification or termination has not yielded significant caselaw.

Enforcement

Successful enforcement requires diligence from the inception of the easement.[44] Before the easement is conveyed to the land trust, baseline data must be gathered and consensus on accuracy must be reached.[45] The property owner must acknowledge and

---

[43] N.Y. Envir. Conserv. Law §49-0307 (1984)(an easement may be modified or extinguished by its own terms, by eminent domain, necessity of public utility or (N.Y. Real Prop. Acts. Law §1951 (1962)) when the benefit intended is not actual or substantial, has already been achieved or changed circumstances dictate); Zachary Bray, *Reconciling Development and Natural Beauty: The Promise and Dilemma of Conservation Easements*, 34 HARV. ENVTL. L. REV. 119, 140 (2010) (noting that merger may include financial risk of sanction or loss of tax exempt status "if the merger is found to confer private benefits on the fee owner.")

[44] Maryanne McGovern, Presentation at the New York State Bar Association Continuing Legal Education Seminar: Planning, Drafting & Administration of Conservation Easements (June 4, 2010) (In order to achieve the conservation objectives and interests of all parties in perpetuity, success begins at the beginning with easements that specifically address the property, the conservation values protected and places restrictions that are within the capacity of the land trust to monitor); Ann Harris Smith, Note, *Conservation Easement Violated: What Next? A Discussion of Remedies*, 20 FORDHAM ENVTL. L. REV. 597, 633 (2010) (arguing that easement drafting is key to enforcement, and provisions should be included related to the remedies for violations, as well as an agreement for collection of attorney's fees in case of enforcement action, to encourage lawsuits for enforcement).

[45] McGovern, *supra*, note 44.; Interview with Seth McKee, Land Conservation Director, Scenic Hudson, Inc. (June 10, 2010) (Baseline documentation is necessarily detailed, and must include not only written descriptions, accurate maps and photos and important landmarks, but each piece of documentation must be signed off on by the landowner and the grantee. Annual monitoring visits are required, and the level of precision and agreement in the baseline documentation facilitates the ability to monitor and enforce the easement over time, beyond the tenure of the original persons involved in the transaction. The level of

validate the baseline data submitted before the easement can take effect.[46] Issues of enforcement are typically noted during annual monitoring visits. If, in the monitoring process, an issue of violation of the easement is found, enforcement action can be brought in court. Enforcement actions related to easements require standing[47] for a court to recognize the party bringing the action, which is standard in civil rules of procedure.[48] The entity identified as the holder of the easement has standing because of the fact that they hold ownership of the easement[49] (Pennington and Rosenberg 2010). Even though conservation easements are granted for conservation purposes that benefit the public, the general public cannot unequivocally obtain standing for enforcement action.[50,51]

---

professional expertise is not necessarily a credentialed one at this point, but requires a team of specialists who understand the purpose of the easement as well as the property itself in order to assess its efficacy.)

[46] *O'Mara v. Town of Wappinger*, 9 N.Y.3d 303, 310—11, 879 N.E. 2d 148,152, 849 N.Y.S. 2d 9, 13 (N.Y. 2007) (holding that while all work to approve the easement had been done, an actual conveyance was not made because the title company hired by the purchasers did not identify the property restriction and the surveyor did not reference the easement in the property description, rendering subsequent attempt at enforcement moot.)

[47] Standing is "[a] party's right to make a legal claim or seek judicial enforcement of a duty or right" it is essentially the legal ability to bring a lawsuit in court. BLACK'S LAW DICTIONARY 1536 (9th ed. 2010).

[48] Restatement (Third) of Property: Servitudes §8.1 (2000).

[49] Mark C. Pennington and Steven Rosenberg, *Conservation Easement Administration*, in PLANNING, DRAFTING & ADMINISTRATION OF CONSERVATION EASEMENTS 39, 48 (New York State Bar Association Continuing Legal Education, 2010) (only the parties to the easement and those designated with a third party right of enforcement may bring enforcement actions. Individuals such as neighbors and adjacent landowners may not enforce the provisions of the easement).

[50] *Hicks v. Dowd*, 157 P.3d 914, 920 (Wyo. 2007) ("The mere fact that a person is a possible beneficiary is not sufficient to entitle him to maintain a suit for the enforcement of a charitable trust."); Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 337, 339—42 (2004) (with exacted easements for habitat conservation purposes authorized under the Endangered Species Act, there is ambiguity in who has the right of enforcement to protect the paramount concern of endangered species protection as the parties to the easement contract may not have been involved in the formation of the easement in any way and the easement owner may choose or not choose enforcement for strategic reasons. The Attorney General may be vested with discretionary power to bring an enforcement suit. California permits property owners and local landowners the right of enforcement for open space easements but does not address exacted conservation easements.)

[51] Interview with Seth McKee, *supra* note 45.

METHODS

This research adopted a multi-faceted approach to analyze conservation easements and their enforceability. Beginning with a literature review focused primarily in legal journals and case law, the search extended to the perspectives and interdisciplinary analyses found in the social sciences literature. Because there is very limited case law and virtually no published opinions regarding the Hudson River and its riparian lands, the search included jurisdictions nationwide. Although the courts of other states do not generally bind property law in New York, an examination of law in other jurisdictions does indicate how this relatively nascent property right should be considered as part of the context of how these rights exist and are utilized. Concurrent with the literature review, outreach and informal interviews with local land trust stakeholders were conducted. These interviews were informational in nature and provided anecdotal context as to the state of conservation easements along the Hudson itself.

Additionally, a brief survey was developed and submitted to all thirty-two land organizations listed on the Land Trust Alliance[52] website[53] located within the Hudson River valley between New York Harbor and the Troy Dam. Excluded were organizations that focused only on community gardens in the boroughs of New York City. The selected organizations ranged from those with a small geographical focus to those with a national presence. Six organizations that did not have any easement holdings listed on

---

[52] http://www.landtrustalliance.org is a national policy and advocacy organization that collectively represents the interests of land trusts, serves as a resource and network, and provides accreditation and education.

[53] http://www.landtrustalliance.org/community/falt. Information on the website was last officially collected through an organizational census in 2005. Some organizations have updated their contact information and statistics, but others have not. As such, a cross-reference check with the organization websites was conducted. The 2010 census is currently in process.

the Land Trust Alliance website directory were nonetheless invited to participate because the information on their own websites indicated they hold easements. None of these six chose to participate. Of the thirty-two identified organizations in the watershed area, four had a national or statewide presence and one had a Hudson Valley regional focus. The survey, administered via surveymonkey.com, sought to identify the types and quantities of conservation easements and other property interests held in the Hudson River and its watershed, when and how they were obtained, purposes of easements, issues of enforcement, and staffing and legal support resources. The survey was administered during June 2010.

The New York State Department of Environmental Conservation (DEC) is directed by statute to provide a place for recording all conservation easements once conveyed. This project included contact with the DEC to identify easements along the Hudson.[54] Other resources accessed include the New York State Bar Association, which held its first Continuing Legal Education (CLE) seminar on conservation easements this summer,[55] the Land Trust Alliance generally, as well as the conservation easement listserv, and the websites of local land trust organizations, many of which include a "Frequently Asked Questions" page that is written in a manner accessible to the general public.[56]

---

[54] N.Y. Envtl. Conserv. Law §49-0305(4) (1983) requires a deed conveying the easement to be duly recorded in the county where the easement is located, and a copy forwarded to the Department of Environmental Conservation for file purposes.

[55] New York State Bar Association Continuing Legal Education, Planning, Drafting & Administration of Conservation Easements, attended June 4, 2010

[56] See, Mohawk Hudson Land Conservancy, http://mohawkhudson.org/faqs.htm; Scenic Hudson, http://www.scenichudson.org/whatwedo/landconservation/privatelandowners; Agricultural Stewardship Association, http://agstewardship.org/index.php?option=com_content&view=category&layout=blog&id=15&Itemid=34 ; Rensselaer Land Trust, http://www.renstrust.org/information-for-landowners/land-preservation?start=2;

RESULTS

The survey was sent to thirty-two organizations all identified as Land Trusts holding easements in the Hudson Valley.[57] Eleven respondents completed the survey yielding a 34% response rate. One respondent declined to participate because his organization does not hold any conservation easements in the Hudson or its watershed, and three respondents communicated their intent to complete the survey in ongoing email discussion, but did not actually complete it. Of the twenty-one who did not complete the survey or decline to participate, fifteen are listed on the Land Trust Alliance website as having no full-time staff, and limited volunteer staff. Among the additional three organization representatives who intended to complete the survey but did not, the reason cited universally was that staff time was needed to attend to the deadline related work of the organization. Extending the survey for those individuals did not yield participation. Of the twenty who did not respond, six were listed on the Land Trust Alliance website as not holding easements, but their individual websites indicated that they might hold easements at this time. As such, the results are limited in their application, but do provide information indicating areas for further research, including the future viability of the necessary monitoring of properties and interests within the land trusts' portfolios. The fact that 75% of those not responding do not have staff is indicative in and of itself of the limitations of volunteer land trust organizations.

Columbia Land Conservancy, http://clctrust.org/faq.htm; Mohonk Preserve, http://mohonkpreserve.org/index.php?howweprotectland; Wallkill Valley Land Trust, http://www.wallkillvalleylt.org/index.php?option=com_content&task=blogsection&id=3&Itemid=28; Dutchess Land Conservancy, http://www.dutchessland.org/landprotection.htm; Hudson Highlands Land Trust, http://hhlt.org/faq.html; Orange County Land Trust, http://orangecountylandtrust.org/options.htm#easement; Westchester Land Trust, http://www.westchesterlandtrust.org/easements-qa.

[57] http://www.ltanet.org/landtrustdirectory/state.tcl?state_id=newyork36 (accessed Aug. 11, 2010).

The DEC database is compiled on a county level and as such, challenging to search for geographic correlations between the easements themselves and the Hudson River and watershed location.[58] According to Timothy Reynolds, who maintains the database, prior to 2006, compliance with the statutory requirement for filing an easement with the DEC was sporadic at best.[59] In 2006, when the tax credit went into effect, DEC saw a significant increase in recording of easements.

The DEC itself holds five easements along the Hudson: two in Dutchess County, one purchased with Environmental Quality Bond Act grant funding in 1985, constituting three parcels and 99.01 acres and the second consisting of 26.37 acres of waterfront granted in 1989, two in Ulster County, both easements conveyed by Scenic Hudson to the DEC in 1990, consisting of 6.97 and 12.93 acres, and one riverfront parcel in Westchester consisting of 50.11 acres, conveyed in 2001. These easements, as is typical, specify that the DEC has the right of enforcement, as well as the right of limited access to monitor.

Although there are many tools for protecting property, the primary mechanisms of land acquisition employed along the Hudson and within the watershed are conservation easements encumbering use but retaining private ownership, or fee simple ownership of the parcel (Figure 1). Covenants are a "formal agreement or promise . . . . to do or not do a particular act."[60] Land Trusts acquire these types of interest to protect property because they can be less expensive, but are also not intended in perpetuity and are a lesser interest

[58] Please note that the DEC database is not publicly accessible and searches of this database are subject to staff availability.
[59] Telephone Interview with Timothy Reynolds, Conservation Easement Database Manager, NYS Department of Environmental Conservation (June 30, 2010).
[60] BLACK'S LAW DICTIONARY 419 (9th ed. 2010).

than conservation easements.

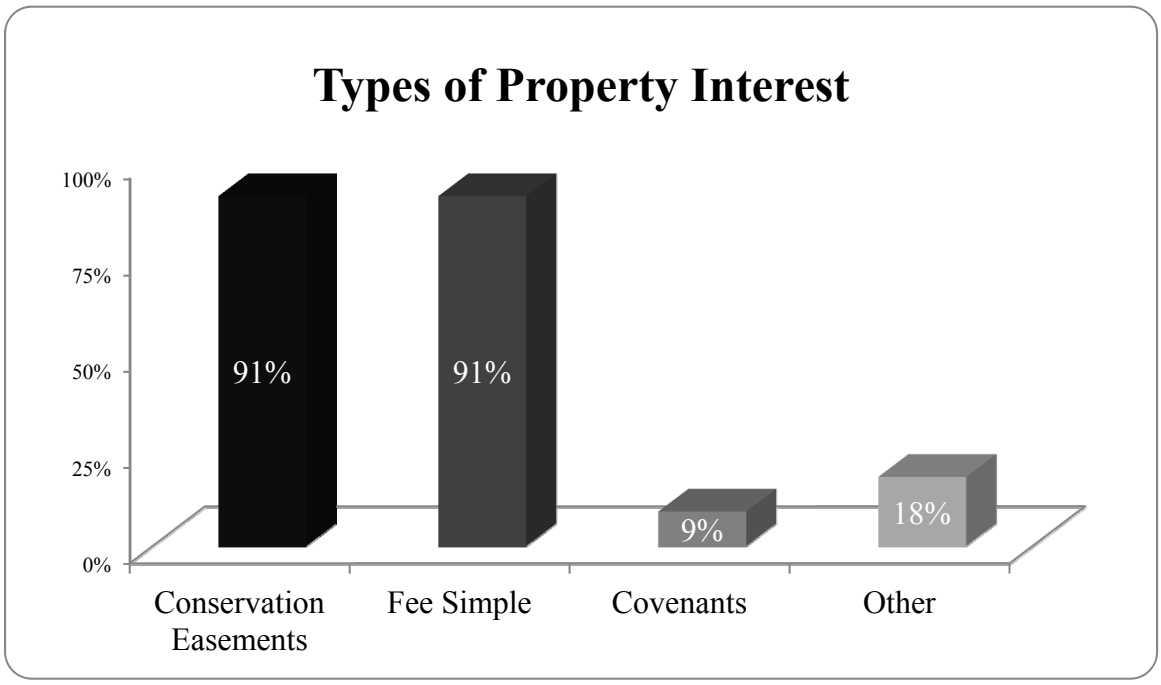


Figure 1 - Types of Property Interests held by the Land Trusts. ******Note that the question asked respondents what types of property interests the Land Trust holds. Land trusts can hold a portfolio of different types of property interests and as such, the interests do not add up to 100%.***

For each easement owned, annual monitoring by the easement holder is required.[61] As such, with a mean number of easements of 47.7, and a median of 15.5, this represents a significant workload (Figure 2). Monitoring begins with comprehensive baseline reporting, and annual reporting that requires site visits, assessing status in relation to the encumbered interest and analysis of potential violations.

[61]26 I.R.C §170 requires that charitable organizations, of which Land Trusts are a qualifying member, must evaluate and report all assets on an annual basis. Easement interests are considered a charitable asset and are therefore subject to annual monitoring regardless of how the interest was acquired. This is distinct from the requirement of conservation purpose that provides a public benefit that is required for the landowner donating the easement to meet in order to qualify for state and federal income tax deductions.

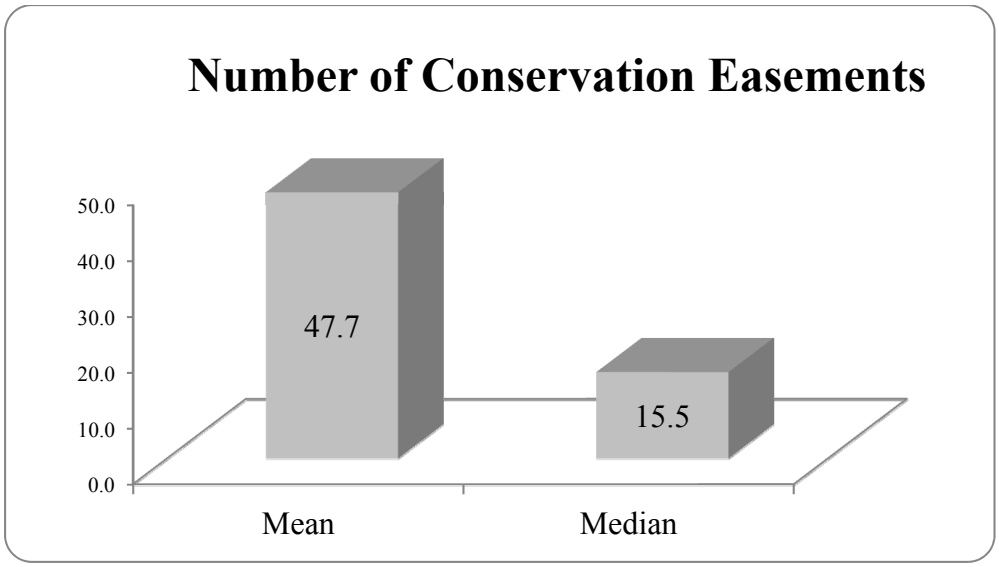


Figure 2 - Number of Conservation Easements. This chart represents the mean and median number of easements held by each organization participating in the survey.

The total number of acres amassed under easement is 30,606 (30,461 acres reported in the survey, plus an additional 145 acres reported by the DEC)[62] (Paden 2010). The variation in mean and median acres under easement demonstrates that there is a high degree of variation amongst land trusts (Figure 3).

[62] Peter R. Paden, *Planning Conservation Easements*, in PLANNING, DRAFTING & ADMINISTRATION OF CONSERVATION EASEMENTS 1, 5 (New York State Bar Association Continuing Legal Education, 2010) ("according to unofficial tabulation of data by the New York LTA office, as of November 2008, 11 Land Trusts in the greater Hudson Valley reported holding 904 conservation easements on approximately 78,000 acres of land.); As such, the limitations of the survey should be clear, accounting for well under half of the total reported just eighteen months ago.

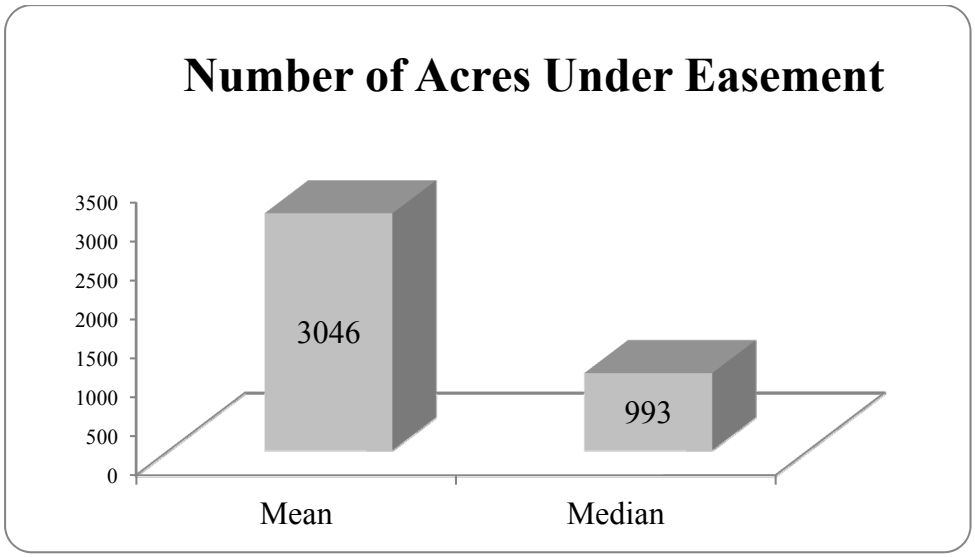


Figure 3 - Number of Acres Under Easement. This chart represents the mean and median numbers of acres under easement held by each organization participating in the survey.

In fact, the number of acres within an individual land trust's easement portfolio varies from a low of 13.3 acres to a high over 10,879 acres. Two land trusts hold the bulk of the easements, accounting for nearly 22,000 acres between them. Additionally, the character of land use in the Hudson Valley, and the northeast in general, has developed in a manner that allows even small parcels to be subdivided and developed. The average size of easements, with a mean of 57.3 acres and a median of 30 acres, further illustrates this point that the relative size of properties under easement reflects an overarching pattern of land use (Figure 4).

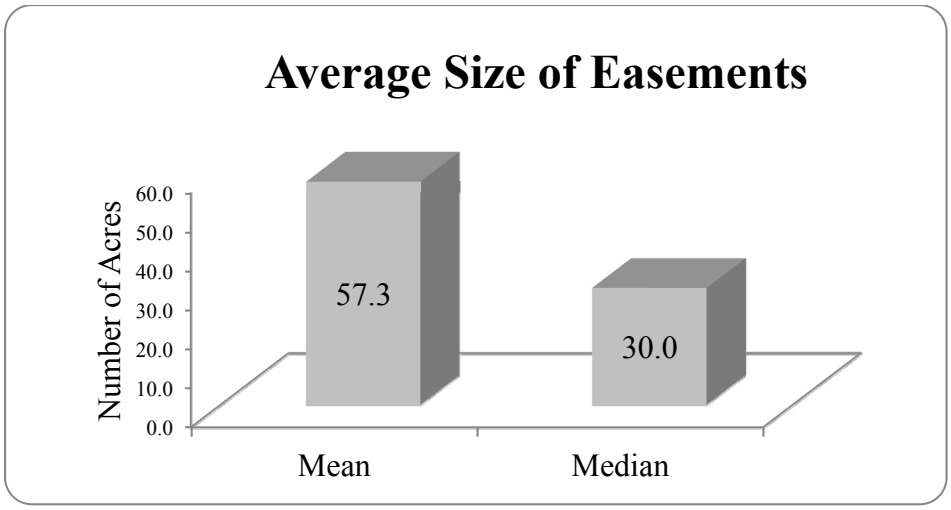


Figure 4 - Average Size of Easements. This chart represents the mean and median number of acres constituting individual easements held by organizations participating in the survey.

Despite discussion and anecdotal reports of the dramatic increase in donated conservations easements following the tax credit, the decade in which the easements were obtained is fairly proportional in the past three decades (Figure 5).

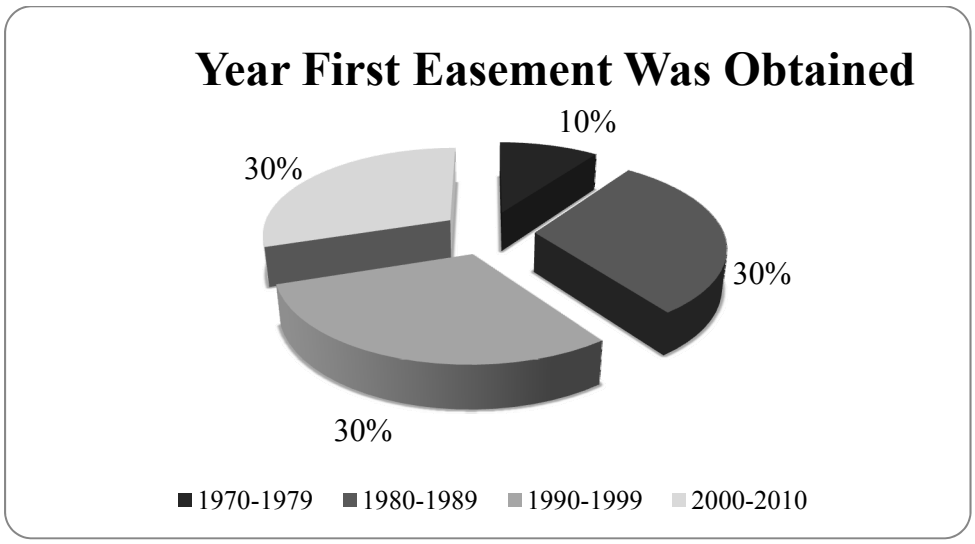


Figure 5 - Year First Easement Was Obtained. This chart represents the percent of total easements held by the decade in which they were obtained.

This statistic looks at the use of conservation easements by land trusts in the Hudson Valley as a whole and does not identify the proportion of donated vs. purchased vs. exacted easements.

Because easements must be donated for a conservation purpose, each property must be encumbered with specific intent to achieve that purpose. Along the Hudson, easements are granted for a variety of purposes but overwhelmingly constitute intentions of ecological/ environmental protection (Figure 6).

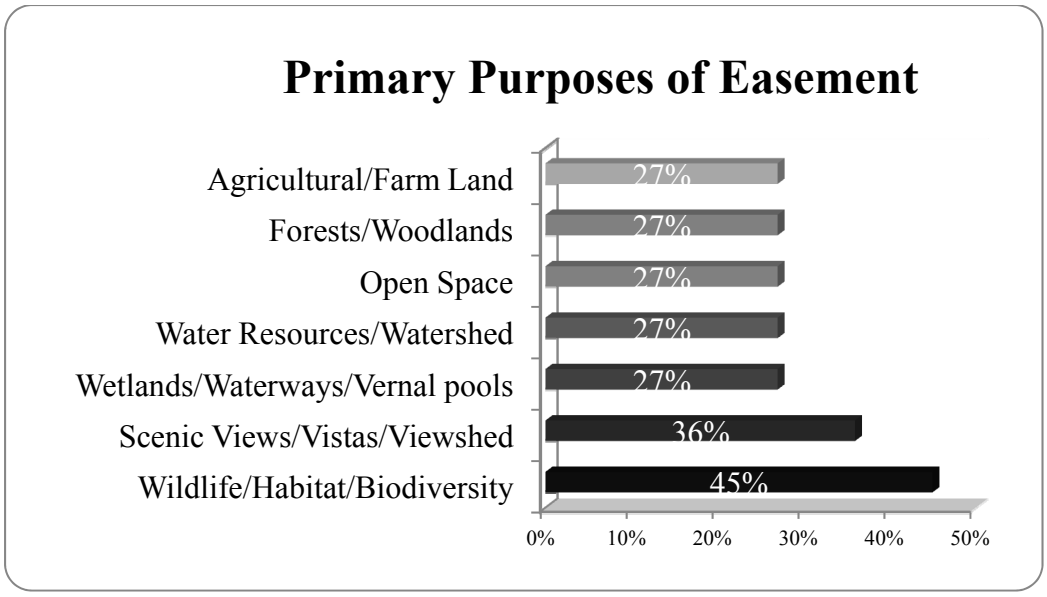


Figure 6 - Primary Purpose of Easement. Though easements must be granted with an intended purpose, there is often more than one reason cited as the "primary" purpose.

These explicit and recorded intentions are an important part of providing ecosystem services that enhance the Hudson and its watershed.

In the Hudson Valley and its watershed, the survey indicates that donation is the overwhelming mechanism for obtaining the easement (Figure 7).

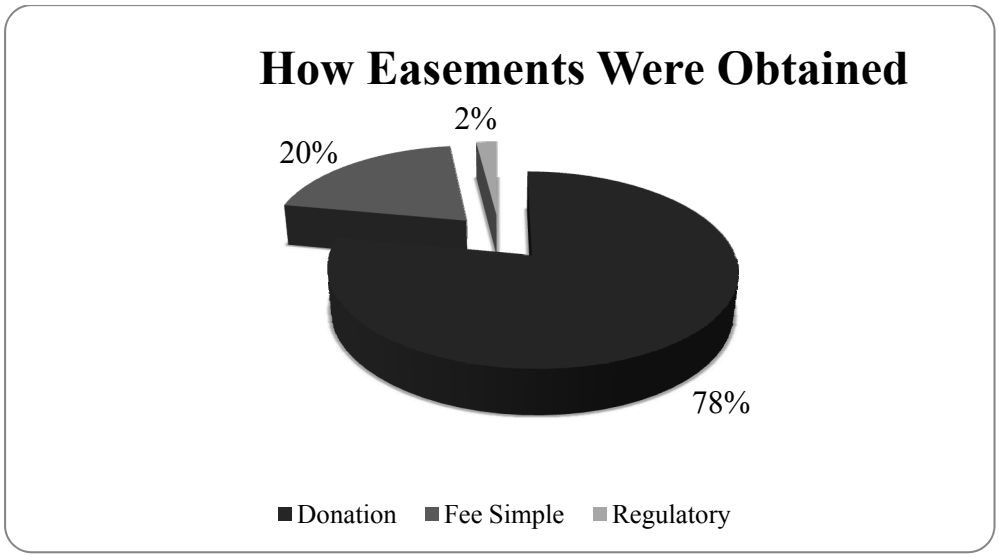


Figure 7 - How Easements Were Obtained. This chart represents the percentage of easements by the mechanism through which the easement was obtained.

Because donation is often tied directly to the availability of the tax credit, the willingness of landowners to provide this property protection may change if the tax credit is reduced and/or discontinued.

The need to enforce easements is evident from the statistic that forty-five percent of conservation easements had a reported violation and an additional twenty-two percent required intervention to prevent a violation (Figure 8). The enforcement provisions suggest that easements will be subject to issues and will need to be enforced in some manner. Such enforcement requires sufficient resources on the part of the land trust organization.

Anecdotally, easements have been enforced when land trust monitoring has found a violation but do not always require court action. For instance, where a property owner cut trees on a parcel with an easement granted to protect the viewshed, and the missing trees adversely affected the viewshed, the land trust required immediate replanting of indigenous species mature enough to effect the goals of the easement, as well as a

significant punitive fine that was paid to the land trust and used to further organizational conservation efforts. The owner complied with the enforcement requirements and legal action was not necessary. Enforcement set strong precedent with this particular easement.[63]

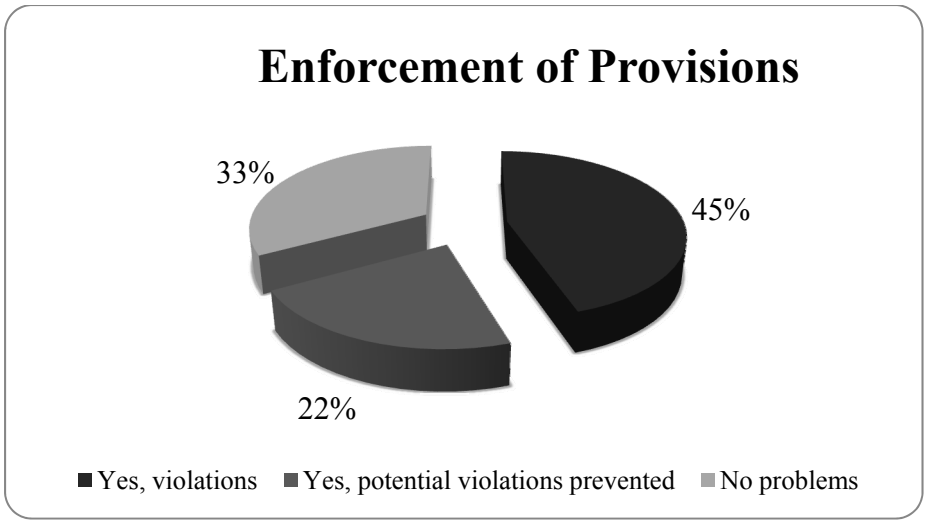


Figure 8 - Enforcement of Provisions. This chart represents the percentage of violations of the easement provisions of the total number of easements held.

Modifications to easements constitute slightly less than a quarter of easements (Figure 9). This is a drain on resources given the fact that any modification in essence requires a new deed (inherently involving legal counsel) and authorization by the board to approve the modification as consistent with the organizational goals.

[63] Interview with Seth McKee, *supra*, note 45.

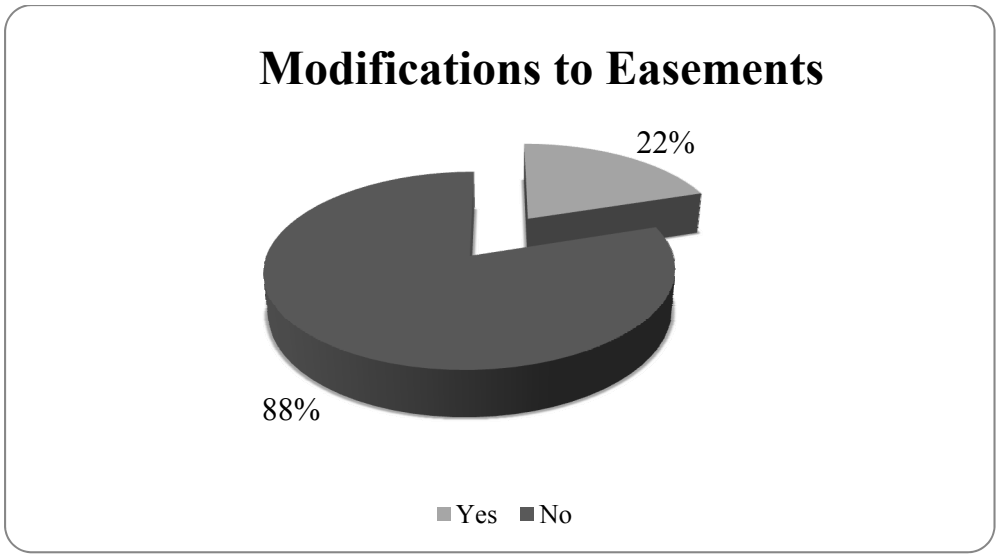


Figure 9 - Modification to Easements. This chart represents the percentage of easements held that have been modified as compared to the percentage that have not been modified.

The organizational structure of land trusts in the Hudson Valley is highly reliant on volunteers. Staff is limited and usually includes full and part time personnel. (Figure 10).

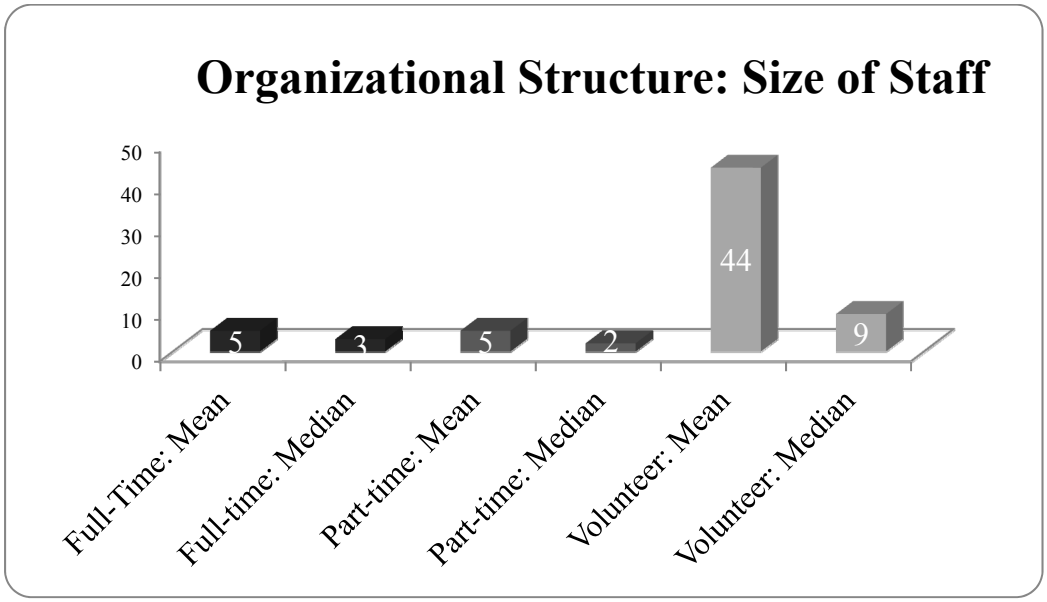


Figure 10 - Organizational Structure: Size of Staff. This chart represents the mean and median number of staff who were full-time, part-time or volunteer from all easement holding organizations participating in the survey.

Only eighteen percent of those completing the survey had legal counsel on staff, and an additional thirty-six percent had counsel on retainer, while eighty-two percent sought counsel as needed (Figure 11). This indicates that there is no exclusive mechanism for providing legal counsel, and though there may be an attorney on staff, others will be consulted depending on the particular situation. One land trust has an attorney in the position of executive director, though they also access outside counsel as necessary. As such, there is a wide range of available legal counsel for land trust organizations.

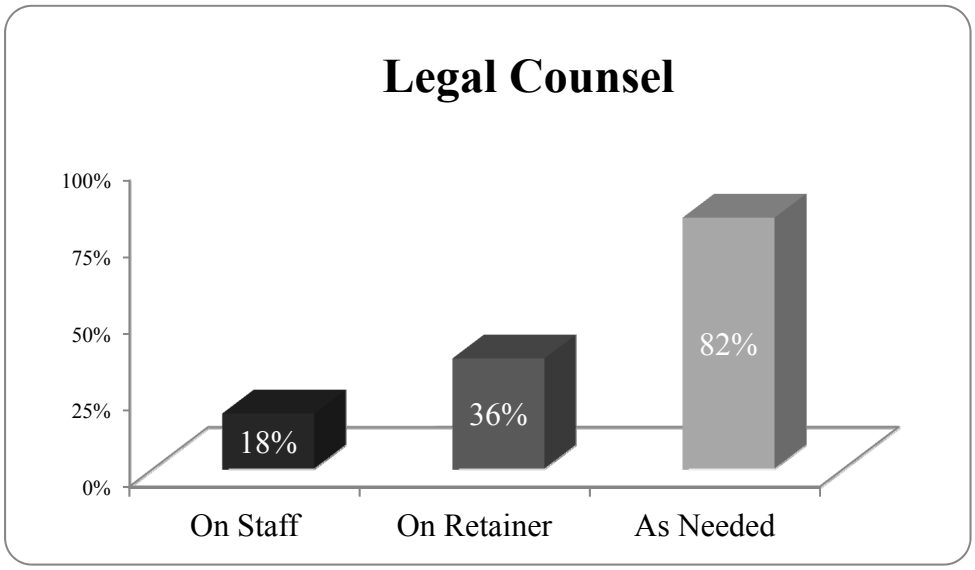


Figure 11 - Legal Counsel. This chart represents the percentage of types of legal counsel arrangements that participating land trusts use.

DISCUSSION

Conservation Easements as an Established Property Interest

Based on the results of the survey, the acquisition of conservation easements as a property right is a substantial part of the portfolio of many local land trusts in the Hudson Valley. Although it is not the only type of interest held, the prevalence of their existence

given the relatively recent use of these tools is indicative of their importance to the Hudson River and its watershed.

The various reasons for granting the easement include explicit protection of the environment. Protection alone implicitly provides an ecological service to the properties protected and their surrounding environs.[64] However, as a relatively new and somewhat untested property right, conservation easements may be subject to judicial tinkering as conflicts eventually find their way to court[65] (Bray 2010). At least, courts will be asked to contextualize the intensity of expertise necessary for monitoring and enforcement and the very novelty of the right to enforce this non-possessory interest. Of course, understanding that the property interest could be subject to change may not have been a mystery at the time the statutes were enacted. Moreover, because of their public-private nature, conservation easements may be subject to future challenges for public rights of access and recreation that are not expressly included in the original intent[66] (Lippmann 2004).

Perpetuity then is the persistent interest in the land, and that interest itself does not have a predetermined termination. Societal, economic, land use and environmental

---

[64] Please note that agricultural easements support working farmland and as such, are somewhat distinct from other conservation easements in terms of ecosystem services. Depending on the scope and nature of the farm operations, there can be an inherent tension between the environmental benefits of producing food and the practices by which produce is cultivated. See also, The Glynwood Center, *Land Trusts and Agricultural Land: Protecting Farmland or Farming?,* available at http://www.glynwood.org/publications-multimedia/land-trusts-and-agricultural-land/ (accessed Aug. 11, 2010).

[65] Zachary Bray, *Reconciling Development and Natural Beauty: The Promise and Dilemma of Conservation Easements*, 34 HARV. ENVTL. L. REV. 119, 137 (2010) (discussing the inefficiency of restrictions without end, and noting that "today's easements may frustrate future legitimate conservation initiatives").

[66] Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 308—09 (2004); *see also*, Zachary Bray, *Reconciling Development and Natural Beauty: The Promise and Dilemma of Conservation Easements*, 34 HARV. ENVTL. L. REV. 119, 163 (2010) (arguing that public access may be a mechanism for sustaining enforcement as it will provide for monitoring compliance without encumbering resources).

considerations all contribute to changed circumstances, which courts will evaluate as to whether or not they are changed sufficiently enough to warrant a modification or termination to the easement. This flexibility is consistent with the interests of conservation purposes, which need to be able to use the property interest as an asset, to best serve the goal of keeping the Hudson viable for the long-term. The Hudson has many functions, of which habitat, industry, commerce, aesthetics and transportation are included. To be able to best serve these divergent purposes, the conservation easement tool must remain as flexible and adaptable as possible over time. Conservation easement proponents must exercise due diligence in ensuring consistency between interests intended to be protected and those actually protected, using the inherent flexibility of the easement as a mechanism to achieve conservation. For example, climate change impacts the environment in ways that are not within the power of a property interest to address. If however, the climate of an easement property, granted to protect the habitat of a particular species, were to change to the extent that the species could no longer persist in that habitat, it may be in the interest of the easement holder to extinguish the easement and allocate resources to other means by which to protect said species. To the extent that the flexibility exists within an easement interest, conservation advocates should recognize it as an asset and understand its potential application as appropriate.[67]

---

[67] Jessica Owley, *Changing Property in a Changing World: A Call for the End of Perpetual Conservation Easements*, 30 STAN. ENVTL. L. J. (In Press), Consider also, the substantial challenges in implementing an easement system that is based on finite terms of interest. Term easements may not be as attractive to donors because they would not necessarily come with tax benefits because the current level of donated easements is in part fueled by its use as a wealth management/ estate planning mechanism. The complexities of dealing with a term that expires may be quite lengthy and expire generations in the future. Courts would also have to consider what injunctive relief would look like if a party could simply wait for a short term easement to expire to conduct an action that was in violation of the intended conservation purpose. This alternative poses a host of new and relevant ambiguities.

Complexity of Land Trust Monitoring and Enforcement of Easement Property Interests

The level of expertise necessary to enforce easements is substantial. Given land trusts' limited resources, the lack of a requirement that funds be dedicated toward the enforcement of the interest[68] and the significant costs involved in litigation,[69] the level of expertise needed could easily be outside of the scope of many organizational budgets.[70] Each donated easement held must be monitored annually.[71] The broad range of property size, the wide variety and complexity of what conservation purposes were intended to be protected and the time commitment by which to monitor the interest annually, document findings, determine if a violation has occurred and then address the violation or negotiate with a landowner prior to bringing an enforcement action requires comprehensive knowledge of the property itself as well as a great range of expertise for which it would be rare that one individual was capable of possessing alone. For land trusts with small conservation easement portfolios, this is not necessarily a significant burden, particularly

---

[68] George M. Covington, *Conservation Easements: A Win/Win for Preservationists and Real Estate Owners*, 84 ILL. B.J. 628, 629 (1996) (In order to comply with IRS regulations, there is no requirement that the grantee organization set aside funds for future enforcement); Interview with Seth McKee, *supra*, note 34 (Scenic Hudson has a policy to request property owners to donate a percentage of the value of the easement to a dedicated stewardship fund for ongoing monitoring and enforcement of the interest. However, the donation is not mandatory. For those easements that are not accompanied by a donation, the Land Trust sets aside stewardship funds in an endowment to assure the viability of protecting the interest over time);

[69] Ann Harris Smith, Note, *Conservation Easement Violated: What Next? A Discussion of Remedies*, 20 FORDHAM ENVTL L. REV. 597, 634 (2010) (analyzing the use of expert witnesses in court cases as effective in securing decisions favoring enforcement based on familiarity with the property as well as significant scientific and historical understandings of particular habitat, species, or historical occurrences necessary to interpret and effect the purpose of the easement).

[70] Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 315 (2004) (Because many land trusts are new and have few staff, "[i]t is not clear whether these groups have enough capacity to both monitor and enforce complex easements well into the future."); http://www.landtrustalliance.org/conserve/conservation-defense/conservation-defense-insurance/background (accessed Aug. 11, 2010) ("estimates that the litigation costs would range from $70,000 to $100,000 for a typical trial in a typical jurisdiction, $35,000 for summary judgment motions and $150,000 for appeal. The average historic cost of all claims was $38,000 including those that did not go to a full trial.")

[71] IRS Code §170(h) (requiring annual documentation of value of donated conservation easement).

if there are board members or volunteers who can provide consultation where expertise is needed. The results of the survey demonstrate that with an average easement portfolio of 15.5 properties, and a median of nearly 50, that even for the smallest land trust there are substantial staffing and monetary requirements for the ongoing maintenance of these easements. What may be sufficient now may not be enough to secure the resources for future enforcement needs, creating an enforceability problem. Given a trend of increasing the number of easements conveyed, a high probability that the original grantor may convey the possessory interest to a third or fourth party and an intent to keep easements in perpetuity, the burden will increase.[72]

Legal and Organizational Challenges to the Perpetual Nature of the Easement Property Interest

The legal question of whether conservation easements are enforceable in perpetuity is answered in the affirmative, though individual easement interests are subject to consideration of extinguishing such interest on a case-by-case basis, taking into account the context of statutory requirements and property law. An easement drafted with the intent of perpetuity, authorized as such by statute will still be subject to circumstances that may modify or extinguish it, even though the interest itself continues. While long-term control is often desirable in the marketplace ("Our favorite holding period is forever" ~ Warren Buffet), it is not necessarily realistic ("Do not try to live forever. You will not succeed." ~ George Bernard Shaw).

[72] http://www.landtrustalliance.org/conserve/conservation-defense/conservation-defense-insurance/threats-to-permanence-why-take-action (accessed Aug. 11, 2010) ("Although land trusts have had relatively few legal challenges, research shows that as property values rise, incentives to disrupt or void easements grow as well, and so does trespass on land trust property.")

Perpetuity presents future challenges because the primary mechanism for enforcement relies on land trusts. Where land trusts are staffed by volunteers and part time personnel,[73] there are limited resources to address issues of monitoring and enforcement, in an increasingly technical process that requires the expertise of many different types of professionals (Lippmann 2004). The Land Trust Alliance has developed a program to address this,[74] but it is not clear that the smaller and more predominately volunteer-based organizations will have the wherewithal to participate, creating a greater disparity in capacity between land trusts[75] (Snow 1991). The survey indicates that access to legal counsel takes on a myriad of arrangements, from executive director, staff, retainer or as-needed, all of which represent different capacities for the legal approach to easements over time. It is important to note that easement monitoring and enforcement is only one function of a land trust's organizational objectives and responsibilities. Educational programming, fundraising, sustaining other property interests and general organizational operations also require personnel and resources.

The ability to provide long-term protection of ecological resources and meet conservation objectives is the organizational strength of the land trusts. An

---

[73] Jessica Owley Lippmann, *Exacted Conservation Easements: The Hard Case of Endangered Species Protection*, 19 J. ENVTL. L. & LITIG. 293, 315, 336 (2004) (discussing the unknown capacity of land trusts and the anticipation of vulnerability if the expertise is insufficient as properties change hands through repeated subsequent ownership.)

[74] http://www.landtrustalliance.org/conserve/conservation-defense/conservation-defense-insurance (accessed Aug. 11, 2010) (The Land Trust Alliance has started an initiative to provide insurance that would cover costs associated with litigation in defense of conservation easement enforcement, as well as fee-simple owned properties. Begun in 2009, the initiative required land trusts to commit their entire portfolio to the program, but did not require accreditation for participation. The Alliance will charge a per-parcel premium and anticipates the program will require $4 million in capital to begin. Currently, over 17,000 parcels from 425 land trusts in 47 states have committed to the program, indicating substantially more interest than was needed to launch the program, which is anticipated to be available in the upcoming year).

[75] DONALD SNOW, INSIDE THE ENVIRONMENTAL MOVEMENT: MEETING THE LEADERSHIP CHALLENGE 6 (1991)(Reflective of the history and culture of the environmental movement supported by non-profits and reliant on volunteers. Such organizational underpinnings and charitable reliance is a market reality); *See also*, James L. Huffman, *The Past and Future of Environmental Law*, 30 ENVTL. L. 23, 24—25 (2000).

organizational framework that lasts forever has yet to be achieved in any field or institution. Even governments change over time. Collaboration between land trusts that acknowledges and accounts for merger will likely occur over time and is a normal market pattern[76] (Hempel 1999). Assemblage value of these organizations and their protected properties portfolio, from an ecological standpoint, is probably desirable because the aggregated management alone can serve to more cogently enforce the easement provisions in order to provide ecosystem services.

Long-term strategic planning for such merger and acquisition will allow for the efficient and appropriate management of existing resources as well as identify properties and parcels that will allow the aggregation of benefits, i.e. adjacent parcels.

Questions for Future Research

- Should the ancient property system persist at all, given its assumption that perpetuity exists without limits on its face, in light of the fact that there are different and competing interests?
- What properties remain unencumbered and how should they be protected, including the long-term strategic organizational plans necessary to ensure that interests are protected?
- What is the extent of geographic and intended purpose connectivity between and among encumbered properties? To what extent does proximity or lack thereof affect the ability to effect the intended purpose by which they were granted?

[76] Lamont C. Hempel, *Conceptual and Analytical Challenges in Building Sustainable Communities*, in TOWARD SUSTAINABLE COMMUNITIES: TRANSITIONS AND TRANSFORMATIONS IN ENVIRONMENTAL POLICY 43, 45 (Daniel A. Mazmanian, Michael E. Kraft eds., 1999).

- What does merger of land trust organizations look like, how does it happen, and what can the Hudson Valley do to prepare for, negotiate and use mergers to best meet conservation objectives?
- How do issues related to public access affect encumbered properties now and in the future?

ACKNOWLEDGEMENTS

The author wishes to thank Professor Keith Hirokawa for his encouragement and support in proposing and developing this project, as well as in editing and critiquing the written report, Seth McKee of Scenic Hudson Land Trust for discussing the issues and his generosity of time, to the late Seymour Gordon, whose work with farmland preservation in the Town of Warwick, mentoring and support with landowners and easements have given me a very practical understanding of implementation of this property tool, Maria Koenig Guyette and Ellen Hamilton Newman for editing, and kt Tobin Flusser of the Center for Regional Research and Education Outreach for her support with statistical analysis and graphical representations.

LITERATURE CITED

Blackie, J.A. 1989. Conservation easements and the doctrine of changed conditions. Hastings Law Journal 40:1187.

Bray, Z. 2010. Reconciling development and natural beauty: the promise and dilemma of conservation easements. Harvard Environmental Law Review 34: 119-177.

Bruce, J. W. and J. W. Ely, Jr. 2001. The Law of Easements And Licenses in Land. West Group, Danvers, MA.

Cheever, F. 1996. Public good and private magic in the law of land trusts and conservation easements: a happy present and a troubled future. Denver University Law Review 73:1077.

Collins, R.B. 1996. Alienation of conservation easements. Denver University Law Review 73:1103-1106.

Covington, G.C. 1996. Conservation easements: a win/win for preservationists and real estate owners. Illinois Bar Journal 84: 628-633.

Dana, A. and M. Ramsey. 1989. Conservation easements and the common law. Stanford Environmental Law Journal 8:38-39.

Hempel, L.C. 1999. Conceptual and analytical challenges in building sustainable communities. Pages 43-45 in D.A. Mazmanian and M.E. Kraft, editors. Toward Sustainable Communities: Transitions and Transformations In Environmental Policy. MIT Press, Cambridge.

Hylton, J. G., D.L. Callies, D. R. Mandelker and P.A. Franzese. 2007. Property Law and the Public Interest: Cases and Materials. LexisNexis, Newark. 3rd edition.

Kastely, A., D.W. Post and N.Ota. 2006. Contracting Law. Carolina Academic Press, Durham. 4th edition.

Korngold, G. 1984. Privately held conservation servitudes: a policy analysis in thecontext of in gross real covenants and easements. Texas Law Review 63:441-442.

Laitos, J.G., S.B. Zellmer, M.C. Wood and D.H. Cole. 2006. Natural Resources Law. Thomson West, St. Paul.

Lippmann, J.O. 2004. Exacted conservation easements: the hard case of endangered species protection. Journal of Environmental Law and Litigation. 19: 293-355.

McLaughlin, N.A. 2002. The role of land trusts in biodiversity conservation on private lands. Idaho Law Review 38: 460-468.

McLaughlin, N.A. 2005. Rethinking the perpetual nature of conservation easements. Harvard Environmental Law Review 29: 421-521.

Nolan, J. R. 2001. Well-grounded: Using local land use authority to achieve smart growth. Environmental Law Institute, Washington, D.C.

Owley, J. In Press. Changing property in a changing world: A call for the end of perpetual conservation easements. Stanford Environmental Law Journal.

Paden, P. 2010. Planning conservation easements. Pages 1-36 in Planning, Drafting & Administration of Conservation Easements. New York State Bar Association. Albany.

Pennington, M.C. and S. Rosenberg. 2010. Conservation easement administration. Pages 37-70 in Planning, Drafting & Administration of Conservation Easements. New York State Bar Association. Albany.

Smith, A.H. 2010. Conservation easement violated: what next? a discussion of Remedies. Fordham Environmental Law Review 20:600-602.

Snow, D. 1991. Inside The Environmental Movement: Meeting the Leadership Challenge. Island Press, Washington, D.C.

Tapick, J. 2002. Threats to the continued existence of conservation easements. Columbia Journal of Environmental Law 27: 266-272.

RESOURCES

- Land Trust Alliance – landtrustalliance.org
- Conservation & Preservation Counsel, LLC – landprotect.com
- Hudson Valley Land Trust Sites:
    - Agricultural Stewardship Association – www.agstewardship.org
    - Columbia Land Conservancy – www.clctrust.org
    - Dutchess Land Conservancy – www.dutchessland.org
    - Hudson Highlands Land Trust – www.hhlt.org
    - Mohawk Hudson Land Conservancy – http://www.mohawkhudson.org
    - Mohonk Preserve – www.mohonkpreserve.org
    - Orange County Land Trust – www.orangecountylandtrust.org
    - Rensselaer Land Trust – www.renstrust.org
    - Scenic Hudson – www.scenichudson.org
    - Wallkill Valley Land Trust – www.wallkillvalleylt.org
    - Westchester Land Trust – www.westchesterlandtrust.org

APPENDIX 1- SURVEY

Conservation Easements in the Hudson River Estuary

1. How many types of property interests does your land trust acquire or receive? (e.g. conservation easements, fee simple, covenants).

a. conservation easements

b. fee simple

c. covenants

d. other

2. If known, what is the number of conservation easements held? How many acres are under easement? What is the average size of the easement parcel?

a. Number of conservation easements

b. Number of acres under easement

c. Average size of easement

3. If known, of the conservation easements held, how many (and what acreage) are located on Hudson River riparian land? How many are located within the Hudson River estuary? How many non-riparian easements are held?

a. Hudson River riparian land

b. Hudson River estuary riparian land

c. Non-riparian land

4. Year first conservation easement obtained?

5. What are the primary ecological services/ purposes for which the conservation easements are granted?

6. How were the conservation easements obtained? Please note percentage of easements.
a. Donation Fee Simple

b. Regulatory (i.e. zoning requirement)

c. Other

d. Conservation Easements in the Hudson River Estuary

7. If known, what is the market value of your easement portfolio? What is the tax base of your easement portfolio?

a. Market value

b. Tax base

8. Have you tried to enforce provisions in your conservation easements? What result? Have there been any modifications?

9. Organizational Structure

a. Number of full-time staff

b. Number of part-time staff

c. Number of volunteer staff

d. Legal counsel on staff?

e. Legal counsel on retainer?

f. Legal counsel as needed?

10. Would you be willing to discuss this information further? If so, please identify the best way and time for me to contact you.

Thank you for your participation!

STABLE ISOTOPE ANALYSIS IN THE HUDSON RIVER MARSHES – IMPLICATIONS FOR HUMAN IMPACT, CLIMATE CHANGE, AND TROPHIC ACTIVITY

A Final Report of the Tibor T. Polgar Fellowship Program

Thien Khoi V. Nguyen

Polgar Fellow

Department of Earth and Environmental Sciences
Columbia University
New York, NY 10027

Project Advisor:

Dorothy M. Peteet
Lamont-Doherty Earth Observatory
NASA Goddard Institute for Space Studies
Columbia University
Palisades, NY 10964

Nguyen, T. K. V. and D. M. Peteet. 2012. Stable Isotope Analysis in the Hudson River Marshes – Implications for Human Impact, Climate Change, and Trophic Activity. Section II: 1-29 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

Heightened anthropogenic activities such as land-use change and nutrient loading have been shown to affect both the biodiversity and sedimentation dynamics of wetlands, but how have the marshes of the Hudson River Valley been affected by these changes? The study of stable carbon and nitrogen isotopes provides useful records of eutrophication, carbon cycle balance, biological productivity shifts, and trophic linkages pertaining to the wetlands of the Hudson River Valley. To answer the proposed question, records of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in sediment cores from the marshes of Iona Island, Piermont, Staten Island, and Jamaica Bay were measured using an isotope ratio mass spectrometer (IRMS). Results suggest that $\delta^{15}N$ levels in the marshes have increased over time since the first European contact due to agricultural and wastewater input, but decreased in the 1970s due to the increase in the use of synthetic fertilizers. Increasing human populations, however, have possibly caused $\delta^{15}N$ to continue to rise in the past couple of decades. The $\delta^{13}C$ signal decline in the marshes parallels the disturbance characterized by a rise in the settlement indicator ragweed (*Ambrosia*). However, the signal fluctuates with time, reflecting shifts in the dominant plant species composition within the respective marshes. There also exist gradients in the $\delta^{15}N$ and $\delta^{13}C$ levels along the transect of the Hudson River. From north to south, the signals for both $\delta^{15}N$ and $\delta^{13}C$ are enriched, probably due to population increases and sewage effluent in the south and C3 plant dominance in the north and C4 plant dominance in the south, respectively. Results of this research provide important background information for future studies on trophic dynamics in the Hudson River.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Often considered the most productive and biologically diverse ecosystems on Earth, tidal river marshes function as habitats for both estuarine and marine life, flood control, and shoreline protection (Morris and Bradley 1999). With intensified anthropogenic alterations in surrounding regions, the need to understand the effects on biological and geological processes in wetlands becomes increasingly more important (Mitsch and Gosselink 2007). Regional land use changes, such as land clearance and nutrient loading, have been shown to affect both the biodiversity and sedimentation dynamics of lakes and marshes (i.e., Chang et al. 2009; Hubeny et al. 2009). However, it is difficult to assess the health of these marshes because baseline data of pre-anthropogenic estuarine conditions are lacking.

Fortunately, the high depositional rates of Hudson River wetlands allow for high-resolution analysis of vegetation shifts and climate change archived in sediment cores (Pederson et al. 2005; Peteet et al. 2006). These characteristics make the marshes useful study sites for paleoenvironmental reconstruction, where a rich and continuous historical record is needed to understand the past.

The study of stable carbon and nitrogen isotopes and C:N ratios, in particular, provides useful records of eutrophication (Hubeny et al. 2009), biological productivity shifts (Hubeny et al. 2009; Chang et al. 2009), the carbon cycle balance (Morris and Bradley 1999), climate shifts (Minckley et al. 2009), and trophic positions (Abrantes and Sheaves 2010; Litvin and Weinstein 2003; Schiesari et al. 2009; Weinstein et al. 2009). A knowledge of all of these factors throughout a long time scale is important because they determine the health and functioning of an ecosystem, and any significant changes

that are noted may indicate a response to either natural or anthropogenic forcings. Stable isotope analysis can help identify many of these signals. This study examined the ^{13}C, ^{15}N, and C:N records in sediment cores from the marshes of Iona Island, Piermont, Staten Island, and Jamaica Bay (Figure 1) to analyze the implications of human impact, climate change, and the food web in the Hudson River Valley. The variability of these isotopes down each core was compared with existing pollen, macrofossil and elemental X-ray fluorescence spectrometer (XRF) data (Kenna et al. 2011) to analyze vegetation shifts, land-use changes and pollution history in these sites . Multi-proxy information provides useful evidence of human impact in the region, ranging from European colonization to land clearance and the use of fertilization in modern society. The differences between biochemical processes in fresh and saltwater marshes were also targeted in this study.

Historic record of land use

It is important to understand the historic timeline of anthropogenic contact with the Hudson River Marshes to correlate land use changes with stable isotope ratios. The Europeans first colonized the Hudson River Valley region in A.D. 1683 (NERR 2009). The clearance of land was a common activity for farms and homes, and vegetation was also cleared for use in construction, fuel, and defense. Iona Island, for example, was used for commercially growing fruits (NERR 2009). During the Revolutionary War, forests in the Hudson River Valley were not only cleared for battles, but also burned to produce charcoal used in the production of iron (Cronon 1983). In Rockland County, where Piermont Marsh and Iona Island are located, the population increased from a total of 219 people in 1693 to a total of 6000 in a 1790 census (Cole 1884). Later on, the Industrial Revolution and national expansion of agriculture would further transform the ecology of

the marshes (Cronon 1983).

Stable Isotopes

The study of $\delta^{15}N$ and $\delta^{13}C$ provide biochemical information on organic matter in the marsh and estuary ecosystems (i.e., Hubeny et al. 2009). These δ values, in ‰, are calculated using the equation $(R_{sample}/R_{standard} - 1)*1000$, where R is the ratio of rare versus abundant isotope of either C or N with the respective standard (PD-Belemnite and air respectively). Nitrogen is a necessary element for plant, algal, and microbial production. Previous research has indicated that increases in plant biomass and height could result from excess nitrogen in the estuary (Morris 1991). Thus, studying nitrogen stable isotopes is vital in order to gain a better understanding of nutrient changes and to determine whether or not they can be associated with anthropogenic forcings. The study of the stable nitrogen isotope provides a historical record of eutrophication in the regions of interest.

Most of the carbon in marshes is *in situ* and reflects the local dominant vegetation (Brickerurso et al. 1989; Connor et al. 2001; Rooth et al. 2003). Thus the $\delta^{13}C$ signature in the marshes reflects the local marsh vegetation composition and decomposition within the marshes. Differences in photosynthetic pathways of two different groups of plants – C3 and C4 plants – result in different degrees of fractionation of atmospheric CO_2 (Smith and Epstein 1971). Consequently, the two groups of plants have distinct ranges of $\delta^{13}C$. C3 plants have ranges from -23 to -34‰ while C4 plants range from -9 to 17‰ (Chmura and Aharon 1995). Since $\delta^{13}C$ in sediments in marshes is mainly derived from the local dominant vegetation (Chmura and Aharon 1995), changes in species composition of vegetation in the marshes will reflect in the shifts in the stable isotope profile. A more

enriched C4 source (i.e. *Spartina*) would have a high $\delta^{13}C$ value, whereas $\delta^{13}C$ in C3 plants (i.e. *Phragmites*) and aquatic algae sources fluctuate at lower values (Hubeny et al, 2009; Varekamp et al. 2010; McKinley et al. 2009). Stable isotopes of carbon have also been used to better understand the importance of salt marsh primary production in the flux of nutrients to higher consumers (Litwin and Weinstein 2003). The same authors showed that although anthropogenic inputs from upriver may play a minor role in Delaware Bay, local inputs in tidal estuaries are important and detectable. In this study, the organic component of carbon, which required the removal of carbonates, was used to detect the $\delta^{13}C$ signature of the sediment, and how it has changed through time.

METHODS

1. Study Sites

Along the 240-km stretch of the Hudson River estuary between New York City and Troy lie a range of wetland habitats. Iona Island is a component site of the Hudson River National Estuarine Research Reserve (HRNERR). Located the furthest north of the four study sites, about 72 km from the Atlantic, the wetlands of Iona Island are slightly brackish (NERR 2009). The salinity values at Iona range from 3-6 ppt (Winogrond 1997). There, habitats include brackish intertidal mudflats, brackish tidal marsh, freshwater tidal marsh, and deciduous forested uplands (NERR 2009). The vegetation of Iona Island's marshes is dominated by narrowleaf cattail (*Typha angustifolia*), common reed (*Phragmites australis*), and swamp rose mallow (*Hibiscus moscheutos*) (Buckley and Ristich 1976; NERR 2009). Deciduous forest also covers the island and mainland slopes, including species such as red oak (*Quercus rubra*), chestnut oak (*Quercus prinus*), and pignut hickory (*Carya glabra*) (NERR 2009).

Piermont Marsh , another HRNERR component site, is located approximately 40 km north of the river's mouth (Pederson et al. 2005). There, mean salinity is also 3-6 ppt (Winogrond 1997), and the habitats include brackish tidal marsh, shallows, and intertidal flats (NERR 2009). The marsh vegetation of Piermont is dominated by invasive *Phragmites australis,* but species such as saltmarsh cordgrass (*Spartina alterniflora*), saltmeadow cordgrass (*Spartina patens*), big cordgrass (*Spartina cynosuroides*), sturdy bulrush (*Schoenoplectus robustus*), and chairmaker's bulrush *(Schoenoplectus americanus*) are present (Lehr 1967; Blair and Nieder 1993).

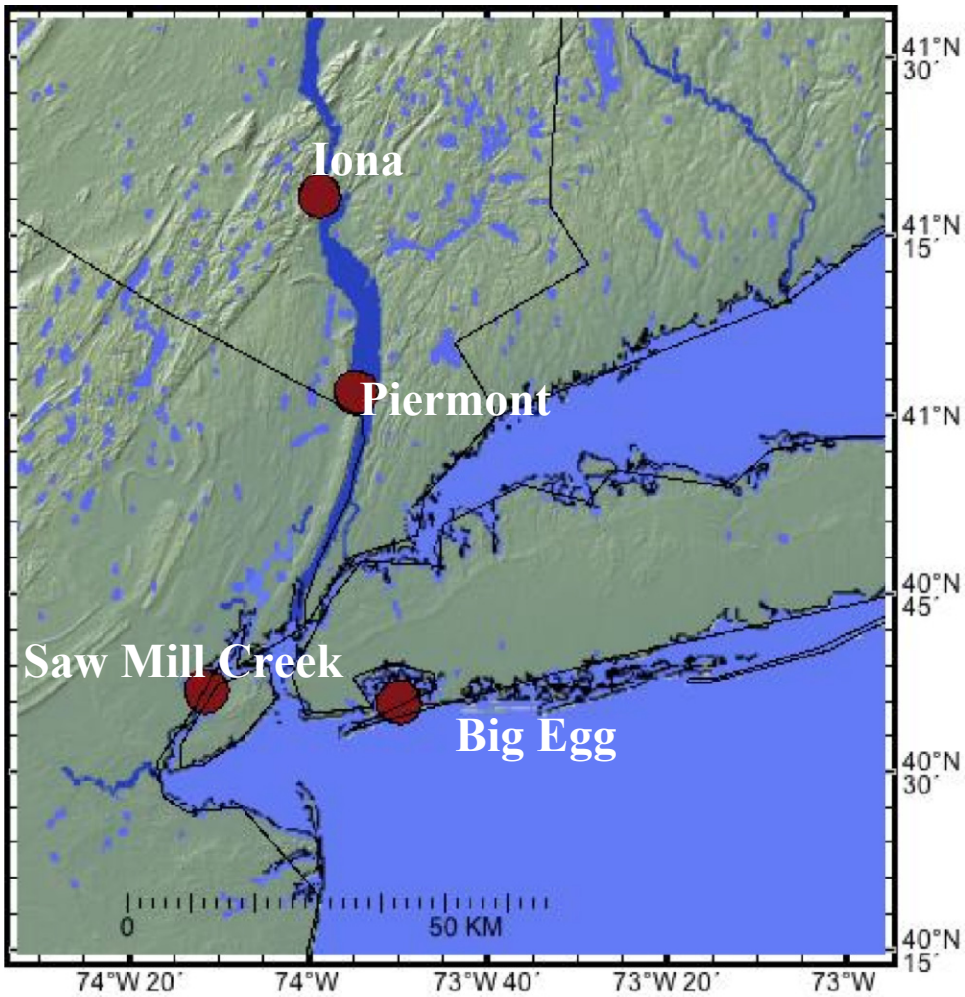


Figure 1. Map of Iona Island marsh, Piermont marsh, Saw Mill Creek marsh, and Big Egg marsh (GeoMapApp, Ryan et al., 2009).

In comparison, the salt marshes of Staten Island are located along the lower transect of the Hudson River and closer to the Atlantic Ocean, thus having higher salinity values. Saw Mill Creek, located in the northwestern part of Staten Island, is part of the Arthur Kill Complex, which has salinity values ranging from 17 to 27 ppt (U.S. Fish and Wildlife Service 2005). The marshes of Staten Island make contact with the Atlantic Ocean, and are composed of broad expanses of salt meadow fringed by low marsh, forested uplands, rock outcrops, a swamp forest, and many small, spring-fed ponds (DEC 2010). Saw Mill Creek marsh, in particular, is dominated by *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*, and *Phragmites australis*, which were affected by the oil spill into the marsh in 1990 (DEC 2010).

Lastly, Jamaica Bay, which is the southernmost site, is a back-barrier lagoon containing salt marshes with an average salinity range of 20.5 to 26 ppt (Waldman 2008). These marshes make contact with the Atlantic Ocean via Rockaway Inlet and the system has been designated as a National Wildlife Refuge since 1972 (NY Harbor Parks 2010). However, much of the original tidal wetlands of Jamaica Bay have disappeared as a result of infrastructure development and other contributing factors (Hartig et al. 2002). The marsh is also home to over 330 species of birds, over 60 species of butterflies, and has one of the largest populations of horseshoe crabs in the northeast (NY Harbor Parks 2010). The low marsh is dominated by *Spartina alterniflora*; *Spartina patens* characterizes the high marsh. Where disturbed, invasive *Phragmites australis* is present. The high marsh also has a greater variety of species, such as salt grass (*Distichlis spicata)*, black grass (*Juncus gerardii*), glasswort (*Salicornia spp*.), and sea lavender (*Limonium carolinianum*) (Mack and Feller 1990). Big Egg, a high marsh that is part of

the Jamaica Bay marsh system, is the study site. The contrast of the marshes of Staten Island and Jamaica Bay against the less saline marshes of Iona Island and Piermont allows for comparative data in the stable isotope analysis, which provides useful information of stable isotope signatures

Sediment Coring

Sediment cores (each 1 meter in length) were collected from the marshes of Iona Island, Piermont, Staten Island, and Jamaica Bay with a Dachnowski corer from 2007 to 2008. These cores were stored in PVC half pipe liners and D-tube containers with a wet sponge to preserve moisture levels and kept in a refrigerator. The 1 m cores span approximately 1000 years, thus include the pre-European and post-European contact, providing information prior to and after anthropogenic impact. Table 1 summarizes the cores that were obtained for each marsh.

Isotope Analysis

Approximately 5 g of wet sediment was extracted at the surface and every 4 cm for each core (26 samples each) and then freeze-dried overnight. The dry sediment was then ground and homogenized using a ceramic mortar and pestle. Samples were then treated for the removal of carbonates through leaching with 1 M hydrochloric acid. After 24 hours, the samples were rinsed and centrifuged to remove the acid and the water was evaporated overnight at 75°C. Approximately 1 g of the dry residue was homogenized using a mortar and pestle and weighed into tin capsules for stable carbon and nitrogen isotope ratio analysis.

The samples were prepared at the Lamont-Doherty Earth Observatory, while the stable isotope analysis was sent to the Cornell University Isotope Laboratory, where an isotope ratio mass spectrometer (IRMS) was utilized. For quality control, duplicates and standard reference samples were also measured.

Dating

Radiocarbon dating has already been determined on macrofossils from Piermont (Pederson et al. 2005), Iona (Chou and Peteet 2010), and Saw Mill Creek (Peteet et al., unpublished data). Macrofossils were selected from sediment cores at these three sites and were radiocarbon dated using an Accelerator Mass Spectrometer (AMS) at Lawrence Livermore National Laboratory. Radiocarbon dates were calibrated using the CALIB program, version 6.0 of Stuiver and Reimer (1993) to determine the calendar ages of the samples.

Table 1: List of Sediment Cores and Chronology Sources

Marsh	Sediment Core	Chronology	Source
Piermont	07-Piermont-RC01	Carbon-14 dating; XRF	Pederson et al., 2005
Iona	07-Iona-RC01	Carbon-14 dating; XRF	Chou and Peteet, 2010
Staten Island (Saw Mill Creek)	08-SMC-RC01	Carbon-14 dating, XRF	Kleinstein, D., MS Thesis, 2004; Peteet et al., unpublished data
Jamaica Bay (Big Egg Marsh)	08-Big Egg-RC2	XRF	Sritrairat et al., in prep

RESULTS

Figures 2 and 3 are downcore profiles of the specified normalized stable isotope versus the respective standard for the four study sites. Each plot has been labeled with calibrated ages based on earlier data (Table 1). The depth at which Pb from XRF results (Sritrairat et al., unpublished) peaked were also marked on the $\delta^{13}C$ results of the cores, labeled as the 1970s when Pb in gasoline was banned in the Hudson River Valley region. For all graphs, the y-axis represents the average of the sampling interval of the depth of the sediment core. The x-axis is the normalized stable isotope value in ‰.

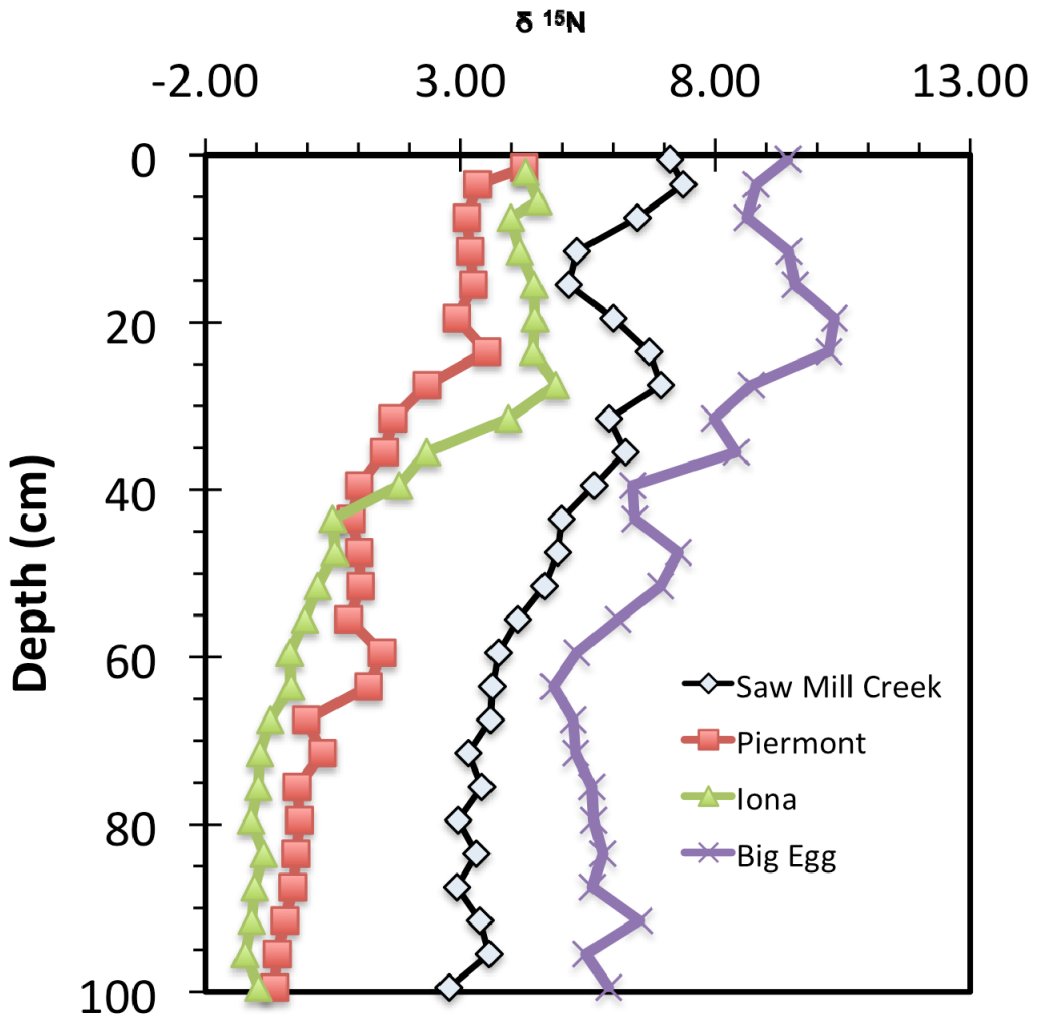


Figure 2: $\delta^{15}N$ results

Nitrogen

Figure 2 shows that the $\delta^{15}N$ value for the Iona core at the bottom of the meter core is 0.96‰ (the most negative value) and remains stable until about 70 cm. The values increase between 70 and 30 cm, decline slightly between about 30 and 8 cm below the surface, then slightly increase at 5 cm, before decline from 5 cm to the surface. Iona's nitrogen signal is more negative in comparison to the Piermont signal at the bottom of the core, but is more positive than Piermont above 40 cm.

At Piermont**,** Figure 2 also shows that $\delta^{15}N$ initially are -0.64‰ for the Piermont core, and the overall trend is an increasing one. The profile of the stable isotope remains somewhat stable until a depth of about 80 cm, then sharply increases up to 60 cm, then stabilizes from 60 cm to 40 cm but increases again from 40 cm to 20 cm. The values stabilize from 20 cm to 5 cm, but then increase at the surface.

At Saw Mill Creek, Figure 3 shows that $\delta^{15}N$ begins at a higher value of 2.78‰ and the overall trend is increasing. A shift is noticeable at 60cm and reaches a peak at 30 cm depth, where values decrease between 30 and 20 cm. The increasing pattern returns above 10 cm.

Finally, the initial value of $\delta^{15}N$ at Big Egg is depicted to be 5.91‰ – the highest of all four marshes. The overall trend is again the increasing $\delta^{15}N$ values toward the surface. The rise in $\delta^{15}N$ is most evident at just below 60 cm below the surface. The increase in $\delta^{15}N$, however, changes at about 20 cm below the surface between 10 and 20 cm, when values decline. Above 10 cm, $\delta^{15}N$ again increase.

Carbon

Figure 3a shows that the $\delta^{13}C$ value for the Iona core begins at -27.39‰. The values oscillate below 60 cm, but the oscillation trends towards the left and increases in magnitude above 60 cm.

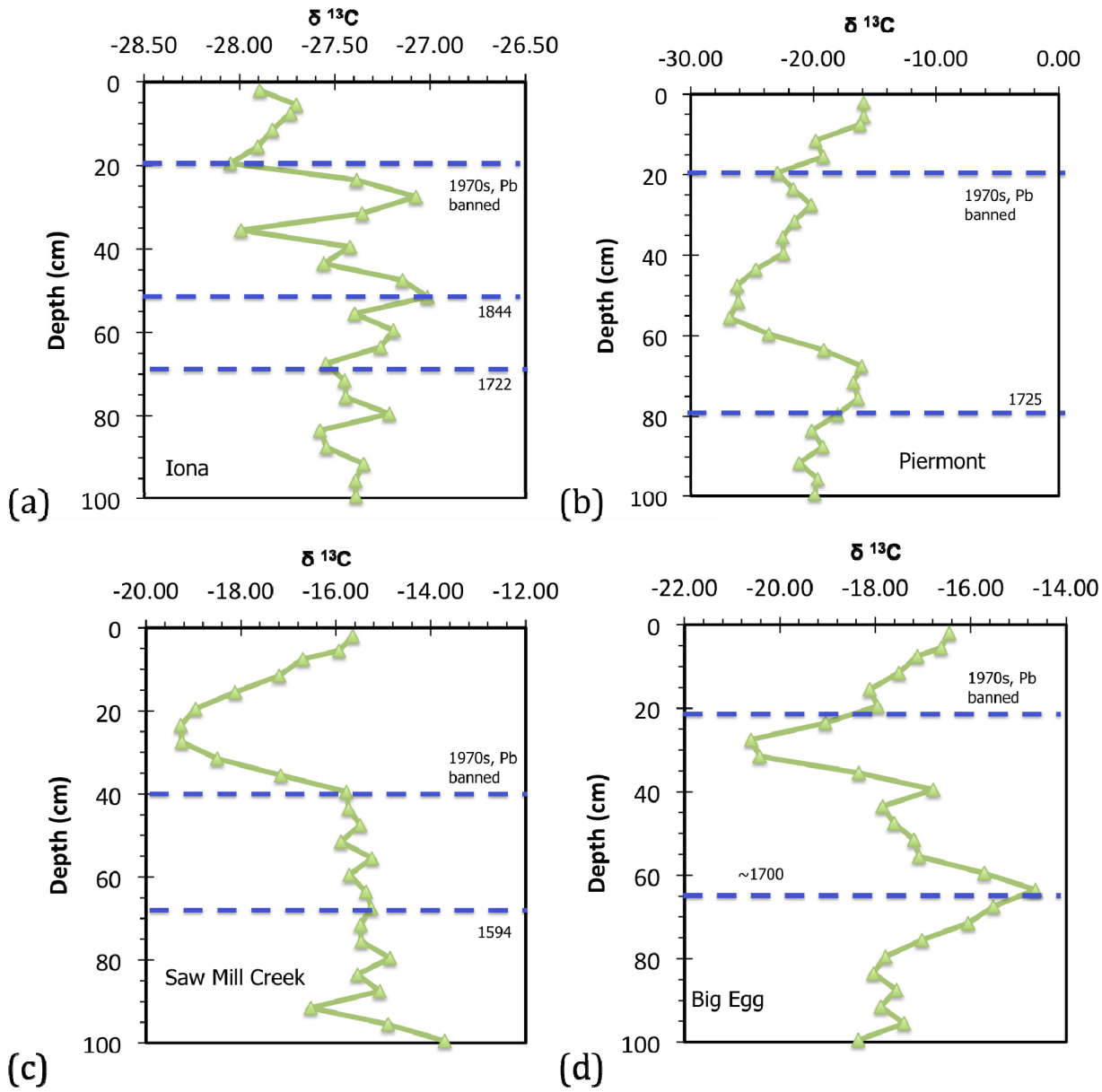


Figure 3: Delta 13C results with years obtained from C-14 dating and XRF results: (a) 07-Iona-RC01(b) 07-Piermont-RC01 (c) 08-SMC-RC01(d) 08-Big Egg-RC2

In the Piermont results, the $\delta^{13}C$ value starts at -19.91‰ (Figure 3b). The values oscillate at the bottom of the core, but there is a trend towards higher values until about 70 cm. Above 70 cm, the $\delta^{13}C$ values decrease from -16.06 to -26.83 ‰ (at 55 cm). The values then increase above this depth until about 30 cm. Between 20-30 cm below the surface, the values decrease a small amount, but increase again above 20 cm. Above 30 cm, the values have reached a level that is more positive than the initial values.

Figure 3c depicts the δ13C profile with depth for Saw Mill Creek. The starting value at 100 cm below the surface is -13.71‰, and values remain close to -15‰ up to 40 cm. The values then decline to -19‰ at 25 cm. Above 25 cm, the values increase back to -15‰.

For Big Egg marsh, the value of $\delta^{13}C$ is -18.36‰ at 100 cm below the surface (Figure 3d). The values increase between 100 and 60 cm below the surface, decrease between 60 and 30 cm below the surface, and then increase above 30 cm.

DISCUSSION

Marked changes in the stable isotope profiles are observed throughout the sediment record for the marshes of Piermont, Iona Island, Staten Island, and Jamaica Bay. These shifts in the profiles of the stable isotopes reflect changing conditions in the environment at specific points in the sediment record. The observed perturbations are likely to be indicative of changes in climate and human alterations of the marshes that affect species composition and sediment dynamics in the marshes.

Stable Nitrogen Isotope

The chronology associated with the stable isotope profiles suggests that an isotopically heavy source of nitrogen dominated the marshes after European contact with the Hudson River Valley in the 1700s at between 80 and 65 cm depth. Manure and septic effluent is enriched in $\delta^{15}N$ (Aravena et al. 1993; Bedard-Haughn et al. 2003), and if released into the environment can result in an output of higher $\delta^{15}N$ in the marsh vegetation (Cole et al. 2004). Increasing livestock and human population after European settlement in the Hudson River Valley intensified wastewater input and agricultural runoff, and probably contributed to the significant increase in $\delta^{15}N$ that was observed in all of the marshes after the 1700s.

The significant increase in the usage of nitrogen in the United States (Vitousek et al. 1997) has also resulted in eutrophication throughout many bodies of water in the country (Howarth et al. 1991). Eutrophication results in algal blooms and consequently anoxic or hypoxic waters in many estuaries and coastal seas, and poses a serious threat to marine and estuarine wildlife (Bonsdorff et al. 1997; Howarth et al. 1991; Price et al. 1985). Eutrophication furthermore can increase denitrification through anoxic conditions (Childs et al. 2002; Cole et al. 2004), thus enriching $\delta^{15}N$ in macrophytes and marsh sediments even further (Cole et al. 2005; Cole et al. 2004).

Amongst all of the four marshes, Big Egg Marsh at Jamaica Bay had the highest initial level of $\delta^{15}N$ at 5.91‰, which reached a maximum of 10.34‰ at 19.5 cm below the surface (Figure 2). Saw Mill Creek Marsh in Staten Island had the second highest starting level at 2.78‰ (Figure 2), with Iona Island and Piermont marshes having relatively similar starting values at -0.96 and -0.64‰ respectively (Figure 2). Big Egg

Marsh perhaps has the highest levels because of wastewater collection in the marsh due to its location inside a cove that is nearby extensive wastewater treatment plants. The difference in the levels of $\delta^{15}N$ may indicate an influence by species composition or salinity levels in the marshes. The $\delta^{15}N$ levels for Iona Island exceeded that of Piermont just below 40 cm, perhaps due the rapid increase in population in the 20th century in regions local to the Iona Island marsh. However, more research would be necessary to better understand this change as well as to better understand the differences in $\delta^{15}N$ levels that exists along the transect of the Hudson River.

In all of the marshes there is a reversal in the rise of $\delta^{15}N$ near the top of the cores, corresponding to about the 1970s. This perhaps reflects the increase in the use of synthetic fertilizers, which has increased by almost twenty-fold over the latter end of the 20th century (Glass 2003). Synthetic fertilizers have a lower $\delta^{15}N$ range than manure and septic effluent, as synthetic fertilizers range from -5 to 5‰ while manure and septic effluent range from 10 to 20‰ (Bottcher et al. 1990). The increase of synthetic fertilizer consumption may have decreased the $\delta^{15}N$ levels in marsh sediments, but the $\delta^{15}N$ profile also shows an increase in $\delta^{15}N$ levels again above 5 cm for most of the marshes (Figure 2). This may indicate an even higher increase in wastewater and agriculture due to increasing population in the region in recent decades.

Stable Carbon Isotopes

The stable carbon isotope profiles in the four marshes reflect a more complicated history. With time, degradation of organic material can result in an enrichment of $\delta^{13}C$ in the sediment. Furthermore, decomposition preserves ^{13}C-depleted components such as

lignin at the surface (Chmura and Aharon 1995; Hornibrook et al. 2000). The resulting effect would be low $\delta^{13}C$ values near the surface of the core, and higher values with increasing depth below the surface. However, the stable isotope profiles do not reflect this pattern. Since the situation was more complicated than initially hypothesized, individual marshes will be examined to better understand the stable isotope profiles.

The pollen record for Iona Marsh shows an increase in ragweed pollen (*Ambrosia*) at 60 cm (Chou and Peteet 2010), which dates to the early 1800s. *Ambrosia* requires plentiful sunlight, and as a result a disturbance such as land clearance that perturbs the forests would allow for *Ambrosia* to thrive (Pederson et al. 2005). This suggests that the 60 cm mark in the Iona sediment core signifies the beginning of major anthropogenic alterations in the region, and this same anthropogenic alteration affects the nutrient balance in the estuary, with the decrease of $\delta^{13}C$ in the sediment core record (Figure 3a). The decrease in the carbon signal possibly also reflects increased C3 runoff from the uplands into the marsh. However, the shifts in the carbon isotopes are minor, with the total around 1‰. The fluctuations in the $\delta^{13}C$ profile are indicative of the change in species composition in the marshes, and Iona has many species. Pollen and macrofossil records for the marsh suggest that the marsh was predominantly *Scirpus americanus* (-26.0‰), and may have shifted to *Typha* species (27-28‰) (Chmura and Aharon 1995) above 60 cm, which decreased overall $\delta^{13}C$ levels. The subsequent shifts back to enriched levels up to 30 cm are difficult to understand without the known values of all species from the marsh.

Macrofossil and pollen data for Piermont marsh shows a rise in ragweed at 80 cm (Pederson et al. 2005), and a decrease in $\delta^{13}C$ levels occurs at 70 cm (Figure 3b). The

decline in the signal can also be due to invasive C3 species from the uplands entering the marsh as the region was cleared. Above 40 cm, the signal becomes enriched, suggesting that C4 plants had a greater influence on the marsh. Interestingly, the rise of *Phragmites* (-24.6‰ to -29.4‰) in the marsh (Pederson et al. 2005; Chmura and Aharon 1995) would deplete the signal, but enrichment in the $\delta^{13}C$ signal was seen towards the top, suggesting other species are affecting the signal, possibly including more algae.

At Saw Mill Creek, preliminary pollen results indicate that the sustained rise in *Ambrosia* occurred at the same depth interval as the decline in $\delta^{13}C$ (Kleinstein 2004), suggesting more of a C3 signal in the marsh, possibly from uplands in the 1800s as the region was heavily modified. This is also possibly concurrent with a vegetation shift to C3 salt marsh plants such as *Salicornia* (-26‰) and *Atriplex patula* (-25.0‰ to -27.8‰) (Chmura and Aharon 1995) that have more depleted isotopic signatures than the original C4 grasses present in the marsh. The fluctuation at 30 cm below the surface to increased $\delta^{13}C$ levels, however, suggests that C4 grasses dominate the marsh again as they do at the surface today (Figure 3c), and there is possibly less input from land clearance.

For Big Egg Marsh at Jamaica Bay, anthropogenic influence in the marsh begins at 60 cm below the surface, but the shift from background levels of Pb in the XRF data to a significant rise is after 40 cm (Figure 3d). The decrease in $\delta^{13}C$ perhaps represents the dominance of invasive species such as *Phragmites australis* and *Typha* or other upland influence as land was cleared. The increase in $\delta^{13}C$ above 30 cm, however, is perhaps indicative of increased algae species in the waters due to increased wastewater input into the New York Harbor or a rise of C4 plants such as *Spartina*.

The carbon stable isotope pattern also reveals the salinity gradient in the plants

dominant in the marshes along the transect of the Hudson River. The two marshes in the lower part of the river (Saw Mill Creek and Big Egg) are more enriched in $\delta^{13}C$ compared to the two marshes in the upper transect (Iona and Piermont) (Figure 3). This agrees with the occurrence of C4 plants that are more prominent in the southern marshes, while C3 plants dominate the two northern marshes.

Prospects for Trophic Activity

Recent studies have also used stable isotope ratios to determine trophic activity in marsh ecosystems (Abrantes and Sheaves 2009, 2010; Litvin and Weinstein 2003; Schiesari et al. 2009; Weinstein et al. 2009), and thus this research can act as a foundation for future research on the subject for the Hudson River estuary. Trophic positions in the food web can be identified by looking for a consistent relationship between the isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ in organisms and primary producers. Researchers have shown 1) that variations exist in trophic linkages along the salinity gradient of the Delaware Bay (Litvin and Weinstein 2003), 2) the existence of significant feeding niche differentiation in species from six different wetlands on the University of Michigan's E.S. George Reserve (Schiesari et al. 2009), 3) the trophic relationships between *P. australis* and resident mummichog (*Fundulus heteroclitus*) and marine transient species from Sandy Hook Bay, New Jersey (Weinstein et al. 2009), and 4) the variations in trophic positions and trophic lengths between the primary producers, primary consumers and secondary consumers of the Ross River estuary in northern Australia (Abrantes and Sheaves 2010).

The role of "specialized" habitats such as Piermont Marsh in the trophic activities

of Hudson fish warrants further investigation, as these marshes may serve as overwintering areas for specific species (Weinstein et al. 2009). The isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ in the sediment record can be compared to present isotopic signatures in existing vegetation in the Hudson River wetlands. Thus, isotopic results from this study can consequently function as background data in future research identifying the role of wetland producers in the trophic activities of Hudson River fish throughout the transect of the estuary.

The Hudson River Valley has provided the structural framework for human development over hundreds of years by linking communities economically, culturally and ecologically. Increased anthropogenic activities, however, can be detrimental to estuarine health, thus requiring a more thorough understanding of the chemistry and organic matter of the wetlands and how these environments behave in response. This research project has shed light on the effects of anthropogenic forcings, such as the use of fertilizers and land development, and upon the dynamics of the nitrogen and carbon cycles in Hudson River wetlands on a much longer time scale than instrumental measurement allows. Understanding the natural responses of these diverse ecosystems to human activities will provide a history of biochemical processes and physical and ecological shifts as a result of human activities. A more thorough understanding of the natural responses of the wetland ecosystem to human activities will not only document the historical effects of anthropogenic forcings, but also yield public awareness and facilitate conservation and restoration efforts.

CONCLUSIONS

(1) There exists a gradient in the $\delta^{15}N$ levels along the transect of the Hudson River. From north to south, the signal enriches, probably due to population increases and sewage effluent in the south.

(2) $\delta^{15}N$ levels in Hudson River marshes have increased since European impact due to agricultural and wastewater input. These human-induced changes have also increased eutrophication levels in marshes,further enriching $\delta^{15}N$ in marsh sediments through denitrification. Anthropogenic alterations in marshes have therefore changed nutrient dynamics.

(3) Increased use of synthetic fertilizers has apparently decreased the $\delta^{15}N$ signal in marshes during the 1970s, since synthetic fertilizers have a lower $\delta^{15}N$ range than manure and septic effluent. With increasing population levels, however, and more wastewater and agricultural inputs, it appears that the $\delta^{15}N$ signal has increased again.

(4) The $\delta^{13}C$ signal is enriched from North to South along the transect of the Hudson River, as expected from the present distribution of increased salinity and the gradient in plant composition from fresh (depleted) to brackish to salt marsh species (enriched) at the mouth.

(5) The rise in ragweed (*Ambrosia*) in the uplands parallels an initial decrease in the $\delta^{13}C$ value in marshes, sometimes due to invasive influence, possibly from uplands, or possibly from increased deposition of eroded upland plant material.

(6) Fluctuations in $\delta^{13}C$ since European impact indicates a complex sequence of changing species composition, and further research on the isotopic signature of marsh species will help us define these shifts.

ACKNOWLEGEMENTS

We would like to thank the Tibor T. Polgar Fellowship Program of the Hudson River Foundation for funding and supporting this project. We are also very grateful to Sanpisa Sritrairat for her help, support, guidance and advice throughout this whole research project. Thank you to Kim Sparks at the Cornell University Stable Isotope Laboratory for her guidance and expertise with the IRMS. Lastly, special thanks also go to Sriya Sundaresan, Kathrin Sears, Cleo Chou, Jonathan Nichols, Peter Isles, Baruch Tabanpour, Max Perez, Zhehan Huang, and Tim Kenna for their help and support both in the lab and field.

REFERENCES

Abrantes, K., and M. Sheaves. 2009. Sources of nutrition supporting juvenile penaeid prawns in an Australian dry tropics estuary. Marine and Freshwater Research 60:949-959.

Abrantes, K., and M. Sheaves. 2010. Use of a delta C-13-delta N-15 relationship to determine animal trophic positions in a tropical Australian estuarine wetland. Austral Ecology 35: 96-103.

Aravena, R., M.L. Evans, and J.A. Cherry. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water 31: 180-186.

Bedard-Haughn, A., J.W. van Groenigen, and C. van Kessel. 2003. Tracing N-15 through landscapes: potential uses and precautions. Journal of Hydrology 272: 175-190.

Blair, E.A., W.C. Nieder, 1993. Mapping of the Hudson River NERR: creating tools for tidal wetland research, management, and education. Final report to the Hudson River Foundation. New York, NY.

Bonsdorff, E., E.M. Blomqvist, J. Mattila and A. Norkko. 1997. Coastal eutrophication: causes, consequences and perspectives in the Archipelago areas of the northern Baltic Sea. Estuarine Coastal and Shelf Science 44: 63-72.

Bottcher, J., O. Strebel, S. Voerkelius, and H.L. Schmidt. 1990. Using isotope fractionation of nitrate nitrogen and nitrate oxygen for evaluation of microbial denitrification in a sandy aquifer. Journal of Hydrology 114: 413-424.

Brickerurso, S., S.W. Nixon, J.K. Cochran, D.J. Hirschberg, and C. Hunt. 1989. Accretion rates and sediment accumulation in Rhode-Island salt marshes. Estuaries 12: 300-317.

Buckley, E.H. and S.S. Ristich. 1976. Distribution of rooted vegetation in the brackish marshes and shallows of the Hudson River estuary. Paper number 20 in Hudson River Ecology, 4th Symposium on Hudson River Ecology, March 28-30, 1976. The Hudson River Environmental Society, Inc.

Chang, C. C. Y., P.W. McCormick, S. Newman, and E.M. Elliott. 2009. Isotopic indicators of environmental change in a subtropical wetland. Ecological Indicators 9: 825-836.

Childs, C. R., N.N. Rabalais, R.E. Turner, and L.M. Proctor. 2002. Sediment denitrification in the Gulf of Mexico zone of hypoxia. Marine Ecology-Progress Series 240: 285-290.

Chmura, G. L., and P. Aharon. 1995. Stable carbon-isotope signatures of sedimentary carbon in coastal wetlands as indicators of salinity regime. Journal of Coastal

Research 11: 124-135.

Chou, C., D. Peteet. 2010. Macrofossil evidence for Middle to Late Holocene vegetation shifts at Iona Island Marsh, Hudson Valley, NY. Final Report of the Tibor T. Polgar Fellowship Program, 2009. Hudson River Foundation.

Cole, D., 1884. History of Rockland County New York. J.B. Beers and CO., New York.

Cole, M. L., I. Valiela, K.D. Kroeger, G.L. Tomasky, J. Cebrian, C. Wigand, R.A. McKinney, S.P. Grady, and M.H.C. da Silva. 2004. Assessment of a delta N-15 isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality 33: 124-132.

Cole, M. L., K.D. Kroeger, J.W. McClelland, and I. Valiela. 2005. Macrophytes as indicators of land-derived wastewater: application of a delta N-15 method in aquatic systems. Water Resources Research 41.

Connor, R. F., G.L. Chmura, and C.B. Beecher. 2001. Carbon accumulation in Bay of Fundy salt marshes: implications for restoration of reclaimed marshes. Global Biogeochemical Cycles 15: 943-954.

Cronon, W., 1983. Changes in the land: Indians, colonists, and the ecology of New England. Hill and Wang.

Department of Environmental Conservation. 2010. "Sawmill Creek wetlands," New York State Department of Environmental Conservation. http://www.dec.ny.gov/outdoor/55407.html (accessed 29 Oct 2010).

Glass, A. D. M. 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Critical Reviews in Plant Sciences 22: 453-470.

Hartig, E. K., V. Gornitz, A. Kolker, F. Mushacke, and D. Fallon. 2002. Anthropogenic and climate-change impacts on salt marshes of Jamaica Bay, New York City. Wetlands 22: 71-89.

Hornibrook, E. R. C., F.J. Longstaffe, and W.S. Fyfe. 2000. Factors influencing stable isotope ratios in CH4 and CO2 within subenvironments of freshwater wetlands: implications for delta-signatures of emissions (vol 36, pg 151, 2000). Isotopes in Environmental and Health Studies 36: U1-U1.

Howarth, R. W., J.R. Fruci, and D. Sherman. 1991. Inputs of sediment and carbon to an estuarine ecosystem - influence of land-use. Ecological Applications 1: 27-39.

Hubeny, J., King, J., and M. Cantwell. 2009. Anthropogenic influences on estuarine sedimentation and ecology: examples from the varved sediments of the Pettaquamscutt River Estuary, Rhode Island. Journal of Paleolimnology 41: 297-314.

Kenna, T. C., F. O. Nitsche, M. M. Herron, B. J. Mailloux, D. Peteet, S. Sritrairat, E. Sands, and J. Baumgarten. 2011. Evaluation and calibration of a field portable X-Ray Fluorescence spectrometer for quantitative analysis of siliciclastic soils and sediments. Journal of Analytical Atomic Spectrometry.

Kleinstein, D. Paleoenvironmental change during the last millennium at Saw Mill Creek Marsh, Staten Island. 2004. M.S. thesis. Columbia University, New York, New York.

Lehr, J.H., 1967. The marshes at Piermont, New York: a field report. Sarracenia 11: 31–34.

Litvin, S. Y., and M.P. Weinstein. 2003. Life history strategies of Estuarine Nekton: the role of marsh macrophytes, benthic microalgae, and phytoplankton in the trophic spectrum. Estuaries 26: 552-562.

Mack, L., and J.M. Feller. 1990. Salt marshes of New York City. City of New York, Parks and Recreation & Natural Resource Group, New York, NY.

Minckley, T. A., M.T. Clementz, A. Brunelle, and G.A. Klopfenstein. 2009. Isotopic analysis of wetland development in the American Southwest. Holocene 19: 737-745.

Mitsch, W.J. and J.G. Gosselink. 2007. Wetlands. John Wiley & Sons, Inc., Hoboken. 4th edition.

Morris, J. T., and P.M. Bradley. 1999. Effects of nutrient loading on the carbon balance of coastal wetland sediments. Limnology and Oceanography 44: 699-702.

National Estuarine Research Reserve. 2009. "Four treasures on the Hudson," National Estuarine Research Reserve System. http://www.nerrs.noaa.gov/NERRSReserve.aspx?ID=131&ResID=HUD (accessed 16 Feb 2010).

NY Harbor Parks. 2010. "Jamaica Bay Wildlife Refuge," National Parks of New York Harbor Conservancy. http://www.nyharborparks.org/visit/jaba.html (accessed 16 Feb 2010).

Pederson, D. C., D.M. Peteet, D. Kurdyla, and T. Guilderson. 2005. Medieval Warming, Little Ice Age, and European impact on the environment during the last millennium in the lower Hudson Valley, New York, USA. Quaternary Research 63: 238-249.

Peteet, D., D. Pederson, D. Kurdyla, and T. Guilderson. 2006. Hudson River paleoecology from marshes, Chapter 11, pp 113-128 in: Waldman, J.R., K.E. Limburg, and D. Strayer, Editors. Hudson River Fishes and their Environment. American Fisheries Society Symposium 51.

Price, K. S., D.A. Flemer, J.L. Taft, G.B. Mackiernan, W. Nehlsen, R.B. Biggs, N.H. Burger, and D.A. Blaylock. 1985. Nutrient enrichment of Chesapeake Bay and its impact on the habitat of striped bass - a speculative hypothesis. Transactions of the American Fisheries Society 114: 97-106.

Rooth, J. E., J.C. Stevenson, and J.C. Cornwall. 2003. Increased sediment accretion rates following invasion by Phragmites australis: the role of litter. Estuaries 26: 475-483.

Ryan, W.B.F., S.M. Carbotte, J.O. Coplan, S. O'Hara, A. Melkonian, R. Arko, R.A. Weissel, V. Ferrini, A. Goodwillie, F. Nitsche, J. Bonczkowski, and R. Zemsky. 2009. Global multi-resolution topography synthesis, Geochemistry Geophysics Geosystems, 10: Q03014,

Schiesari, L., E.E. Werner, and G.W. Kling. 2009. Carnivory and resource-based niche differentiation in anuran larvae: implications for food web and experimental ecology. Freshwater Biology 54: 572-586.

Smith, B. N., and S. Epstein. 1971. 2 Categories of C-13/C-12 ratios for higher plants. Plant Physiology 47: 380.

Sritrairat, S., D. M. Peteet, R. Sambrotto, K. Griffin, S. Chillrud, T. Kenna. 2009. "Past vegetation, climate, sediment, and nutrients dynamics at Tivoli North Bay, Hudson River, New York." In preparation for Ecological Applications

Stuiver, M. and P. J. Reimer. 1993. Extended C-14 data-base and revised calib 3.0 C-14 age calibration program. Radiocarbon 35: 215-230.

U.S. Fish and Wildlife Service. 2005. "Significant habitats and habitat complexes of the New York Bight Watershed – Arthur Kill Complex," U.S. Fish and Wildlife Service Conservation Library. http://library.fws.gov/pubs5/web_link/text/akc_form.htm (accessed 29 April 2011)

Varekamp, J., E. Thomas, M. Buchholtz ten Brink, M. A. Altabet, and S. Cooper. 2010. "Environmental change in Long Island Sound in the recent past: eutrophication and climate change." Final Report of Long Island Sound Research Fund Grant. Connecticut Department of Environmental Protection.

Vitousek, P. M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7: 737-750.

Waldman, J. 2008. Research opportunities in the natural and social sciences at the Jamaica Bay unit of Gateway National Recreation Area. Jamaica Bay Institute.

Weinstein, M. P., S.Y. Litvin, and V.G. Guida. 2009. Essential fish habitat and wetland restoration success: a tier III approach to the biochemical condition of common

Mummichog *Fundulus heteroclitus* in Common Reed *Phragmites australis*- and Smooth Cordgrass *Spartina alterniflora*-dominated salt marshes. Estuaries and Coasts 32: 1011-1022.

Winogrond, H.G., 1997. Invasion of *Phragmites australis* in the tidal marshes of the Hudson River, M.S. thesis. Bard College, Annandale-on-Hudson, New York.

QUANTIFICATION AND IDENTIFICATION OF ANTIBIOTIC RESISTANT MICROBES IN THE HUDSON RIVER AND FLUSHING BAY

A Final Report of the Tibor T. Polgar Fellowship Program

Suzanne Young

Polgar Fellow

Department of Earth and Environmental Sciences
Queens College, City University of New York
Flushing, NY 11367

Project Advisor:

Gregory O'Mullan
Department of Earth and Environmental Sciences
Queens College, City University of New York
Flushing, NY 11367

Young, S. and G. O'Mullan. 2012. Quantification and Identification of Antibiotic Resistant Microbes in the Hudson River and Flushing Bay. Section III: 1-29 pp. *In* S.H. Fernald, D. J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

Microorganisms resistant to tetracycline and ampicillin, two commonly used antibiotics, were detected in the Hudson River and other urban waterways of New York City. Culture dependent approaches were used to quantify the abundance of antibiotic resistant microbes and to examine their correlation to raw sewage inputs, while 16S rRNA gene sequences were used for taxonomic identification of microbes found to be resistant. Higher frequency sampling was conducted at Flushing Bay, NY in order to examine the patterns of antibiotic resistant microbes under both dry and wet weather conditions. Ten additional sites in the Hudson River Estuary were sampled during monthly research cruises to examine spatial variability in resistant microbes. Resistant microbes were detected at all sampling sites. Analysis of 16S rRNA genes amplified and sequenced from resistant colonies identified a phylogenetically diverse group of bacteria, including the genera *Aeromonas*, *Pseudomonas*, *Stenophomonas*, and *Escherichia.* All of these genera include opportunistic pathogens and have been associated with antibiotic resistant infections, especially in immuno-compromised individuals. The abundance of ampicillin and tetracycline resistant bacteria, in paired samples, were positively correlated with one another and both groups of microbes were found in greater abundance following precipitation events. The abundance of *Enterococcus,* a sewage indicating microbe, was also found to be positively correlated with levels of resistance, suggesting a shared sewage-associated source for the indicator microorganism and the phylogenetically diverse resistant bacteria.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Antibiotics are often referred to as miracle drugs and considered to be one of the greatest achievements in public health. Penicillin, among the first antibiotics to be investigated for use in medicine, was named and characterized by Alexander Fleming in 1928 (Overbye and Barrett 2005). Soon after, penicillin and other newly discovered antibiotics became widely used to treat and prevent infections. The prevalence of antibiotic use has caused many bacteria to become resistant to commonly used antibiotics. In the decades that followed the early use of penicillin, a greater number of resistant infections began to appear in hospitals throughout the developed world, as more and more antibiotics were used and sometimes over-prescribed. In the United States alone, approximately 2 million people a year now acquire an antibiotic-resistant infection, and approximately 90,000 of these cases are lethal (Overbye and Barrett 2005). Antibiotic resistance has become one of the most pressing and urgent public health crises in the world (Wise et al. 1998). The spread of antibiotic resistance in the environment is also a growing concern that has implications for ecosystem functions and public health.

Antibiotics and antibiotic resistant bacteria can enter surface water, groundwater systems, or drinking water sources in association with human and animal waste. Antibiotics are not completely metabolized by the human or animal body (Costanzo et al. 2005) and consequently, intact and functional compounds can enter waterways through the waste products of humans or animals who have ingested antibiotics. Waste can be efficiently disinfected at a treatment facility, but the antibiotic compounds are not efficiently removed from the effluent because even the most modern wastewater

treatment plants (WWTPs) are not designed to remove antibiotics present in small concentrations. Therefore, even treated effluent from WWTPs can contribute to antibiotic loading into waterways.

Other major sources which contribute to waterway contamination are concentrated animal farm operations (or CAFOs) where antibiotics are used as prophylactics to avoid disease and promote rapid growth. Concern over antibiotic use in CAFOs relates both to the release of antibiotic compounds associated with waste products from these facilities into the environment and to the evolution and stimulation of antibiotic resistant bacterial strains from their overuse. The European Union has banned prophylactic use in CAFOs, and a bill was introduced into the United States Congress in early 2010 to do the same. Measures such as these demonstrate the growing consensus that overuse of antibiotics is contributing to greater occurrence of resistant infections, affecting animal husbandry, human health and the environment (Conly 1998).

The presence of antibiotics in waterways leads to an increase in bacterial resistance to those antibiotics through selective pressure and horizontal gene transfer (Alonso et al. 2001). Selective pressure in the presence of antibiotics occurs due to the enhanced persistence or growth of bacterial strains carrying resistance genes that block a mechanism of cellular damage caused by antibiotic compounds. These resistance genes are often located on plasmids prone to horizontal gene transfer, accounting for the main pathway by which antibiotic resistant genes are spread. Plasmids are extrachromosomal gene structures that can be mobile, allowing for plasmid encoded genes to be transferred across cell membranes between bacterial cells. This mode of genetic transfer allows the abundance and diversity of resistant bacteria to increase rapidly in aquatic environments

exposed to frequent contamination with antibiotic compounds, and waterways may become reservoirs or incubators for resistant bacteria (Alonso et al. 2001).

The predominant source of antibiotics and resistant bacteria in the lower Hudson River Estuary (HRE) is thought to be human sewage (Kim et al. 2010), although this connection has yet to be carefully tested in the waterways of New York. New York City has as a combined sewer system, which means that sanitary sewers in homes and businesses are connected to storm drain sewers (City of New York 2011a). The benefit of such a system is that during dry weather, street runoff and other drainage can be treated before being released into local waterways, whereas separated systems would simply release storm drainage into the water untreated. Negative consequences of the combined sewer system become apparent during wet weather, when the excess volume, caused by rainwater combined with sanitary sewage, overwhelms the capacity of treatment plants and is diverted and released through combined sewer outfalls (CSOs) into the surrounding waterways (e.g. Hudson River, Harlem River, East River, Flushing Bay, and others) to avoid backups of drains and plumbing systems. This results in bacteria-laden human sewage, which may contain antibiotics or antibiotic resistant microbes, entering local waterways without treatment.

The presence of antibiotics and antibiotic resistant microorganisms in aquatic environments has been extensively documented and studied (Guardabassi et al. 1998; Goni-Urriza et al. 2000; Reinthaler et al. 2003), but no studies have been completed in urban NYC watersheds that may point to the possible environmental and public health risks presented by antibiotic contamination and the consequential promotion of antibiotic resistance. Due to the combined sewer system, sewage inputs associated with rainfall

events are predicted to contribute to the presence of antibiotics and antibiotic resistant microbes in the HRE.

The health of HRE ecosystem may also suffer when increased levels of antibiotics are introduced to aquatic communities. Bacteria that are essential to nutrient cycling are not immune to the effects of antibiotics, and studies have shown that microbial mediated nutrient cycling (e.g. denitrification rates) can be altered in the presence of antibiotics (Costanzo et al. 2005). In a system such as the HRE, which is known to experience excessive nutrient loading (Lampman et al. 1999), the disruption of microbial nutrient cycling could decrease the removal of gaseous nitrogen from the system, thereby increasing the intensity of eutrophication. Eutrophication can have deleterious effects on aquatic plant communities, invertebrates, and fish, and represents a large scale alteration to the aquatic ecosystem including increased potential for hypoxia or anoxia associated with the decomposition of microalgae blooms.

The goals of this study are to examine the spatial and temporal distribution of resistant microbes in correlation with the sewage indicating bacterium, *Enterococcus,* and to phylogenetically identify the species of bacteria in the HRE that were found to be resistant to two commonly used antibiotics: ampicillin and tetracycline. This research tested the hypothesis that the occurrence of antibiotic-resistant microbes is positively correlated with sewage loading in wet weather conditions indicated by the presence of *Enterococci*. This study is a preliminary step in understanding the patterns of antibiotic resistant bacteria in the lower HRE, and highlights the need for improvements in integrated approaches to stormwater management and public health risk assessment.

METHODS

Sampling

Samples for microbiological analysis were collected from surface waters into sterile 50 ml plastic tubes that were also triple rinsed with the sample water before collection. Sampling containers were immediately stored on ice, away from sunlight, in a cooler and transported to the lab within 12 hours for processing. Hach (www.hach.com) handheld sensor systems were used to measure levels of oxygen, temperature, salinity (HACH HQ40d) and turbidity (HACH 2100Q portable turbidimeter) at the time of sampling. Precipitation data were recorded over the prior 5 days from LaGuardia Airport and Central Park; wet days were classified by having >0.5 inches of rain within 2 days of sampling, based on the Weather Underground historical data (www.wunderground.com).

The majority of water samples were collected from Flushing Bay near College Point, Queens, a site in close proximity to Queens College campus where frequent access was feasible. The proximity of this site allowed water samples to be collected under both dry weather and following rain events. Water samples were also collected from various points in the HRE in coordination with the Riverkeeper monthly sampling cruises on the Hudson River, from June to October.

Study Site: Flushing Bay

Flushing Bay is located in Northern Queens, and surrounded by various industrial and commercial establishments, as well as the World's Fair Marina and LaGuardia Airport. The sampling site for this study is east of the marina, near a frequently-used kayak and boat launch. After some exploration of the area, this site was found to be the most conducive shore point on the Bay for the requirements of this project. During the

sampling, police boats were seen using the bay as a training site for water rescue, fishermen were frequently on the dock (who also said they swim in the Bay in hot weather), and other recreational boats and kayaks were launched. The recreational use of this area was one desired characteristic in selecting a sampling site, along with access to the water and proximity to the lab at Queens College.

Study Site: Riverkeeper Sampling Sites

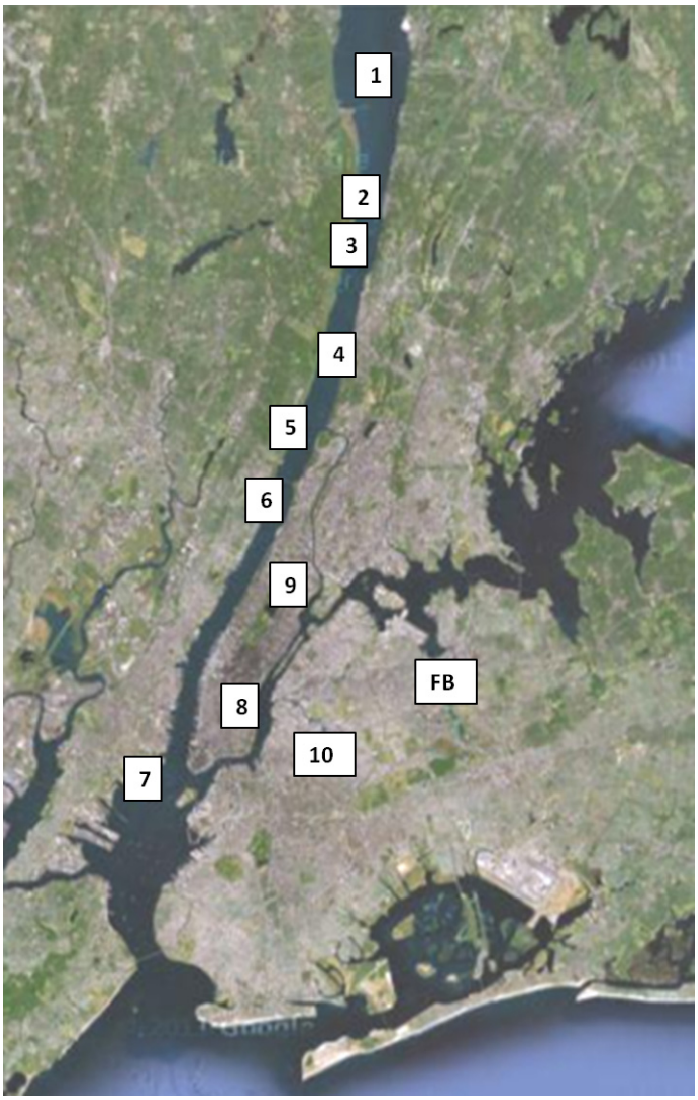
Sample collection from ten sampling sites throughout the lower HRE was performed in coordination with Riverkeeper's monthly water quality survey (www.riverkeeper.org/water-quality), The sites listed from North to South, and shown in Figure 1 are: Tappan Zee, Piermont Pier, Piermont Outfall, Sawmill River, Harlem River, 125th St. Outfall, Harlem Piers, East River, Newtown Creek, Battery.

Sites were chosen to provide a breadth of environments within the river based on location characteristics and historical data collected over the last five years. Samples were collected using the same procedure described above except that in some cases, *Enterococci* sewage indicator counts were processed while still aboard the boat because of time constraints.

Laboratory Procedures

Sewage Indicators

The procedure for analysis of the sewage indicating bacteria, *Enterococci,* in water samples was performed using the IDEXX Enterolert methodology (www.Idexx.com). All sampling stations were from brackish water sites and therefore a one in ten dilution of sample water in sterile DI water was performed prior to selective enrichment and enumeration in accordance with the manufacturer suggested protocol for



1-Tappan Zee
2-Piermont Pier
3-Piermont Outfall
4-Sawmill River
5-North River WWTP
6-125th St. Pier
7-Battery
8-East River
9-Harlem River
10-Newtown Creek
FB- Flushing Bay

Figure 1. Map of the ten Riverkeeper sampling sites around Manhattan and throughout the lower HRE, and key for numbered sites. Additional sampling station of Flushing Bay marked as "FB". (Markers are not exact to GPS references, but represent approximate locations.)

Enterolert. Processing was always done within six hours of sample collection. The samples were added to liquid media, sealed in a Quanti-tray (IDEXX), and incubated at 41°C. After 24 hours incubation, the Quanti-tray was exposed to UV light and the number of wells displaying blue color in the trays was recorded used to calculate a most probably number (MPN) of *Enterococci* cells per volume of sample water.

Colony-based approaches for microbial counts

Quantification of heterotrophic bacteria and antibiotic resistant heterotrophic bacteria required the preparation of solid nutrient-rich R2A agar, and the addition of dry mass of antibiotics (Reasoner 2004). Media was autoclaved, then placed in a 55°C water bath to cool before the addition of antibiotics. Antibiotics were added to media for determination of antibiotic resistance in the following proportions: 50 mg/L ampicillin and 10 mg/L tetracycline. Plates were poured in a hepa filtered laminar flow hood and once liquid media solidified, plates were seasoned overnight at room temperature before being stored in a refrigerator until future use. For sample processing, two to four ten-fold dilutions of the sample water were created, using autoclaved and then 0.2 μm filter sterilized sample water as dilution water, and 100 μl of the dilutions was spread onto the plates using aseptic technique in a laminar flow hood. For each sample, plates were inoculated for growth on R2A agar with no antibiotic added, R2A agar with: 1) no antibiotic added (referred to as Heterotrophic or "Het" plates in the text); 2) with ampicillin added (referred to as "Amp" plates); and 3) with tetracycline added (referred to as "Tet" plates, in masses per volume as described previously). Control plates were created using sterile water spreads as a method blank. Inoculated plates were then incubated at 28˚C for three days.

Colonies were counted after three days of incubation, and then stored in the refrigerator for future molecular analysis. Counts of less than 300 colony forming units (CFUs)/100 μl were deemed "countable" numbers, and when multiple dilutions were processed, plates with less than 300 colonies were utilized in data analysis. Results were recorded in units of CFU/100 μl of initial sample water.

Molecular Techniques for Taxonomic Identification

Molecular analysis involved isolating colonies grown on R2A agar from the plate counts described above, including Het, Amp and Tet plates. Colonies were picked off of the media, in a sterile biosafety hood, using sterile pipet tips, and transferred into 40 µl of Hyclone molecular-grade sterile water in 0.2 ml stripe tubes. Strip tubes containing picked colonies were boiled at 95°C for 5 minutes using an Eppendorf thermocycler to lyse cells and then stored at -20°C into additional processing could be completed.

Polymerase Chain Reaction method (PCR) was used to amplify 16S ribosomal RNA genes from the DNA of lysed cells using the universal bacterial primers 8F and 1492R (Lane 1991) and cycling conditions as follows: 95° C for 10 minutes; 30 cycles of 95° C for 1 min, 55° C for 30 secs, 72°C for 1 min; 72° C for 5 mins; 4° soak. Amplification products were separated using gel electrophoresis in 1% (w/v) agarose gels, stained with SyberSafe dye (Invitrogen), and visualized using UV light and a Syngene gel documentation system. Amplification product sizes were estimated relative to a 1 kb ladder (Invitrogen) and products of approximately 1500 bp were selected for gene sequencing. Amplification product size was quantified using a Qubit fluorometer (Invitrogen), normalized in concentration and sent for DNA sequencing to SeqWright Inc. (Houston, TX). The sequence output files were edited using FinchTV (www.geospiza.com) and Geneious (www.geneious) software packages. Edited sequence files were exported in FASTA format and uploaded to the Ribosomal Database Project webserver (http://rdp.cme.msu.edu/) for alignment and classification, to the level of genus.

Statistical Analyses

Prism statistical analysis software (Version 4C, May 13, 2005) was used in order to perform non-parametric tests for statistically significant differences between wet and dry weather antibiotic resistance. Non-parametric tests were used because microbial counts were non-normally distributed. Spearman's coefficient was used to evaluate the relationship between sewage indicators and antibiotic resistant microbes. Values of zero were replaced with values of 0.1 when calculating geometric means for *Enterococci* measurements.

RESULTS

Spatial Variation

Ampicillin resistant colonies were detected at all of the eleven sampling sites in the HRE (Figure 2 and Table 1), including the ten sites sampled during Riverkeeper monthly water quality patrols and shore based sampling of Flushing Bay. Tetracycline resistant colonies were detected at all sites except the Battery. During monthly June to October Riverkeeper sampling cruises, 83% of samples collected at the ten sites were found to contain ampicillin resistant microbes and 36% of samples were found to contain tetracycline resistant microbes (Table 1). Ampicillin resistant heterotrophs were found more frequently (86% of the samples) than tetracycline resistant heterotrophs (28% of the samples) at all sites and were found to have a higher maximum abundance than tetracycline resistant heterotrophs at all sites except at the North River treatment plant (Figure 2 and Table 1). Newtown Creek and the Harlem Piers samples contained the highest maximum number of ampicillin resistant heterotrophs detected during monthly

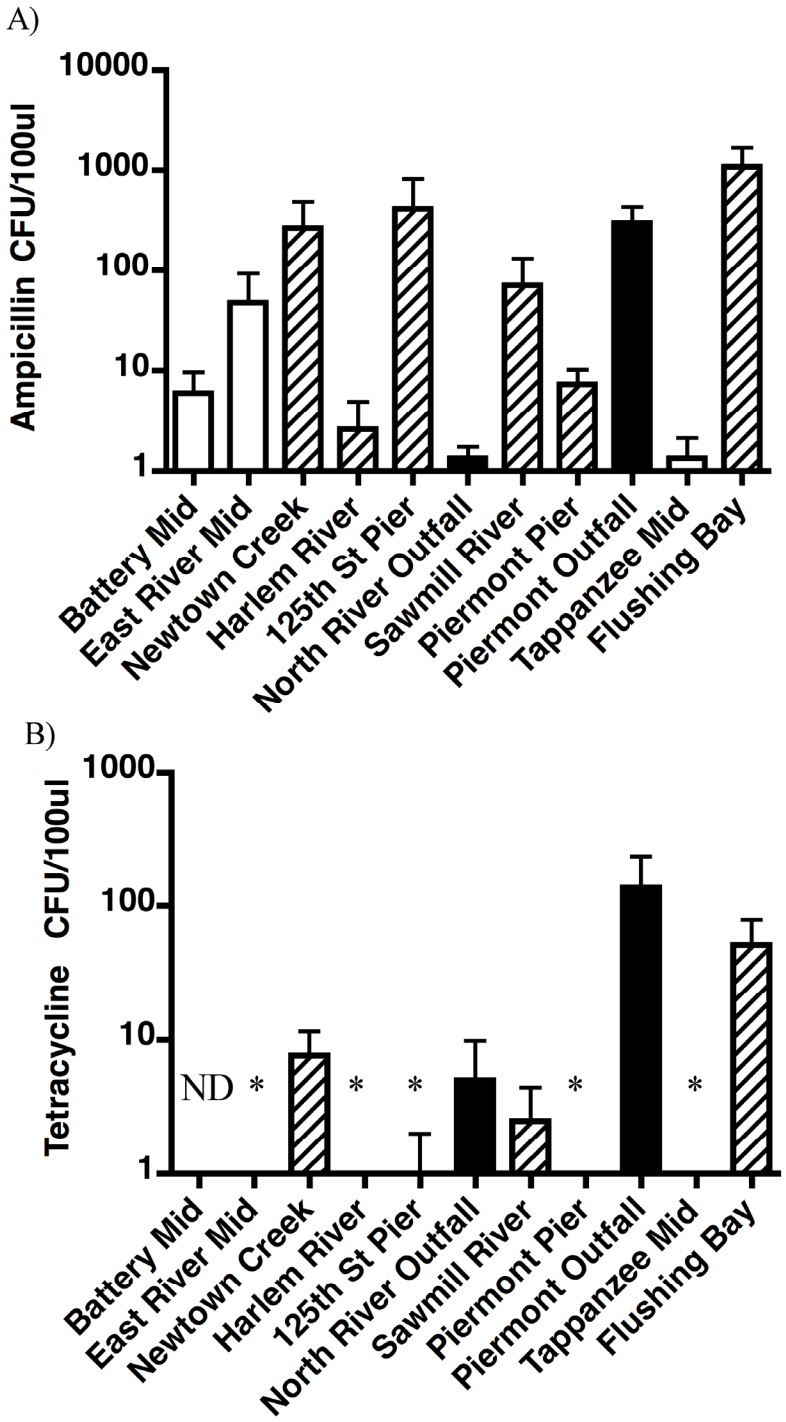
Riverkeeper sampling patrols. In contrast, the highest maximum numbers of tetracycline resistant heterotrophs were detected at the two wastewater treatment outfalls.

Geometric means of *Enterococci* calculated over the entire sampling period did not exceed monthly geometric mean standards (35 CFU/100 ml) except at one site, Newtown Creek. Maximum values of measured *Enterococci* exceeded single sample maximum standards (104 CFU/100 ml) at four sites: Newtown Creek, Sawmill River, Piermont Outfall and Piermont Pier. Across sites, the geometric mean of *Enterococci* at a site was found to be positively correlated with the percentage of samples found to contain tetracycline resistant microbes (Spearman $r = 0.837$; $p = 0.003$), but not with the percentage of samples with ampicillin resistant microbes (Spearman $r = 0.563$; $p = 0.096$).

Temporal Variation: Flushing Bay

Higher frequency sampling was conducted in Flushing Bay, NY (n=16, Table 1) to investigate patterns of temporal variation in antibiotic resistant microbes and correlations with environmental conditions, especially rainfall and sewage loading. Seven samples were collected following dry weather and nine samples were collected after rainfall.

The geometric mean for *Enterococci* at this site (250.87 CFU/100 ml, Table 1) exceeded the EPA recommended monthly geometric mean standard and the maximum recorded *Enterococci* also exceeded single sample maximum standard. All samples from Flushing Bay contained ampicillin resistant microbes and 88% contained tetracycline resistant microbes (Table 1).



Figures 2A and 2B. Antibiotic resistant heterotrophs (A: ampicillin, B: tetracycline) in the HRE are variable based on location of sampling at eleven sites. White bars represent mid-channel stations, hatched bars are near-shore stations and black bars are WWTP outfall stations. In Figure 2B, ND indicates that no resistant organisms were detected on any of the sampling collection days and the asterisk (*) indicates a mean value of less than one.

site	#	% samples w/amp resistance	% samples w/tet resistance	max amp-resistant (CFU/ml)	max tet-resistant (CFU/ml)	geomean ENT (CFU/100 ml)	max ENT (CFU/100 ml)
Battery	6	83%	0%	240	0	0	0
E.River	6	83%	17%	2800	10	0.82	31
Newtown	6	100%	67%	13800	210	58.34	3448
Harlem River	5	40%	20%	120	10	2.29	63
Harlem Piers	5	100%	20%	20600	50	3.49	86
North River WWTP	6	83%	33%	30	290	5.21	20
Sawmill	6	83%	50%	3800	120	27.71	1274
Piermont Outfall WWTP	6	100%	83%	7220	5880	20.74	134
Piermont Pier	6	100%	33%	210	30	25.92	740
Tappan Zee	6	50%	33%	50	10	1.52	41
Total	*58*	*83%*	*36%*				
Flushing Bay	16	100%	88%	9810	439	250.87	24196

Table 1. Microbial data collected from surface water samples at ten Riverkeeper patrol boat sampling sites show total antibiotic resistance (tetracycline or ampicillin), determined from heterotrophic plate counts on R2A media with and without antibiotics added. Percentages are based on the total number of samples with any resistant colonies grown divided by the total number of samples collected at the site over the study period.

The abundance of antibiotic resistant microbes in Flushing Bay increased significantly following rain events (Figure 3; Mann Whitney, ampicillin p=0.005, tetracycline p=0.007). The proportion of culturable heterotrophs that are resistant to antibiotics appears to increase in wet weather compared to periods of dry weather, but the difference was not statistically significant with the current sampling effort (Figure 4; Mann-Whitney, ampicillin p=0.173, tetracycline p=0.211).

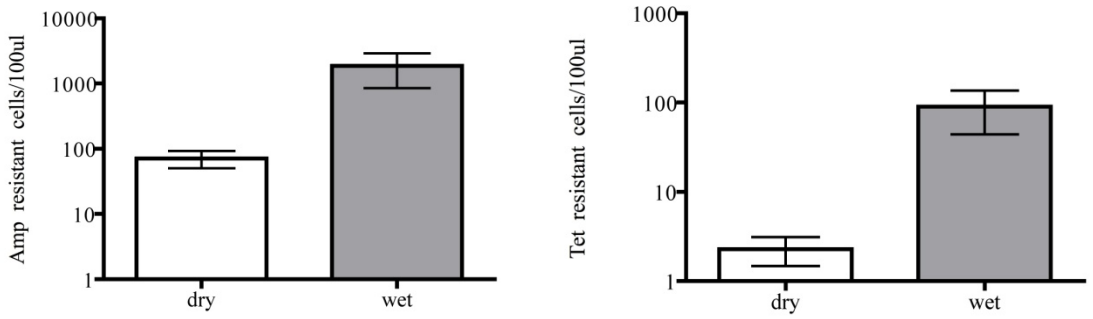


Figure 3. Abundance of cultured antibiotic resistant microbes from surface water samples at Flushing Bay following periods of dry weather (n=7) and wet weather (n=9).

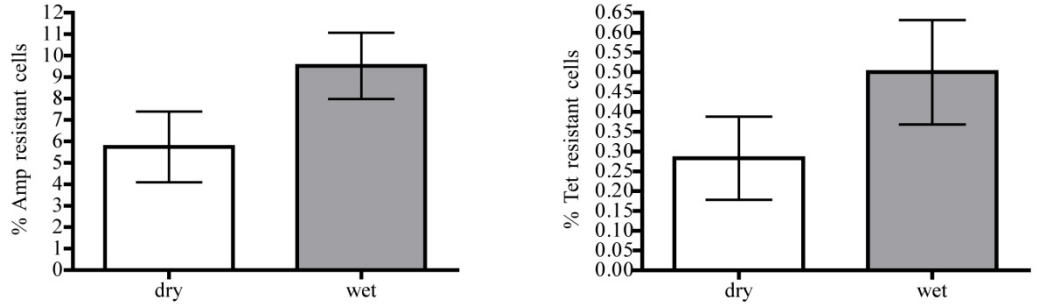


Figure 4. Proportions of culturable antibiotic resistant heterotrophs as a percentage of total culturable heterotrophs in Flushing Bay following periods of dry weather (n=7) and wet weather (n=9).

However, abundance of the sewage indicator *Enterococci* was positively correlated with resistant microbes (Figure 5; Spearman, ampicillin r=0.867 and p=0.005, tetracycline r=0.9 and p=0.002). Similarly, the abundance of ampicillin and tetracycline resistant cells were positively correlated with one another in Flushing Bay (Figure 6; Spearman r=0.918, p<0.001).

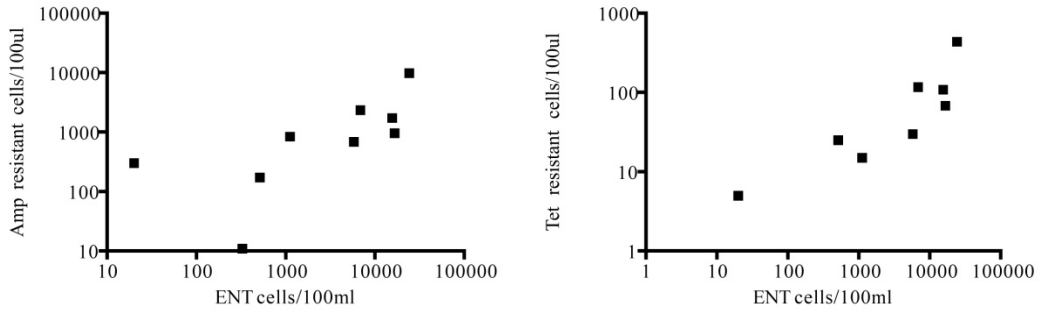


Figure 5. Positive association of antibiotic resistant heterotrophs (Amp or Tet) and sewage indicators *Enterococci (ENT)*. Ampicillin resistant microbes had a greater magnitude (note scale of axes) than tetracycline resistant microbes.

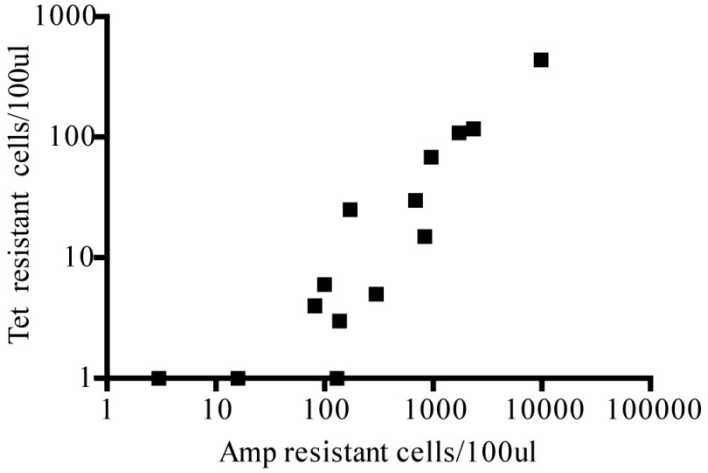


Figure 6. The abundance of both ampicillin and tetracycline resistant microbes at Flushing Bay were positively correlated.

Identification of Microbes

Proteobacteria were the most abundant phylum detected for all three types of culturable heterotrophs sequenced (Table 2). Proteobacteria accounted for 83% of total heterotroph sequences, 100% of ampicillin resistant heterotrophs, and 88% of tetracycline resistant heterotrophs. Of the total Proteobacteria sequenced from het plates, the most abundant genus was *Sphingobium*. Of the ampicillin resistant, the most abundant genus

was *Aeromonas* and of the tetracycline resistant, the most abundant genus was *Escherichia/Shigella*.

Type	Phylum	#	Genus	conf.
All heterotrophs n=23	Proteobacteria (19)	6	*Sphingobium*	80%
		4	*Acinobacter*	
		2	*Shewanella*	
		1	*Aeromonas*	
		1	*Pseudomonas*	
		1	*Psychrobacter*	
		1	*Azospirillum*	
		1	*Erythrobacter*	
			unclassified	
		1	*Gammaproteobacteria*	
	Actinobacteria (2)	1	*Arthrobacter*	
		1	*Brachybacterium*	
	Bacteroidetes (2)	2	*Flavobacterium*	
Amp-resistant heterotrophs n=60	Proteobacteria (60)	24	*Aeromonas*	95%
		19	*Pseudomonas*	
		7	*Stenotrophomonas*	
		2	*Brevundimonas*	
		2	*Comamonas*	
		1	*Variovorax*	
		1	*Acidovorax*	
		1	*Delftia*	
		1	*Escherichia/Shigella*	
		1	*unclassified Enterobacteriaceae*	
		1	*unidentified Xanthomonadaceae*	
Tet-resistant heterotrophs n=16	Proteobacteria (14)	2	*Actinobacter*	95%
		1	*Klebsiella*	
		1	*Citrobacter*	
		6	*Escherichia/Shigella*	
		3	*Stenotrophomonas*	
		1	*unidentified Xanthomonadaceae*	
	Bacteroidetes (2)	1	*Chryseobacterium*	
		1	*Flavobacterium*	

Table 2. Classifications of 16S rRNA sequences picked from Flushing Bay surface water samples based on Ribosomal Database Project (www.rdp.msu.edu). Results are reported at highest confidence interval at which the genus could be identified: 80% for het, 95% for amp and tet.

Aeromonas and *Pseudomonas* accounted for 72% of the ampicillin resistant sequences identified. The most abundant genera of resistant bacteria identified, *Aeromonas, Pseudomonas, Stenophomonas*, and *Escherichia/Shigella*, all contain opportunistic pathogens that have been associated with antibiotic resistant infections (e.g. Varley et al. 2009).

DISCUSSION

Spatial Variation

Antibiotic resistance was found to be wide spread and highly variable throughout the lower HRE. Of the 56 total water samples collected, a majority (86%, Table 1) showed some level of antibiotic resistant bacteria, indicating that antibiotic microbes are commonly present across most of the lower estuary. The scope of this study does not specifically address where or how these resistant microbes develop resistance, but as discussed in the introduction, waterways may act as incubators for conferring resistance genes through horizontal gene transfer. The spread of resistance may be fostered in sewer systems, treatment plants and in near-shore environments where raw sewage is released.

The greatest mean values of ampicillin resistant microbes (Figure 2) and the highest maximum values of sewage indicators (Table 1) were recorded at three sites with historically high sewage contamination based on a four year sewage loading dataset collected by Riverkeeper (www.riverkeeper.org/water-quality). These sites are considered hot spots for contamination and include two tributaries (Newtown Creek and Sawmill River) and the Orangetown WWTP effluent at the Piermont Outfall. Maximum

levels of *Enterococci* were the highest in Newtown Creek and Sawmill River, both tributaries of the Hudson River. Microbial loads (het, Tet, Amp, and Entero counts) increased in tributaries following rainfall events and runoff. The high levels of *Enterococci* found at Newtown Creek and Sawmill River confirm that urban tributaries were a source of sewage loading into the major Hudson River during the course of this study.

Point sources near the shore also appear to contribute sewage to the Hudson River, evidenced by the high levels of sewage indicators measured from near-shore environments as compared to mid-river sampling sites (Figure 2). One such point source is a CSO, where wet weather release of combined sewage occurs. All monitoring cruises occurred during dry weather except for August sampling, which occurred after significant rainfall. Wet weather causes the delivery of sewage through CSOs, so the analysis of grouped data from the samples collected in this study are likely underestimated due to the predominance of dry weather during the Riverkeeper sampling days used for this study. The abundance of antibiotic resistant heterotrophs increased after wet weather events based on data collected from Riverkeeper sites. The highest maximum level of ampicillin resistance was recorded at the 125th Street Harlem Piers, following the wet weather in August. Raw data show either no resistance or low levels of resistance on dry sampling days at the Harlem Piers. The sampling point is directly adjacent to a CSO, and the extremely high value of resistant microbes recorded during wet weather is likely related to the release of sewage through the CSO during a rain event.

The highest measurements of tetracycline resistance and the third highest measurements of ampicillin resistance were recorded at the Piermont Outfall (maximum

values reported in Table 1). The Piermont Outfall site is located directly at the effluent release from the Orangetown WWTP in Orange County, NY. This plant is known to have significant problems with disinfection processes (State of New York 2010) and is known to commonly have high fecal indicator bacteria counts associated with effluent (Riverkeeper 2011a; Michaels 2008). The other wastewater treatment plant sampled, North River (125th Street WWTP) had much lower levels of both antibiotic resistance and sewage indicators. Although the North River WWTP serves most of northern Manhattan and receives a much larger sewage load than the Orangetown plant, this result is not surprising since the North River WWTP is among the newest wastewater treatment plants in NYC and has been found to have low *Enterococcus* signals over the last four years relative to the Piermont Outfall (Riverkeeper 2011b). The efficiency of disinfection can vary widely across treatment plants and some have been suggested to be reservoirs causing the spread of antibiotic resistance that can then be released into the environment. Kim et al. (2010) found that tetracycline resistant bacteria were less efficiently removed by NYC WWTPs using UV versus chlorination for disinfection. The primary clarifier effluent in NYC WWTPs sampled by Kim et al. (2010) was found to contain tetracycline resistant bacteria in concentrations ranging from 10^4 to 10^5 CFUs per milliliter. Based on Kim's data, sewage in CSOs from the NYC area may contain tetracycline resistant microbes at concentrations from 100 to 10,000 times the maximum surface water concentrations detected at most sites in this study. Kim et al. (2010) also found that, despite disinfection, the effluent from NYC WWTPs still contained tetracycline resistant bacteria in concentrations from 10^1 to 10^3 CFUs per ml. Given the predominance of dry weather during our sampling, it is not surprising that the highest tetracycline resistant

counts were found near the effluent of waste water treatment plants. It also suggests that the Orangetown WWTP's Piermont outfall, where a concentration of 5880 CFUs per ml was detected (Table 1), is inefficiently removing antibiotic resistant bacteria and is further evidence that disinfection procedures at this plant require additional attention.

Ampicillin resistance was found in much greater abundance than tetracycline resistance estuary-wide. Both antibiotics have been on the market for a similar length of time, so exposure time is not a likely explanation for the difference. The frequency of prescription or a difference in inherent resistance may explain greater abundance of organisms resistant to ampicillin than tetracycline.

Temporal Variation: Flushing Bay

Flushing Bay is an outlet for multiple CSOs and is surrounded by a highly urbanized industrial region, as well as LaGuardia Airport. In Flushing Bay, analyses showed significantly higher levels of resistant microbes and sewage indicators following wet weather as compared to dry weather (Figure 3). Over the course of the sampling period, *Enterococci* counts increased during wet weather, as would be predicted from earlier studies in the HRE (e.g. Young and Bower 2008). Diverted raw sewage from combined sewers can be detected at outfalls during rain events and antibiotic resistant microbes are in greater abundance during these overflow events. Total heterotrophs and resistant heterotrophs increased significantly during wet weather, demonstrating elevated microbial inputs from sewage loading.

The correlation of *Enterococcus* and antibiotic resistance (Figure 5) in combination with the increased levels of antibiotic resistance following wet weather (Figure 3) strongly suggests that sewage loading through CSOs introduces antibiotic

resistant microbes into Flushing Bay. The correlation of antibiotic resistance with sewage indicators, such as *Enterococcus*, indicates the value in fecal indicator monitoring programs. It also suggests the ability of fecal indicators to predict the abundance of other potentially harmful bacteria, such as resistant microbes, that provide a concern for public health.

The correlation of tetracycline resistant and ampicillin resistant microbes (Figure 6) also supports a shared source and may suggest that some microbes could be multi-drug resistant. Based on these data, testing for resistance to other antibiotics, such as vancomycin or streptomycin, would be predicted to produce similar patterns of distribution and environmental association to wet weather and sewage loading. Further molecular testing or characterization of cultures would be necessary to assess the extent of multi-drug resistance in the environment and in the colonies isolated in this study.

Identification of Microbes

These data provide a preliminary investigation of the diversity and identity of antibiotic resistant microbes in the HRE. The most abundant genera identified are known to contain opportunistic pathogens (Table 2) suggesting that these antibiotic resistant microbes are of potential concern to recreational users, especially immuno-compromised individuals. Potential human pathogens were isolated from both Amp and Tet plates. These include: *Aeromonas, Pseudomonas, Arthrobacter, Klebsiella* and *Escherichia/Shigella*. Many *Aeromonas* species, such as *Aeromonas hydrophilia*, are known to be associated with gastrointestinal disease in humans and infections in fish. The most common tetracycline resistant genus was *Escherichia/Shigella*, enteric bacterial groups that include known pathogens and are found in high density within human and

animal waste. Based on this limited sampling of sequences, the ampicillin resistant colonies were found to be less diverse (all Proteobacteria) than the heterotrophs (Proteobacteria, Actinobacteria and Bacteroidetes) and the tetracycline resistant bacteria (Proteobacteria and Bacteroidetes). However, larger sequence libraries would be needed to make additional quantitative statements about the relative diversity of the resistant bacteria or the connection of diversity patterns to environmental conditions. This is an area that the authors expect to pursue with future studies. These data also demonstrate the importance of conducting future risk assessment studies of human exposure to these resistant pathogens, especially in recreational waterways during warm weather where contact is more likely to occur.

RECOMMENDATIONS

This work has confirmed that antibiotic resistant microbes are widespread in the HRE system. The study has clear management implications related to water quality and public health, given the promotion of on-water recreational activities along the waterfront in New York City. Decades of historical records exist for *Enterococci* and other fecal indicator organisms in the New York Harbor Water Quality Surveys dating back to 1909 (City of New York 2011b). These measurements are common and the correlation of antibiotic resistance with fecal indicators (*Enterococci*) strongly supports the value of measuring such indicators as representative of other sources of sewage related concern in the system. The persistence and transport of resistant pathogens in the HRE is currently unclear, and potential reservoirs within the system have yet to be confirmed.

The extent of the public health threat requires additional research and epidemiological study.

Urban stormwater management policy should integrate the linkage between wet weather discharges and potential spread of antibiotic resistant infections. Serious investigation should be pursued regarding the sources of antibiotics and antibiotic resistant microbes and possible solutions including: reduced CSO volumes through sustainable and green infrastructure; more discriminating prescription practices by the local healthcare community; potential upgrades to WWTPs to attempt to remove antibiotics and other emerging contaminants of great concern to society.

ACKNOWLEDGEMENTS

This report would not have been possible without the generous support provided by: The Hudson River Foundation, the Tibor T. Polgar committee, The Wallace Foundation and Riverkeeper. The authors gratefully acknowledge the assistance of Captain John Lipscomb, Carol Knudsen, Dr. Andy Juhl, and the members of the O'Mullan Lab at Queens College of CUNY: Eli Dueker, Brian Brigham, Maren Mellendorf, Liz Bisbee and Simon Lax.

REFERENCES

Alonso, A., P. Sanchez, and J.L. Martinez. 2001. Environmental selection of antibiotic resistance genes. Environmental Microbiology 3: 1-9.

City of New York. 2011a. Combined Sewer Overflows, New York City Department of Environmental Protection. http://www.nyc.gov/html/dep/html/ stormwater/combined_sewer_overflow.shtmlh (accessed Feb 3 2011).

City of New York. 2011b. New York Harbor Water Quality Surveys, New York City Department of Environmental Protection. http://www.nyc.gov/html/ dep/html/harborwater/harborwater_quality_survey.shtml (accessed Feb 3 2011).

Conly, J. 1998. Controlling antibiotic resistance by quelling the epidemic of overuse and misuse of antibiotics. Canadian Family Physician 44: 1769-1773.

Costanzo, S. D., J. Murby, and J. Bates. 2005. Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Bulletin 51: 218-223.

Goni-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin. 2000. Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp. Applied Environmental Microbiology 66: 125-132.

Guardabassi, L., A. Petersen, J.E. Olsen, and A. Dalsgaard. 1998. Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Applied Environmental Microbiology 64: 3499-3502.

Kim, S., H. Park, and K. Chandran. 2010. Propensity of activated sludge to amplify or attenuate tetracycline resistance genes and tetracycline resistant bacteria: A mathematical modeling approach. Chemosphere 78: 1071-1077.

Lampman, G. G., N. F. Caraco, and J.J. Cole. 1999. Spatial and temporal patterns of nutrient concentration and export in the tidal Hudson River. Estuaries 22: 285-296.

Lane, D. J. 1991. 16S/23S rRNA sequencing, p. 115–174. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chishester, United Kingdom.

Michaels, C. 2008. Swimmable River: Towards Better Water Quality Monitoring in the Hudson River Estuary. Final Report to Riverkeeper. New York, NY. http://www.riverkeeper.org/wp-content/uploads/2009/06/Swimmable_ River_ Report_final.pdf (accessed online Feb 3 2011).

Overbye, K. M. and J. F. Barrett 2005. Antibiotics: where did we go wrong. Drug Discovery Today 10: 45-52.

Reasoner, D. J. 2004. Heterotrophic plate count methodology in the United States. International Journal of Food Microbiology 92: 307-315.

Reinthaler, F. F., J. Posch, G. Feierl, G. Wust, D. Haas, G. Ruckenbauer, F. Mascher, and E. Marth. 2003. Antibiotic resistance of E. coli in sewage and sludge. Water Research 37: 1685-1690.

Riverkeeper. 2011a. Piermont Wastewater Treatment Plant Outfall, Water Quality. http://www.riverkeeper.org/water-quality/locations/rockland-westchester/piermont-stp// (accessed Feb 3 2011).

Riverkeeper. 2011b. 125th Street Wastewater Treatment Plant Outfall, Water Quality. http://www.riverkeeper.org/water-quality/locations/nyc-hudson-bergen/125th-st-wt/ (accessed Feb 3 2011).

State of New York. 2010. Town of Orangetown Wastewater Capital Project Report of Examination, Office of the Comptroller. http://www.orangetown.com/docs/Orangetown_T.pdf (accessed Feb 7 2011).

Varley, A., H. Williams, and S. Fletcher. 2009. Antibiotic resistance in the intensive case unit. Continuing Education in Anaesthesia, Critical Care and Pain 9: 114-118.

Wise, R., T. Hart, O. Cars, M. Streulens, R. Helmuth, P. Huovinen, and M. Sprenger. 1998. "Antimicrobial resistance. Is a major threat to public health." British Medical Journal 317: 609-610.

Young, S. and P. Bower. 2008. Measurements of *Enterococci* bacteria in the Hudson River: Environmental health issues and policy recommendations for combined sewer overflow. Section II-1-39 in C.A. McGlynn and J. Waldman (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2007. Hudson River Foundation, New York, New York.

INVASIVE-SPECIES REMOVALS AND NITROGEN-REMOVAL ECOSYSTEM SERVICES IN FRESHWATER TIDAL MARSHES

A Final Report to the Tibor T. Polgar Fellowship Program

Mary Katherine Alldred

Polgar Fellow

Ecology and Evolution
Stony Brook University
Stony Brook, NY 11794

Project Advisor:

Stephen B. Baines
Ecology and Evolution
Stony Brook University
Stony Brook, NY 11794

Alldred, M. K. and S. B. Baines. 2012. Invasive-Species Removals and Nitrogen-Removal Ecosystem Services in Freshwater Tidal Marshes. Section IV: 1-23 pp. In S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation

ABSTRACT

Two major management goals in the Hudson River Estuary are the control of invasive species such as *Phragmites australis* and the maintenance of water quality. Species invasions and invasive species removals have the potential to alter the fraction of nitrogen that is removed from wetland sediments via denitrification. Several small-scale *Phragmites australis* removals were performed in Ramshorn Marsh, a freshwater tidal marsh of the upper Hudson River, in late September 2010. This report includes data from two pre-removal sampling efforts in late August of 2009 and 2010, with the ultimate goal of characterizing the response of denitrification potential to the removal of an invasive plant community. Pre-removal data indicate that the selected reference sites, West Flats and Brandow Point, do not differ from Ramshorn Marsh in terms of denitrification potential or important sediment characteristics and will therefore be useful as reference sites for post-removal comparisons. Differences in denitrification potential were not detected between sediments dominated by native and invasive vegetation; however, significant interannual variations in denitrification potential and organic content of sediments were detected in Ramshorn Marsh. These results suggest that repeated sampling efforts throughout the growing season will be essential to understanding plant-mediated effects on sediment dynamics.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Two major management goals in the freshwater tidal marshes of the upper Hudson River are the control of invasive species and the maintenance of water quality. Nitrogen pollution from non-point sources is a major concern in the Hudson watershed (USACE 2009). Delivery of excess nitrogen to downstream salt marshes and estuaries can result in eutrophication, hypoxia, and harmful algal blooms, all of which can have severe consequences for the economy and human health (Vitousek et al. 1997; Bertness et al. 2002; Hinga et al. 2005). Marshes provide a valuable ecosystem service as sites of active denitrification, a microbial process in which nitrate is permanently removed from an ecosystem as nitrogen gas (Zedler 2003).

Marsh plants are generally understood to play an important role in nitrogen removal, either directly by assimilating nitrate and ammonium, or indirectly by altering sediment characteristics to favor denitrification (Caffrey et al. 2007). Denitrification requires that anoxic conditions (or very low oxygen concentrations) accompany the presence of organic carbon (as an energy source) and nitrate (as an oxidant) in sediments. Marsh plants may alter denitrification rates by directly or indirectly affecting the availability of any of these three components. The degree to which plants alter organic matter and nitrogen availability will depend on their own nutrient demands, their productivity, and the quality of the litter they produce. Denitrification could be depressed if plants have high nitrogen demands or if they produce low quality litter to promote immobilization of inorganic nitrogen by sediment microbes, as *Phragmites* has been suggested to produce (Meyerson et al. 2000b). Alternatively, plants that produce more

easily decomposed litter with a high nitrogen content could enhance denitrification by increasing the availability of inorganic nitrogen and organic carbon substrates.

Plants and sediment microbes are also likely to influence denitrification through the daily cycle of oxygen concentrations in sediments. Because sediments that are saturated with water tend to become anoxic, the major mineralized form of inorganic nitrogen in marsh sediments tends to be ammonium (the reduced form of mineral nitrogen). Denitrification cannot occur unless nitrate diffuses into the anoxic sediments, or the ammonium in those sediments is converted to nitrate via nitrification, which requires the presence of oxygen. Therefore, denitrification tends to occur at maximum rates at oxic/anoxic boundaries, where nitrate can diffuse into anoxic sediments, or where sediments alternate between oxygenated and non-oxygenated states over time (Seitzinger et al. 2006). Near plant roots, oxygen increases during the day, while at night this oxygen is rapidly consumed by sediment organisms. The diel pumping of oxygen into plant roots increases the spatial and temporal variability of oxygen in sediments, thus maximizing the potential for denitrification to occur (Bodelier et al. 1996).

Because marsh plants differ in many of the key characteristics expected to affect denitrification rates (e.g. productivity, nutrient demands, and root-zone oxygenation) (Windham and Meyerson 2003), species invasions and invasive-species removals may be expected to modify nitrogen-removal ecosystem services that marshes provide. In freshwater tidal marshes of the Hudson, replacement of the native marsh grasses, predominantly *Typha angustifolia*, by the invader *Phragmites australis* has caused considerable decreases in the native diversity of plants, insects, birds, and other wildlife (Chambers et al. 1999; Meyerson et al. 2000a). Control and removal of *Phragmites* is

thus an important management goal for the Hudson River Estuary (Kiviat 2006). Removal of *Phragmites* from small patches of freshwater marshes is often successful in promoting the regrowth of native plants and restoring native plant diversity (Farnsworth and Meyerson 1999; Meyerson et al. 2000a). However, the effects of *Phragmites* removals on nitrogen-removal ecosystem services have been less well-documented (Findlay et al. 2003).

A glyphosate-herbicide removal was performed in several small (<2 acre) patches of *Phragmites australis* in Ramshorn Marsh (also Catskill Marsh), a freshwater tidal marsh of the upper Hudson River. Here, the results of two pre-removal monitoring efforts are reported, which were conducted in late August of 2009 and 2010, with the purpose of characterizing sediment characteristics and denitrification potential of the site of future *Phragmites* removal, as well as two similar marshes not undergoing removal that will serve as reference sites. Data collected thus far will be used to answer three major questions:

1) Do West Flats and Brandow Point differ from Ramshorn Marsh in denitrification potential or any sediment characteristics, such that they would be inappropriate for use as reference sites?
2) Do denitrification potentials or sediment characteristics differ between *Phragmites*- and *Typha*-dominated sediments within Ramshorn Marsh?
3) Do denitrification potentials and sediment characteristics in Ramshorn Marsh differ interannually?

These results will lay the basis for a larger study that will examine the effects of *Phragmites australis* removal, and subsequent recolonization by the native vegetation, on

denitrification potential, and thus the capacity of marsh sediments to act as a sink for nitrogen.

METHODS

STUDY DESIGN AND SITES

Ramshorn Marsh was first sampled in August 2009, with the goal of adequately assessing the variation in denitrification potential and sediment characteristics for each *Phragmites* patch prior to removal with herbicide. Due to a delay in herbicide treatment, the pre-removal assessment of Ramshorn Marsh was repeated in August 2010, and additional data were collected on two reference sites, West Flats and Brandow Point (Figure 1).

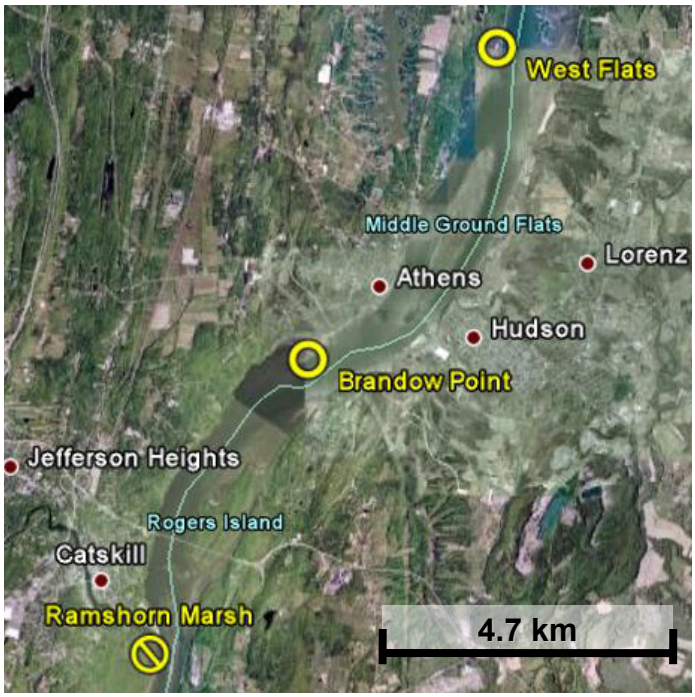


Figure 1: Study sites sampled in August 2010. Ramshorn Marsh (also Catskill Marsh) was the site of *Phragmites* removals, and West Flats and Brandow Point were sampled as reference *Phragmites* stands that will not undergo removal.

In August 2009, four locations were sampled within each *Phragmites* patch of Ramshorn Marsh (Fig. 2A). In August 2010, two locations were sampled within each *Phragmites* patch of West Flats (Fig. 2B) and Brandow Point (Fig. 2C). For the Ramshorn Marsh patches (Fig. 2A), one location was sampled in each *Phragmites* patch and in an adjacent patch of native vegetation (predominantly *Typha angustifolia*). For each sample location in 2009 and 2010, one nutrient profile was obtained using a PVC

equilibrator, and duplicate sediment cores, each with a total depth of about 15 cm, were collected for organic-content and denitrification-potential measurements.

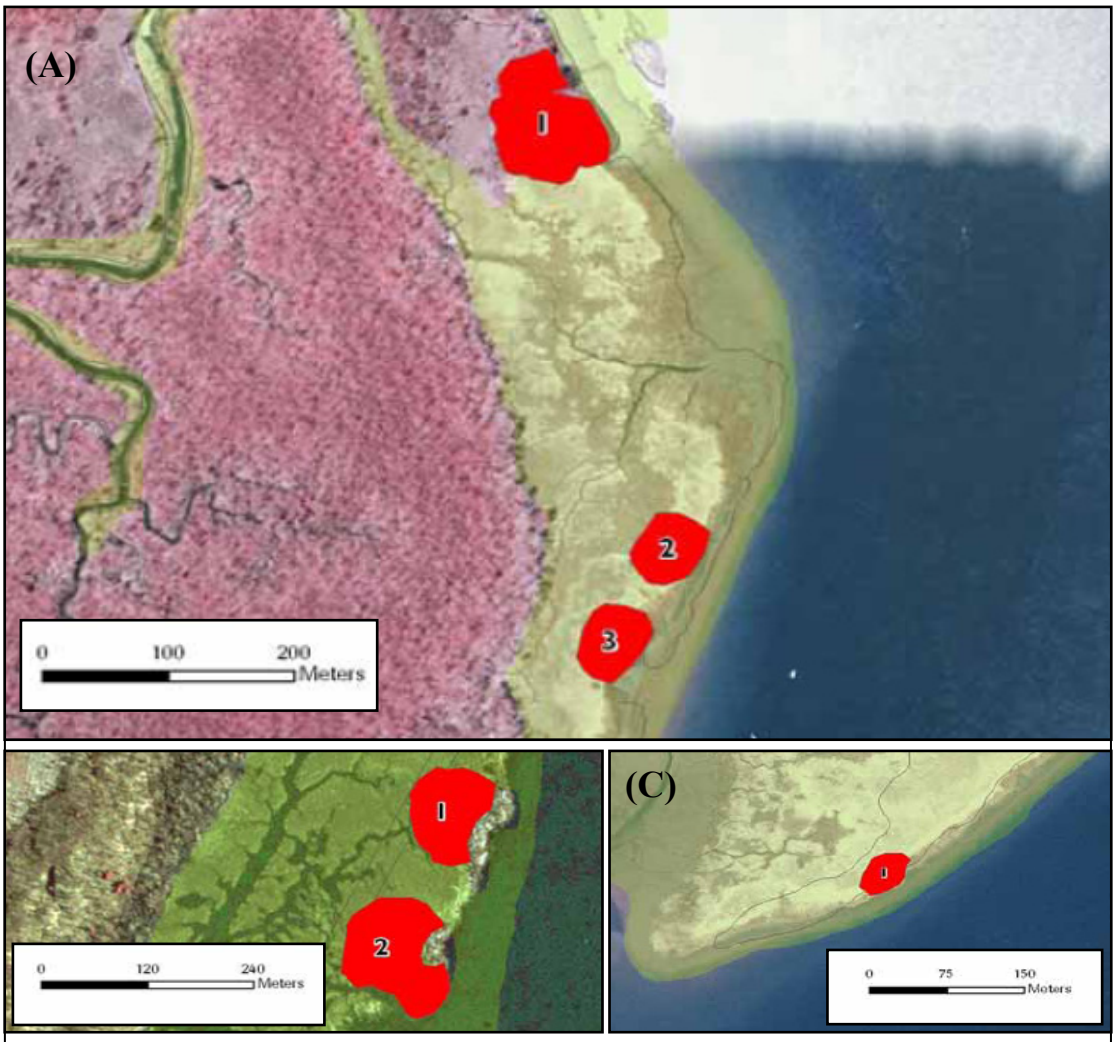


Figure 2: Extent of *Phragmites australis* stands sampled for this study in (A) Ramshorn Marsh, (B) West Flats, and (C) Brandow Point. Maps modified from Zimmerman and Shirer (2009).

SEDIMENT ANALYSES

Pore-water samples were collected at each sampling location using PVC equilibrators, each containing two 12 ml sampling cavities spaced vertically at 3 cm intervals. Equilibrators were prepared in the laboratory using deoxygenated water and Spectr-Por cellulose membranes and allowed to equilibrate in the vegetation sites for

eight days. Pore water was collected immediately upon removal from sediments using a syringe, after which it was acidified and stored in 20 ml scintillation vials. In August 2009, samples were analyzed for nitrate and ammonium via ion chromatography using a Lachat Flow Injection Analyzer (Lachat, Loveland, CO). In August 2010, samples were analyzed for nitrate and ammonium using standard colorimetric techniques (Jones 1984; Parsons et al. 1984). Each duplicate sediment core was homogenized and a ~5.0 g subsample analyzed for sediment water content and total organic matter. Organic content was measured as loss after combustion at 450° C for 4 hours.

DENITRIFICATION POTENTIALS

Denitrification enzyme activity (DEA) measurements provide an estimate of the maximum potential of the microbial community to perform denitrification under ideal conditions (Smith and Tiedje 1979). Because these measurements are less sensitive to transient nutrient and oxygen availability in the field, they are useful in comparing responses in denitrification (or the denitrifier community) among field sites and experimental treatments (Groffman et al. 2006). For this analysis, sediment subsamples from duplicate sediment cores (~5.0 g) were amended with KNO_3, glucose, chloramphenicol, and acetylene, and incubated under anaerobic conditions for 90 minutes, with gas sampling at 30 and 90 minutes. Gas samples were stored in evacuated septum vials and analyzed for N_2O by electron-capture gas chromatography.

STATISTICAL ANALYSES

Organic content and denitrification potential were analyzed using two-level, nested ANOVAs with unequal samples size. The three independent variables analyzed were site (Ramshorn, West Flats, and Brandow Point), vegetation (native and invasive),

and year (2009 and 2010). Patches were nested within these variables to determine whether variation was greater among the variables, or among patches within each variable. Percent organic matter was arcsine-square-root transformed prior to analysis to achieve homoscedasticity. Pore-water ammonium and nitrate were analyzed using three-level, nested ANOVAs with unequal sample size. The same independent variables were analyzed; however, depth within the sediment was added as an additional level, nested within sampling patch. Because the sampling design was necessarily unbalanced, Satterthwaite approximations were used to calculate error degrees of freedom and mean squares for "site" and "year." All analyses were performed in SAS (SAS 2002-2008).

RESULTS

TREATMENT AND REFERENCE SITES

Pore-water ammonium ranged between 0 and 0.5 mg/L and did not vary significantly among the three sites (Figure 3), nor among patches within sites (Table 1). Nitrate concentrations did not vary significantly among sites or patches (Table 2), and mean concentrations remained below detectable limits across all sampling locations.

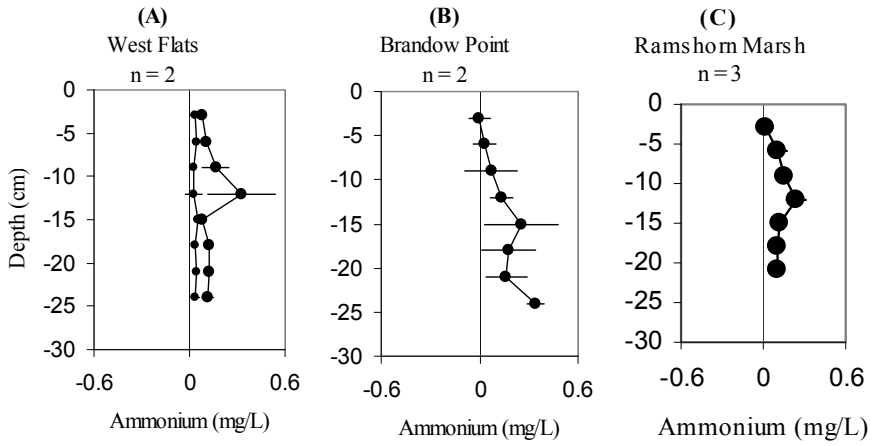


Figure 3: Ammonium sediment-pore-water profiles for (A) West Flats, (B) Brandow Point, and (C) Ramshorn Marsh in August 2010. Error bars show standard error.

Table 1: ANOVA Table: Pore-water ammonium in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.019311	0.009655	0.21	0.8401
Error	0.8868	0.040126	0.045247		
Patch	1	0.039549	0.039549	3.06	0.0945
Depth	21	0.270955	0.012903	0.99	0.4924
Error	44	0.573220	0.013028		

Table 2: ANOVA Table: Pore-water nitrate in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.053782	0.026891	0.31	0.8082
Error	0.5976	0.051727	0.086559		
Patch	1	0.092118	0.092118	1.44	0.2436
Depth	21	1.344206	0.064010	0.54	0.9341
Error	44	5.191765	0.117995		

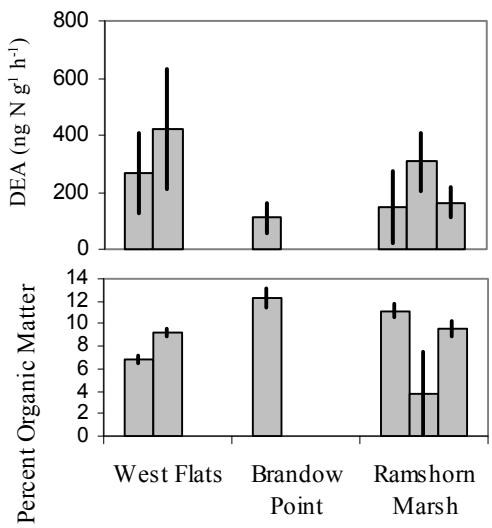


Figure 4: Percent organic matter and denitrification potential in reference and treatment patches (n_i = 4, 4, and 2).

Sediment organic matter did not differ among reference and treatment sites but did differ significantly among patches within sites (p = 0.021, Table 3). Though the F-value for sites appears large (F = 5.40, Table 4), no significant difference was detected in denitrification potential among sites. This result may be due to a lack of power. However, mean values for DEAs and organic matter in Ramshorn Marsh generally fall within the range of those measured at the two reference sites (Fig. 4).

Table 3: ANOVA Table: Sediment organic matter in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	0.0222	0.0111	0.56	0.627
Error	2.75	0.0551	0.0200		
Patch	3	0.0501	0.0167	4.75	0.021
Error	12	0.0422	0.0035		

Table 4: ANOVA Table: Denitrification potential in reference and treatment sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Site	2	159276	79683	5.40	0.424
Error	0.57	8466	14741		
Patch	3	78000	26000	0.37	0.779
Error	12	852419	71035		

NATIVE AND INVASIVE SITES

Pore-water ammonium did not differ between *Phragmites*- and *Typha*-dominated locations in Ramshorn Marsh (Table 5); however, they did differ significantly by depth ($p = 0.014$, Table 5), with the largest ammonium concentrations occurring approximately 12 cm from the sediment surface (Fig. 5). Nitrate did not differ significantly between vegetation types, nor among patches or depths (Table 6).

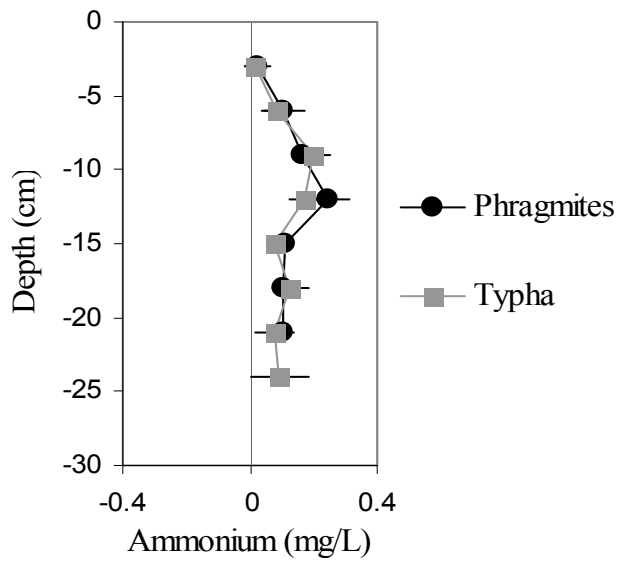


Figure 5: Ammonium sediment-pore-water profiles for *Phragmites*- and *Typha*-dominated patches in Ramshorn Marsh ($n_i = 3$).

Organic matter content and denitrification potential were similar across vegetation types (Fig. 6). Organic matter content (Table 7) and denitrification potential (Table 8) did not vary significantly between *Phragmites*- and *Typha*-dominated locations within Ramshorn Marsh, nor did they vary among patches within sites.

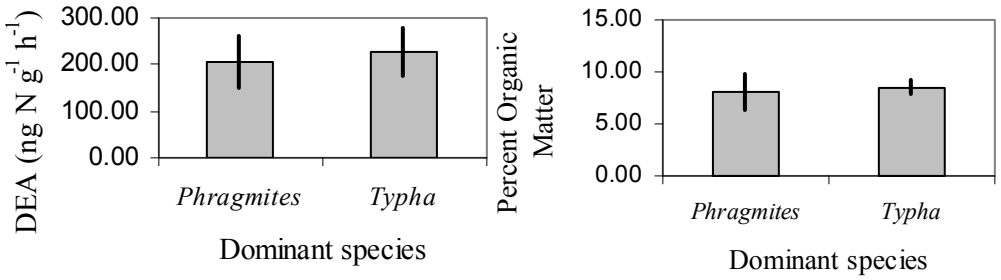


Figure 6: Denitrification potential and percent organic matter of *Phragmites*- and *Typha*- dominated patches in Ramshorn Marsh (n_i = 6).

Table 5: ANOVA Table: Pore-water ammonium in *Phragmites*- and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0014	0.0014	0.05	0.839
Error	2.00	0.0516	0.0258		
Patch	2	0.0517	0.0258	2.65	0.095
Depth	20	0.1944	0.0097	2.83	0.014
Error	19	0.0651	0.0034		

Table 6: ANOVA Table: Pore-water nitrate in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0132	0.0132	0.84	0.456
Error	2.00	0.0315	0.0158		
Patch	2	0.0316	0.0158	2.47	0.110
Depth	20	0.1279	0.0064	1.70	0.126
Error	19	0.0714	0.0038		

Table 7: ANOVA Table: Sediment organic matter in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	0.0033	0.0033	0.27	0.630
Patch	4	0.0492	0.0123	1.80	0.247
Error	6	0.0409	0.0068		

Table 8: ANOVA Table: Denitrification potential in *Phragmites* and *Typha*-dominated sites.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Vegetation	1	1256	1256	0.14	0.728
Patch	4	36002	9000	0.41	0.797
Error	6	132021	22004		

INTERANNUAL VARIATION

Ammonium and nitrate concentrations did not differ between years in *Phragmites*-dominated patches of Ramshorn Marsh (Fig. 7), and ammonium concentrations were similar among replicate patches and across depths (Table 9). While nitrate concentrations differed significantly among replicate patches (p = < 0.0001, Table 10) and depths (p = 0.046, Table 10), average concentrations were near or below detectable limits (Fig. 7); however, nitrate was occasionally detected in individual samples, normally at shallow depths (Fig. 7). Percent organic matter (p = 0.017, Table 11) and denitrification potentials (p = < 0.0001, Table 12) differed significantly between sampling years, with an average 78% decrease in denitrification potential and 54% decrease in organic matter between 2009 and 2010 (Fig. 8). These large differences in denitrification potential and organic matter between sampling years were consistent across all *Phragmites*-dominated patches in Ramshorn Marsh (Tables 11 and 12).

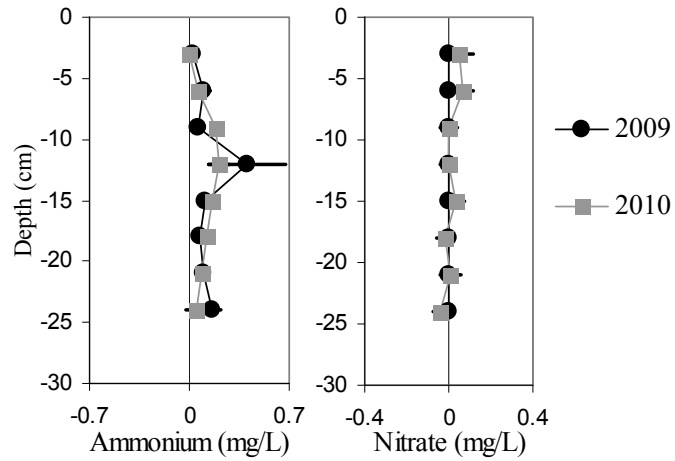


Figure 7: Ammonium profiles for *Phragmites*-dominated patches in Ramshorn Marsh in 2009 (n_i = 12) and 2010 (n_i = 3).

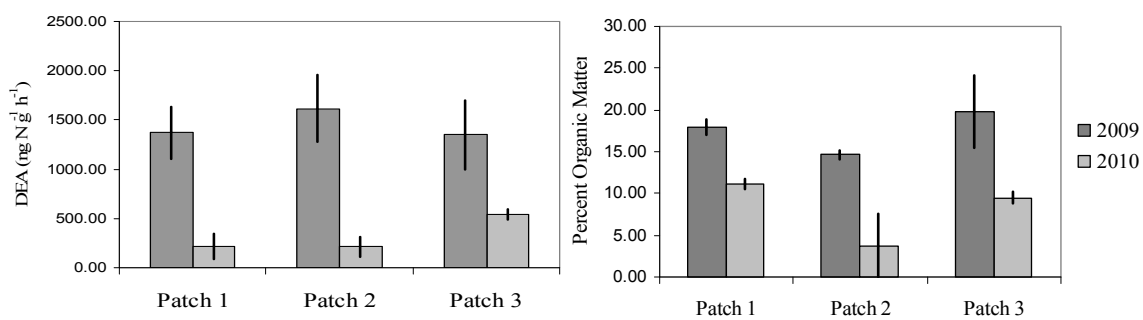


Figure 8: Denitrification potentials and organic matter content in Ramshorn Marsh in 2009 (n_i = 8) and 2010 (n_i = 2).

Table 9: ANOVA Table: Pore-water ammonium in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.0018	0.0018	0.53	0.536
Error	2.22	0.0076	0.0034		
Patch	2	0.0065	0.0033	0.03	0.969
Depth	21	2.1732	0.1034	0.89	0.609
Error	88	10.2830			

Table 10: ANOVA Table: Pore-water nitrate in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.0020	0.0020	0.09	0.788
Error	2.00	0.0423			
Patch	2	0.0423	0.0212	22.87	< 0.0001
Depth	21	0.0194	0.0009	1.70	0.046
Error	88	0.0479	0.0005		

Table 11: ANOVA Table: Percent organic matter in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	0.1192	0.1192	8.79	0.017
Error	8.28	0.1123	0.0136		
Patch	4	0.0579	0.0145	1.21	0.332
Error	24	0.2868	0.0120		

Table 12: ANOVA Table: Denitrification potential in *Phragmites*-dominated patches of Ramshorn Marsh in 2009 and 2010.

Source of Error	df	Sum of Squares	Mean Square	F Value	p
Year	1	7502020	7502020	22.98	<0.0001
Error	27.10	8849708	326526		
Patch	4	421485	105371	0.15	0.963
Error	24	17272550	719690		

DISCUSSION

Results confirm that West Flats and Brandow Point do not differ from Ramshorn Marsh in sediment nutrient concentrations, organic matter, or denitrification potential. Given the similarity among these sites, West Flats and Brandow Point will serve as useful reference sites for testing the response in denitrification potential and sediment characteristics to the *Phragmites*-removal treatment at Ramshorn Marsh next summer.

Additionally, the variation in pore-water ammonium and sediment organic content was found to be greater among the patches of a single site than among the different sites (Tables 1 and 3, respectively). This within-site variation will be useful in the future because it will allow for assessing variation in the response of sediment characteristics and denitrification potential, if any, to the removal of *Phragmites*, relative to native vegetation patches and intact *Phragmites* patches.

Despite differences between *Phragmites* and *Typha* in characteristics that may be expected to influence sediment characteristics and denitrification potential (Windham and Meyerson 2003), no differences in these variables between *Phragmites*- and *Typha*-dominated sediments were detected (Fig. 5-6). This lack of difference between *Phragmites*- and *Typha*-dominated sites may result from sufficient similarity between these two species, such that abiotic factors (temperature, sediment type, etc.) (Boyer et al. 2006) are more important drivers than plant-mediated factors in driving nutrient availability and denitrification rates. However, the lack of a difference between *Phragmites*- and *Typha*-dominated stands may also be an artifact of collecting field measurements at the end of August, which coincides with the end of growing season in these systems. Differences in plant characteristics (especially productivity, nutrient uptake, and root-zone oxygenation) may vary as biomass accumulates and as peak biomass is reached (Vitousek and Reiners 1975). Such seasonality has been observed in agricultural soils, in which vegetated soils experience peak denitrification rates during the early growing season, with these rates steadily decreasing as the plant community reaches peak biomass (Parsons et al. 1991).

Perhaps the most surprising result of this study was a very large difference in denitrification potential and sediment organic content in Ramshorn Marsh between 2009 and 2010 (Fig. 8). Given the consistency of this effect across all three patches (Tables 11-12), the similarity in organic-matter measurements across all sampling sites (Fig. 4), and the simplicity of organic-matter measurement methods, this interannual variation is probably a true difference and not a result of measurement error. One possible explanation for this difference is the large difference in summer weather between 2009 and 2010. In 2009, mean and maximum summer temperatures (May-August) were lower (19.97°C and 25.76°C, respectively), relative to mean and maximum summer temperatures in 2010 (21.78°C and 28.28°C, respectively) (NOAA 2009-2010). Total monthly precipitation during the summer of 2009 (174.1 cm/month) was nearly double that of 2010 (88.1 cm/month) (NOAA 2009-2010). Lower temperatures may have resulted in lower decomposition rates in the summer of 2009, leaving a larger amount of organic matter remaining at the end of August, providing more energy for sediment denitrifiers, and resulting in higher denitrification potentials in 2009, compared to 2010. Conversely, higher temperatures and drier conditions in 2010 would result in heating of the sediments and faster decomposition rates, leaving less organic matter for denitrifiers by the end of August.

In future summers, Ramshorn Marsh will be revisited to assess the response in denitrification potential and sediment characteristics to the removal of *Phragmites*, relative to the reference sites, as well as responses that occur as the native vegetation recolonizes devegetated patches. The hypothesis to be tested is that following the removal of vegetation, pore-water ammonium should accumulate due to lack of

assimilation by plants, as well as decreased rates of nitrification in the absence of plant-mediated oxygenation of sediments. The resulting decrease in nitrate availability is expected to cause a decrease in denitrification potentials, relative to the reference sites. As vegetation recolonizes, a decrease in ammonium and a recovery in denitrification potential should be observed, and thus the ability of the wetland to act as a nitrogen sink should increase. Similar responses have been observed following *Phragmites* removals in Connecticut (Findlay et al. 2003).

Based on pre-removal results, repeating measurements throughout the growing season (May through August) will be essential to understanding the dynamics of plant-sediment interactions in freshwater tidal marshes. Differences in sediment characteristics and denitrification potential between *Phragmites*- and *Typha*-dominated sediments will also be revisited to assess important plant characteristics that could be responsible for differences in denitrification potential. Sediment and plant measurements will be repeated throughout the growing season to assess variation in denitrification, as well as variation among plant communities, over time. Differences among plant communities, if they exist, are expected to be more easily detected earlier in the growing season as biomass accumulates and plants are more physiologically active. This information will greatly assist efforts to predict responses in nitrogen-removal ecosystem services to invasive-species-management practices.

ACKNOWLEDGEMENTS

We would like to think our collaborators at the Cary Institute of Ecosystem Ecology, particularly Dr. Stuart Findlay and David Fischer, as well as Troy Weldy and Melissa Kalvestrand of The Nature Conservancy. Stony Brook University undergraduates Diana Lenis and Michael Tong assisted greatly with laboratory nutrient analyses. This research was supported by The Nature Conservancy and a grant from the Hudson River Foundation Tibor T. Polgar Fellowship program.

LITERATURE CITED

Bertness, M. D., P. J. Ewanchuk, and B. R. Silliman. 2002. Anthropogenic modification of New England salt marsh landscapes. Proceedings of the National Academy of Sciences of the United States of America 99:1395-1398.

Bodelier, P. L. E., J. A. Libochant, C. Blom, and H. J. Laanbroek. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Applied and Environmental Microbiology 62:4100-4107.

Boyer, E. W., R. B. Alexander, W. J. Parton, C. Li, K. Butterbach-Bahl, S. D. Donner, R. W. Skaggs, and S. J. D. Grosso. 2006. Modeling denitrification in terrestrial and aquatic ecosystems at regional scales. Ecological Applications 16:2123-2142.

Caffrey, J. M., M. C. Murrell, C. Wigand, and R. McKinney. 2007. Effect of nutrient loading on biogeochemical and microbial processes in a New England salt marsh. Biogeochemistry 82:251–264.

Chambers, R. M., L. A. Meyerson, and K. Saltonstall. 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Botany 64:261-273.

Farnsworth, E. J. and L. A. Meyerson. 1999. Species composition and inter-annual dynamics of a freshwater tidal plant community following removal of the invasive grass, *Phragmites australis*. Biological Invasions 1:115-127.

Findlay, S. E. G., P. M. Groffman, and S. Dye. 2003. Effects of *Phragmites australis* removal on marsh nutrient cycling. Wetlands Ecology and Management 11:157-165.

Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, and M. A. Voytek. 2006. Methods for measuring denitrification: Diverse approaches to a difficult problem. Ecological Applications 16:2091-2122.

Hinga, K. R., A. Batchelor, M. T. Ahmed, O. Osibanjo, N. Lewis, and M. Pilson. 2005. Chapter 15: Waste Processing and Detoxification. Pages 417-439 in N. Faruqui and A. Wagener, editors. Millennium Ecosystem Assessment. World Resources Institute, Washington, DC.

Jones, M. N. 1984. Nitrate reduction by shaking with cadmium: Alternative to cadmium columns. Water Research 18:643-646.

Kiviat, E. 2006. *Phragmites* Management Sourcebook for the Tidal Hudson River. Hudson River Foundation, New York, New York, USA.

Meyerson, L. A., K. Saltonstall, L. Windham, E. Kiviat, and S. E. G. Findlay. 2000a. A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. Wetlands Ecology and Management 8:89-103.

Meyerson, L. A., K. A. Vogt, and R. M. Chambers. 2000b. Linking the success of *Phragmites* to the alteration of ecosystem nutrient cycles. Pages 827-844 in M. P. Weinstein and D. A. Kreeger, editors. Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic, Norwell, MA.

NOAA. 2009-2010. Surface Data, Monthly, Hudson Correctional Facility Station, Hudson, NY, United States. NOAA Satellite and Information Service. National Climate Data Center, US Department of Commerce, Washington, DC, USA.

Parsons, L. L., R. E. Murray, and M. S. Smith. 1991. Soil denitrification dynamics: spatial and temporal variations of enzyme activity, populations, and nitrogen gas loss. Soil Science Society of America Journal 55:90-95.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. 1.4 Determination of Ammonia (Alternative Method). Pages 14-17 in A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Elmsford, New York.

SAS. 2002-2008. SAS 9. SAS Institute Inc. Intel Corporation, Cary, NC, USA.

Seitzinger, S., J. A. Harrison, J. K. Bohlke, A. F. Bouwman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and waterscapes: A synthesis. Ecological Applications 16:2064-2090.

Smith, M. S. and J. M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biology & Biochemistry 11:261-267.

USACE. 2009. Hudson-Raritan Estuary Comprehensive Restoration Plan. US Army Corps of Engineers (USACE) and the Port Authority of NY and NJ, in partnership with the New York/New Jersey Harbor Estuary Program.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277:494-499.

Vitousek, P. M. and W. A. Reiners. 1975. Ecosystem succession and nutrient retention: A hypothesis. BioScience 25:376-381.

Windham, L. and L. A. Meyerson. 2003. Effects of Common Reed (*Phragmites australis*) expansions on nitrogen dynamics of tidal marshes of the Northeastern U.S. Estuaries 26:452-464.

Zedler, J. B. 2003. Wetlands at your service: reducing impacts of agriculture at the watershed scale. Frontiers in Ecology and the Environment 1:65-72.

Zimmerman, C. and R. Shirer. 2009. Hudson River Invasive Plant Pre-Treatment Monitoring Report. The Nature Conservancy, Albany, New York, USA.

THE EFFECTS OF AN URBANIZED ESTUARY ON THE PHYSIOLOGY AND METAL STORAGE OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

A Final Report of the Tibor T. Polgar Fellowship Program

Allison S. Mass

Polgar Fellow

Biology Department- Ecology, Evolution, Behavior, and Systematics
The Graduate Center, City University of New York

Biology Department- Rm 6S 143
The College of Staten Island
2800 Victory Blvd
Staten Island, NY 10314

Project Advisor:

Dr. William Wallace
Biology Department- Rm 6S 143
The College of Staten Island
2800 Victory Blvd
Staten Island, NY 10314

Mass, A.M. and W.G. Wallace. 2012. The Effects of an Urbanized Estuary on the Physiology and Metal Storage of the Eastern Oyster, *Crassostrea virginica*. Section V: 1-47 pp. *In:* S.H. Fernald, D. J. Yozzo, and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT:

Prior to the urbanization of the Hudson River Estuary (HRE), New York City was the center of a flourishing oyster industry. The Eastern oyster (*Crassostrea virginica*) was found in large reefs and was a significant contributor to the ecological and economic health of the region. However, due to overharvesting, pollution, and declining water quality, this ecosystem engineer has become 'functionally extinct' in the lower HRE. Recent efforts to restore the important bivalve have yielded mixed results, possibly due to the unique suite of contaminants seen in the HRE. Exposure to contaminants, particularly metals, can lead to alterations in the physiology of the oyster. Disruptions in mitochondrial energetics, changes in the relative percentages of energy stores (proteins, carbohydrates), and binding of metals to specific fractions within the cell can all lead to changes in the overall health of the oyster, and may prevent growth, reproduction, and survival. A field-based study examining the condition, biochemistry, and metal storage of juvenile *C. virginica* at a variety of sites within the urbanized HRE was conducted in the summer of 2010. Juvenile oysters were placed in the subtidal areas of four sites along a contamination gradient, and subsampled every two weeks to determine the changes in physiology and metal storage over time. Site-specific differences in condition index, biochemistry, and Cd accumulation were seen over time (Factorial ANOVA; $p < 0.05$). These results indicate that restoration of *C. virginica* to the urbanized HRE must take into account the differences in energy budgets and condition of juveniles. Future studies on adult physiology and biochemistry will complement this research.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Urbanization of estuaries along the Atlantic coast has lead to the destruction of ecological niches and the loss of many important species. In the Hudson River Estuary (HRE), sewage pollution (leading to lowered dissolved oxygen content in the water column) and overharvesting of the population were most likely the primary causes for the loss of the Eastern oyster, *Crassostrea virginica.* The input of various contaminants (e.g., metals, pesticides, PCBs and other organics) and the destruction of estuarine habitat (e.g., marsh loss, bulkheading of canals, dredging of the harbor) have also contributed to the continued loss of this ecological engineer (Yozzo et al. 2004; Wakeman & Themelis 2001; Franz 1982)

Oysters are able to filter large amounts of water through their gills, removing suspended particulates from the water column and depositing feces or pseudofeces onto the benthos, thus acting as benthic-pelagic couplers within the ecosystem (Newell 2004; Brumbaugh et al. 2000; Gerritsen et al. 1994). This action allows for increasing amounts of light and oxygen to penetrate the water column and become available to other organisms, affecting the entire estuarine community (Nelson et al. 2004; Coen & Lukenbach 2000). Dense populations of oysters, such as those that historically occurred in the HRE, therefore have the ability to control phytoplankton assemblages, nutrients, and suspended matter within an estuary (Nelson et al. 2004; Coen & Lukenbach 2000; Gerritsen et al. 1994).

Alteration of the HRE over the past centuries has lead to an urbanized environment, which makes it very different from the one present when oysters were abundant (Yozzo et al. 2004; Wakeman & Themelis 2001; Franz 1982). Increased

pollution (including sewage run-off, organic, and inorganic pollutants), dredging of the harbor, removal of fringing marshes, and other impacts of urbanization have led to lowered dissolved oxygen levels, increased nutrients, and increased sediment loads within the HRE (Yozzo et al. 2004; Crawford et al. 1994). PCBs and PAHs have also been found at levels exceeding NOAA's ER-M (Effects Range Median) guidelines (Dimou et al. 2006; Feng et al. 1998). The HRE ranks second behind the San Francisco Bay Estuary in terms of metal contaminant levels within an estuary (Levinton & Waldman 2006). Cd, Cu, Hg, and Zn are found in elevated quantities throughout the HRE (Yozzo et al. 2004; Feng et al. 1998; Crawford et al. 1994). All of these contaminants have been shown to effect metabolism, causing mortality, reproductive failure, and decreased growth rates, as well as alterations in cellular responses (i.e., lysosomal destabilization) in oysters and other bivalves (Cherkasov et al. 2007; Ringwood et al. 1998; Roesijadi 1996).

Contaminants, such as metals (i.e., Cd, Cu, and Hg), may have profound effects on the physiology of oysters, and may have played a part in their demise in urbanized estuaries. Oysters are able to bioconcentrate metals up to several orders of magnitude higher than ambient concentrations before experiencing toxicity (O'Connor 2002; Mouneyrac et al. 1998). Accumulated metals can be separated within a variety of subcellular compartments, including heat denatured proteins (HDP), heat stable proteins (HSP), insoluble metal-rich granules (INS), organelles (ORG), and cell debris (CD) (Wallace et al. 2003), after which the metal can be sequestered, eliminated, or transferred along the food chain. At low concentrations, metals may be stored in subcellular compartments or eliminated with no ill-effects to

the oyster (Geffard et al. 2002). Metals may be bound to metallothionein proteins or granular hemocytes in large quantities, which presumably render the metal biologically inert (Amiard et al. 2006; Wallace et al. 2003; Roesijadi 1996).

The exposure to, and storage of, metals within oysters can have drastic impacts on energy stores (Pridmore et al. 1990). Increased exposure to non-essential metals (i.e., Cd) has negative effects on growth and reproduction of oysters (Volety 2008; Ringwood et al. 2004). The amount of energy reserves within the oyster, reproductive condition, and environmental parameters (e.g., food, temperature, salinity) can affect the amount of metallothioneins present within the oyster, and thus the amount of metal that can be successfully sequestered (Erk et al. 2008; Amiard et al. 2006). If excess metal is present in the environment, accumulations from the environment can outpace detoxification mechanisms, leading to toxicity and mortality (Amiard et al. 2006).

Understanding the relationship between environmental contaminants and oyster health is a critical aspect of restoration efforts within the HRE. Linking the effects of the environment (including contaminants) to changes in oyster physiology (i.e., energy stores, reproduction) will allow for a greater understanding of how oysters can be restored in urbanized estuaries. In many cases, a suite of contaminants as well as various environmental stresses are typically responsible for adverse effects on organisms and it is often difficult to discern which variables are the primary causes for stress. Oysters that sequester and eliminate toxins may deal with physiological trade-offs including lowered reproductive output, respiration and metabolic rates, and smaller size (Lanning et al. 2008; Hartwell et al. 1991).

Crassostrea gigas living in a polluted estuary with high Cu and Zn concentrations were found to have lower glycogen content and reduced overall condition than those living in a cleaner area (Pridmore et al. 1990). Additionally, the binding of metals onto different subcellular fractions may adversely impact oyster metabolism and energy budgets, leading to mortality. For example, cadmium (Cd) has been found to bind to mitochondria in oysters even at low concentrations, which can lead to disruption of the electron transport system and alterations of the oysters' metabolism (Cherkasov et al. 2009). Mitochondrial bioenergetics may be affected at low levels of Cd exposure, including those that are environmentally relevant within the HRE (Sokolova et al. 2005). If metals are able to preferentially bind with the HDP fraction (which contains enzymes), energy storage and reproduction may be impaired (Blanchard et al. 2009; Wallace et al. 2003). Considering the many different environmental variables (i.e., pollutants, dissolved oxygen, temperature and salinity) occurring within the HRE, a field study examining linkages between important environmental variables and metal toxins would allow for a more thorough comparison of metal uptake and biochemistry of transplanted oysters. Field studies would allow for the examination of multiple variables, at environmentally relevant concentrations.

The objectives of this study are 3-fold: (1) to determine the effects of a highly urbanized environment (the HRE) on the physiology of the Eastern oyster, *Crassostrea virginica*; (2) to determine the body burden of accumulated metals, and the internal storage of these metals to different subcellular fractions, incurred by *C. virginica* while living in an urbanized estuary; and (3) to determine the relationships

among physiology, as measured by overall condition and biochemistry (carbohydrates, proteins, and lipids), the storage of metals within the oyster tissue (both total body burdens, and in different metal fractionations), and the environmental variables present at each site. It is hypothesized that oysters placed at impacted sites will exhibit a lower overall condition, lower energy reserves, and higher total body burdens of metals (Cd, Cu, Hg) than oysters placed at a reference site. Furthermore, oysters placed at impacted sites are expected to accumulate metals in different subcellular compartments. Linkages between energy reserves and the partitioning of metals to different subcellular compartments should differ among oysters from contaminated sites and oysters from a clean reference site.

METHODS

Field Deployment of Oysters:

Oysters were placed at the field sites from July 12-15, 2010. Three chronically contaminated sites within the Hudson River Estuary were chosen based on (1) high levels of contaminants, (2) potential or current usage in oyster restoration projects within New York Harbor, and (3) probability that native oysters were historically found at the site (Waldman 1999; Kurlansky 1996; Franz 1982). The sites chosen were (1) Spring Creek, a small dead-end creek located at the Northern end of Jamaica Bay, Queens, NY (**JB**); (2) Floyd Bennett Field, along the Rockaway Inlet (between Jamaica Bay and New York Bay, Brooklyn, NY; **FBF**); and (3) Soundview Park, located at the confluence of the Bronx River and the East

River, Bronx, NY (**SVP**). A clean reference site, located outside the urbanized HRE, was also chosen at Rutgers Marine Field Station, Tuckerton, NJ (**TK**) (Figure 1). The three impacted sites have been shown to have elevated concentrations of pollutants, including essential and non-essential metals (Bopp et al. 2006; Wirgin et al. 2006; Feng et al. 1998; Adams et al. 1996; Seidemann 1991). Sediment samples from 2009 concur with previous data showing that the three impacted sites have higher burdens of important metals (Cd, Cu, Hg, and Zn) (Mass, unpubl. data). Tuckerton has been used as a local reference site in several other studies concerning metal uptake by invertebrates (Khoury et al. 2009).

Juvenile oysters (18-25mm) were obtained from a local hatchery (Aeros Cultured Oyster Co., Southold, NY) on July 13, 2010, and transported on ice to the various sites. At each location there were two polyethylene mesh bags (Aquatic Ecosystems, FL) of juvenile oysters, each with approximately 350 oysters, for a total of 700 oysters per site. At the three impacted sites, the bags were secured to a cinderblock and placed in the subtidal zone; at TK the bags were suspended off a dock in the subtidal area. A subsample of the oysters was taken prior to being placed in the field (July 12-14, 2010), and then on bi-weekly intervals until October 18-22, 2010 (7 sampling events). The bags were retrieved during a low tide, and a random subsample of 35 juvenile oysters was removed from each bag. All oysters were placed on ice, and immediately transported back to the laboratory. Of the oysters subsampled, 25 were used to determine overall condition and biochemistry (carbohydrates, lipids, and proteins), 5 were used to estimate ICP metal burdens and subcellular fractionation (Cd, Cu), and another 5 to estimate total Hg. Additionally,

salinity, temperature, and dissolved oxygen readings were taken in the field with a hand-held YSI Pro-20 water quality probe during each field sampling. Water samples were collected, then filtered back in the laboratory for total suspended particulates, and total and size-fractionated (5-28 µm, <5 µm) chlorophyll *a* analysis.

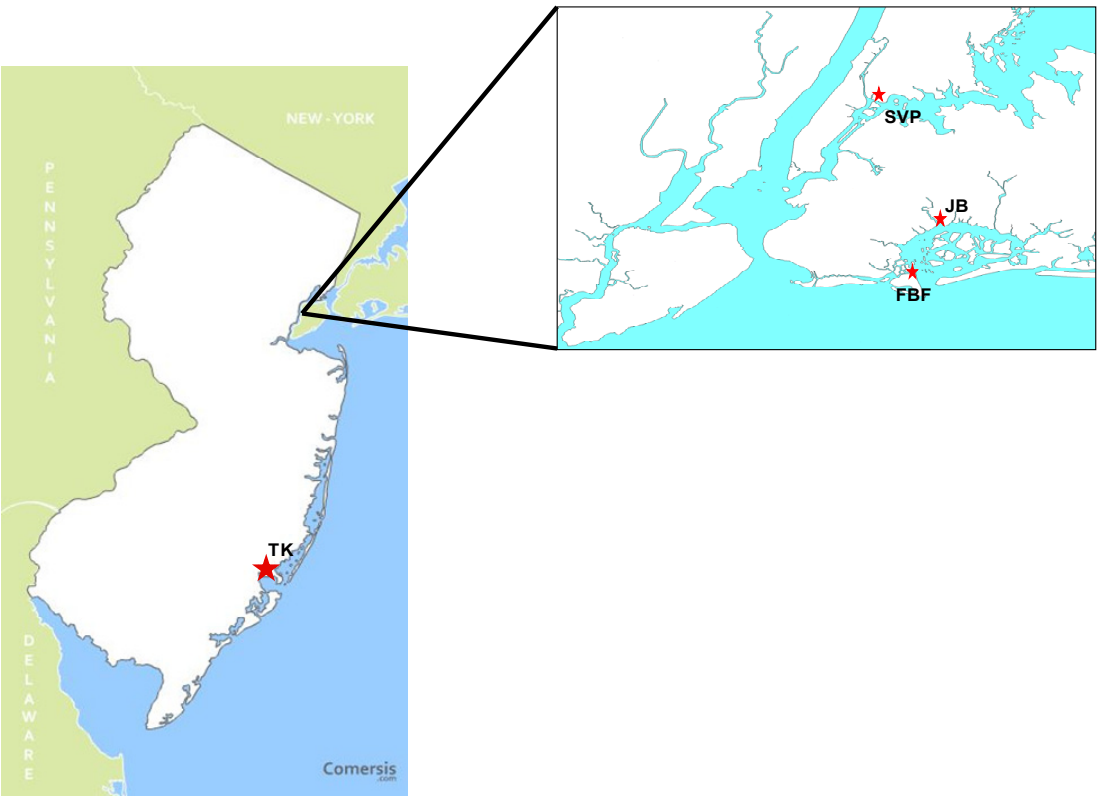


Figure 1: Map of study sites. TK= Tuckerton, NJ (Rutgers University Marine Field Station), FBF= Floyd Bennett Field, Brooklyn, NY (Gateway National Recreation Area, National Parks Service), JB= Spring Creek, Jamaica Bay, Queens, NY (Gateway National Recreation Area, National Parks Service), SVP= Soundview Park, Bronx River, Bronx, NY (NYC Department of Parks and Recreation).

Laboratory Assays

All oysters were brought back from the field sites and stored at -80°C until the assays were performed. Prior to flash-freezing on dry ice, oysters collected for metal body burden and subcellular fractionation were depurated in clean water (DI water mixed with Reef crystals; salinity 25ppt, 23°C) for 24-48 hours, to rid the gut of any foreign particles, and then stored in the –80°C freezer. At each site, 25 oysters were measured for total length (mm), shucked, and dried to determine the condition index (Crosby & Gale 1990). Dried tissue was then pooled into groups (n=25), and used to determine carbohydrate, lipid, and protein levels of the oysters. Lipid levels were determined using chloroform-methanol extraction technique (Bligh & Dyer 1959). Total protein content was determined using a CHN elemental analyzer (Perkin Elmer 2400 Series II CHNS/O Analyzer), and adjusted using stoichiometric ratios to determine the protein content (Gnaiger & Bitterlich 1984). Total carbohydrates were determined using the phenol-sulfuric acid method (Dubois et al. 1956).

To determine total body burdens and metal allocation within different subcellular fractions, four juvenile oysters were weighed for wet weight (g), and homogenized with TRIS buffer (pH 7.6). A 0.8 ml subsample was then removed to determine total body burden (TOT). Then, sequential centrifugation and heat treatment steps were used to separate the oyster tissue into operationally-defined fractions (ORG, CD, HDP, HSP, and INS) (Wallace et al. 2003). All fractions (including TOT) were placed in a drying oven for 2-3 days, digested with trace-metal grade nitric acid, resuspended in 2% nitric acid, and analyzed on an Atomic

Absorption Spectrophotometer (Perkin Elmer 3100) to estimate the amount of Cd present (Brown & Luoma 1995). Analysis is pending on an additional five oysters, which will be used to determine total Hg (THg) body burdens (Klajovic-Gaspic et al. 2006; Hatch and Ott 1968). Tissue samples will be homogenized and digested (HNO_3), and THg concentrations will be determined by Cold-Vapor Atomic Absorption Spectrophotomy (Perkin Elmer FIMS-100 Hg analyzer) using standard techniques ($SnCl_2$ will be added to digested tissue prior to analysis).

Water samples (1-3 L) from each site were filtered through a 1.8 μm GF/F glass fiber filter to collect total suspended matter. The filter was then dried in a 60°C oven to determine the total particulate matter (TPM), and subsequently ashed in a muffle furnace at 450°C to determine the ash-free dry weight (total organic matter, TOC/POM) (Bayne 2002). Additional water samples at each site (100-500 ml) were filtered through a 0.45 μm nitrocellulose filter, digested in 90% acetone, and run on a UV-spectrophotometer (GENESYS 10) to determine total and size-fractionated chlorophyll *a* (Parsons et al. 1984).

Statistics:

Data was analyzed to compare biochemistry, metal accumulation and subcellular distribution, and environmental variables at each site and to identify relationships between these variables. Regressions were used to determine linkages between variables. Two-way ANOVAs were performed to compare the effects of site and time on the different variables. Post-Hoc tests (Tukey or Unequal N HSD) were performed to identify all significant differences (Zar 1999).

RESULTS

Condition Index:

Juvenile oysters grow quite rapidly in size and body mass. The condition index (ratio of wet flesh weight to total body weight) is a measure of the overall health of the oyster (Crosby & Gale 1990). The condition index values differed significantly among sites over time. While each site showed significant changes in condition index over the summer (ANOVA, $p<0.05$), amongst site differences give us more information as to the site-specific trends in overall oyster health. As the summer began, SVP and TK were significantly different than JB and FBF; however, as time at each site increased, condition index values at SVP became significantly lower than the other three sites, while TK began to display similar condition index values as FBF and JB (Factorial ANOVA, $p< 0.05$) (Figure 2).

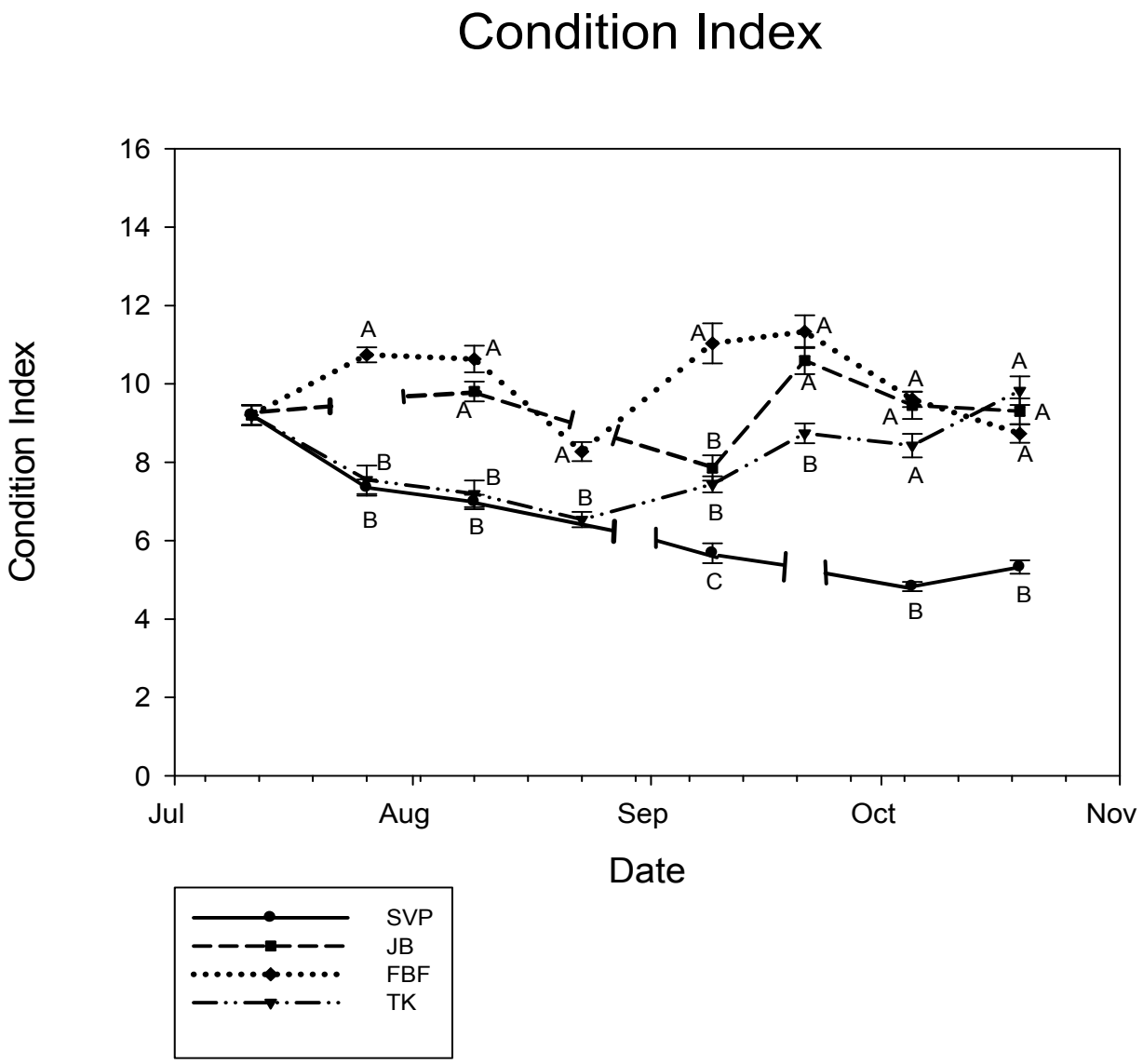


Figure 2: Condition Index of juvenile *Crassostrea virginica* at four field sites over time. Condition index values expressed as means (*n*=25) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, $p<0.05$) differences between sites at each sampling event.

Biochemistry:

The proportion of carbohydrates, lipids, and proteins within oyster tissue is an indicator of the health of the juvenile. Juveniles will put more energy into growth while they are small, meaning more of the energy gained from feeding will end up as carbohydrates within the body, versus protein and lipid.

Within sites, significant changes in carbohydrate storage over time were seen at all four sites (ANOVA, $p < 0.05$, arc-sin transformation of data). SVP showed a steady decline in carbohydrate storage from 8/9/10 through 10/7/10, while TK showed a steady increase of carbohydrate storage from 8/23/10 through 10/22/10. FBF and JB oysters had more variable carbohydrate storage, with fluctuating levels from 7/13/10 through the end (although with an upward trend). Significant differences between sites were seen over all dates (Factorial ANOVA, $p < 0.05$, arc-sin transformation of data) (Figure 3).

Protein storage also showed significant changes in stored proteins within each site over time (ANOVA, $p < 0.05$, arc-sin transformation of data). The percent of protein per gram dry weight was much higher than that of carbohydrate or lipids (40-60% of tissue). All sites showed a decline of protein storage over time, with FBF declining to the lowest levels of protein storage by the end of the summer. JB maintained the highest percentage of stored protein over the summer, while SVP and TK fluctuated as well. Significant differences between sites were found on 7/26/10, 8/9/10, 9/9/10, and 10/22/10 (Factorial ANOVA, $p > 0.05$, arc-sin transformation of data).

The amount of lipids found in tissue was significantly different over time within SVP; however, within the other three sites, no significant change was seen between 7/13/10 and 10/22/10 (ANOVA, $p>0.05$, arc-sin transformed data). SVP oysters steadily decreased the percentage of lipids within tissues as the summer went on, as TK oysters steadily increased lipid stores from 8/22/10 to 10/22/10. FBF oysters displayed fluctuating lipid stores, with sharp drops on 8/22/10 and 9/22/10 (with recovery periods after). JB oysters displayed a similar pattern as FBF oysters, with a sharp decline in lipid storage on 10/7/10. Across sites, significant differences in lipid storage were seen over the summer (except 7/26/10 and 8/22/10; Factorial ANOVA, $p> 0.05$, arc-sin transformed data) (Figure 5).

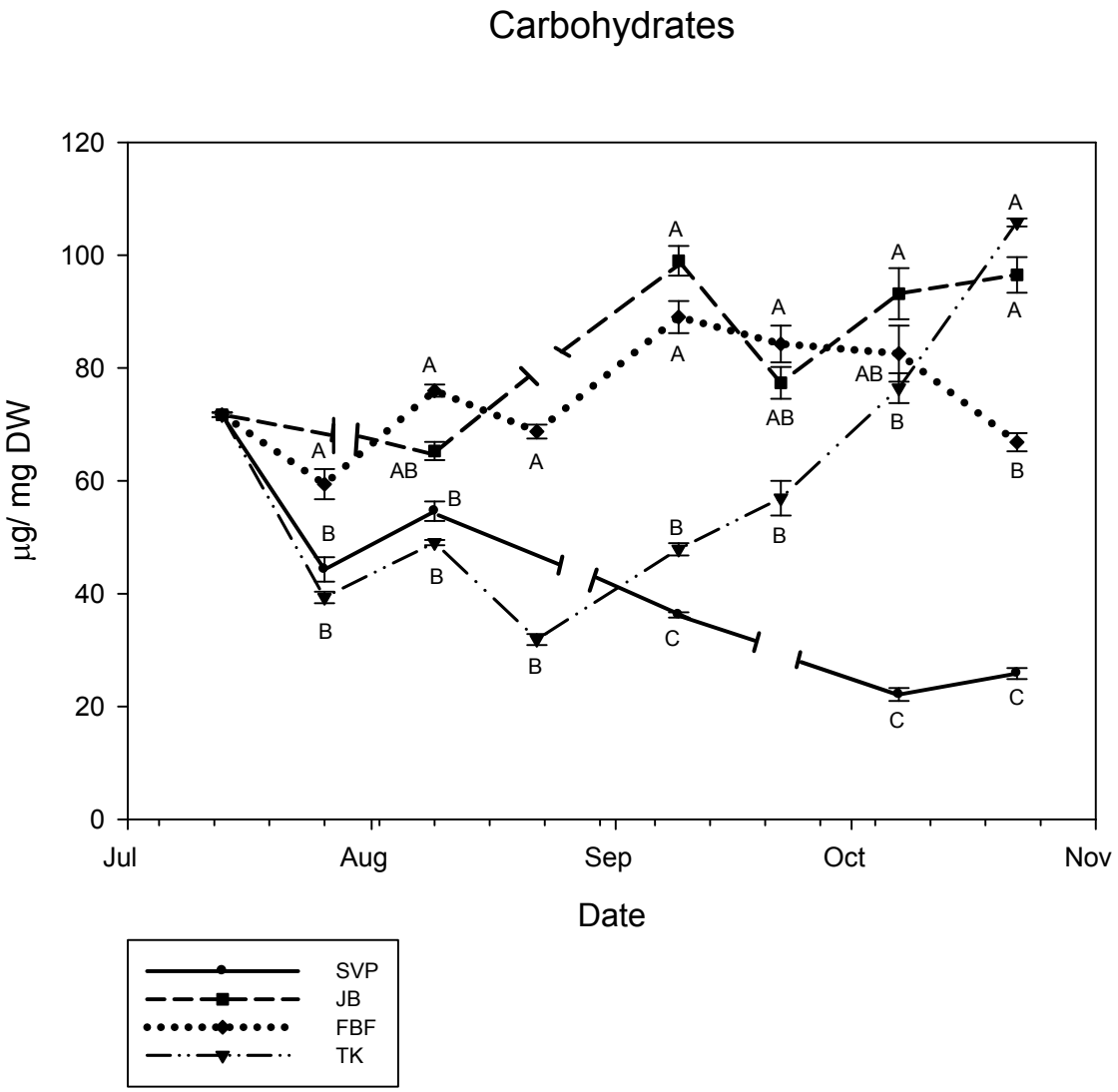


Figure 3: Carbohydrates (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Carbohydrate values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, $p<0.05$) differences between sites at each sampling event.

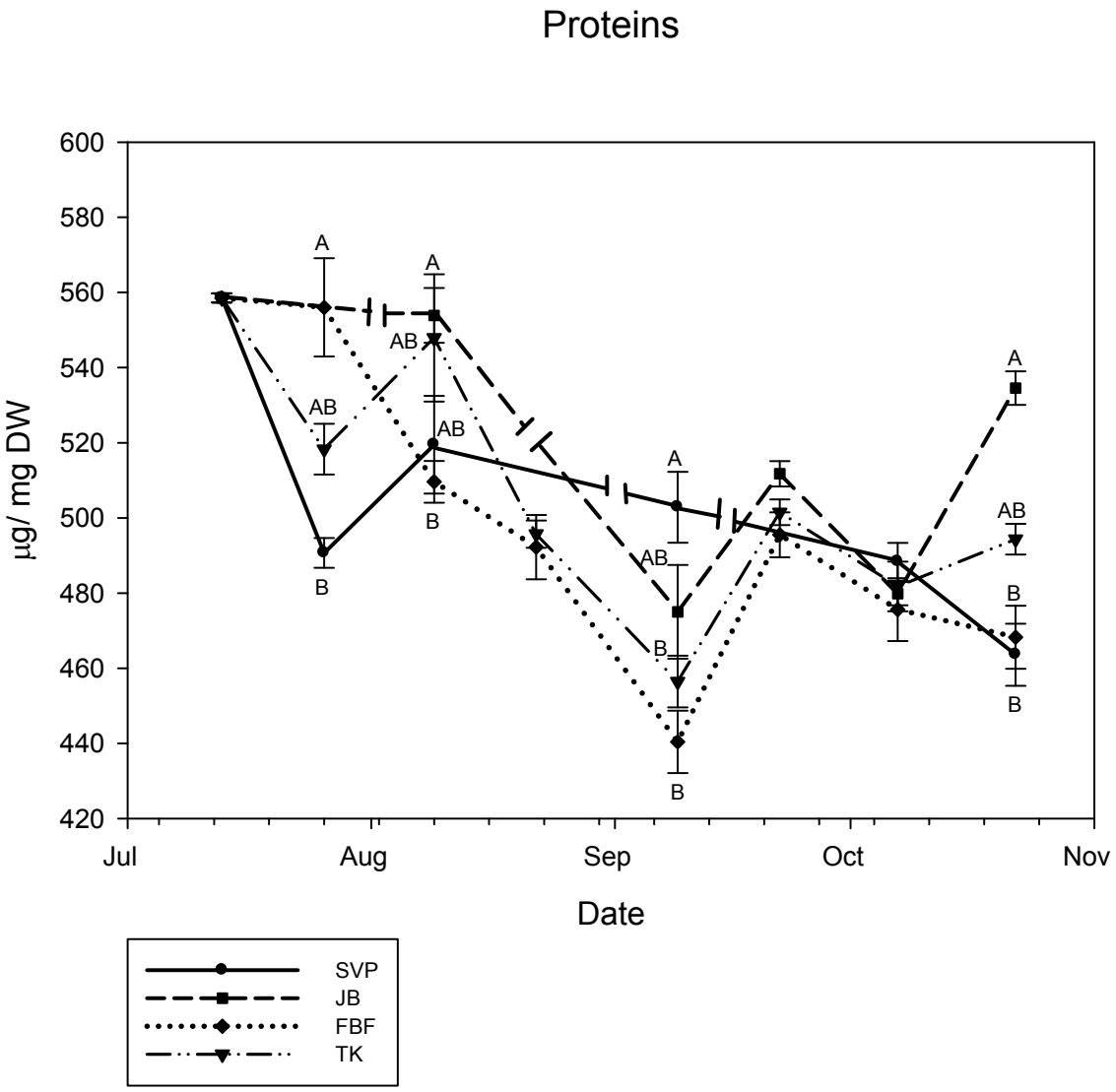


Figure 4: Protein (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Protein values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B) represent statistically significant (Factorial ANOVA, $p<0.05$) differences between sites at each sampling event.

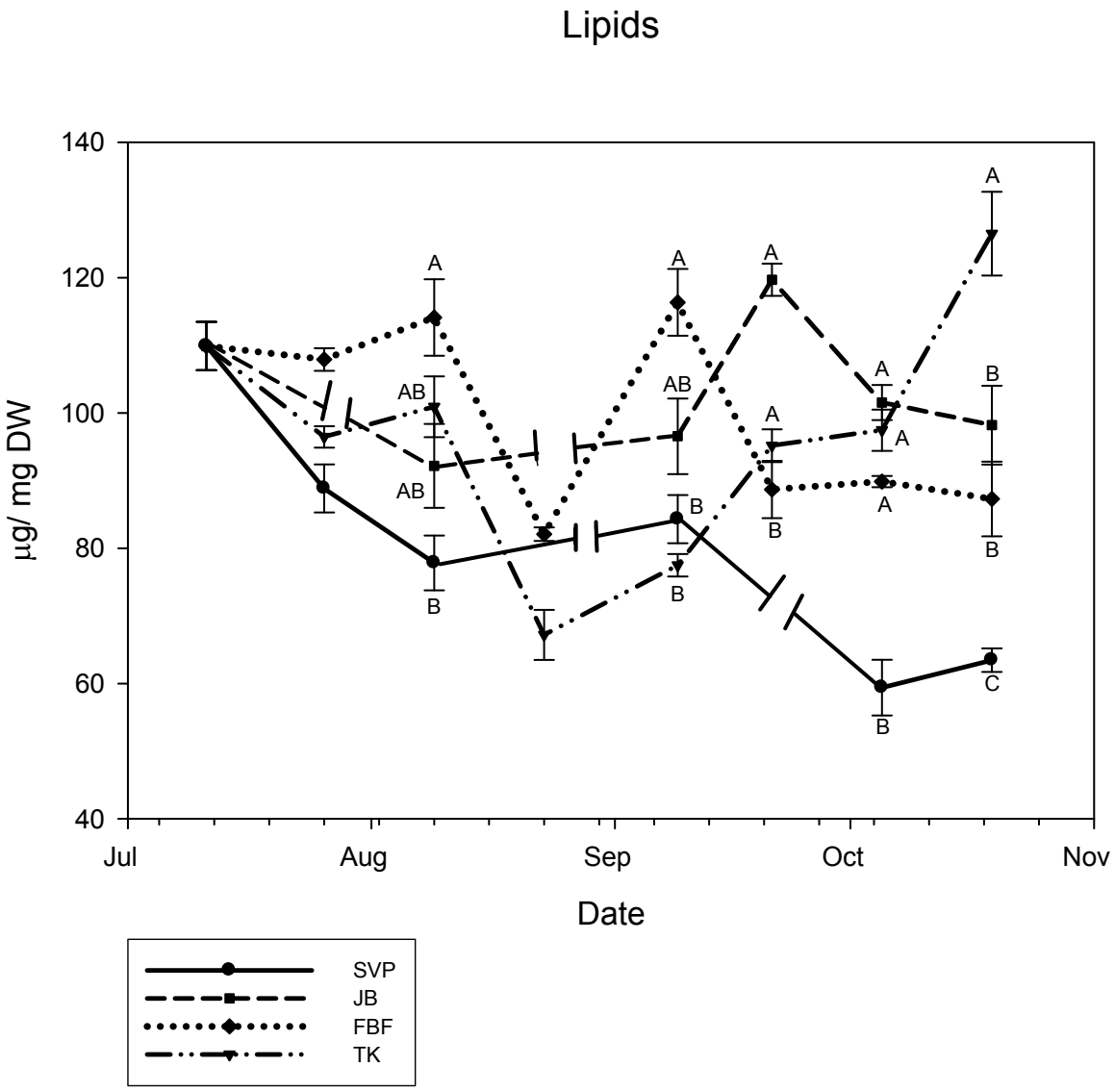


Figure 5: Lipids (microgram per gram dry weight) of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Lipid values are expressed as means (*n*=3) with standard error bars. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather (represented with broken lines). At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction (represented with broken lines). Letters (A, B, C) represent statistically significant (Factorial ANOVA, $p<0.05$) differences between sites at each sampling event.

Water Quality:

Environmental data was taken during each sampling event. Water collected for chlorophyll *a* was filtered and processed for both total and size-fractionated chlorophyll. Total chlorophyll *a* values reached a wide range at each site, due to the annual summer bloom of algae that occurs in the HRE (Taylor et al., 2003; Sambrotto 2001). JB recorded the highest chlorophyll *a* values during the peak on 7/26/10 (120.32 mg/L), while the other sites recorded much lower values (SVP- 60.075 mg/L, FBF- 63.792 mg/L, TK- 19.693 mg/L) during this date. Significant differences among sites with regards to total chlorophyll *a* were found (Factorial ANOVA, $p>0.05$) on all sampling dates except 9/9/10 and 10/7/10 (Figure 6). At each site, the chlorophyll *a* measurements for the 5-28 μm fraction of phytoplankton follow the same pattern as the total chlorophyll *a* measurements. The 5-28 μm fraction is 40% or higher of the total chlorophyll a at each site (Figure 6).

Water was filtered through a preweighed GF/F filter and dried at 60°C to determine the total particulate matter (TPM) at each site. Significant differences among sites with respect to TPM were seen (Factorial ANOVA, $p>0.05$, log10 transformed data). SVP water had the highest amounts of TPM throughout the entire sampling season (Figure 8). After being weighed for dry weight, filters were then put into a muffle oven and ashed, and the particulate organic matter (POM) calculated from the difference between the ash weight and the dry weight. Again, statistically significant differences were seen in the POM among sites (Factorial ANOVA, $p>0.05$, log10 transformed data) (Figure 9).

MacDonald & Ward (1994) quantified seston quality as the percent of particulates in the water column that are photosynthetic (algae) particles. The amount of chlorophyll *a* (μg) per gram of suspended particulate (TPM) can act as a proxy for the amount of nutritional particles being ingested by the oyster. A measure above 1 μg chl *a* g TPM^{-1} quantifies as "good" seston quality; below 1 μg chl *a* g TPM^{-1} is considered "poor" seston quality (Figure 10). SVP had consistently poor seston quality, only reaching above 1 μg chl *a* g TPM^{-1} on 7/26/10. FBF and JB had high seston quality most of the sampling season, only falling below 1 μg chl *a* g TPM^{-1} after 10/7/10.

Water was filtered through GF/F filters, and a sample run on the CHNS/O elemental analyzer to determine the ratio of carbon to nitrogen in the water, which can indicate seston quality as well. SVP had a significantly higher C:N ratio throughout the sampling season than all other sites (Factorial ANOVA, $p>0.05$, arc-sin transformed data) (Figure 11).

At each sampling event, environmental parameters (temperature, salinity, and dissolved oxygen) were taken with a handheld YSI probe (Table 1). Environmental parameters are within ranges typically seen at these sites (Mass, unpubl. data).

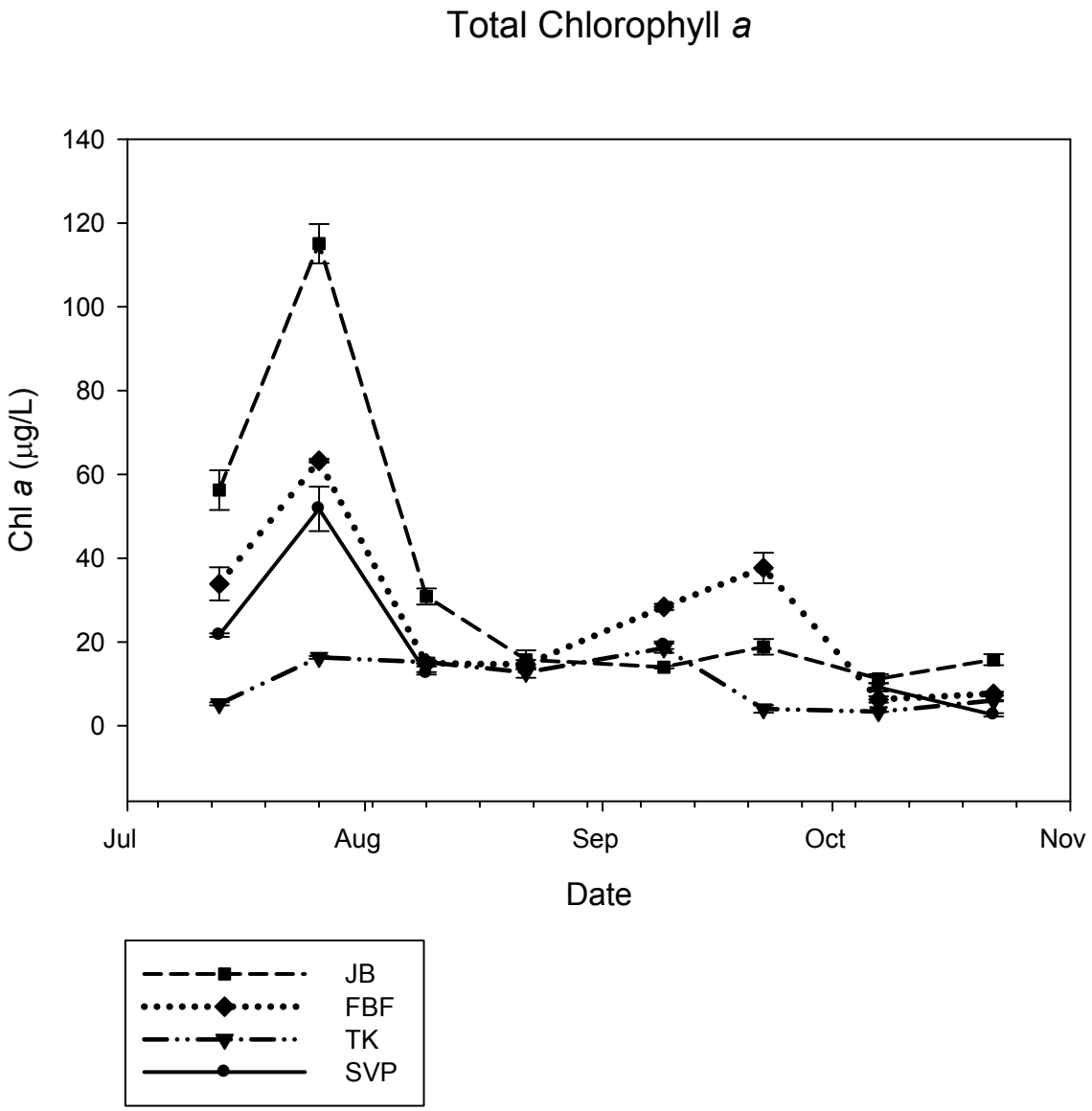


Figure 6: Total chlorophyll *a* (µg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

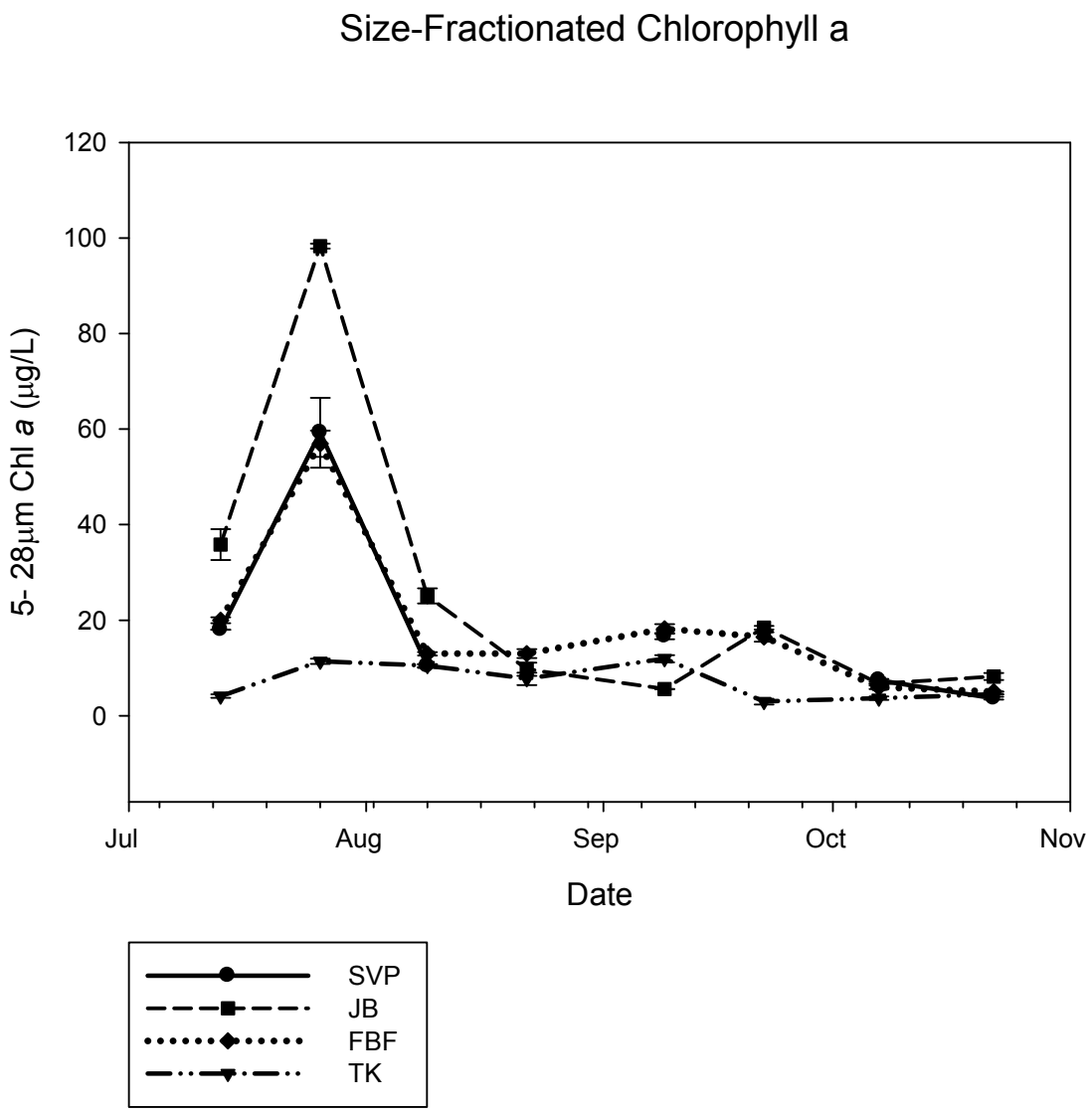


Figure 7: Size-fractionated (5-28µm) chlorophyll *a* (µg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

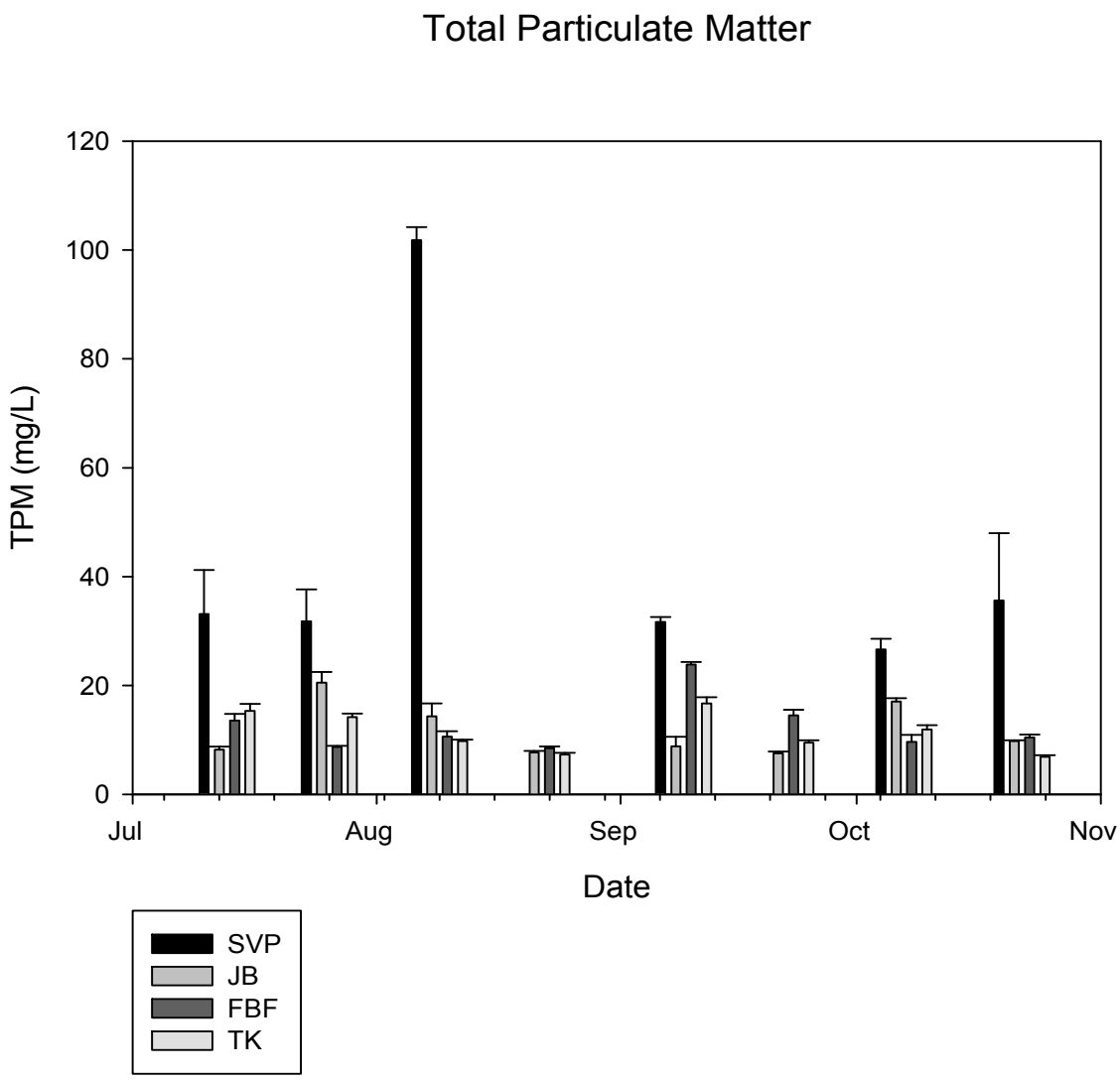


Figure 8: Total particulate matter (mg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

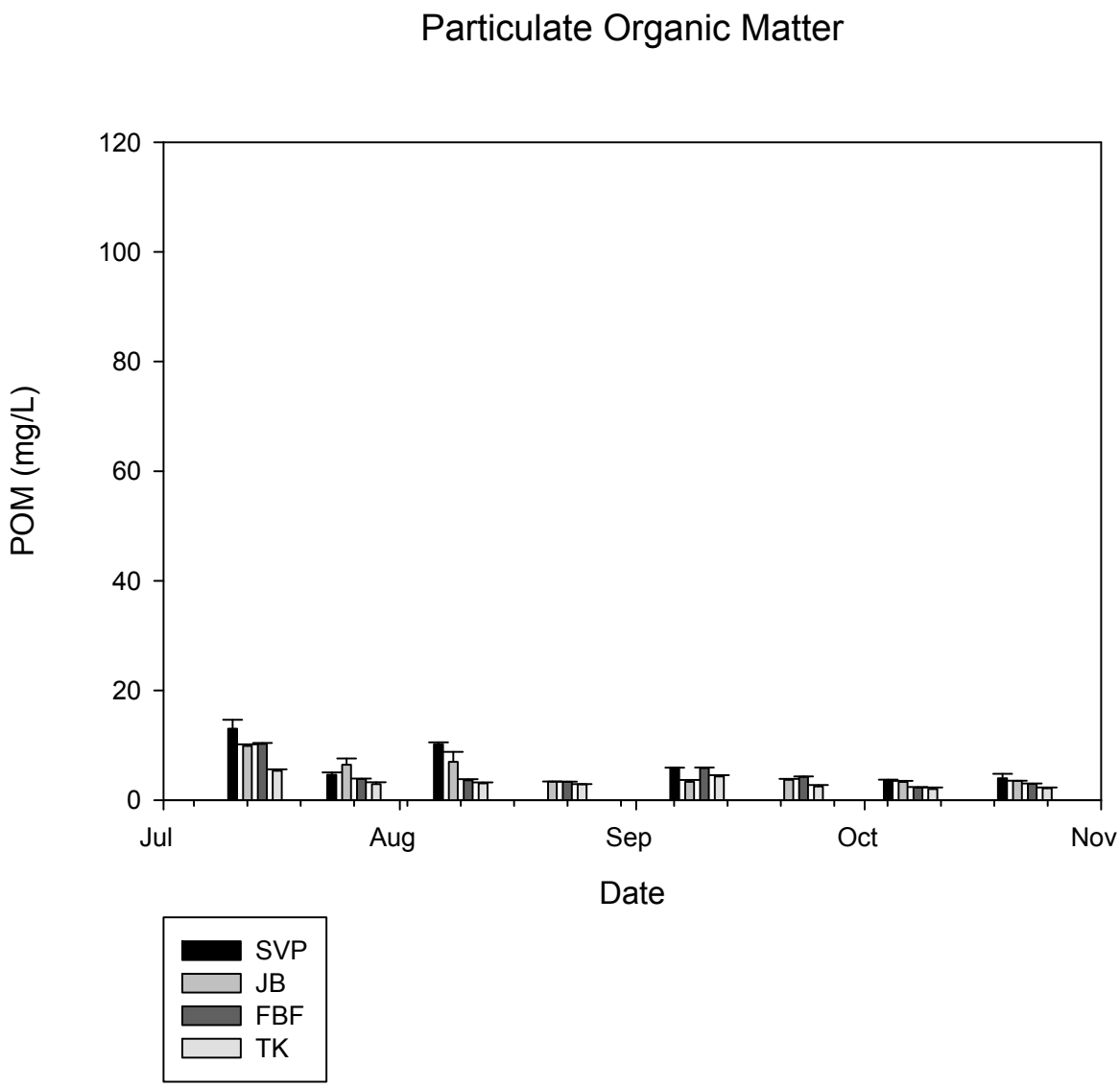


Figure 9: Particulate organic matter (mg/L) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather. The scale was kept the same as TPM to highlight relative percentage of TPM that is POM.

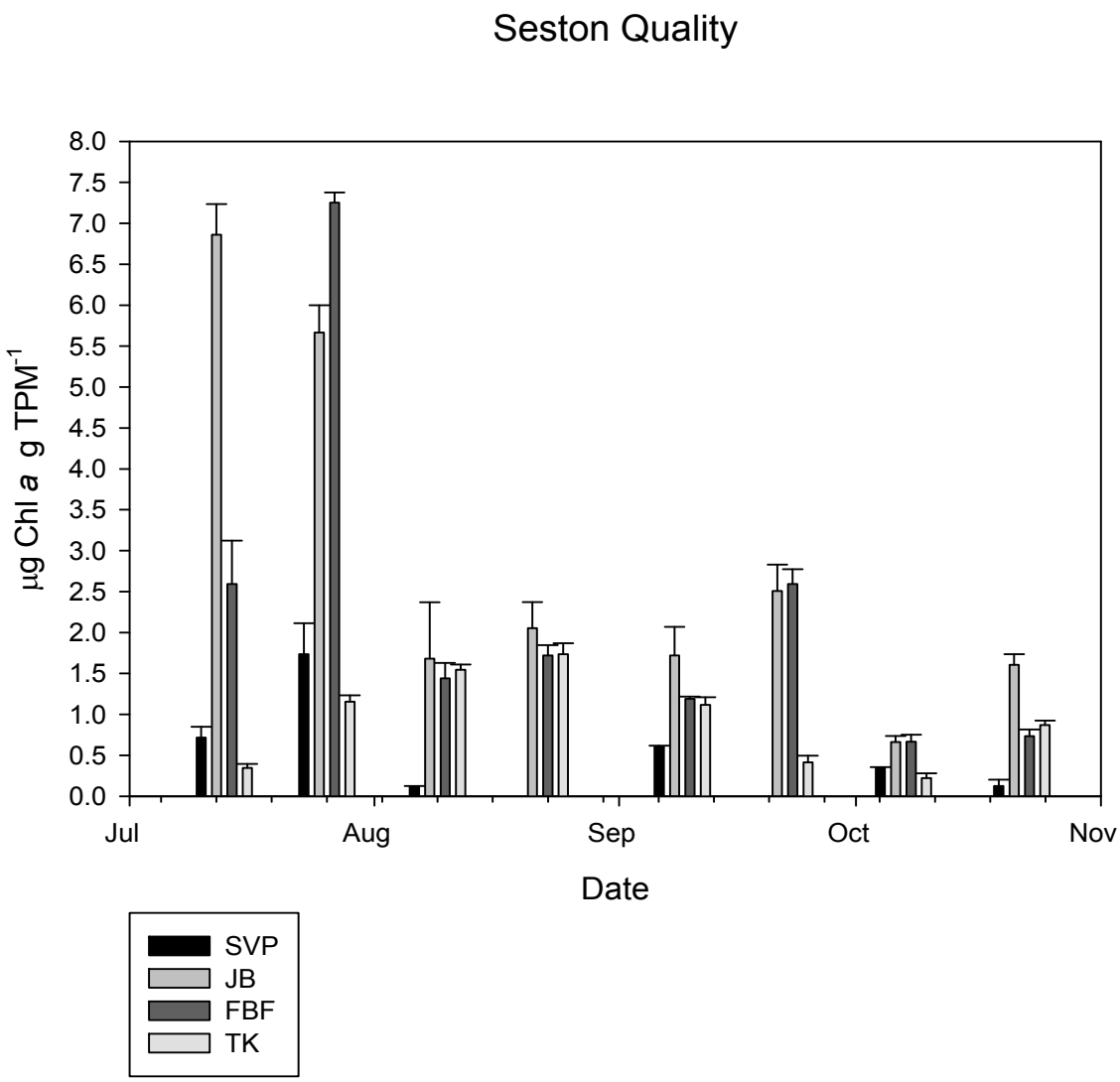


Figure 10: Seston quality (µg Chl *a*/ g TPM) at four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather. Values above 1.0 µg Chl *a*/ g TPM indicate high quality seston.

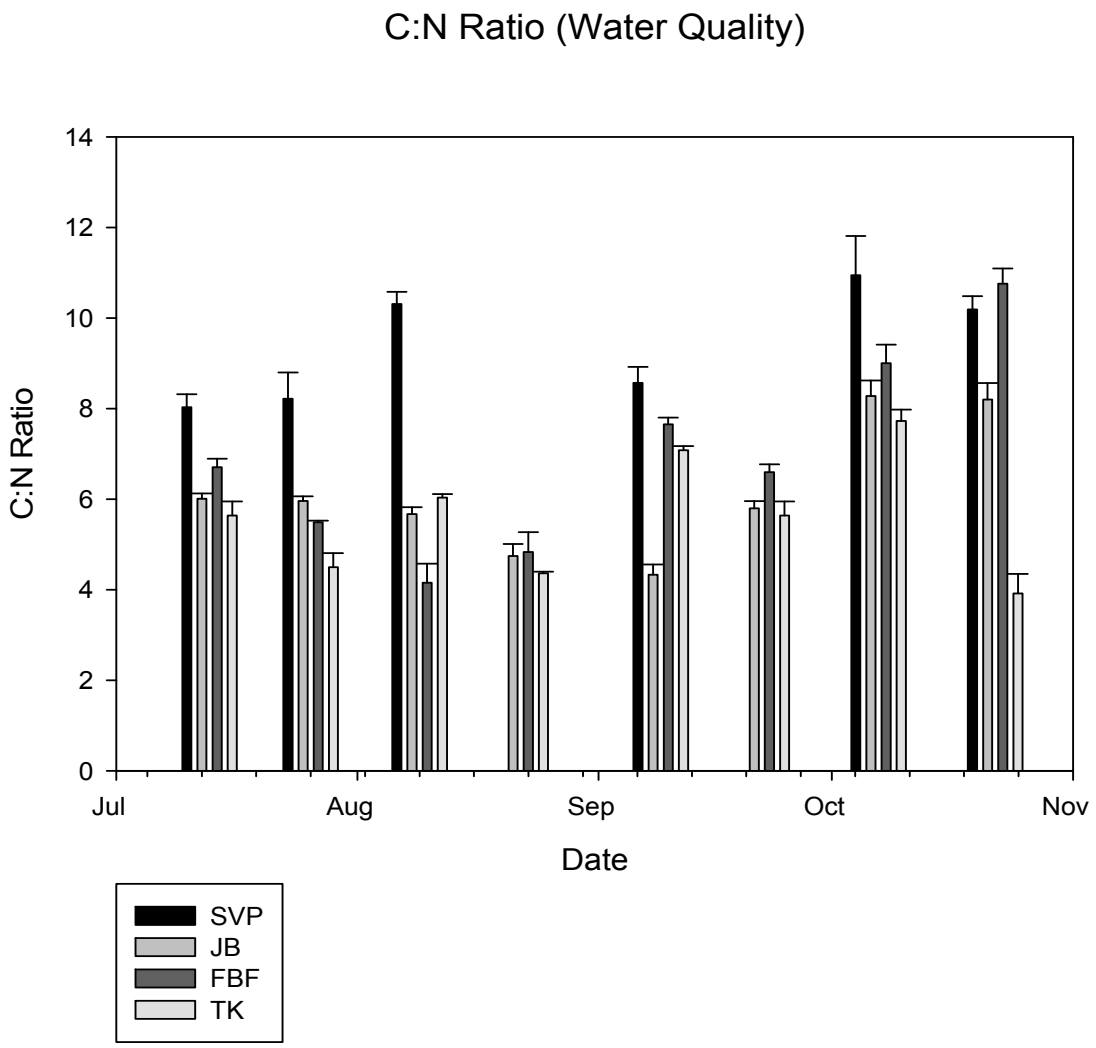


Figure 11: Ratio of elemental carbon to elemental nitrogen in water samples from four field sites during the summer of 2010. Data is expressed as mean (*n*=3) values with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/22/10 and 9/22/10) due to inclement weather.

	DATE	7/13/2010	7/26/2010	8/9/2010	8/22/2010	9/9/2010	9/22/2010	10/7/2010	10/23/2010
SVP	TEMPERATURE (^oC)	22.6	23.5	23.6	--	22.4	--	18	16.5
	SALINITY (ppt)	25	25	25	--	23	--	30	18
	D.O. (mg/L)	3.78	1.75	3.76	--	6.5	--	6.12	9.63
JB	TEMPERATURE (^oC)	25.4	24.9	29.3	21.9	21.1	23.2	19.2	12.3
	SALINITY (ppt)	28	26	26	28	29	27	29	29
	D.O. (mg/L)	2.95	8.63	12.01	6.11	7.07	12.03	8.4	10.19
FBF	TEMPERATURE (^oC)	25.9	25.6	24.9	23	20.1	23.1	18.3	14.2
	SALINITY (ppt)	28	28	30	31	32	30	30	31
	D.O. (mg/L)	6.98	9.61	7.21	6.09	8.01	10.61	7.05	8.66
TK	TEMPERATURE (^oC)	28.7	27.6	27.6	24.7	25.5	21.1	16	15.1
	SALINITY (ppt)	30	30	30	29	30	33	33	32
	D.O. (mg/L)	5.91	7.36	7.36	8.72	7.32	7.78	7.69	7.66

Table 1: Environmental parameters (temperature, salinity, dissolved oxygen) at four field sites from 7/13/2010- 10/23/10.

Metals:

Metal body burdens were determined using Atomic Absorption Spectrophotometry. Whole body burdens of Cd were highest in SVP oysters, peaking on 10/7/10 at 55.05 μg Cd g dry tissue^{-1} (Figure 12). Statistical differences between sites were seen on 10/7/10 (Factorial ANOVA, $p < 0.05$), with SVP having significantly more Cd within tissues than TK. JB accumulated the least amount of Cd throughout, with only 10.25 μg Cd g dry tissue^{-1} on 10/23/10 (Figure 12).

At SVP, sequential centrifugation steps to separate different subcellular cytosolic partitions yielded differences in Cd burdens. More Cd was seen in the INS fraction than ORG, HDP, or HSP. The least amount of Cd accumulation was found

in the HSP fraction, which has metallothioneins. Statistically significant differences in Cd burdens within fractions were seen on 7/15/10, 9/9/10, and 10/7/10 (Figure 13).

Total body burdens of Hg were analyzed using Cold-Vapor Atomic Absorption Spectrophotometry. Body burdens of juvenile *C. virginica* were elevated at the initial sample, obtained from the hatchery on Long Island (0.756 μg/ g dry weight). At SVP, body burdens decreased as the season progressed, but detectable levels of Hg remained in tissues until the last sampling event (0.101 μg/ g dry weight on 10/23/10). Oysters in JB and FBF had increases in Hg body burdens in September 2010, but then quickly decreased to undetectable levels in October 2010. Oysters at TK remained at low, but detectable, body burdens through September, before becoming undetectable (Figure 14).

Correlations:

Correlations were calculated between variables to determine any linkages. At SVP, condition index was positively correlated with carbohydrate and lipid percentages in tissue, but not with the amount of protein stored in tissue (Pearson correlation, $p < 0.05$). At JB, environmental parameters were correlated with carbohydrate storage. Salinity was positively correlated with the amount of carbohydrates present, while temperature showed a negative correlation with carbohydrate storage (Pearson correlation, $p < 0.05$). FBF oysters had protein storage positively correlated to both temperature and seston quality, but negatively correlated with salinity (Pearson correlation, $p < 0.05$). At TK, condition index was positively

correlated with carbohydrate and lipid storage in tissues, but not protein (Pearson correlation, $p < 0.05$). Condition index was not significantly correlated with either total chlorophyll *a* or seston quality at any of the sites. Cd body burdens were not correlated with any physiological (condition index, carbohydrates, lipids, proteins) or environmental (chlorophyll *a*, seston quality, temperature, salinity, dissolved oxygen) at any of the sites. Hg body burdens were positively correlated with condition index, carbohydrates, and lipid levels at SVP, but not at any of the other sites (Pearson correlation, $p<0.05$).

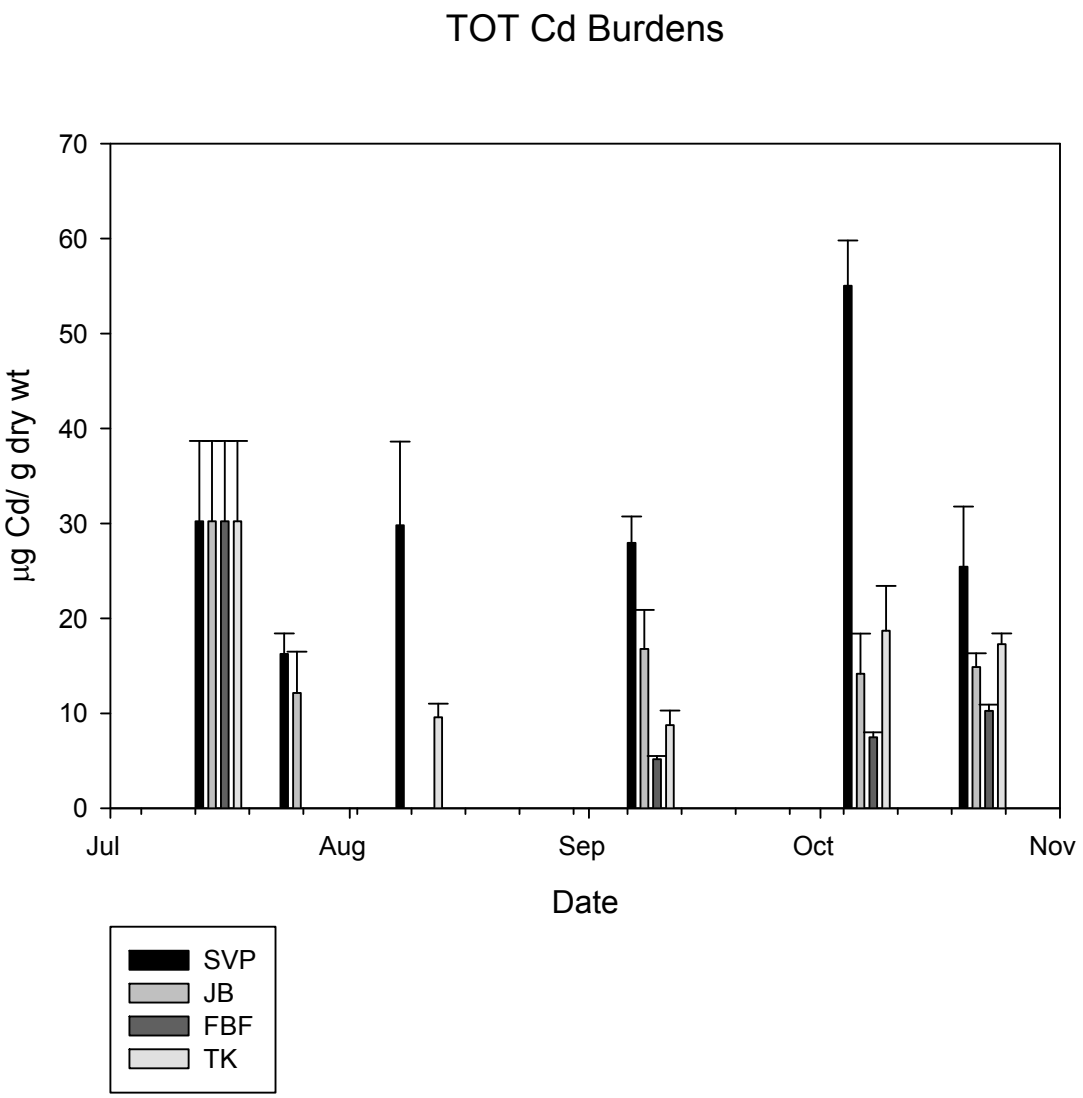


Figure 12: Total body burdens of Cd (µg) per unit dry weight of juvenile oysters, *Crassostrea virginica*, at four field sites during the summer of 2010. Cd burdens are expressed as means (*n*=4) with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction. At TK, oysters from the 2nd sampling event (7/26/10) were preserved incorrectly and not useable for analysis. Body burdens from missing FBF (8/10/10, 8/22/10, 9/22/10), JB (8/10/10, 9/22/10), and TK (8/22/10, 9/22/10) dates were not analyzed yet due to AA machine problems.

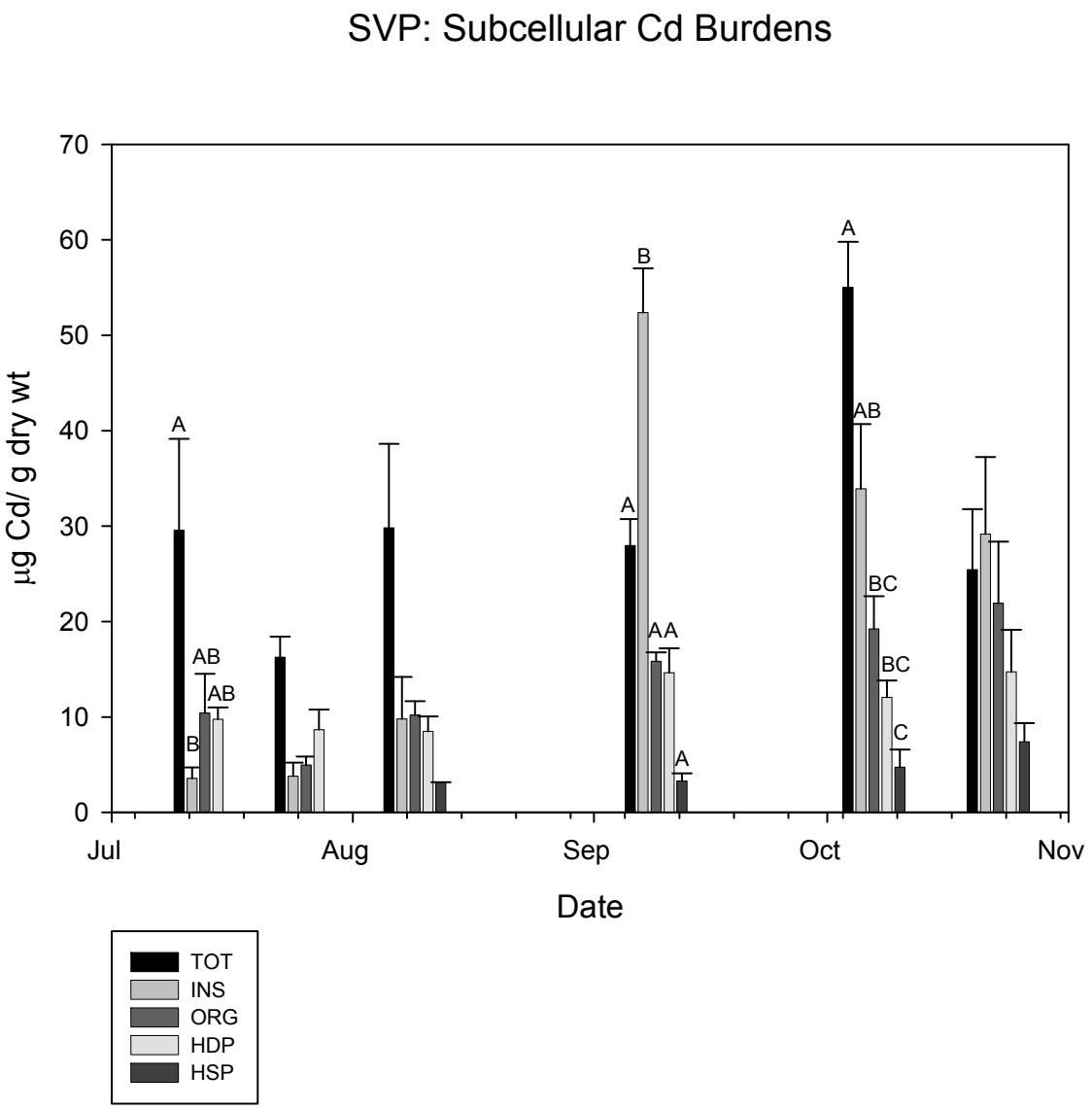


Figure 13: Subcellular Cd burdens found in different cytosolic fractions at SVP during 2010. Cd burdens are expressed as means (*n*=4) with standard error. Data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. TOT= total body burden, INS= insoluble granules, ORG= organelles, HDP= heat-denatured proteins (i.e., enzymes), HSP= heat-stable proteins (i.e., metallothioneins). No detectable Cd burdens were found in the HSP fraction on 7/15/10 and 7/26/10. Letters (A,B,C) represent significant differences between fractions on that date (Factorial ANOVA, $p < 0.05$).

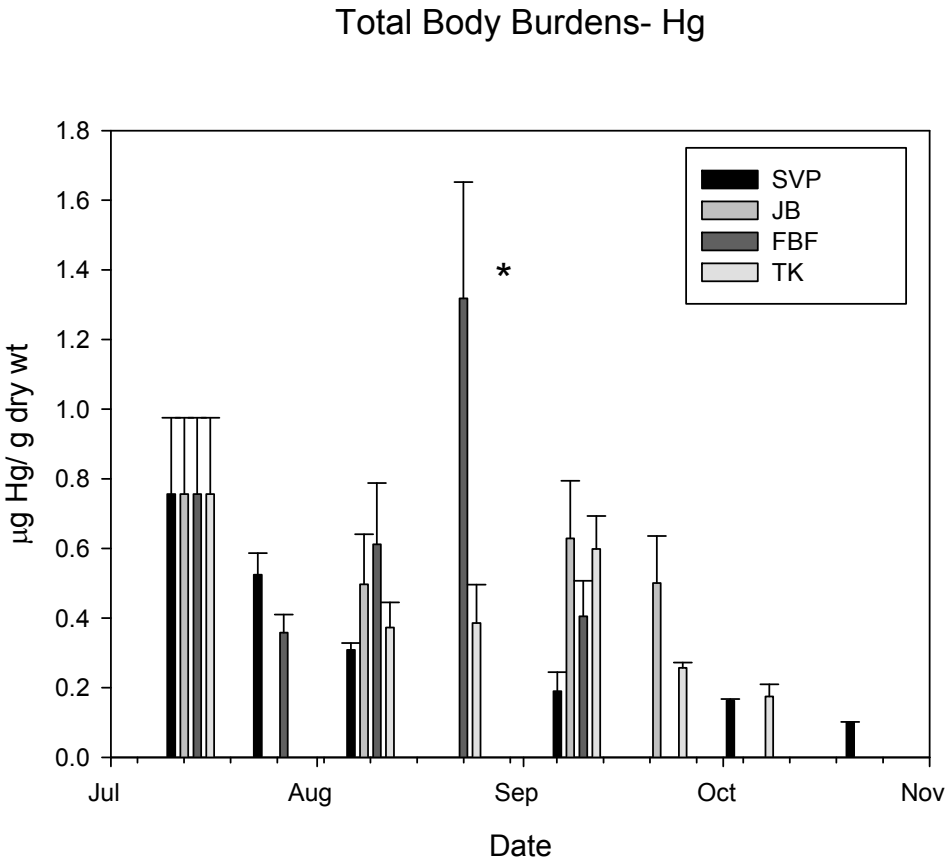


Figure 14: Total body burdens of Hg in juvenile *C.virginica* from field sites during 2010. Hg burdens are expressed as means (*n*=4) with standard error. At SVP, data was not collected at the 4th and 6th sampling events (8/23/10 and 9/21/10) due to inclement weather. At JB, data was not collected at the 2nd and 4th sampling events (7/26/10 and 8/23/10) due to tidal height restriction. At TK, oysters from the 2nd sampling event (7/26/10) were preserved incorrectly and not useable for analysis. Other missing values indicate undetectable levels of Hg in tissues. A * indicates a significant difference between sites (Factorial ANOVA, $p < 0.05$).

DISCUSSION

Previous findings have shown elevated concentrations of pollutants (including Cd, Cu, and Hg) within the HRE (Mass, unpubl. data; Bopp et al. 2006; Wirgin et al. 2006; Feng et al. 1998; Adams et al. 1996; Seidemann 1991). Analysis of sediments from SVP and JB revealed similar concentrations of Cd (0.170 μg/L Cd at SVP; 0.167 μg/L Cd at JB; Mass, unpubl. data). These concentrations have been shown to cause deleterious physiological effects in oysters in lab exposures (Ivanina et al. 2009). Exposure to 0.05 μg/L of Cd led to significant accumulation of Cd within tissues, and also increased expression of heat-sensitive proteins and metallothioneins (Ivanina et al. 2009). After an initial loss phase, oysters at SVP were shown to accumulate Cd within tissues throughout the summer, significantly more than the other three sites (Factorial ANOVA, $p>0.05$). Oysters had significantly higher TOT Cd burdens on 10/7/10 and 10/23/10 than the initial condition (ANOVA, $p< 0.05$). As the summer progressed, and oysters continued to filter water and particulates through their gills, exposure to Cd ions and increased accumulation became more likely. Cadmium burdens increased through October, coinciding with a decrease in condition index (Figure 2 & 12). Abbe et al (2000) found that increases in Cd burdens occurred in Chesapeake Bay oysters as the condition index was decreasing, which could be due to loss of tissue mass or retention of metals in insoluble granules or metallothioneins. SVP oysters also showed increased Cd burdens in both the INS and HSP fractions as the summer progressed (Figure 13). More Cd was accumulated in the INS fraction, where granular hemocytes are found (Roesijadi 1996). These insoluble granules may help

to detoxify oysters by binding the free Cd within tissues and sequestering it, thus helping to detoxify the organism (Roesijadi 1996). Among the fractions, oysters accumulated more Cd into the ORG and HDP fractions than HSP (Figure 13). Organelles (such as mitochondria; ORG) and enzymes (HDP) necessary for metabolism, defense, and growth may be impacted by the accumulated Cd. Metals binding to mitochondria and important enzymes may denature the enzymes, and alter the metabolic regime of the oyster. Differences in energy budgets can lead to large-scale effects (i.e., lowered reproductive output, slower filtration rate) that will affect potential restoration of healthy oyster reefs (Sokolova et al. 2005). Preliminary data (Mass, unpubl.) on filtration rate of juvenile oysters at the four field sites (from 2010) has shown a significant decrease in filtration rate as Cd accumulates within SVP oysters. This may be due to the binding of metals to sensitive intracellular targets within ORG and HDP that control filtration activity for *C. virginica*. More investigation into the alteration of physiological variables such as filtration and assimilation is necessary to determine if changes are due solely to Cd accumulation.

No significant correlations were seen between TOT Cd burdens and any physiological or environmental parameters, which was surprising. Cd complexes easily with free chlorine ions to form $CdCl_2$, a form that is not bioavailable for oysters to uptake (Blackmore & Wang 2003); therefore, salinity was predicted to affect the accumulation of Cd by oysters at the various sites. JB is located furthest from the mouth of Jamaica Bay in an enclosed creek, where salinities may become higher due to low flushing and dry summers (as seen in 2010). On the other hand, TK was located on a well-flushed inlet, yet had higher salinity due to its proximity to

the Atlantic Ocean. Additionally, a correlation between TOT Cd and condition index was predicted, since the loss of tissue mass may affect the μg Cd per unit dry mass (if the Cd is not being offloaded, due to spawning activities or sequestration). Future analysis is needed to determine if metal accumulation is the root cause for the differences seen in physiology at these sites, including a laboratory exposure and further metal analysis.

Total body burdens of Hg were not significantly different between sites over time except for 8/22/10 when elevated concentrations of Hg in tissues at FBF were seen (Factorial ANOVA, $p<0.05$; Figure 14). After this date, there was a decline in condition index that indicates possible spawning by the oysters (Figure 2). Formation of gonad tissue was found in FBF oysters at this date and prior sampling as well (pers. obsv.). A spawning event may have provided the oysters with a way to detoxify; Hg will easily bind with gonad tissue, allowing for depuration as the oyster spawns (Kehrig et al. 2006; Gagne et al. 2002). Gagne et al (2002) found high levels of class IIb metals (which include Hg and Cd) in gonads of female *Mya arenaria* along with elevated condition index values which indicate the clam is getting ready to spawn. A positive correlation between condition index, carbohydrate and lipid content, and Hg concentrations found at SVP support this statement; as the oysters were building up gonad tissue, Hg was accumulating (Figures 2, 3, 5, & 14).

Condition index values at FBF and JB increased over time from 7/13/10-8/3/10, followed by a sharp decrease from 8/22/10- 9/9/10, and again a significant increase until 10/7/10. This fluctuation in condition index levels may indicate a

spawning event, where the tissue mass of the oyster would decrease quickly over time due to the release of gametes (Li et al. 2009). The decreasing condition index values correspond with decreasing lipid levels (although not significantly correlated) at FBF as well. This decrease in lipid storage may also indicate spawning because oysters store large quantities of lipid in gametes (Pazos et al. 1996). Post- spawning, recovery occurs with increasing quantities of lipids. Both SVP and TK showed condition indices correlated with carbohydrate and lipid levels; however, no correlation between condition index and lipid or carbohydrate levels was seen at FBF or JB, and no correlation with protein levels was seen at any of the sites (Pearson correlation; $p < 0.05$). Oysters use carbohydrate stores first during somatic and gametic growth phases, followed by lipid stores. Protein percentages within tissue were at the lowest levels on 9/9/10, as condition index values were increasing after sharp decreases the previous weeks (Figures 2 & 4). This may be due to large quantities of carbohydrates and lipids being depleted, and oysters depending on proteins for somatic growth as food supplies diminish. The decrease in condition index occurs along with the decline in food quantity (total chlorophyll *a*) (Figure 2 and 6). Juvenile oysters rapidly feed on algae when it is available, putting ingested energy towards shell growth and tissue growth. Oysters are able to particle-sort on the velum, and are able to reject particles that are less nutritious into their pseudofeces (Baldwin & Newell 1995), which may be why at lower concentrations of algae, the oysters had lower condition indices. As the condition index declined, the amount of stored carbohydrates increased. Storing carbohydrates when food availability is lower is a way to ensure energy during low-food times (such as

winter), when oysters will use energy for basic metabolic demands rather than for growth and reproduction.

It was observed that the shells at SVP and TK were much thinner than those at FBF and JB, which may be due to the diversion of energy more towards general metabolism and less towards shell growth at these two sites, both of which had the lowest condition index values throughout the season (Figure 2). Both sites also saw a decrease in stored biochemical compounds (carbohydrates, proteins, and lipids) until 9/9/10; however, carbohydrate and lipid levels remained low in SVP while TK oysters were able to recover and increase beyond what was seen in the initial sample (Figures 3-5). This change in percentages of biochemical storage compounds indicates that the oysters were transferring energy from growth and reproduction into stored compounds for metabolic demands. JB and FBF oysters did not see a sharp decline in carbohydrates, nor as much lost from protein and lipid storage as seen at other sites. This may be due to higher chlorophyll *a* amounts available, or better seston quality (Figures 6 & 10). No statistically significant correlations between food availability or quality and overall condition index were found at JB or FBF; however, these correlations do not take into account the food availability seen between bi-monthly sampling events. A more consistent chlorophyll/ seston quality monitoring program would show more temporal trends in food availability, and help to explain why thinner shells were seen at the two sites outside of Jamaica Bay.

Environmental parameters (i.e., seston quantity and quality, temperature, salinity, oxygen levels) can have a profound effect on oyster physiology (Paterson et al. 2003; Pridmore et al. 1990; Ivanina et al. 2009). Salinity can influence the

binding state of metal ions and thus the amount of metal that is accumulated in the tissue. Changes in temperature, especially elevated temperatures, can increase metabolic demands and increase the expression of heat-shock proteins and metallothionein proteins, which can affect the accumulation and detoxification of metals (Cherkasov et al. 2007; Ivanina et al. 2009). Between June-August, 2010, the HRE experienced a very hot and dry summer, with temperatures reaching a peak of 25.5^{o}C and salinity between 23-32 ppt (Table 1). Along with an increase in temperature comes a decrease in dissolved oxygen levels; sites experienced values between 1.75- 12.03 mg/L. Values below 2.5 mg/L indicate hypoxic conditions, which may affect oyster metabolism and lead to higher metal body burdens (Baker & Mann 1994).

Evaluation of oyster physiology when placed at various field sites along a contamination gradient will allow for a greater understanding of bivalve responses to urbanization. While numerous lab-based studies have examined a single variable and the oyster's response, this situation allows us to determine the interactions of multiple variables on oyster condition, energy budgets, and storage of harmful contaminants. A suite of contaminants exists in the HRE, including organic pollutants (PAHs, PCBs) which can alter oyster physiology, and this study will provide a starting point for discussions on restoration. Various institutions and organizations in New York City and New Jersey have begun oyster restoration projects within the HRE, and knowledge of how our unique system affects the growth, reproduction, and larval dynamics of *Crassostrea virginica* is imperative for the success of such projects.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Chester Zarnoch for his assistance and expertise with field work, as well as advisement of A. Mass' PhD dissertation work. Pawel Pieluszynski, Narendra Paramanand, Roland Hagen and Dr. Timothy Hollein provided field assistance. Dr. David Seebaugh provided laboratory assistance and advice during the project. This project was primarily supported by a Tibor T. Polgar Fellowship from the Hudson River Foundation to A. Mass. Additional support was provided by the Jamaica Bay Institute (research grant to A. Mass), and NSF research grant (to C. Zarnoch). Site use was generously provided by the Rutgers University Marine Field Station, Tuckerton, NJ; New York City Parks Department, Soundview Park, Bronx, NY; and Gateway National Recreation Area, National Parks Department, Brooklyn, NY.

LITERATURE CITED

Abbe, G.R., G.F Riedel, and J.G. Sanders. 2000. Factors that influence the accumulation of copper and cadmium by transplanted eastern oysters (*Crassostrea virginica*) in the Patuxent River, Maryland. Marine Environmental Research 49: 377-396.

Adams, D.A., J.S. O'Connor, and S.B. Weisberg. 1996. Sediment quality of the NY/NJ Harbor system- an investigation under the Regional Environmental Monitoring and Assessment Program. Final report to the US EPA.

Amiard, J.-C., C. Amiard-Triquet, S. Barka, J. Pellerin, and P.S. Rainbow. 2006. Review: Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquatic Toxicology 76: 160-202.

Baker, S.M, and R. Mann. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. Journal of Experimental Marine Biology and Ecology 181: 239-253

Baldwin, B.S., and R.I.E Newell. 1995. Feeding rate responses of oyster larvae (*Crassostrea virginica*) to seston quantity and composition. Journal of Experimental Marine Biology and Ecology 189: 77-91.

Bayne, B.L., 2002. A physiological comparison between Pacific oysters *Crassostrea gigas* and Sydney rock oysters *Saccostrea glomerata*: food, feeding and growth in a shared estuarine habitat. Marine Ecology Progress Series 232, 163–178.

Blackmore, G., and W.-X. Wang. 2003. Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. Aquatic Toxicology 62: 205-218.

Blanchard, J., K. Brix, and M. Grosell. 2009. Subcellular fractionation of Cu exposed oysters, *Crassostrea virginica,* and Cu accumulation from a biologically incorporated Cu rich oyster diet in *Fundulus heteroclitus* in fresh and sea water. Comparative Biochemistry and Physiology, Part C. 149: 531-537.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

Bopp, R.F., S.N. Chillrud, E.L. Shuster, and H.J. Simpson. 2006. Contaminant chronologies from the Hudson River sedimentary records. pp. 383-397 *in*: Levinton, J.S. and J.R. Waldman (Eds). The Hudson River Estuary. Cambridge University Press, New York.

Brown, C.L., and S.N. Luoma. 1995. Use of the euryhaline bivalve *Potamocorbula amurensis* as a biosentinel species to assess trace metal contamination in San Francisco Bay. Marine Ecology Progress Series 124: 129-142.

Brumbaugh, R.D., L.A. Sorabella, C.O. Garcia, W.J. Goldsborough, and J.A. Wesson. 2000. Making a case for community-based oyster restoration: an example from Hampton Roads, Virginia, USA. Journal of Shellfish Research 19: 397– 400.

Cherkasov, A.S., S. Grewal, and I.M. Sokolova. 2007. Combined effects of temperature and cadmium exposure on haemocyte apoptosis and cadmium accumulation in the eastern oyster *Crassostrea virginica*. Journal of Thermal Biology 32: 162-170

Cherkasov, A.S., C. Taylor, and I.M. Sokolova. 2009. Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. Aquatic Toxicology 97: 68-78.

Coen, L.D., and M.W. Lukenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? Ecological Engineering 15: 323-343.

Crawford, D.W., N.L Bonnevie, C.A. Gillis, and R.J. Wenning. 1994. Historical changes in the ecological health of the Newark Bay Estuary, New Jersey. Ecotoxicology and Environmental Safety 29: 276-303.

Crosby, M.P., and L.D. Gale. 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. Journal of Shellfish Research 9: 233–237.

Dimou, K.N., T.-L. Su, R.I. Hires, and R. Miskewitz. 2006. Distribution of polychlorinated biphenyls in the Newark Bay Estuary. Journal of Hazardous Materials 136: 103-110.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350-356.

Erk, M., B.T.A. Muyssen, A. Ghekiere, and C. Janssen. 2008. Metallothionein and cellular energy allocation in the estuarine mysid shrimp *Neomysis integer* exposed to cadmium at different salinities. Journal of Experimental Marine Biology and Ecology 357: 172-180

Feng, H., J.K. Cochran, H. Lwiza, B.J. Bronawell, and D.J. Hirschberg. 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: The Hudson River. Marine Environmental Research 45: 69-88.

Franz, D.R. 1982. *A historical perspective on mollusks in lower New York Harbor, with emphasis on oysters. In*: G.F. Mayer. (Ed.) Ecological Stress and the New York Bight: Science and Management. Estuarine Research Foundation, Columbia, S.C.

Gagne, F., C. Blaise, J. Pellerin, and S. Gauthier- Clerc. 2002. Alteration of the biochemical properties of female gonads and vitellins in the clam *Mya arenaria* at contaminated sites in the Saguenay Fjord. Marine Environmental Research 53: 295-310.

Geffard, A., J.C. Amiard, and C. Amiard-Triquet. 2002. Kinetics of metal elimination in oysters from a contaminated estuary. Comparitive Biochemistry and Physiology, Part C. 131: 281-293.

Gerritsen, J., A.F. Holland, and D.E. Irvine. 1994. Suspension-feeding bivalves and the fate of primary production: an estuarine model applied to Chesapeake Bay. Estuaries 17: 403–416.

Gnaiger, E., and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologica 62: 289-298.

Hartwell, S.I., D.A. Wright, R. Takacs, and C.H. Hocutt. 1991. Relative respiration and feeding rates of oyster and brackish water clam in variously contaminated waters. Marine Pollution Bulletin 22: 191-197.

Hatch, W.R., and W.L. Ott, 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Analytical Chemistry 40: 2085-2087.

Ivanina, A.V., C. Taylor, and I.M. Sokolova. 2009. Effects of elevated temperature and cadmium exposure on stress protein response in eastern oysters *Crassostrea virginica* (Gmelin). Aquatic Toxicology 91: 245-254.

Kehrig, H.A., M. Costa, I. Moreirac and O. Malma. 2006. Total and Methyl Mercury in Different Species of Molluscs from Two Estuaries in Rio de Janeiro State. Journal of the Brazilian Chemical Society 17: 1409-1418.

Khoury, J.N., E. Powers, P. Patnaik, and W.G. Wallace. 2009. Relating disparity in competitive foraging behavior between two populations of fiddler crabs to subcellular partitioning of metals. Archives of Environmental Contamination and Toxicology 56: 489-499.

Klajovic-Gaspic, Z., N. Odzak, I. Ujevic, T. Zvonaric, M. Horvat, and A. Baric. 2006. Biomonitoring of mercury in polluted coastal areas using transplanted mussels. Science of the Total Environment 368: 199-209.

Kurlansky, M. 2006. The Big Oyster: History on the Half Shell. Random House, New York, NY.

Lanning, G., A.S. Cherkasov, H.O. Portner, C. Bock, and I.M. Sokolova. 2008. Cadmium-dependent oxygen limitation affects temperature tolerance in eastern oysters (*Crassostrea virginica* Gmelin). American Journal of Physiology: Regulated, Integrative, and Comparative Physiology 294: 1338-1346.

Levinton, J.S. and J. R. Waldman. 2006. The Hudson River Estuary: Executive Summary. pp. 1-13 *in*: Levinton, J.S. and J.R. Waldman (Eds). The Hudson River Estuary. Cambridge University Press, New York.

Li, Y., J.G. Qin, X. Li, and K. Benkendorff. 2009. Monthly variation of condition index, energy reserves, and antibacterial activity in Pacific oysters, *Crassostrea gigas*, in Stansbury (South Australia). Aquaculture 286: 64-71.

MacDonald, B.A., and J.E. Ward. 1994. Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus* (Mollusca: Bivalvia). Marine Ecology Progress Series 108: 251-264.

Mouneyrac, C., J.C. Amiard, and C. Amiard-Triquet. 1998. Effects of natural factors (salinity and body weight) on cadmium, copper, zinc, and metallothionein-like protein levels in resident populations of oysters *Crassostrea gigas* from a polluted estuary. Marine Ecology Progress Series 162: 125-135.

Nelson, K.A., L.A. Leonard, M.H. Posey, T.D. Alphin, and M.A. Mallin. 2004. Using transplanted oyster (*Crassostrea virginica*) beds to improve water quality in small tidal creeks: a pilot study. Journal of Experimental Marine Biology and Ecology 298: 347-368.

Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. Journal of Shellfish Research 23: 51-61.

O'Connor, T.P. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. Marine Environmental Research 53: 117-143.

Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Paterson, K.J., M.J. Schreider, and K.D. Zimmerman. 2003. Anthropogenic effects on seston quality and quantity and the growth and survival of Sydney rock oyster (*Saccostrea glomerata*) in two estuaries in NSW, Australia. Aquaculture 221: 407-426.

Pazos, A.J., C. Ruiz, O. Garcia- Martin, M. Abad, and J.L. Sanchez. 1996. Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N.W. Spain. Comparative Biochemistry and Physiology 114B: 171-179.

Pridmore, R.D., D.S. Roper, and J.E. Hewitt. 1990. Variation in composition and condition of the Pacific oyster, *Crassostrea gigas*, along a pollution gradient in Manukau Harbour, New Zealand. Marine Environmental Research 30: 163-177.

Ringwood, A.H., D.E. Conners, and A. DiNovo. 1998. The effects of copper exposures on cellular responses of oysters. Marine Environmental Research 46: 591-595.

Ringwood, A.H., J. Hoguet, C. Keppler, and M. Gielazyn. 2004. Linkages between cellular biomarker responses and reproductive success in oysters- *Crassostrea virginica.* Marine Environmental Research 58: 151-155.

Roesijadi, G. 1996. Environmental Factors: Response to Metals. pp. 515-538 *in*: Kennedy, V.S., R.I.E. Newell, and A.F. Eble (Eds.) The Eastern Oyster, *Crassostrea virginica*. Maryland Sea Grant College. College Park, MD.

Sambrotto, R. 2001. Nitrogenous Nutrients and Plankton Production in Jamaica Bay. *in*: Integrated Reconnaissance of the Physical and Biogeochemical Characteristics of Jamaica Bay: Initial Activity Phase (A Coordinated Program of the Gateway National Recreational Area and the Columbia Earth Institute). Final Report.

Seidemann, D.E. 1991. Metal pollution in sediments of Jamaica Bay, New York, USA- An urban estuary. Environmental Management 15: 73-81.

Sokolova, I.M., E. P. Sokolov, and K. M. Ponnappa. 2005. Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Aquatic Toxicology 73: 242-255.

Taylor, G.T, J. Way, and M. I. Scranton. 2003. Planktonic carbon cycling and transport in surface waters of the highly urbanized Hudson River estuary. Limnology and Oceanography 48: 1779-1795.

Volety, A.K. 2008. Effects of salinity, heavy metals, and pesticides on health and physiology of oysters in the Caloosahatchee Estuary, Florida. Ecotoxicology 17: 579-590.

Wakeman, T.H., and N.J. Themelis. 2001. A basin-wide approach to dredged material management in New York/ New Jersey Harbor. Journal of Hazardous Materials 85: 1-13.

Waldman, J. 1999. Heartbeats in the Muck. Lyons Press, New York, NY.

Wallace, W.G., B.G. Lee, and S.N. Luoma. 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. 1: Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Marine Ecology Progress Series 249: 183-197.

Wirgin, I., J.S. Weis, and A.E. McElroy. 2006. Physiological and Genetic Aspects of Toxicity in Hudson River Species. pp. 441-460 in: Levinton, J.S. and J.R. Waldman (Eds). The Hudson River Estuary. Cambridge University Press, New York.

Yozzo, D.J., P. Wilber, and R.J. Will. 2004. Beneficial use of dredged material for habitat creation, enhancement, and restoration in New York- New Jersey Harbor. Journal of Environmental Management 73: 39-52.

Zar, J.H. 1999. Biostatistical Analysis. Upper Saddle River, NJ. Fourth edition.

IMPACT OF SILVER NANOPARTICLE EXPOSURE ON CRAYFISH (*Orconectes virilis)* GROWTH, CHEMISTRY AND PHYSIOLOGY IN CONTROLLED LABORATORY EXPERIMENT AND HUDSON RIVER ECOSYSTEM

A Final Report of the Tibor T. Polgar Fellowship Program

Allen Clayton

Polgar Fellow

School of Science, Department of Biology
Marist College
Poughkeepsie, NY 12601

Project Advisor:

Dr. Zofia Gagnon
School of Science, Department of Environmental Science
Marist College
Poughkeepsie, NY 12601

Clayton, A.C. and Z.E. Gagnon. 2012. Impact of Silver Nanoparticle Exposure on Crayfish (*Orconectes virilis*) Growth, Chemistry and Physiology in Controlled Laboratory Experiment and Hudson River Ecosystem. Section VI: 1-36 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

The use of nanotechnology has become widespread in commercial, industrial and medical applications; however, there is concern that their high level of reactivity may also pose risks to human health and the environment. Previous studies involving metal nanoparticles have shown them to be toxic and destructive to DNA and metabolic pathways. Silver nanoparticles (AgNPs) have recently received much attention for their growing role in biotechnology and life sciences.

Crayfish (*Orconectes virilis*), a common inhabitant of the Hudson River and its tributaries, were used as an experimental model in this project. A colloidal solution of AgNPs was synthesized from chemical reduction of silver nitrate ($AgNO_3$) by sodium borohydride ($NaBH_4$), and organisms were exposed for 10 days to different concentrations of colloidal AgNP suspended in Hudson River water. The following AgNP concentrations were used: 0.0, 0.05, 0.107, 0.16, and 0.214 mg/L. Control treatments of $AgNO_3$ and $NaBH_4$ were established in the same concentrations used for synthesis of the AgNP treatments. Additional control treatments were established using untreated Hudson River water, and cages placed directly in the Hudson River (river control). Crayfish were harvested and examined for silver accumulation, DNA damage, and pathological changes. Silver accumulation in major organs was determined by atomic absorption using a ThermoElemental Solaar M5 spectrophotometer in graphite furnace mode. DNA damage was examined via single cell gel electrophoresis (comet assay).

The bioaccumulation of Ag in crayfish liver, muscle, and green gland tissues was detected in all AgNP and $AgNO_3$ treatments. DNA damage was found to be statistically significant in all laboratory specimens when compared to the river control.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

The use of nanotechnology has grown dramatically in commercial, industrial, medical, and consumer products in the last decade. The National Science and Technology Council in 2001 defined the scale of nanotechnology as atomic, molecular or macromolecular levels in the range of ~ 1 – 100 nanometers (Hornyak et al. 2009). The National Nanotechnology Initiative, established in 2001, led to public funding for nanoparticle (NP) research in the United States to develop new material applications and to investigate new commercial applications for NP antimicrobial capability (Ahamed et al. 2008). Metal oxide NPs currently have wide industrial applications in photocatalytic water purification systems (Hagfeldt and Graetzel 1995), solar cells (Usui et al. 2004), electronics, and many everyday products such as sunscreens and cosmetics. NPs also have exceptionally desirable biological, fungicidal, bacteriological, and algicidal properties. Today, development of a NP application is frequently considered an advancement of modern science.

Silver nanoparticles (AgNPs) have recently received broad attention for their growing role in biotechnology and life science. Among 580 consumer nanotechnology-based products, the most common material mentioned in product descriptions is silver-based nanoparticles (Woodrow Wilson Center 2007; Henig 2007). AgNPs have a large surface area relative to their volume and, as a result of their size, easily interact with other particles (Ying 2001). AgNPs are used to technologically enhance products such as bandages, clothing, cosmetics, food, and toys (Woodrow Wilson Center 2007; Henig 2007). The antibacterial effect of AgNPs has brought about their extensive use in health, electronic, and home goods. Recently, AgNPs have been used in the production of

clothing and food products to reduce bacterial growth (Chau et al. 2007; Vigneshwaran et al. 2007). AgNPs are used as an antimicrobial agent in consumer goods such as disinfectants, deodorants, toothpaste, shampoo, and humidifiers (Woodrow Wilson Center 2007; Henig 2007). AgNPs are proven to be very effective bacterial filters. It has been suggested that AgNPs be added to aquaculture systems for use as anti-bacterial and anti-fungal agents in wastewater treatment plants. However, researchers have found that nanoparticles also eliminate helpful bacteria that remove ammonia, and lethal toxicity levels of NPs are still under debate (Choi and Hu 2008).

AgNPs are also of interest to defense and engineering programs for new material applications (Ringer and Ratinac 2004). There is a potential for AgNPs to be an ingredient in the treatment of diseases that need constant drug concentration in the blood or to target specific cells or organs (Moghimi et al. 2001; Panyam and Labhasetwar 2003). *In vitro* tests have shown that AgNPs can be used to inhibit binding of the HIV-1 virus to host cells (Ahamed et al. 2008; Elechiguerra et al. 2005). Medicinally, antimicrobial activity of AgNPs has been used to reduce infections in burn treatment (Kim et al. 2007; Ulkur et al. 2005) and to reduce the risk of infection by treating the surface of catheters (Samuel and Guggenbichler 2004), prostheses (Gosheger et al. 2004), and human skin (Paddle-Ledinek et al. 2006).

Regardless of the prevalent application of AgNPs, there is a lack of information relating to their toxicity at the organismal, cellular, and molecular level (Ahamed et al. 2008). The concentrations at which AgNPs become toxic are currently being determined. Mnyusiwalla et al. (2003) expressed concern that NPs could have potentially adverse effects on human health and the environment. The high surface to volume ratio gives NPs

catalytic qualities, and their size allows them to pass through cell membranes with currently unknown biological effects (The Center for Food Safety 2006).

Sung et al. (2008) conducted an extensive study on inhalation exposure of AgNPs using Sprague-Dawley strain rats. Histopathological examinations indicated that inflammatory cell infiltrate, chronic alveolar inflammation, and small granulomatous lesions were proportionally correlated to AgNP dose. The exposure influenced minimal bile-duct hyperplasia in males and females, chronic alveolar inflammation and macrophage accumulation in the lungs of males and females, and erythrocyte aggregation in females. However, the authors of the study reported that an exposure level of 100 $\mu g/m^3$ had no adverse effect on experimental animals, which was consistent with the American Council of Government Industrial Hygienists silver dust threshold limit value (TLV).

In studies on mammal germ line stem cells, AgNPs have been shown to decrease mitochondrial activity and increase membrane leakage. In addition, AgNPs were found to increase the creation of reactive oxygen species (ROS), reduce antioxidant activity of glutathione (GSH), and diminish mitochondrial function in Buffalo rat liver (BRL-3A) cells (Ahamed et al. 2008; Braydich-Stolle et al. 2005; Hussain et al. 2005). Studies were done to observe DNA damage in response to polysaccharide surface functionalized (coated) and non-functionalized (uncoated) AgNPs in two types of mammalian cells, including mouse embryonic stem (mES) cells and mouse embryonic fibroblasts (MEF) (Ahamed et al. 2008). The experiment showed more severe damage in coated AgNPs, suggesting that genotoxicity may be affected by different AgNP surface chemistry (Ahamed et al. 2008).

Another experiment on AgNPs studied the lipid-based dispersion of NPs, sometimes helpful in reducing nanoparticle toxicity and in developing therapeutic agents (Bothun 2008). Accommodation of large hydrophobic NPs in lipid bilayers was confirmed. It appears that this is done by distortion of lipid bilayers relative to the thickness of the bilayer (Bothun 2008).

Blood hematology and biochemistry were analyzed and the results found significant dose-dependent changes on alkaline phosphates and cholesterol values for both male and female rats, implying slight liver damage from AgNP inhalation exposures of more than 300 mg (Kim et al. 2007). The study showed accumulation of AgNPs was more significant in female kidneys than in male kidneys. Conclusions were made that prolonged AgNP inhalation exposure would significantly increase the occurrence of lung inflammation, at much lower mass dose concentrations, when compared to submicrometer particles (Sung et al. 2008).

The safety of NP topical use has also become of great interest. There is very little known about their potential to penetrate the skin. Larese et al. (2009) evaluated *in vitro* skin penetration of AgNPs coated with polyvinylpirrolidone (PVP). The experiments were done using the Franz diffusion cell method with intact and damaged human skin. AgNP absorption through intact and damaged skin was detectable by electron microscopy in the stratum corneum and the outermost surface of the epidermis (Larese et al. 2009).

The very limited knowledge on toxicological risk assessment of engineered NPs to biological systems raises public and scientific concern. Questions regarding NPs associated with commonly used nanotechnology, particularly how much eventually enters

the environment, remain unanswered. Of even greater concern, and poorly understood, is the potential effect on human health and the fate of nanomaterials in terrestrial and aquatic environments (Hornyak et al. 2009). Since there are no existing methods for removal of AgNPs from wastewater effluents (Hornyak et al. 2009), it is especially urgent to learn how much silver (Ag) from colloidal AgNP suspensions is being introduced into waterways.

The purpose of this project was to study the effect of AgNP exposure on an aquatic animal, using crayfish (*Orconectes virilis*) as experimental model. Crayfish is a common inhabitant of the Hudson River Watershed and known not to tolerate polluted water. In this study, crayfish were exposed to varying levels of colloidal AgNPs in Hudson River water culture media to test the hypothesis that exposure would be correlated with bioaccumulation of Ag in animal tissues, pathological changes, and DNA damage. An additional control group of caged crayfish was placed directly in the Hudson River (river control) during the experimental period to compare laboratory findings with a natural environment.

METHODS

Experimental Organism

The crayfish (*Orconectes virilis*), a common inhabitant of the Hudson River and its tributaries, was chosen as the experimental organism because it lives in the sediment where most pollutants accumulate. The stock used in the experiment consisted of crayfish specimens purchased from Northeastern Aquatics in Rhinebeck, NY.

Hudson River Water Dechlorination and Filtration

The aquaria were filled with 20 L of raw, unfiltered Hudson River water. Upon trial tests of the addition of $AgNO_3$, $NaBH_4$, and AgNP, the $AgNO_3$ water became light pink which progressed to red and eventually black. The CRC Handbook of Chemistry and Physics was consulted, and it was determined that the coloration was due to the formation of the chemical, AgCl. A colormetric test was conducted on untreated Hudson River water, and free Cl was found to be 0.070mg\L. It turns out that the Marist College River Lab is located approximately 300 yards down river from the Poughkeepsie Water Treatment Plant, the apparent source of the chlorine. Due to $AgNO_3$'s high reactivity, all water used in the experiment was allowed to dechlorinate for one week. A separate aquarium was completely filled with raw Hudson River water to serve as a fresh supply of dechlorinated water to keep each tank's water level at 20 L. The contents of the tanks were filtered during the declorination period with a Lee's Economy Corner Filter filled with filter floss for particulates only. Filter floss was replaced on the second, fourth, and seventh days. Due to its highly absorptive nature and ability to remove metals, activated charcoal filters were avoided in the experiment (Bansal and Goyal 2005). The filters were powered using Tetra Whisper aquarium tank air pumps.

Aquaria Preparation

After the declorination period, three cups of quarter inch aquarium stone were placed on the bottom as substrate. Three five-inch pieces of 2 in. diameter PVC cut in half lengthwise were placed on the substrate as a shelter for the crayfish.

Crayfish Acclimation

Upon arrival, three experimental organisms were placed in each tank and allowed to acclimate to the laboratory and Hudson River conditions for one week. From the 120 crayfish purchased, 96 specimens were chosen at random, while at the same time trying to obtain organisms of relatively the same age and size. Crayfish were fed dry cat food (one piece per organism). Any excess food was immediately removed from the aquarium. No ammonia filtration was required due to the low amount of liquid waste produced by the crayfish and the short, ten day exposure time.

Nanoparticle Synthesis

During the acclimation period, the AgNPs were synthesized. A slightly modified Creighton method of AgNP synthesis was used for the experiment (Creighton and Eadon 1991). AgNPs were synthesized through the chemical reduction of silver nitrate ($AgNO_3$) using sodium borohydride ($NaBH_4$) as a reducing agent. AgNPs are an aggregation of elemental Ag that forms together in a spherical structure, each structure from 1 to 100 nm. An $AgNO_3$ solution (3.4 mg in 20 ml deionized water) cooled to approximately 10°C

Table 1. The schematic of compound amounts used in the synthesis of colloidal AgNPs and AgNP concentrations obtained in the process of synthesis.

Synthesis Formula			Concentration
$AgNO_3$ (mg)	$NaBH_4$ (mg)	AgNP (ml)	Ag mg/L
3.4	4.5	80	0.107
Breakdown of Concentrations (mg/L)			Final Concentrations
1.7	2.3	40	0.05
3.4	4.5	80	0.107
5.1	6.8	120	0.16
6.8	9.0	160	0.214

was added drop wise with constant stirring to a $NaBH_4$ solution (4.53 mg in 60 ml deionized water) pre-cooled to 2°C. Table 1 summarizes compound amounts used to synthesize the resulting Ag concentrations used for treatments. Stirring continued for about 45 minutes. A 250 ml Erlenmeyer flask in which the reaction was taking place was wrapped in aluminum foil to block light (the reaction is light sensitive).

Following the synthesis process, the solution remained on ice and stirred for 1h. The ice bath was removed and the AgNPs remained on the stir plate until they reached room temperature. All treatment solutions were then stored at 5°C.

Experimental Setup

After the acclimation period, each tank was refilled with dechlorinated Hudson River water up to the 20 L mark and the weight of each organism was recorded to the nearest tenth of a gram. Specimens in each tank were marked with a red dot, a yellow dot, or no color to distinguish between the three specimens. As noted in Table 1, Ag concentrations of 0.05, 0.107, 0.16, and 0.214 mg/L were established by adding the following amounts of stock AgNP solution, 40.0 ml, 80.0 ml, 120.0 ml and 160.0 ml, to 20 L of Hudson River water. The corresponding control treatments of $AgNO_3$ and $NaBH_4$ (parental compound) in the same concentrations as AgNP were also established, as shown in Table 2. Each treatment was applied to two separate tanks resulting in six total crayfish replicates per treatment. There were eight tanks per generic treatment resulting in 24 treated tanks. Along with the two control tanks containing only filtered and dechlorinated Hudson River water (laboratory control), there were also two sets of three specimens in cages suspended in the Hudson River which served as a natural, non-laboratory control (river control). In addition to the laboratory and river controls,

controls were established for the paternal materials, $AgNO_3$ and $NaBH_4$, from which AgNPs were synthesized. This was done because there is some evidence that AgNPs in colloidal solution can deaggregate to form their original compounds. Deaggregates can be identified in the solutions as described by Jayabalan et al. (2008). The concentrations of these controls were calculated based on the amount used for the synthesis of AgNPs.

Table 2. Treatments and Experimental Design

Treatment No.	Treatment Concentration	Number of Specimens
Laboratory Experiment		
1	0.050 mg/L AgNP[1]	6
2	0.107 mg/L AgNP	6
3	0.160 mg/L AgNP	6
4	0.214 mg/L AgNP	6
5	Laboratory Control (Hudson River water)	6
	$AgNO_3$ Controls	
6	0.085 mg/L $AgNO_3$[2]	6
7	0.170 mg/L $AgNO_3$	6
8	0.270 mg/L $AgNO_3$	6
9	0.340 mg/L $AgNO_3$	6
	$NaBH_4$ Controls	
10	0.115 mg/L $NaBH_4$[3]	6
11	0.225 mg/L $NaBH_4$	6
12	0.340 mg/L $NaBH_4$	6
13	0.450 mg/L $NaBH_4$	6
Hudson River Control		
14	Cages suspended directly in Hudson River	6

[1] 100 ppm concentration of AgNP was determined in the experiments as LD_{50} (lethal dose to 50% of chick embryos in earlier preliminary experiments conducted at Marist College)
[2] $AgNO_3$ control concentrations were based on concentration of Ag ion, see: Synthesis of Silver Nanoparticles (AgNPs) and Controls.
[3] $NaBH_4$ control concentrations based on B (boron) ion, see: Synthesis of Silver Nanoparticles (AgNPs) and Controls.

Gross and Behavioral Observations

The lab specimens were checked for responsiveness to a threat and to food. A piece of sinking wafer food was broken in half and dropped in front of each crayfish. Responsiveness was gauged not by whether or not the food was immediately eaten, but rather by the movement of the mouth. If the mouth appendages began to flutter when food was placed in the tank, they were said to be responsive to food. Fear response was gauged by poking a 12 inch glass stirring rod at the crayfish. If the specimen flipped its tail quickly in a manner similar to escape, they were said to be responsive to fear. Each day the tanks were refilled to the 20 L mark and the specimens were fed and checked for any change in behavior or for death. Any dead organism found within the acclimation period was replaced. Organisms that died after the treatments were administered were removed from the tank and not processed in the results. The replacement crayfish were weighed before being placed into the tank. On the final day of the exposure period all crayfish were again weighed using the same method as with the initial weights.

Tissue Sampling

The experiment was terminated on the 10th day of exposure. On the morning of harvest, all crayfish were put into bags labeled according to their tank and treatment and placed on ice for 1 minute to anesthetize them. A specimen was removed from a bag and surgically decapitated within 5-10 seconds to avoid major stress. The organs harvested included: brain, gills, liver, green gland, nerve ganglia, heart, and tail muscle. Samples of brain and liver chosen at random were immediately processed and analyzed for DNA damage. Samples of liver, green glands, and tail muscles were placed in a drying oven at $80^{o}C$ for chemical analysis of Ag content.

DNA Analysis by Single Gel Electrophoresis (Comet Assay)

At the time of dissection, one brain and one liver sample per tank (two per treatment) were immediately processed for DNA damage. The tissue was minced in a 20 mM solution of EDTA in PBS to release cell nuclei. Minced tissue was placed in fresh solution of 20 mM EDTA and centrifuged for eight minutes. Ten microliters of supernatant was drawn off immediately above the pellet. This was mixed with 90 µl of low melting agar. The 100 µl solution was placed on a pre-treated comet assay slide (Trevigen®). The slide was placed in an alkaline solution of pH 13 to unwind the DNA for 30 minutes. Slides were then placed in CometAssay™ Electrophoresis System (Trevigen®) in electrophoresis buffer (pH >13) and was carried out for 30 minutes. After this time, the slides were placed in 70% alcohol for five minutes and allowed to air dry overnight. Extracted DNA on the slides was stained with SYBR-green™, which emits fluorescent light within the 425-500 nm region. The slides were analyzed under a mercury lit epifluorescent microscope. A Magnafire SP Digital Camera was used to take pictures of comets viewed with the microscope. Length of DNA (comet) migration was measured using Image-Pro® Plus software. All nuclei and their comets on the slides were measured (~50). Distribution of DNA between the tail and head of the comet was used to evaluate the degree of DNA damage. Measurements were recorded on nuclei with clearly defined tail boundaries.

Atomic Absorption Analysis

Samples of muscle, liver, and green gland tissue were oven dried for 72 hours at 80°C. The tissue was ground to a fine powder using mortar and pestle, and samples were weighted to obtain ~0.1g.

In preparation for chemical digestion, tissues were placed in an Xpress vessel with 5 ml of high purity nitric acid (Fisher Scientific, Optima Grade). Chemical digestion was performed using the MARS Xpress microwave (CEM). The process parameters were set to 180 °C operating temperature, 80 watts, ten minute ramp to temperature, and ten minute run time. The digestion program was run for 30 minutes.

Silver content analyses were conducted via atomic absorption (AA) spectrometry using a ThermoElemental Solaar M5 atomic spectrophotometer in graphite furnace mode (GFAAS). Reference 1.0 ppm Ag standard was prepared using a 1.0×10^3 ppm Ag solution purchased from Fisher Scientific (Lot # CL4-132AG) and certified by SPEX CertiPrep. The absorption wavelength of Ag was 328.1 nm, and the temperature was 2500°C. Ag concentrations were established through external calibration standards using a least squares fit regression curve. Results given by the AA computer output were in units of µg/L. Calculations were made to determine the Ag content in the 1 g dry weight of the sample [g (AgNP)/g tissue].

Trace levels of Ag in $NaBH_4$ treatment samples was observed. It was determined that the NPs persisted in the nalgene cuvettes after the digestion process. Ag contamination was essentially eliminated by rinsing cuvettes twice with 5 ml of 50% HNO_3, then microwaving the empty cuvettes. Cuvettes were then rinsed twice with deionized water.

Statistical Analysis

The SPSS (ver. 16.0, 2007) statistical package was used to analyze data collected on comet tail length (DNA damage) and Ag content in the tissue. Analyses of AgNP accumulation for the different treatment concentrations in the liver, green gland, and muscle tissue were also performed. Analysis of variance (ANOVA) followed by the

Student-Newman-Keuls multiple comparison procedure was used to conclude the variation in comet tail length and accumulation of Ag levels at probability level $\alpha \leq 0.05$.

RESULTS

Crayfish Mortality

Figure 1 represents the mortality of crayfish exposed to different treatments. The highest mortality was observed in $AgNO_3$ treatments. A total of 10 crayfish died in $AgNO_3$ treatments, three crayfish died in AgNP treatment exposures, one died in the $NaBH_4$ treatments, and one in the laboratory control treatment. No deaths occurred in the river control.

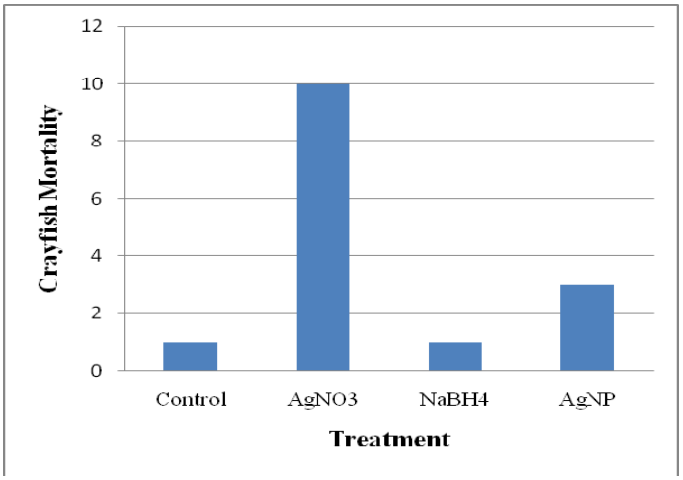


Figure 1. Mortality of crayfish in different experimental treatments. The bar graphs represent total number of organisms that died during treatment exposure. The values were obtained by pooling all the organisms that died at specific concentrations. All deaths were recorded after treatments were administered.

Table 3. Average weight loss of crayfish in different experimental exposures. The values in the table represent average measurements of all 6 organisms per treatment recorded at the beginning of the experiment (June 20) and compared to the weight at the end of the experiment (June 30).

Treatment	Initial Avg Weights (g)	Final Avg Weights (g)	Weight Change (g)
Lab Control	26.2	25.4	-0.8
$AgNO_3$	20.3	19.3	-1.0
$NaBH_4$	17.1	16.7	-0.4
AgNP	16.2	14.2	-2.0

Gross Pathology

Our observations revealed that crayfish in AgNP demonstrated the largest change in weight (Table 3).

Behavioral Changes

Recorded visual observations and external stimuli showed the crayfish in the $AgNO_3$ became very lethargic and unresponsive to both food and threat. Organisms in the $NaBH_4$ sporadically would not respond to food, but always to threat. Organisms treated with AgNP remained responsive to food and threat throughout the experiment.

DNA Analysis

*Brain***:** Results of exposure to treatments, AgNP and the paternal materials $AgNO_3$ and $NaBH_4$, are represented in Figures 2, 3 and 4. All figures show the statistically significant difference in DNA damage between the river control and the laboratory (lab) control as measured by DNA migration (comet tail length). DNA damage was not observed in the river control samples.

As shown in Figure 2, the extent of DNA migration in the AgNP treatments increased significantly in all concentrations in comparison to the controls ($\alpha \leq 0.05$). There was no statistically significant difference in comet tail length between the 0.05,

0.107, and 0.214 AgNP treatment concentrations; however, comet tail length in the 0.16 mg/L AgNP treatment concentration increased significantly when compared to the other three AgNP treatment concentrations ($\alpha \leq 0.05$). Comet length in the 0.16 mg/L AgNP treatment concentration increased 46% in comparison to the lab control.

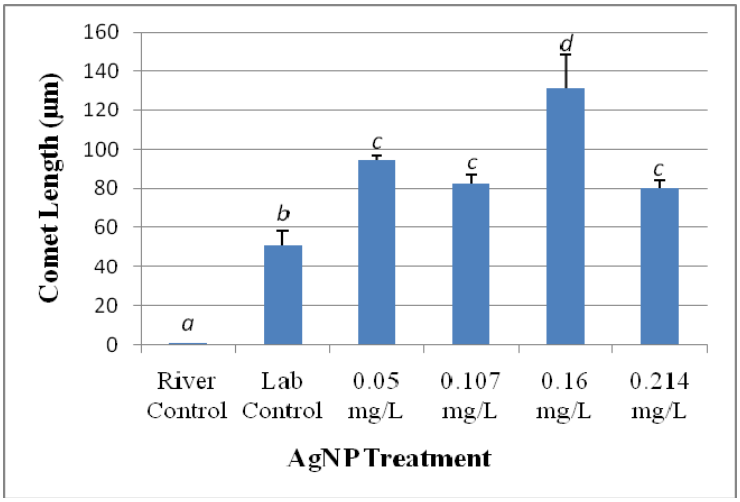


Figure 2. AgNP induced DNA damage in crayfish brain tissues expressed as length of DNA comet tail. The bar graphs represent comet length means ±SD of ~50 nuclei per sample from each specimen. Columns with different letters (*a, b, c, d*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0).

Figure 3 shows that when comparing the $AgNO_3$ treatments to the lab control, there was a statistically significant difference between the comet tail lengths measured in the 0.085, 0.17, and 0.34 mg/L treatments ($\alpha \leq 0.05$). However, comet tail lengths in the 0.27 mg/L $AgNO_3$ treatment were not significantly different to those measured in lab control or to the other three $AgNO_3$ treatments.

As shown in Figure 4, there was a statistically significant increase in comet tail length when comparing the lab control to the 0.115 and 0.225 mg/L $NaBH_4$ treatments; however, there was no significant difference between those two $NaBH_4$ treatments. DNA

damage in the 0.34 and 0.45 concentrations was too extensive and the resulting comet tail was too diffuse for an image to be measured by the Image-Pro® Plus software.

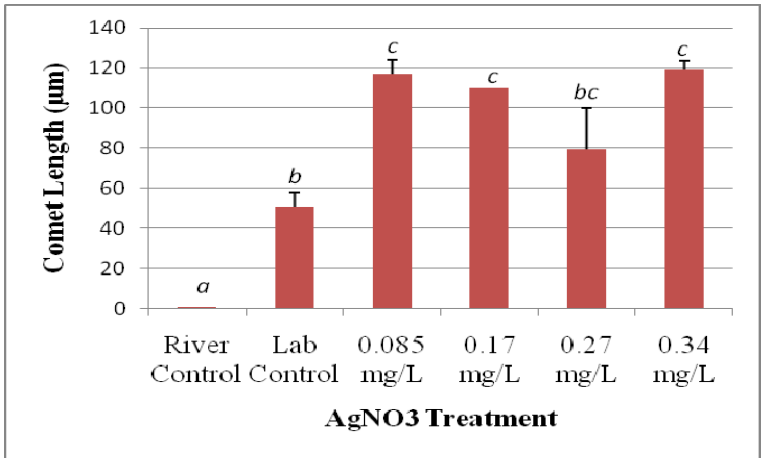


Figure 3. $AgNO_3$ induced DNA damage in crayfish brain tissues expressed as length of DNA comet tail. The bar graphs represent comet length means ±SD of ~50 nuclei per sample from each specimen. Columns with different letters (*a, b, c, d*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0).

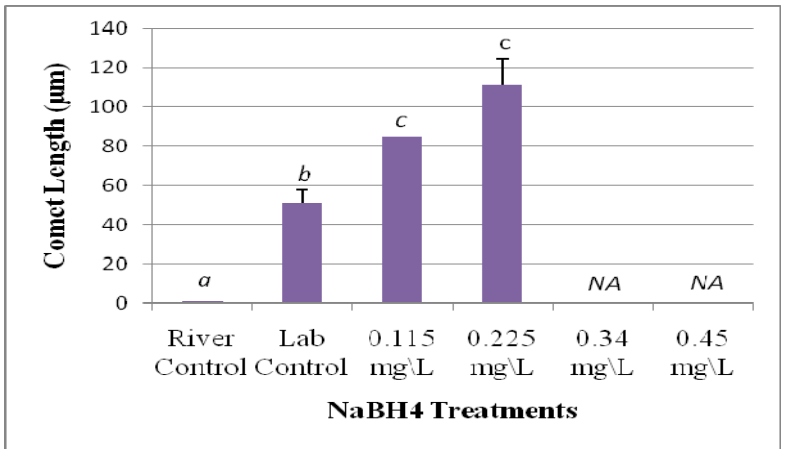


Figure 4. $NaBH_4$ induced DNA damage in crayfish brain tissues expressed as length of DNA comet tail. The bar graphs represent comet length means ±SD are average length of ~50 nuclei per sample from each specimen. Columns with different letters (*a, b, c*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Measurements in the *NA* concentrations were immeasurable with available equipment because of extreme dispersal of DNA.

Atomic Absorbtion Analysis

The results of atomic absorption analysis of Ag content in liver, green gland, and muscle tissues are shown in Figures 5a, 5b, 5c, 6a, 6b, 6c, 7a, 7b, and 7c. Each sample was measured three times, and the absorbance values were averaged before extrapolating the Ag concentration from the calibration curve. Calibrations were performed using acid-matched standard solutions of Silver Standard. Metal concentrations were determined through external calibration, with standards using least-squares fit of regression curves. Chemical analysis of the liver, green gland, and muscle tissues in river control specimens did not detect any presence of Ag.

Liver: Figures 5a-5c represent the Ag content determined in crayfish liver samples. The figures show that there was no statistically significant difference in Ag content detected in lab control tissue samples when compared to river control samples.

Figure 5a illustrates results from the $NaBH_4$ treatments. There was a trace amount of Ag detected in liver tissue in the highest concentration, 0.214 mg/L, although no Ag was in the treatment. The amount of Ag in $NaBH_4$ could be the result of contamination during tissue processing.

Figure 5b presents Ag content in liver tissue in the $AgNO_3$ treatments. There was statistically significant accumulation of Ag in the liver tissue in the lowest (0.085 mg/L) and the highest (0.34 mg/L) $AgNO_3$ treatments when compared to the controls and remaining treatments ($\alpha \leq 0.05$). There was no significant difference in Ag accumulation between the river control, lab control, 0.17 mg/L, and 0.27 mg/L treatments. In addition, there was a statistically significant difference in Ag accumulation in the 0.34 mg/L concentration treatment when compared to the 0.085 mg/L treatment concentration.

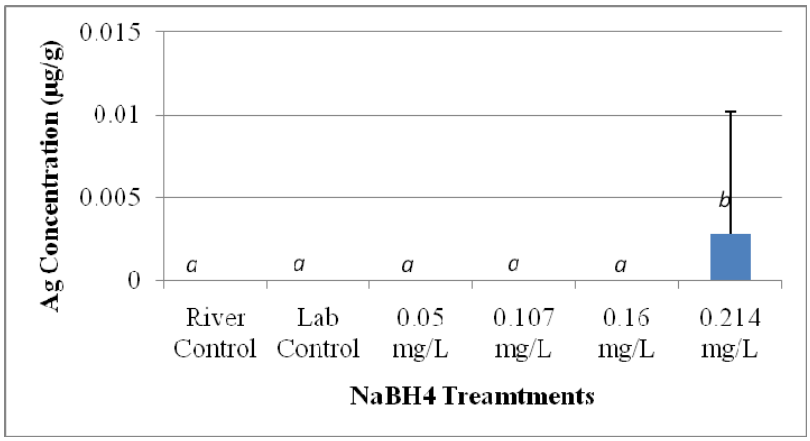


Figure 5a. Total amount of Ag (µg/g dry w.) accumulated in crayfish liver tissue in $NaBH_4$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0).

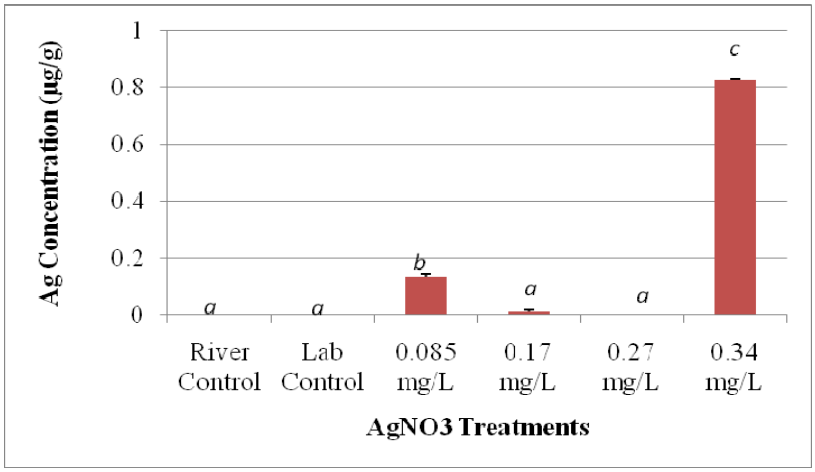


Figure 5b. Total amount of Ag (µg/g dry w.) accumulated in crayfish liver tissue in $AgNO_3$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0).

Atomic absorbtion analysis demonstrated increasing Ag accumulation in the crayfish liver tissues with increasing AgNP treatment concentration (Figure 5c). The

accumulation was not statistically significant for the 0.05 mg/L AgNP treatment concentration when compared to the river and lab controls ($\alpha \leq 0.05$); however, Ag accumulation was statistically significant for the 0.107, 0.16, and 0.214 mg/L concentration treatments. Additionally, each AgNP treatment demonstrated statistically significant accumulation results when compared to the other three AgNP treatments.

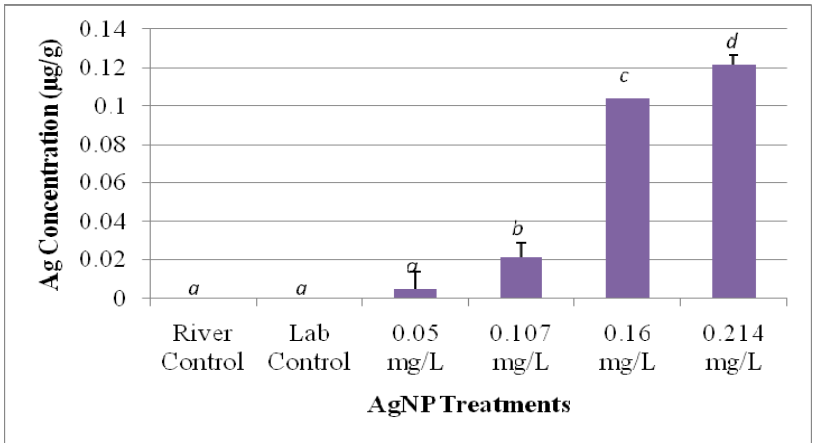


Figure 5c. Total amount of Ag (µg/g dry w.) accumulated in crayfish liver tissue in AgNP treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c, d*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0).

Green Gland: Figures 6a-6c represent Ag content in the crayfish green gland tissues. The pair of kidney-like green glands play a very important excretory function. The figures show that there was a statistically significant difference in Ag content detected in lab control samples when compared to river control samples.

Figure 6a shows that there was Ag present in the green glands of crayfish in the $NaBH_4$ treatments. It may be that accumulation of Ag in these treatments resulted from sample contamination.

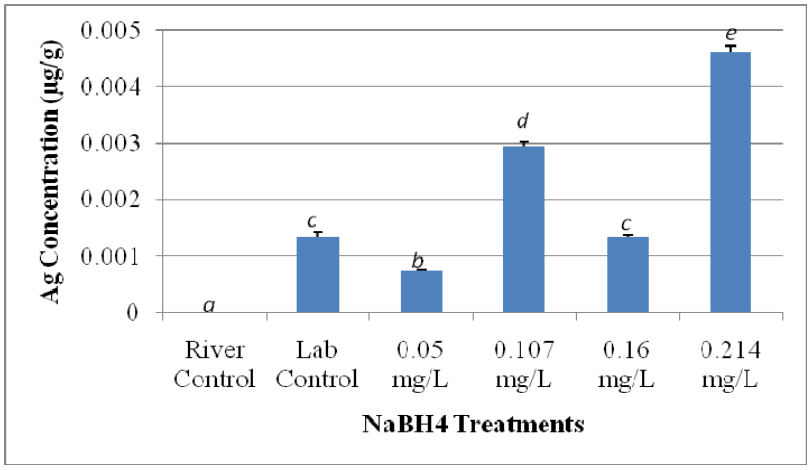


Figure 6a. Total amount of Ag (µg/g dry w.) accumulated in crayfish green gland tissue in $NaBH_4$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b,c,d,e*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

As can be seen in Figure 6b, the $AgNO_3$ treatments demonstrated statistically significant differences in Ag content in the 0.085, 0.27, and 0.34 mg/L concentration treatments when compared to the river control ($\alpha \leq 0.05$). No statistically significant difference was found between the lab control and the 0.27 mg/L concentration treatment. Additionally, each $AgNO_3$ treatment demonstrated statistically significant accumulation results when compared to the other three $AgNO_3$ treatments.

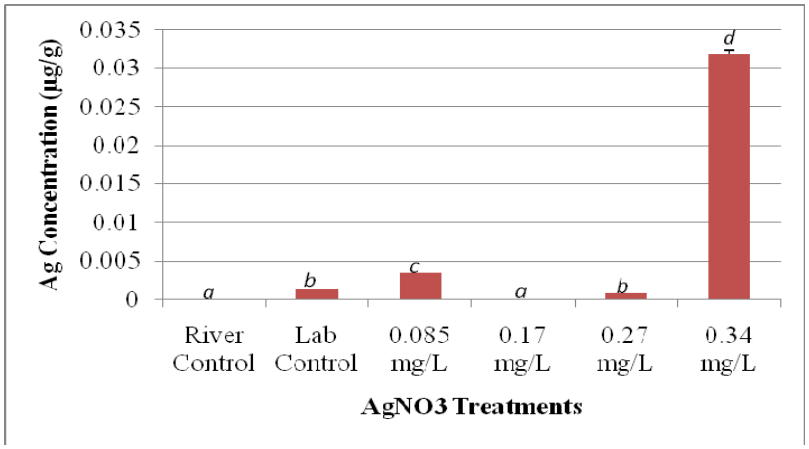


Figure 6b. Total amount of Ag (µg/g dry w.) accumulated in crayfish green gland tissue in $AgNO_3$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c, d*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

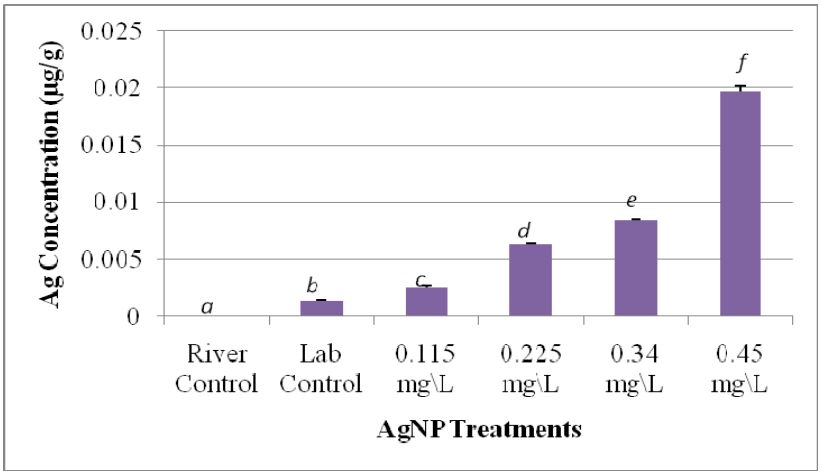


Figure 6c. Total amount of Ag (µg/g dry w.) accumulated in crayfish green gland tissue in AgNP treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c, d, e, f*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

Figure 6c shows statistically significant different Ag content levels in all AgNP treatments when compared to river control and lab ($\alpha \leq 0.05$). Additionally, each AgNP treatment demonstrated statistically significant accumulation results when compared to the other three AgNP treatments.

Muscle: Figures 7a-7c represent Ag accumulation in the crayfish tail muscle. The figures show that there was a statistically significant difference in Ag content detected in lab control tissue samples when compared to river control samples. A trace amount of silver was detected in the $NaBH_4$ treatments (Figure 7a), which may be attributed to contamination.

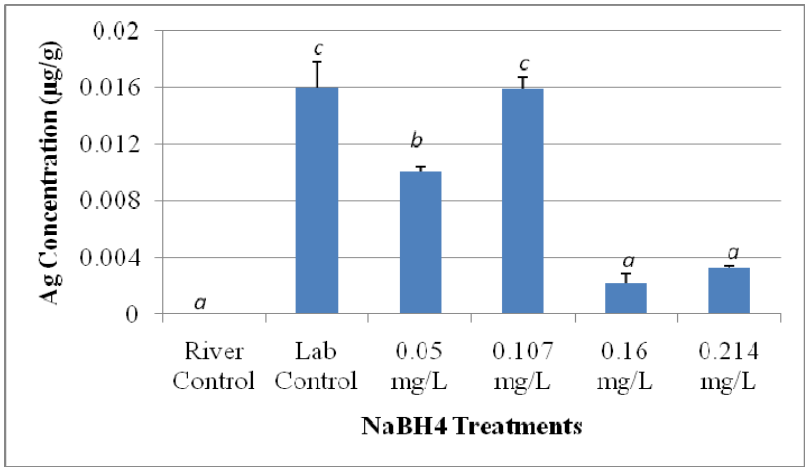


Figure 7a. Total amount of Ag (µg/g dry w.) accumulated in crayfish muscle tissue in $NaBH_4$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

It can be seen in Figure 7b that all $AgNO_3$ treatments demonstrated statistically significant differences in Ag content when compared to the river control ($\alpha \leq 0.05$). No significant difference was found between the lab control and the 0.085 mg/L

concentration treatment. Additionally, each $AgNO_3$ treatment demonstrated statistically significant accumulation results when compared to the other three $AgNO_3$ treatments.

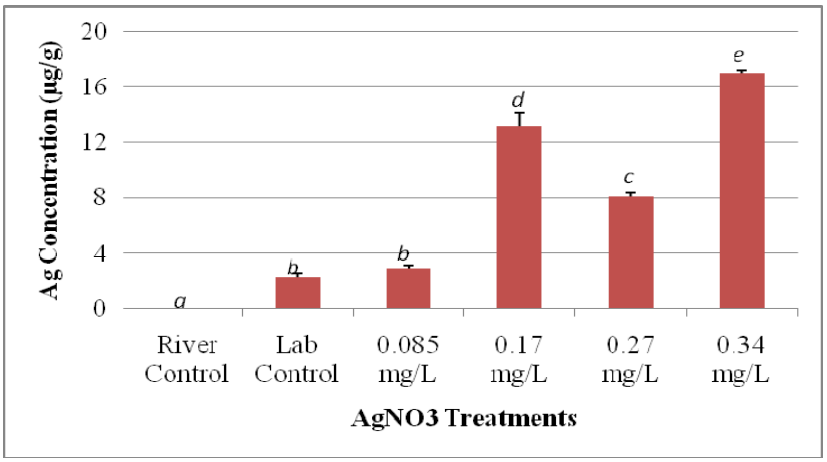


Figure 7b. Total amount of Ag (µg/g dry w.) accumulated in crayfish muscle tissue in $AgNO_3$ treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b*, c, d, e) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

Figure 7c shows significantly different Ag content levels in all AgNP treatments when compared to river control ($\alpha \leq 0.05$). No significant difference was found between the lab control and the 0.115 mg/L concentration treatment. Additionally, each AgNP treatment demonstrated statistically significant accumulation results when compared to the other three AgNP treatments.

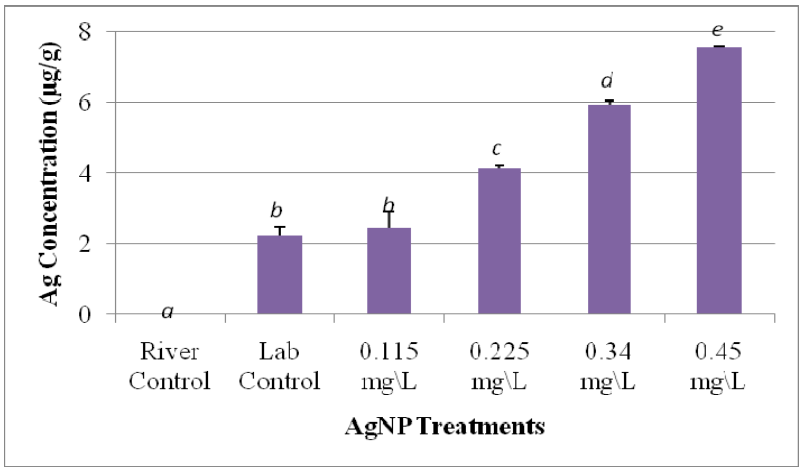


Figure 7c. Total amount of Ag (µg/g dry w.) accumulated in crayfish muscle tissue in AgNP treatments as determined by GFAAS measurements. The bar graphs represent Ag content means ±SD of three measurements. Columns with different letters (*a, b, c, d, e*) are significantly different at probability level $\alpha \leq 0.05$ as determined by multiple comparison test Student-Newman-Keuls (SPSS 16.0). Each value is the mean of three measurements.

DISCUSSION

The results of the comet assay (Figures 5a, 5b, and 5c) show DNA damage in brain nuclei of all treatments. In recent years comet assay has become a standard and reliable method for assessing DNA damage in the biological systems of animals, plants, and humans. The principle of this method, based on single cell gel elecrophoresis, allows for detection of single and double DNA strand breakage (Frenzilli et al. 2006). DNA damage detected in our study on crayfish brain tissue exposed to AgNPs suggests that a similar response could be expected in other living organisms. The observed dose response to all experimental treatments seems to follow a nonconventional dose response. Hodgson (2004) suggests that low doses of chemical exposure stimulate a physiological response that can offset adverse effects. This compensatory response is observed as an effect opposite to toxic response at a higher levels of chemical exposure. At thereshold dose, organisms respond with increased stimulation and overcompensation of toxic effect

demonstrated as a return to the zero response level or decrease in toxic effect. Continuing increased exposure levels overcome the organism's defence ability at the "pseudo" threshold level. In this study, the crayfish body's natural defenses seemed to block toxin uptake as exposure increased and less damage was observed, shown as hormesis in Figure 8. After a certain point, increased treatment exposure concentrations demonstrate the typical dose response to the toxicant, shown graphically as the near linear trend upwards. It is the pseudothreshold that is often mistaken as the actual threshold.

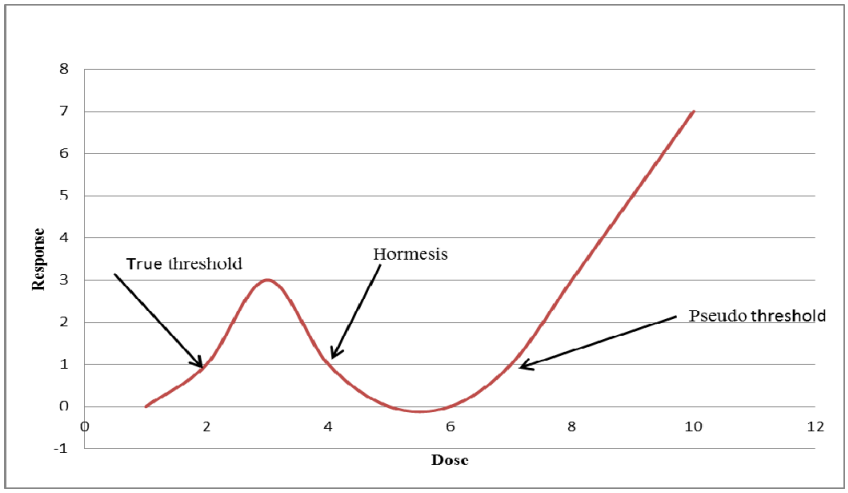


Figure 8. Hypothetical dose response to illustrate a nonconventional dose response.

The treatments resulted in the most damage to brain DNA. Figure 4 is lacking the two highest concentrations responses because the photographs taken of the slides showed either nuclei or DNA comets, but not both. The damage was so great that it was not possible to measure damage at those concentrations. Hyeon-Jin et al. (2009) considers $NaBH_4$ as an effective reducing agent. In the synthesis of AgNPs for this experiment, $NaBH_4$was used as reducing agent to react with $AgNO_3$. Unfortunately there is no literature on the ecological toxicity of $NaBH_4$. There is, however, a large body of

information on toxicity of the other synthesis component, $AgNO_3$, on aquatic (Bianchini and Wood 2003) and terrestrial (Pelkonen et al. 2003) organisms.

Data collected in this study demonstrated that the synthesis components of AgNPs are very toxic given the mortality of crayfish in the $AgNO_3$ treatments (Figure 1), and cause extensive DNA damage (Figures 3 and 4). Jayabalan et al. (2008) has documented that AgNPs in colloidal solution can deaggregate to form the parental compounds. Toxicity of AgNPs in this experiment may also be attributed to the partial deaggregation of AgNPs in the experimental treatments.

Accumulation of Ag in the experimental organisms did not show any specific trend in the $AgNO_3$ treatments and was observed at minimal levels in the $NaBH_4$ treatments. The accumulation of Ag among the AgNP concentrations, however, shows an increase in Ag with increasing treatment concentration, suggesting that as nanoparticle concentration increases the uptake into the organs also increases. Bothun (2008) found that nanoparticles could freely pass through barriers in the body. The DNA damage found in the crayfish neural tissue of our experimental samples supports those findings.

CONCLUSION AND RECOMMENDATIONS

There is a clear trend of Ag accumulation found in the AgNP treatments. The near linear fit suggests that increased exposure leads to increased absorption. It is also clear that the organisms exposed to these nanoparticles showed no outward behavioral changes but were undergoing widespread DNA damage. This can be of concern to humans because extensive DNA damage can be occurring while no apparent effect is being observed.

It is of vital importance both to aquatic organisms and to humans that these materials be studied further. This experiment mirrors others in its findings that AgNPs cause DNA damage. Production of consumer goods containing this product should be placed on hold so more research can be done and the Environmental Protection Agency and the Department of Health and Human Services can issue regulations halting future exposure.

ACKNOWLEDGEMENTS

I would like express my appreciation to the Hudson River Foundation and the Tibor T. Polgar Fellowship Committee for the opportunity to conduct this research and for their support. Completion of this research would not have been possible without the supervision and mentorship of Dr. Zofia Gagnon, and I extend my sincere thanks for all her knowledge, expertise, time, and efforts. I would also like to acknowledge Dr. Neil Fitzgerald for his expertise and support and for providing access to the River Lab and to Marist College School of Science instrumentation. I also express thanks to my peers Anne Quach, Rachel Serafin, and Seth Brittle for their assistance during the research process.

LITERATURE CITED

Ahamed, M., M. Karns, M. Goodson, J. Rowe, S.M. Hussain, J.J. Schlager, and Y. Hong. 2008. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicology and Applied Pharmacology 233: 404-410.

Bansal, R.C. and M. Goyal. 2005. Activated Charcoal Adsorption. Taylor and Francis, Boca Raton, FL.

Bianchini, A. and C.M. Wood. 2003. Mechanism of acute silver toxicity in *Daphnia magna*. Environmental Toxicology and Chemistry 22: 1361-1367.

Bothun, G.D. 2008. Hydrophobic silver nanoparticles trapped in lipid blazers: size distribution, bilayer phase behavior, and optical properties. Journal of Nanobiotechnology 6:13, 10 pp.

Braydich-Stolle, L., S.M. Hussain, J.J. Schlager, and M.C. Hoffman. 2005. *In vitro* cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicological Sciences 88: 412-419.

Chau, C.F., S.H. Wu, and G.C. Yen. 2007. The development of regulations for food nanotechnology. Trends in Food Science Technology 18: 269-280.

Choi, O. and Z. Hu. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environmental Science & Technology 42: 4583-4588.

Creighton, J.A. and D.G. Eadon. 1991. Ultraviolet-visible absorption spectra of the colloidal spectra elements. Journal of the Chemical Society, Faraday Transaction 87: 3881-3892.

Elechiguerra, J.L., J. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara, and M.J. Yacaman. 2005. Interaction of silver nanoparticles with HIV-1. Journal of Nanobiotechnology 3: 6. doi:10.1186/1477-3155-3-6

Frenzilli, G., V. Scarcell, F. Fornal, A. Paparelli, and M. Nigro. 2006. The comet assay as a method of assessement of neurotoxicity. Annals of the New York Academy of Science 1074: 478-480.

Gosheger, G., J. Hardes, H. Ahrens, A. Streitburger, H. Buerger, M. Erren, A. Gunsel, F.H. Kemper, W. Winkleman, and C. von Eiff. (2004). Silver-coated megaendoprostheses in a rabbit model—an analysis of the infection rate and toxicological side effects. Biomaterials 25: 5547–5556.

Hagfeldt, A. and M. Graetzel. 1995. Light-induced redox reactions in nanocrystalline systems. Chemical Reviews 95(1): 49–68. doi: 10.1021/cr00033a003.

Henig, R.M. Our silver-coated future. OnEarth September 1, 2007: 22–29.

Hodgson, E. 2004. A Textbook of Modern Toxicology. Wiley-Interscience, Hoboken, NJ.

Hornyak, G.L., H.F. Tibbals, J. Dutta, and J.J. Moore. 2009. Introduction to Nanoscience and Nanotechnology. CRC Press, Boca Raton, FL.

Hussain, S.M., K.L. Hess, J.M. Gearhart, K.T. Geiss, and J.J. Schlager. 2005. *In vitro* toxicity of nanoparticles in BRL, 3A rat liver cells. Toxicology *in Vitro* 19: 975-983.

Hyeon-Jin, S., K.K. Kim, A. Benayad, S. Yoon, K.P. Hyeon, I. Jung, M.H. Jin, H.K. Jeong, J. Kim, J. Choi, and Y.H. Lee. 2009. Efficient reduction of graphite oxide by sodium borohydride and its effect on electrical conductance. Advanced Functional Materials 19: 1987–1992.

Jayabalan, J., A. Singh, R. Chari, H. Srivastava, P.K. Mukhopadhyay, A.K. Srivastava, and S.M. Oak. 2008. Aggregated nanoplatelets: optical properties and optically induced disaggregation. Journal of Physics: Condensed Matter 20(44). doi: 10.1088/0953-8984/20/44/445222.

Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, and M.H. Cho. 2007. Antimicrobial effects of silver nanoparticles. Nanomedicine 3: 95–101.

Larese, F.F., F. D'Agostin, M. Crosera, G. Adami, N. Renzi, M. Bovenzi, and G. Maina. 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology 255: 33-37.

Mnyusiwalla, A., A.S. Daar, and P.A. Singer. 2003. 'Mind the gap': science and ethics in nanotechnology. Nanotechnology 14(3): 14 R9-R13 doi:10.1088/0957-4484/14/3/201.

Moghimi, S.M., A.C. Hunter, and J.C. Murray. 2001. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacological Reviews 53: 283–318.

Paddle-Ledinek, J.E., Z. Nasa, and H.J. Cleland. 2006. Effect of different wound dressings on cell viability and proliferation. Plastic and Reconstructive Surgery 117: 110S–118S.

Panyam, J. and V. Labhasetwar. 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced Drug Delivery Reviews 55: 329–347.

Pelkonen, K.H., H. Heinonen-Tanski, and O.O. Hanninen. 2003. Accumulation of silver from drinking water into cerebellum and musculus soleus in mice. Toxicology 186: 151-157.

Ringer, S.P and K.R. Ratinac. 2004. On the role of characterization in the design of interfaces in nanoscale materials technology. Microscopy and Microanalysis 10: 324–335.

Samuel, U. and J.P. Guggenbichler. 2004. Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. International Journal of Antimicrobial Agents 23: 75–78.

Sung, J.H., J.H. Ji, J.U. Yoon, D.S. Kim, M.Y. Song, J. Jeong, B.S. Han, J.H. Han, Y.H. Chung, J. Kim, T.S. Kim, H.K. Chang, E.J. Lee, J.H. Lee, and I.J. Yu. 2008. Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles. Inhalation Toxicology 20: 567-574.

The Center for Food Safety. "Nanotechnology: It's a small (and unregulated) world after all." *Food Safety Now!* Autumn 2006.

Ulkur, E., O. Oncul, H. Karagoz, E. Yeniz, and B. Celikoz. 2005. Comparison of silver-coated dressing (Acticoat), chlorhexidine acetate 0.5% (Bactigrass), and fusidic acid 2% (Fucidin) for topical antibacterial effect in methicillin-resistant Staphylococci-contaminated, full-skin thickness rat burn wounds. Burns 31: 874–877.

Usui, H., H. Matsui, N. Tanabe, and S. Yanagida. 2004. Improved dye-sensitized solar cells using ionic nanocomposite gel electrolytes. Journal of Photochemistry and Photobiology A: Chemistry 164: 97-101.

Vigneshwaran, N., A.A. Kathe, P.V. Varadarajan, R.P. Nanchane, R.H. Balasubramanya. 2007. Functional finishing of cotton fabrics using silver nanoparticles. Journal of Nanoscience and Nanotechnology 7: 1893-1897.

Woodrow Wilson International Center for Scholars. 2007. A nanotechnology consumer products inventory. Online at http://www.nanotechproject.org/consumerproducts.

Ying, J. 2001. Nanostructured Materials. Academic Press, New York.

QUANTIFYING LARVAL FISH HABITAT IN SHORELINE AND SHALLOW WATERS OF THE TIDAL HUDSON RIVER

A Final Report of the Tibor T. Polgar Fellowship Program

Claire E. Stouthamer

Polgar Fellow

Department of Natural Resources
Cornell University
Ithaca, NY 14853

Project Advisor:

Mark B. Bain
Department of Natural Resources
Cornell University
Ithaca, NY 14853

Stouthamer, C. E. and M. B. Bain. 2012. Quantifying Larval Fish Habitat in Shoreline and Shallow Waters of the Tidal Hudson River. Section VII: 1-25 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

Shoreline and backwater nursery areas are important for spawning and early life development for many fishes. During the larval stage, or 'critical period,' mortality often exceeds 90%. Nursery areas provide abundant food and cover, which has been shown to be more favorable habitat for developing fishes. Shoreline and shallow water habitats have been structurally and biologically altered along much of the Hudson River. The effect of changes in microhabitat conditions on larval habitat occurrence has not been quantified. The purpose of this study was to quantify larval fish occurrence within a range of shallow water microhabitats in order to determine the importance of habitat variables for larval fish distribution.

Larval fish samples were collected from Tivoli North Bay and the Magdalen Island shallow waters using a w-fold throw trap and mesh seine from May 25 – July 1, 2010. Samples were preserved in 10% buffered formalin and successively transferred to 70% ethanol. Fish were measured and identified to family level. A MANOVA test was applied to microhabitat variables according to larval presence by family. Results indicate microhabitat differentiation by families Moronidae, Clupeidae, Cyprinidae, and Fundulidae. Differential shallow water habitat use between larval fish taxa has implications for fish habitat restoration and shoreline development.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

The larval period is characterized by high mortality, and considered a 'critical period' in the population dynamics of many fish species (Hjort 1914; Marr 1956; May 1974). The post-yolk sac larval period starts when fishes are able to capture food organisms and extends to the size and age marked by the formation of the axial skeleton and the development of the embryonic finfold into fins with rays and spines. During the post-yolk sac larval stage, organs and fins are still forming, limiting locomotion and rendering larvae particularly susceptible to predation and starvation (Moyle and Cech 2004). Due to this increased susceptibility, larval fish have environmental requirements, behaviors, and habitat needs that are distinctly unlike that of juveniles and adults (Snyder 1990). Larval size plays a larger factor in determining suitable environment and larvae are especially sensitive to their surrounding habitat (reviewed in Snyder 1983).

Low velocity habitats such as backwaters, tributaries, and near-shore areas have been shown to support high densities of larval fish (Odom 1987; Scott and Nielsen 1989). The Hudson River has experienced thousands of hectares of nursery habitat losses from navigation channel development and physicochemical changes (Miller et al. 2006). Shoreline wetlands have been altered by the construction of railroads along the river and dams in the upper drainage (Squires 1992; Schmidt and Cooper 1996). Other changes in the river environment impacting nursery habitat include zebra mussel colonization and plankton reduction (Strayer et al. 1999), water intakes and larval impingement, changing littoral fish assemblages (Strayer et al. 2004; Daniels et al. 2005) and non-native plant invasion of shallow water areas (Schmidt and Kiviat 1988; Coote et al 2001; Findlay et al 2006).

Research on post-yolk sac larvae in the Hudson River by Limburg (1996) found highest fish concentrations in or near aquatic vegetation and quiet waters. Leslie and Timmins (1991) and Penáz et al. (1992) found that river margins were able to support greater abundances of larvae than adjacent offshore habitats. This habitat selection behavior was observed as a mechanism for larval fish to avoid being flushed out of nursery areas. Sustained swimming speed for most fish, including larvae, is 3 to 7 body lengths per second (Webb 1975). Scheidegger and Bain (1995) calculated a maximum current velocity of 8.4 cm/s (i.e., 7 body lengths x 12-mm long larvae) to define the upper velocity limit (i.e., critical maximum velocity) for potential nursery habitat. Precise sampling of fish larvae in waters at or below the critical maximum velocity revealed orientation behavior toward habitat characteristics such as substrate, vegetation, cover density, and water depths, while offshore habitats with current velocities greater than the critical maximum contained fish larvae being passively transported by river currents. Different fish taxa varied in the parameters defining their preferred habitats.

The Hudson River shoreline habitats are tidal and experience a range of current velocities and water depths. Furthermore, there exists great structural heterogeneity along the river's edge. It is not known how varying microscale conditions in tidal rivers influence habitat selection and use by fish larvae. The purpose of this study was to test the hypothesis that post-yolk sac larval fish occurrence within shoreline and shallow waters is correlated with exposure to different localized hydrodynamics and habitat structure in order to inform the ecological implications of future shoreline restructuring decisions.

METHODS

Site Description

Tivoli North Bay is a cattail freshwater marsh composed of a network of tidal creeks and pools located around river km 159 in Dutchess County. As part of the Hudson River National Estuarine Research Reserve, Tivoli North Bay is an important refuge for Hudson River resident marsh flora and fauna. The Tivoli Bays are an important spawning and nursery ground for several anadromous and resident freshwater fish species (Yozzo et al 2005). Tivoli North Bay receives freshwater inputs from both the Hudson River and Stony Creek, which drains into the northern portion of the marsh. The bay is separated from the main channel by the Metro-North railroad and receives tidal water exchange with a diurnal tidal change of >1 m through bridges within the railroad bed.

Magdalen Island is located west of the railroad divide separating the Hudson River and Tivoli North Bay. While the western bank of Magdalen Island is primarily steep, scoured bedrock, the eastern shoreline is characterized by finer substrates and abundant aquatic vegetation. The aquatic habitat on the eastern side of the island is composed of a silty bottom, shallow water, and low velocity pockets.

Site Protocol

Samples were collected from 25 May - 1 July, 2010 in Tivoli North Bay and along the shoreline and shallows of Magdalen Island. Sites were sampled for larval fish by random 'point abundance sampling' (Copp and Penáz 1988; Copp 1990) over the daytime tidal cycle. Larval fish were trapped using a 1 m^2 w-fold throw trap (Cotroneo and Yozzo 2010). A deep-water extension net was attached to the aluminum frame for samples taken in water deeper than 0.5 m.

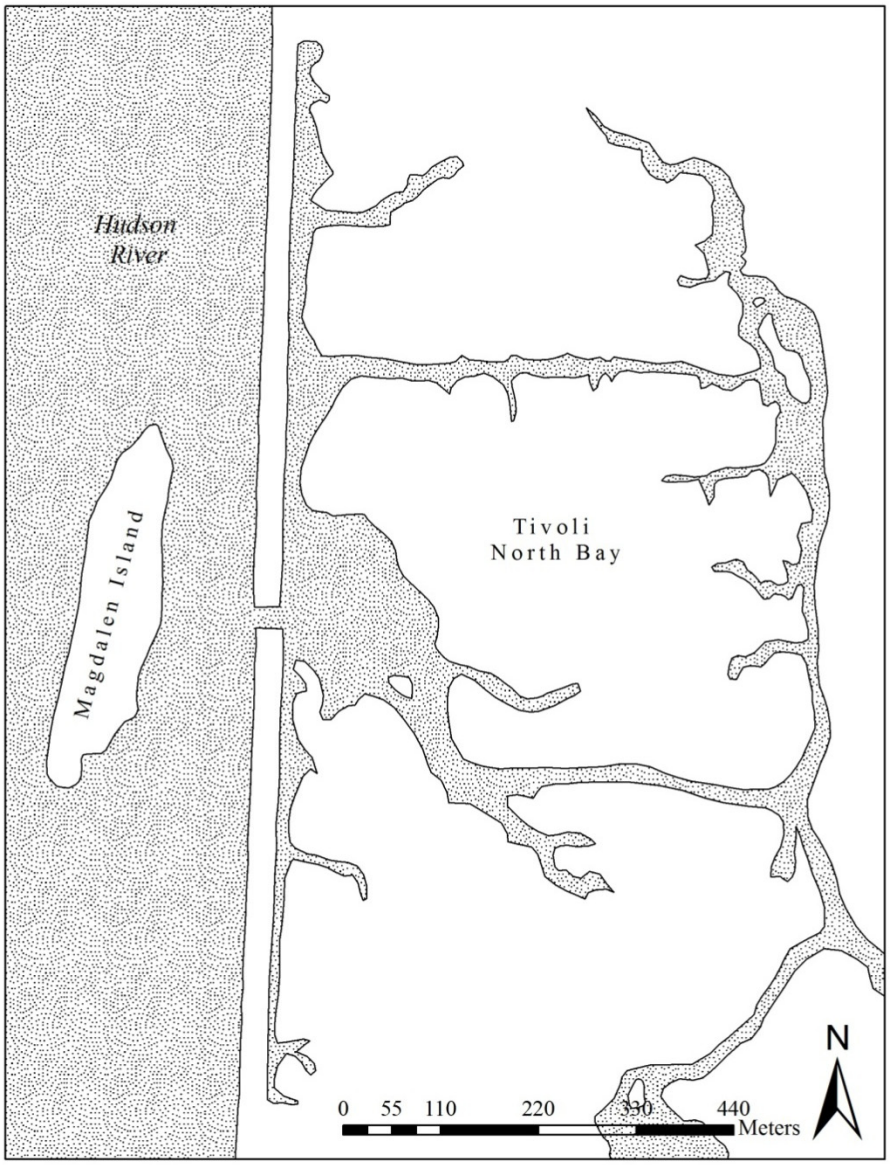


Figure 1. Map of Tivoli North Bay and Magdalen Island study sites

Figure 2. W-fold throw trap and bar seine

Microhabitat conditions were recorded immediately after trap deployment. For each trapping sample, the following habitat attributes were recorded: position (grid point and GPS coordinates), day and time, depth (nearest 0.1 m), current velocity (cm/s at 0.4 depth with a digital flow meter), density of cover, vegetation density and type (Bain and Boltz 1992), and dominant substrate (classes of Bain et al. 1985). Tidal stages were included from tide tables and Hudson River Environmental Conditions Observing System (HRECOS) water depth gauge information for Tivoli North Bay and Tivoli South Bay.

After recording microhabitat variables, fish were removed from the throw trap with a bar seine (Rozas and Odum 1987; Cotroneo and Yozzo 2010). The 1 m^2 500-micron bar seine was reinforced with horizontal crossbars and edged with door sweeps to create a seal with the throw trap and maximize catch efficiency. Larval fish were removed from the bar seine mesh with forceps. Live fish were contained in a separate

vessel with water until all individuals were extracted from the trap. Each trap was seined multiple times until no fish were caught for two consecutive seine passes. All fish larvae and unrecognized fishes were simultaneously euthanized by MS-222 overdose and immediately transferred to 10% buffered formalin. Other known post-larval fish were identified, measured, and released.

Sample Processing

Fish samples were fixed in 70% formalin for a minimum of 2 weeks after collection. After the fixation period, larvae were placed in deionized water for 24 hours and transferred to 70% ethanol for long-term storage and identification. All larvae from each sample were counted and sorted to family level. Subsequently, each fish was measured and classified as yolk sac larva, post-yolk sac larva, or juvenile. Only post-yolk sac larvae were included in this study. Yolk sac larvae and juvenile fish were excluded after classification. Juveniles were identified by the loss of the embryonic finfold and the presence of all spines and rays in each fin. Fish were identified and measured to 0.1 mm tail length (TL) using a dissecting microscope. Fish identifications were based on taxonomic keys by Auer (1982), Kay et al (1994), Jones et al (1978), published taxa descriptions, the Cornell University Shackleton Point biological field station reference collection, and expert consultation (personal communication, Dr. Robert E. Schmidt).

Analysis

A multivariate analysis of variance (MANOVA) was used to test the hypothesis that different microhabitat conditions are related to larval fish presence. Fish presence and habitat variables were coded into numeric values and tested by family. The MANOVA model compares habitat composition differences between groups of samples

with and without fish families present. Significant test results for the MANOVA model indicate microhabitat conditions that were different for present and absent samples for each family. Non-significant whole model results for family presence indicate no distinct habitat association and exclude such taxa from further analysis. The family groups showing distinction in microhabitat occurrence were further tested using analysis of variance (ANOVA) to indicate which habitat variables were significantly different between groups with and without larval fish present. In these analyses, test significance is determined to be $p<0.05$.

A principal component analysis (PCA) was performed to display each family microhabitat distribution in relation to all other microhabitat conditions sampled. The first two components were reviewed with respect to the total data variance captured using habitat variable loadings. Habitat variable scores were plotted along the first two component axes and habitat characteristics were interpreted from each component's eigenvalues.

A standard least squares model was constructed to examine the effects of habitat variables on fish size distribution. This analysis was considered to test the hypothesis that larval fish habitat tolerances change as they grow and their swimming ability increases. All fish size data were entered into the model with their respective sample velocity, distance from shore, and depth measures. Model inputs were analyzed by family and all results were reviewed.

RESULTS

A total of 180 throw trap samples were collected and 2465 post-yolk sac larvae were counted, measured, and identified. Fish sample sizes ranged from 0 - 490 larvae per sample and densities ranged from 0 - 1832 larvae/m^3. There were 104 trap samples collected inside Tivoli North Bay and 76 samples collected around Magdalen Island. A total of 1509 larvae were collected from the Hudson River and 956 larvae were collected from Tivoli North Bay (Table 1). Fundulids and percids were caught in higher densities in Tivoli North Bay and cyprinids were caught in higher densities in the Hudson River.

Table 1. Total catch by family for Hudson River throw trap samples and Tivoli North Bay throw trap samples. Families are Centrarchidae (cen), Clupeidae (clu), Moronidae (mor), Percidae (per), Fundulidae (fun), and Cyprinidae (cyp).

Family	Hudson River	Tivoli North Bay
Cen	345	23
Clu	54	23
Cyp	1042	677
Fun	11	156
Mor	25	25
Per	32	52
All	1509	956

Centrarchid (mostly *Lepomis*) larvae were not collected until the fourth sampling week while cyprinid (including spottail shiner *Notropis Hudsonius*, golden shiner *Notemigonus crysoleucas*, and goldfish *Carassius auratus auratus*), clupeid (river herring, *Alosa*), and percid (tessellated darter *Etheostoma olmstedi*) larvae were collected at a higher frequency earlier in the season. Fundulids (mostly mummichog *Fundulus heteroclitus*) were collected consistently throughout the sampling period (Table 2).

Table 2. Number of post-yolk sac larvae by family collected between Julian date 146 and 182 in the Hudson River and Tivoli North Bay combined. Families are Centrarchidae (cen), Clupeidae (clu), Moronidae (mor), Percidae (per), Fundulidae (fun), and Cyprinidae (cyp).

Family	Julian date						
	146-148	152-155	159-162	166-169	173-176	180-182	Total
Cen	0	0	0	212	153	3	368
Clu	23	25	23	4	2	0	77
Cyp	286	664	596	149	9	15	1719
Fun	1	9	61	29	33	34	167
Mor	0	15	11	21	3	0	50
Per	9	53	5	14	2	1	84
All	319	766	696	429	202	53	2465

Throw trap samples were collected over a range of conditions. Water depth ranged from 0.1 - 1.08 m, water velocity ranged from 0.00 - 0.35 m/s, and distance from shore ranged from 0.05 – 51.0 m. Samples were collected in non-vegetated and vegetated areas; dominant aquatic vegetation included *Vallisneria americana*, *Nuphar luteum*, *Myriophyllum spicatum*, and *Peltandra virginica*. Fish were collected over the tidal cycle.

Non-target collections from throw trapping included over 1500 juvenile fishes, 25 adult American eel, 7-30 cm in size, 2 adult white perch, 3 blue crab, and many adult tessellated darters. The throw trap was successful at capturing fish in all vegetation and substrate types. The upper velocity collection limit for the throw trap was 0.35 m/s. At higher velocities, the trap was not heavy enough to maintain its position in the water column.

The MANOVA analysis indicated significant habitat differentiation for families Clupeidae, Cyprinidae, Fundulidae, and Moronidae. Clupeid and cyprinid presence differentiated by depth, moronids differentiated by water velocity and substrate, and fundulids differentiated by depth and substrate (Table 3). PCA analysis inputs were the

significant variables from the MANOVA analysis: water velocity, water depth, distance from shore, and substrate.

Table 3. Family presence, mean, range, and analysis of variance results for significant habitat variables. Families are Centrarchidae (cen), Clupeidae (clu), Moronidae (mor), Percidae (per), Fundulidae (fun), and Cyprinidae (cyp). Mean and standard error habitat conditions are marked with asterisks if there is a significant distinction between groups with and without family presence. Significant test results are indicated at the mean values and are defined as p<0.001 (***), p=0.001-0.01 (**), and p=0.01-0.05 (*). Insignificant variable relationships within the 90% confidence interval (p=0.05-0.1) are also indicated (+).

Family	Samples	MANOVA *P*	Depth (m)		Velocity (m/s)		DFS (m)		Substrate (coded)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cen	7	0.0971+	0.44	0.109	0.01	0.006	2.28+	0.687	1.11	0.114
Clu	17	0.0003***	0.75**	0.045	0.05	0.010	9.83+	3.145	1.49	0.246
Mor	31	0.0013**	0.52	0.044	0.05**	0.007	6.91	1.936	1.05***	0.025
Per	30	0.1681	0.48	0.043	0.04	0.006	6.12	1.446	1.14	0.080
Fun	38	<.0001***	0.33***	0.031	0.02	0.003	1.89	0.583	1.24***	0.117
Cyp	49	0.0003***	0.36**	0.032	0.02+	0.003	2.34	0.543	1.77	0.150

Variable loading interpretation indicated that the first two components account for 70.27 percent of model variance. Clupeids were found in deeper water over a range of velocities and substrate sizes (Figure 2a). Moronids were found in higher velocity habitats with silty substrate. Depth and distance from shore had no effect on moronid habitat occupancy (Figure 2b). Fundulids were found in shallow areas with silty substrate, while velocity and distance from shore had no effect on their presence (Figure 2c). Cyprinids were also found in shallow water with the same distribution as fundulids. Unlike fundulids, cyprinids were found in coarse and fine sediment habitats (Figure 2d). Non-significant, but notable trends include clupeids occurring in habitat far from shore and cyprinids occurring in low velocity areas.

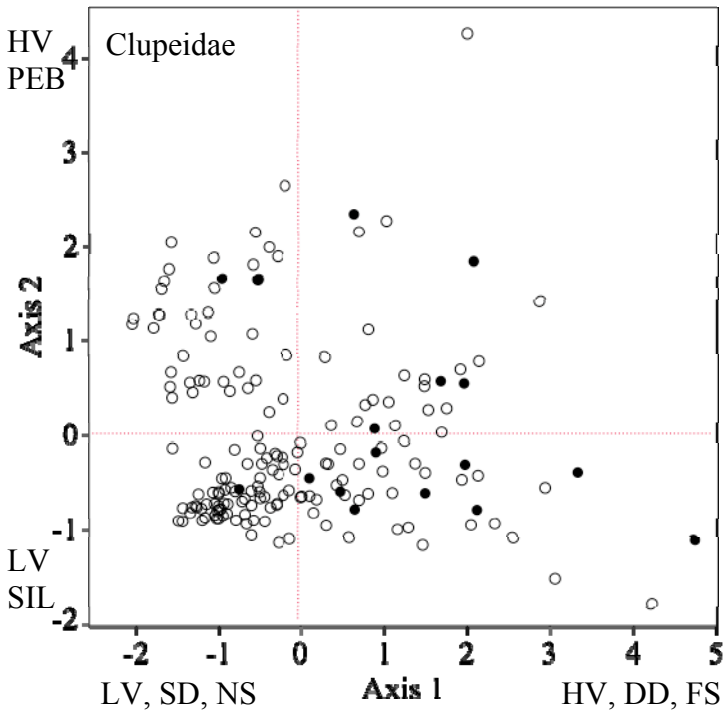
The standard least squares model included velocity, depth, and distance from shore analyzed by family. Clupeidae was the only family showing significant differences

in habitat variables by fish size while also capturing a high amount of model variance (R^2=0.620, $p<0.0001$). Changes in fish size showed significant correlation with changes in velocity ($p<0.0001$), depth ($p>0.0001$), and distance from shore ($p=0.0049$).

DISCUSSION

Study data and the statistical analyses provide strong evidence that larval fish were distributed nonrandomly within the sampled shallow water environments. These results indicate that there are differences in habitat occupancy for larval fish between taxa. The trend of differential occupancy is consistent with past research on littoral and open water larvae in the Tivoli Bays region. Schmidt (1986) found that fundulids, spottail shiners, white perch, and tessellated darters prefer shallow intertidal areas while herrings occupy deeper channels. Furthermore, there is no difference in fish presence between bay and river samples for most taxa, signifying that both shallow water in the river and bay backwaters function similarly in supporting larval fish. Shallow and shoreline waters along the mainstem appear to be as important as protected bays for supporting the region's larval fish community.

a.



b.

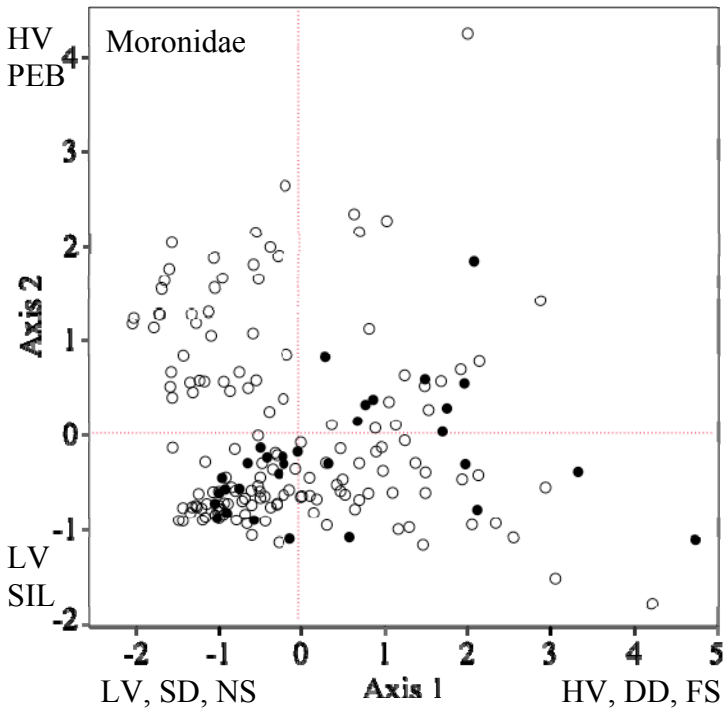
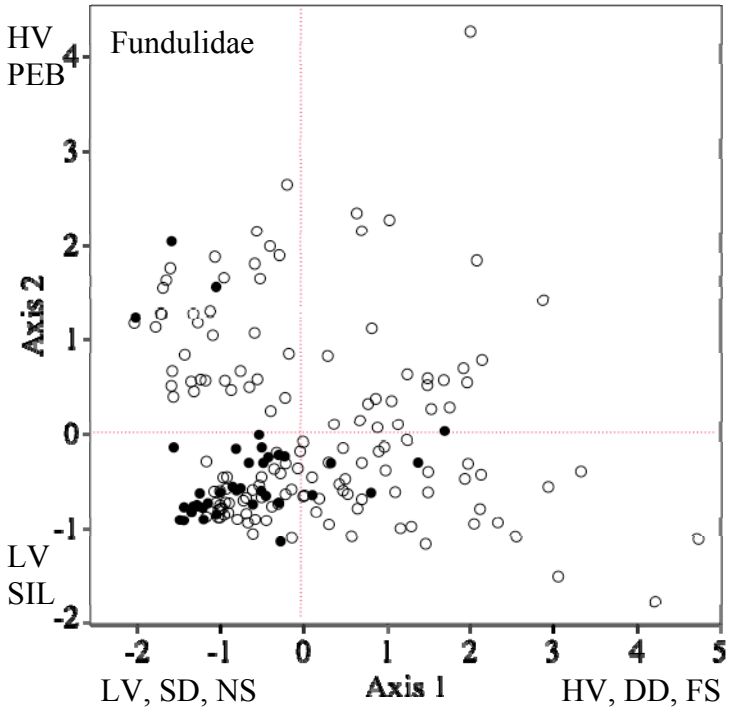


Figure 3ab. PCA analysis of families Clupeidae (a) and Moronidae (b) by depth, velocity, distance from shore, and substrate. The first two components account for 70.27 percent of model variance. LV: low velocity, HV: high velocity, SD: shallow water depth, DD: deep water depth, NS: near to shore, FS: far from shore, PEB: pebbly substrate, SIL: silty substrate.

c.



d.

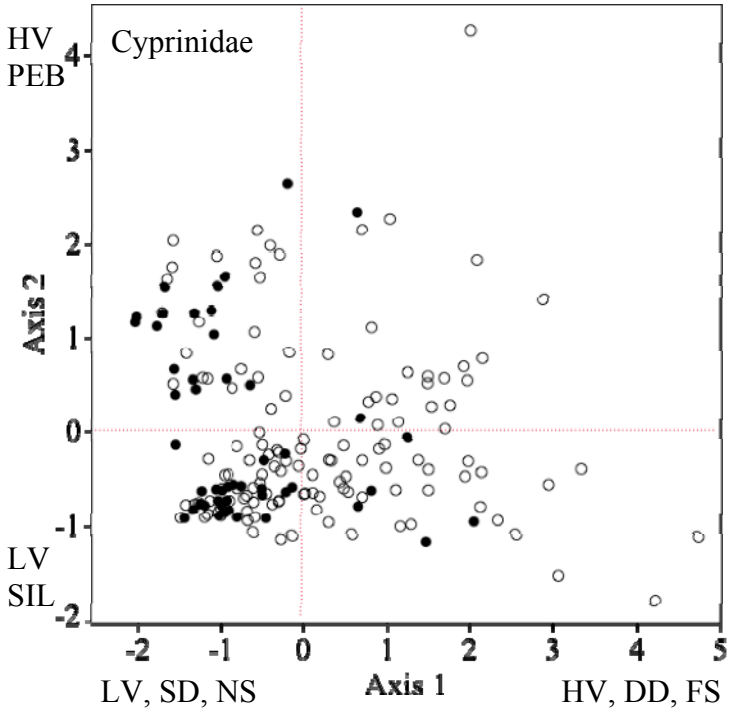


Figure 3cd. PCA analysis of families Fundulidae (c) and Cyprinidae (d) by depth, velocity, distance from shore, and substrate. The first two components account for 70.27 percent of model variance. LV: low velocity, HV: high velocity, SD: shallow water depth, DD: deep water depth, NS: near to shore, FS: far from shore, PEB: pebbly substrate, SIL: silty substrate.

Depth rather than velocity appeared to be the most significant indicator of habitat differentiation. Depth was a significant habitat indicator for three out of the four families distinguished by environmental variables. Velocity was only significant for distinguishing moronid habitat. Other larval fish habitat studies have shown velocity to be a dominant indicator of fish presence and survival (Scheidegger and Bain 1995; Freeman et al 2001; Humphries et al 2002; Niles 2004). In this study, most areas sampled were not exposed to high velocities, which may be why velocity is not a stronger habitat differentiator.

Clupeid larvae were consistently found in deep water and tended to be further from shore. They occurred primarily in the bay and river main channels. Clupeidae was the only family to show significant habitat association by size. Larger fish were found in faster water, deeper habitat, and slightly closer to shore. Clupeid size distribution may support the theory that fish utilize different portions of their habitat as they develop, even in early life stage fishes.

Moronid larvae were present in relatively high velocity water with silty substrate. They occupied habitat up to more than twice the calculated maximum sustainable velocity (8.4 cm/sec). Most of the moronid larvae collected were far into their larval development and would nearly be classified as juveniles. The ability to forage in faster water may be advantageous in a system where most larvae occur in slower water.

Fundulids were primarily found in bay samples. These results suggest that the bay interior has a larger function as a spawning and nursery area for fundulids than the mainstem Hudson. Fundulids were found in shallow water habitat with a silty substrate. Substrate preference is significant because fundulids occurred predominantly in Tivoli

North Bay, which has silty substrate throughout its channels and backwaters. Fundulids were collected throughout Tivoli North Bay backwaters and along the shallow edges of the channels.

Cyprinids were also found in shallow water within the same depth range as fundulids. Unlike fundulids, cyprinid larvae were equally distributed between bay and mainstem samples and were found in habitats with both coarse and fine sediments. Cyprinids appear to occur more frequently in low velocity water. Shallow shoreline areas in both Tivoli North Bay and along the mainstem Hudson were the most suitable collection sites for cyprinid larvae.

Centrarchids and percids did not show significant habitat differentiation. In the case of the centrarchids, this may be due to the low sample presence. Centrarchid larvae occurred in 7 out of 180 trap samples, which is not a sufficient number of samples for a robust MANOVA comparison. Continued sampling until later in the season may have increased centrarchid sample count since centrarchids were not collected until the latter period of sampling. No habitat differentiation was detected for percid larvae, even though they were present in 30 out of 180 samples. This resolution was strong enough to show habitat differentiation in other taxa, but may not be strong enough for percids if their habitat differentiation is more variable than that of other taxa. Another explanation is that percid larval habitat differentiation was broader than the range of conditions sampled so differentiation could not be detected.

Aquatic vegetation stands have been considered important nursery areas for estuarine fishes. Studies conducted within Hudson River backwaters have concluded several cases of larval fishes association with aquatic vegetation. Water-milfoil

(*Myriophyllum spicatum*) and water-celery (*Vallisneria americana*) support a variety of centrarchids and cyprinids (Schmidt and Kiviat 1988). Water-chestnut (*Trapa natans*) stands were found to support greater densities and diversity of larval and juvenile fish (Anderson and Schmidt 1989). High densities of larval and juvenile mummichog were observed in shallow vegetated waters, cattails, and newly established common reed stands (Harm et al 2003). The fact that no significant differences in family presence were detected for vegetated and unvegetated areas is surprising. Changes in sampling design may account for this lack of detection by using paired sampling methods rather than random point abundance sampling.

Differential habitat presence displayed by clupeids, moronids, fundulids, and cyprinids may have implications for habitat restoration and future shoreline development decisions. The Hudson River Estuarine Research Reserve (HRNERR) has an interest in restoring Hudson River backwaters. The aim is not to restore backwaters to their former condition, but to understand the functioning of the current ecosystem with its environmental constraints and human influence (Miller et al 2006). Based on the results of this study, designing habitat appropriate for larval fish would depend on the type of taxa considered for restoration and the range of taxa included in the restoration plan. Efforts aimed towards planning habitat suitable for many groups of fish must take a range of habitats into consideration while efforts to boost individual family larval survival may focus on maximizing taxa-specific habitat parameters.

The Hudson River shoreline around Magdalen Island appeared to be as significant as the bay for larval fish occupancy. Continued shoreline alteration in the Hudson River has the potential to alter or restrict mainstem larval fish sites to be inappropriate for larval

occupancy and development. Even though the presence of larval fish within a given habitat is not a clear indicator of habitat choice or better survival, it is an indicator of how larval fishes are distributed within their aquatic environment. Having more information about larval fish distribution can increase awareness of how future changes may affect microhabitat conditions identified as important to early life fish.

ACKNOWLEDGEMENTS

Utmost thanks to the Polgar Fellowship Committee for this great research opportunity and funding. I am grateful to Daniel Miller, Sarah Fernald and David Yozzo for their invaluable advice and help with fieldwork. Special thanks to Emily Nash and Deidre Hayward who spent long hours traveling and sampling with me. Thanks to Erik Kiviat and Bob Schmidt at Bard College for sharing their knowledge. Finally, I am extremely grateful to my advisor Mark Bain who continues to help me through the research process.

REFERENCES

Anderson, A. B. and R. E. Schmidt. 1989. A survey of larval and juvenile fish populations in water-chestnut (*Trapa natans)* beds in Tivoli South Bay, a Hudson River tidal marsh. Section VI in E. A. Blair and J. R. Waldman (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 1988. Hudson River Foundation, New York, New York.

Auer, N.A. 1982. Identification of larval fishes of the Great Lakes Basin with emphasis on the Lake Michigan Drainage. University of Michigan, Special Publication 82-3, Ann Arbor, Michigan.

Bain, M. B., J. T. Finn, and H. E. Booke. 1985. Quantifying stream substrate for habitat analysis studies. North American Journal of Fisheries Management 5:499–500.

Bain, M. B., and S. E. Boltz. 1992. Effect of aquatic plant control on the microdistribution and population characteristics of largemouth bass. Transactions of the American Fisheries Society 121:94–103.

Cotroneo, C., and D.J. Yozzo. 2010. W-fold throw trap: A folding, transportable 1-m2 throw trap for use in densely vegetated aquatic habitats. Journal of Freshwater Ecology. 25(1):73-78

Copp, G. H. 1990. Shifts in the microhabitat of larval and juvenile roach, *Rutilus rutilus* (L.), in a floodplain channel. Journal of Fish Biology 36:683-692.

Copp, G. H., and M. Penáz. 1988. Ecology of fish spawning and nursery zones in the Floodplain, using a new sampling approach. Hydrobiologia 169:209-224.

Coote, T. W., R. E. Schmidt and N. Caraco. 2001. Use of a periodically anoxic *Trapa natans* (water-chestnut) bed by fishes in the Hudson River. Section IV: 20pp. In J. R. Waldman & W. C. Nieder (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2000. Hudson River Foundation. New York, New York.

Daniels, R. A., K. Limburg, R. E. Schmidt, D. L. Strayer, and R. C. Chambers. 2005. Changes in fish assemblages in the tidal Hudson River, New York. American Fisheries Society Symposium 45:471–503.

Findlay, S., D. Strayer, M. Bain, and W. C. Nieder. 2006. Ecology of Hudson River Submerged Vegetation. Final Report to the New York State Department of Environmental Conservation.

Freeman, M. C., Z. H. Bowen, K. D. Bovee, and E. R. Irwin. 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecological Applications 11(1):179-190.

Harm, L., E. Salak, and D. Osgood. 2003. Effects of *Phragmites australis* on the early life stages of *Fundulus heteroclitus* at Iona Island Marsh, Hudson River, New York. Section IV in J. R. Waldman and W. C. Nieder (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2002. Hudson River Foundation, New York, New York.

Hjort, J. 1914. Remarks on the fluctuations in number and growth in marine populations. Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer 80:1-8.

Humphries, P., L. G. Serafini, and A. J. King. 2002. River regulation and fish larvae: variation through space and time. Freshwater Biology 47:1307-1331.

Jones, P.W., F.D. Martin, and J.D. Hardy, Jr. 1978. Development of Fishes of the Mid-Atlantic Bight. Vol. 1., US Fish & Wildlife Service FWS/OBS-78/12.

Kay, L. K., R. Wallus, and B. L. Yeager. 1994. Reproductive biology and early life history of fishes in the Ohio River drainage, Volume 2: Catostomidae. Tennessee Valley Authority, Chattanooga, Tennessee.

Leslie, J. K., and C. A. Timmins. 1991. Distribution and abundance of young fish in Chenal Ecarte and Chematogen Channel in the St. Clair River delta, Ontario. Hydrobiologia 219:135-142.

Limburg, K. E. 1996. Growth and migration of 0-year American shad (*Alosa sapidissima*) in the Hudson River estuary: otolith microstructural analysis. Canadian Journal of Fisheries and Aquatic Sciences 53:220–238.

Marr, J. C. 1956. The "critical period" in the early life history of marine fishes. ICES Journal of Marine Science/Journal du Conseil International pour l'Exploration de la Mer 21: 160-170.

May, R. C. 1974. Larval mortality in marine fishes and critical period concept, p. 3-19. In J. H. S. Blaxter [ed.] The early life history of fish. Springer-Verlag, Heidelberg.

Miller, D., J. Ladd, and W. C. Nieder. 2006. Channel morphology in the Hudson River Estuary: Historical changes and opportunities for restoration. pg 29-39 In Waldman, J. R., Limburg, K. E., and Strayer, D. L. [ed.] Hudson River Fishes and their Environment. American Fisheries Society Symposium 51.

Moyle, P. B. and J. J. Cech. 2004. Fishes: an introduction to ichthyology. Pearson Benjamin Cummings, San Francisco. 5th ed.

Niles, J. M. 2004. Examination of experimentally engineered larval fish habitat in the Marmet Pool, Kanawha River, West Virginia. Master's Thesis. West Virginia University, Morgantown, West Virginia.

Odom, M. C. 1987. Distribution of larval fishes in the Winfield Pool, Kanawha River, and direct impacts of commercial navigation traffic on larval fish survival. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Penáz, M., A. L. Roux, P. Jurajda, and J. M. Olivier. 1992. Drift of larval and juvenile fishes in a by-passed floodplain of the upper River Rhone, France. Folia Zoologica 41:281-288.

Rozas, L.P. and W.E. Odum. 1987. Fish and macrocrustacean use of submerged plant beds in tidal freshwater marsh creeks. Marine Ecology Progress Series 38:101-108.

Scheidegger, K. J., and M. B. Bain. 1995. Larval fish in natural and regulated rivers: assemblage composition and microhabitat use. Copeia 1995:125–135.

Schmidt, R. E. 1986. Fish community structure in Tivoli North Bay, a Hudson River Freshwater Tidal Marsh. NOAA Technical Report OCRM/SPD, 47pp.

Schmidt, R. E. and E. Kiviat. 1988. Communities of larval and juvenile fish associated with water-chestnut and water-celery in the Tivoli Bays of the Hudson River: A Report to the Hudson River Foundation. Hudsonia, Ltd., Bard College Field Station, Annandale, NY.

Schmidt, R. E., and S. Cooper. 1996. A catalog of barriers to upstream movement of migratory fishes in Hudson River tributaries. Final report to Hudson River Foundation, New York.

Scott, M. T., and L. A. Nielsen. 1989. Young fish distribution in backwaters and main channel borders of the Kanawha River, West Virginia. Journal of Fish Biology 35:21-27.

Snyder D. E. 1983. Fish eggs and larvae. Pages 165-197 in L. Nielsen and D. Johnson [ed] Fishery techniques. American Fisheries Society, Bethesda, MD.

Snyder D. E. 1990. Fish larvae – ecologically distinct organisms. Pages 20-23 in M. B. Bain, ed. Ecology and assessment of warmwater streams: workshop synopsis. US Fish and Wildlife Service Biological Report 90(5), Washington, DC.

Strayer, D. L., N. F. Caraco, J. J. Cole, S. Findlay, M. L. Pace. 1999. Transformation of freshwater ecosystems by bivalves. BioScience 49(1):19-27.

Strayer, D. L., K. A. Hattala, and A. W. Kahnle. 2004. Effects of an invasive bivalve (*Dreissena polymorpha*) on fish in the Hudson River estuary. Canadian Journal of Fisheries and Aquatic Sciences 61:924-941.

Squires, D. F. 1992. Quantifying anthropogenic shoreline modification of the Hudson River and estuary from European contact to modern time. Coastal Management 20:343–354.

Webb P. W. 1975. Hydrodynamics and energetics of fish propulsion. Bulletin of the Fisheries Research Board of Canada. 190:1-159.

Yozzo, D. J., J. L. Anderson, M. M. Cianciola, W. C. Nieder, D. E. Miller, S. Ciparis, and J. McAvoy. 2005. Ecological Profile of the Hudson River National Estuarine Research Reserve. NYSDEC Contract No. C004646, Barry A. Vittor & Associates, Inc., Kingston, New York.

USING STABLE ISOTOPES TO EXAMINE FORAGING ECOLOGY OF NEW YORK HARBOR COLONIAL WATERBIRDS

A Final Report of the Tibor T. Polgar Fellowship Program

Elizabeth C. Craig

Polgar Fellow

Field of Zoology and Wildlife Conservation
Cornell University
Ithaca, NY 14853

Project Advisors:

Paul D. Curtis
Department of Natural Resources
Cornell University
Ithaca, NY 14853

Susan B. Elbin
New York City Audubon Society
New York, NY 10010

Craig, E.C., P.D. Curtis, and S.B. Elbin. 2012. Using stable isotopes to examine foraging ecology of New York Harbor colonial waterbirds. Section VIII: 1-25 pp. *in* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2010. Hudson River Foundation.

ABSTRACT

New York Harbor has become an important breeding area for New York State's colonial waterbird community since their resurgence in the region in the 1970's. Current knowledge of these birds is generally limited to their breeding population sizes and nesting phenology. However, an understanding of foraging behavior is also critical to the conservation of these species. Stable isotope analysis is a valuable tool in the study of diet and foraging ecology of birds. Isotopic signatures observed in feathers reflect the bird's diet and foraging habitat at the time of feather formation. In this study, stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were measured in order to monitor diet, trophic position, and foraging habitat of colonial waterbirds nesting in New York Harbor. During the 2010 breeding season, feathers were collected from nestlings of six colonial waterbird species breeding on four islands within the New York Harbor. The species sampled included the Double-crested Cormorant, Great Black-backed Gull, Herring Gull, Great Egret, Black-crowned Night-Heron, and Glossy Ibis. Significant intra- and inter-specific variation in $\delta^{13}C$ and $\delta^{15}N$ was observed ($p<0.0001$), suggesting differences in foraging habitat (marine versus freshwater), variability of prey selection, and relative trophic position. Stable isotope analysis of feathers has proven to be a powerful and non-invasive tool for studying foraging ecology of colonial waterbirds in urban systems. The findings of this study can help guide resource managers in identifying and protecting important foraging habitat and prey base for these charismatic flagship species.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

The New York-New Jersey Harbor Estuary, with an area of approximately 40,000 km^2, is home to 20 million people. However, it also provides a mosaic of urban habitat types for wildlife, ranging from open water, to fresh- and saltwater marsh, to uplands and the built environment. Small, abandoned islands in the harbor have become important breeding areas for many of New York State's colonial waterbirds since their resurgence in the region in the 1970's. Colonial waterbirds are species that tend to nest in large colonies and forage in aquatic systems. These birds are generally large, conspicuous species that constitute the charismatic megafauna of this urban estuary. Twelve colonial waterbird species are known to reproduce on these islands and forage in the surrounding wetlands and waterways to feed themselves and provision their young (Craig 2010; Elbin and Tsipoura 2010).

Current knowledge of these birds generally concerns their breeding population sizes and nesting phenology (Craig 2010). The Harbor Heron Conservation Plan of the New York-New Jersey Harbor Estuary Program calls for research to fill the information gap about foraging behavior of colonial waterbirds in this system (Elbin and Tsipoura 2010). Recent studies have employed citizen science observations of foraging birds (Tsipoura et al. 2010), and pellet and regurgitant analysis (Grubel and Waldman 2009), to elucidate resource use of focal species within the New York-New Jersey Harbor Estuary. While there are strengths to each of these approaches, both are observational studies that only encompass the diet and foraging habitat of the bird at the moment of observation (Barrett et al. 2007). This study used stable isotope analysis of feathers from nestling birds to elucidate foraging behavior of a suite of colonial waterbird species nesting in the

New York Harbor, focusing on six of the harbor's most numerically abundant colonial waterbird species, including three long-legged wading birds: Black-crowned Night-Heron (*Nycticorax nycticorax*), Great Egret (*Ardea alba*), Glossy Ibis *(Plegadis falcinellus*); and three seabirds: Double-crested Cormorant (*Phalacrocorax auritus*), Great Black-backed Gull (*Larus marinus*), and Herring Gull (*Larus argentatus*).

Stable isotope analysis is a valuable tool in the study of diet and foraging ecology of birds (Hobson and Clark 1992 a & b; Hobson 1999). Isotopic signatures observed in feathers (isotopically inert tissues) reflect the bird's entire diet and foraging habitat at the time of feather formation (Hobson and Clark 1992a). Stable isotope measurements therefore reflect resource use in a more comprehensive manner than behavioral observations or regurgitant and pellet analyses alone. With careful interpretation, isotopic signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be used to monitor diet, relative trophic position, and foraging habitat of birds (Bond and Jones 2009). This study specifically inferred information about the type of diet items the parents used to feed their young, the relative trophic position of the prey items, and the habitat type (marine or freshwater) in which the parents primarily foraged. This approach, in combination with foraging behavior observations and diet studies, will improve the understanding of the foraging landscape used by colonial waterbird species in the harbor.

Carbon isotopic ratios in feathers are primarily indicative of the base of the food web in which the bird has recently foraged. More negative (smaller) $\delta^{13}C$ values can indicate a greater proportion of freshwater resource use, while less negative (larger) $\delta^{13}C$ values can indicate a greater proportion of marine resource use (Mizutani et al. 1990; Hobson and Clark 1992b; Bearhop et al. 1999; Bond and Jones 2009). Inclusion of C4

photosynthetic plant materials in the diet, such as products containing corn or sugar, can also create larger $\delta^{13}C$ values (Farquhar et al. 1989). Such values may indicate use of anthropogenic resources such as human food waste or intensive aquaculture (Hebert et al. 2009).

Nitrogen isotopic ratios increase with every trophic exchange, therefore $\delta^{15}N$ signatures in feathers are primarily indicative of the relative trophic position at which the bird has recently foraged (Hobson and Clark 1992b; Post 2002; Bond and Jones 2009). Higher $\delta^{15}N$ values indicate higher relative trophic position, and lower $\delta^{15}N$ values indicate lower relative trophic position. $\delta^{15}N$ values are also influenced by the $\delta^{15}N$ signature at the base of the food web, which can vary considerably among habitats with different sources of nitrogen and different ecosystem processes. Caution must be taken when interpreting $\delta^{15}N$ in terms of trophic position without prior knowledge of $\delta^{15}N$ signatures at the base of the food web (Post 2002). In this study, $\delta^{15}N$ signatures were compared among populations to infer relative, but not absolute, trophic position.

Feathers of nestling birds were used in this study because these tissues represent the local diet with which adult birds are provisioning their young. New York Harbor colonial waterbirds are generally migratory and can be long-lived, experiencing a multitude of habitats across their migratory range within their lifetimes. Depending on its time of formation, a feather from an adult bird may represent nutrients from a wide range of habitats beyond the breeding grounds. Feathers of nestling birds are therefore most suitable for answering questions about local foraging behavior and diet because they integrate only the resources that the adult bird has provided during the breeding season (Cherel et al. 2000).

Based on a general understanding of the foraging strategies of the focal species of this study, differences in isotopic signatures in species with differing foraging ecologies are to be expected. In general, ibis, egrets, and herons eat fish, crabs, amphibians, and aquatic invertebrates, cormorants eat primarily fish, and gulls eat a variety of items ranging from fish to human garbage (Pierotti and Good 1994; Good 1998; Hatch and Weseloh 1999; Davis and Kricher 2000; McCrimmon et al. 2001; Hothem et al. 2010). The stable isotope approach used in this study allows us to confirm assumed differences in foraging ecology among species. More significantly, the results of this study enhance the understanding of the specific habitat types and forage base that are most important in the diet of individual populations within this urban estuary. This information is critical to the conservation of these species and in determining which areas within the estuary should be prioritized for habitat protection or environmental remediation.

The objectives of this study were to examine the foraging ecology of a suite of colonial waterbird species nesting within New York Harbor, and to identify the key habitats and forage base that were particularly important to each individual population. The hypothesis tested was that colonial waterbird populations would exhibit both inter- and intra-specific variation in foraging behavior and habitat preference, observed through variation in stable isotope signatures, based on differences in their general foraging strategies and on habitat availability near their nesting locations. Identifying the key resources that are most important to these populations is instrumental in the conservation of colonial waterbirds in this urban system.

METHODS

Field Sites

Feather samples were collected at four locations (Figure 1): two in Lower New York Bay (Hoffman Island, Swinburne Island); one in the East River (South Brother Island); and one in Westchester County, New York (Muscoot Reservoir Island).

Hoffman and Swinburne islands are man-made islands located in Lower New York Bay off the east shore of Staten Island, New York (40.578716°N, 74.053752°W). Hoffman Island (approximately 4.5 hectares) and Swinburne Island (approximately 1.5 hectares) were constructed in the mid-1860s as quarantine islands for immigrants carrying contagious diseases. In 1961, all the buildings on Hoffman Island were razed, although many of the structures on Swinburne Island still stand. In 1972 the islands were entrusted to the federal government as part of the National Parks Service Gateway National Recreation Area (Seitz and Miller 1996). Public access is restricted. In 2010, nine species of colonial waterbirds nested on Hoffman Island: Black-crowned Night-Heron, Yellow-crowned Night-Heron, Great Egret, Snowy Egret, Glossy Ibis, Little Blue Heron, Double-crested Cormorant, Great Black-backed Gull, and Herring Gull. In 2010, four species of colonial waterbirds nested on Swinburne Island: Double-crested Cormorant, Great Black-backed Gull, Herring Gull, and Black-crowned Night-Heron (Craig 2010).

South Brother Island is a natural island in the East River situated between Riker's Island and the Bronx, New York (40.796110°N, 73.898060°W). This approximately three-hectare island has historically had only a single residential building, constructed in 1894. Since the destruction of this building by fire in 1909, the island has remained

undeveloped (Seitz and Miller 1996). South Brother Island was acquired by New York City Department of Parks and Recreation in 2007. Public access is restricted. In 2010, eight species of colonial waterbirds nested on South Brother Island: Black-crowned Night-Heron, Yellow-crowned Night-Heron, Great Egret, Snowy Egret, Glossy Ibis, Double-crested Cormorant, Great Black-backed Gull, and Herring Gull (Craig 2010).

Muscoot Reservoir Island is a small island (less than 100 m^2) located in Muscoot Reservoir, 40 km north of New York Harbor, and directly north of the village of Katonah in Westchester County, New York (41.2694°N, 73.7070°W). Muscoot Reservoir is part of the Croton Reservoir system, which provides drinking water to New York City. In 2010, Double-crested Cormorants nested on Muscoot Reservoir Island (personal observation). This colony is monitored by the New York City Department of Environmental Protection.

Figure 1. Map of the New York-New Jersey Harbor and surrounding region. Feather samples were collected at Muscoot Reservoir and Hoffman, Swinburne, and South Brother islands.

Feather Collection

In June and July 2010, colonial waterbird nestlings of five species were captured by hand at nests on Hoffman, Swinburne, South Brother, and Muscoot Reservoir islands in coordination with permitted studies and banding programs conducted by New York City Audubon. Between one and 10 contour feathers per individual were collected while the nestlings were handled for banding. In addition, between June and September 2010, contour feathers were collected from all recently deceased colonial waterbird nestlings of known species identity encountered on each island. All feather samples were stored in individually labeled paper envelopes. A summary of the number of individuals sampled at each location can be found in Table 1.

Table 1. Summary of feather sampling. Feather samples were collected from colonial waterbird nestlings on islands in the New York-New Jersey Harbor Estuary and Muscoot Reservoir from June to September, 2010.

	South Brother Island	*Hoffman Island*	*Swinburne Island*	*Muscoot Reservoir*
Black-crowned Night-Heron	6	9	0	0
Glossy Ibis	0	13	0	0
Great Egret	20	15	0	0
Double-crested Cormorant	21	0	20	7
Great Black-backed Gull	0	20	0	0
Herring Gull	0	6	14	0

Stable Isotope Analysis

A one mg sample (± 0.1 mg) of each feather was encapsulated in tin and analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer with elemental analyzer (EA-IRMS) at Cornell University's Stable Isotope laboratory (COIL).

Statistical Analysis

Single-factor MANOVA and post-hoc ANOVA with Tukey-Kramer HSD were used to determine statistically significant variation in $\delta^{13}C$ and $\delta^{15}N$ signatures using a factor of nesting location or species depending on the analysis. Additionally, a MANOVA of $\delta^{13}C$ and $\delta^{15}N$ was conducted using mortality as the single factor to confirm that isotope signatures did not differ significantly with mortality. The $\delta^{13}C$ and $\delta^{15}N$ signatures of feathers collected from live versus dead nestlings were not found to differ significantly ($p=0.6227$). Therefore, the data were analyzed regardless of mortality for all subsequent analyses.

RESULTS

Intra-specific Variation Among Colonies

Cormorants

As anticipated, $\delta^{13}C$ signatures were found to vary significantly among the three cormorant breeding colonies sampled in this study ($p < 0.0001$; Fig. 2). Cormorants from Muscoot Reservoir Island, located in a purely freshwater environment, had the lowest average $\delta^{13}C$ values (mean ± SD of -27.52 ± 0.85 ‰) indicative of freshwater resource use. Cormorants from Swinburne Island, located in the tidal Lower New York Bay and coastal Atlantic Ocean, had the highest average $\delta^{13}C$ values (-16.95 ± 1.78 ‰) indicative of marine resource use. Cormorants from South Brother Island, located in the tidal East River near freshwater tributaries, exhibited intermediate $\delta^{13}C$ values (-20.53 ± 3.83 ‰). Cormorants from South Brother Island also exhibited the largest standard deviation (3.83 ‰) and range (12.65 ‰) in $\delta^{13}C$ values, indicating a larger range and variety of foraging

habitat use in birds from this location. No significant differences in $\delta^{15}N$ were observed among cormorant colonies.

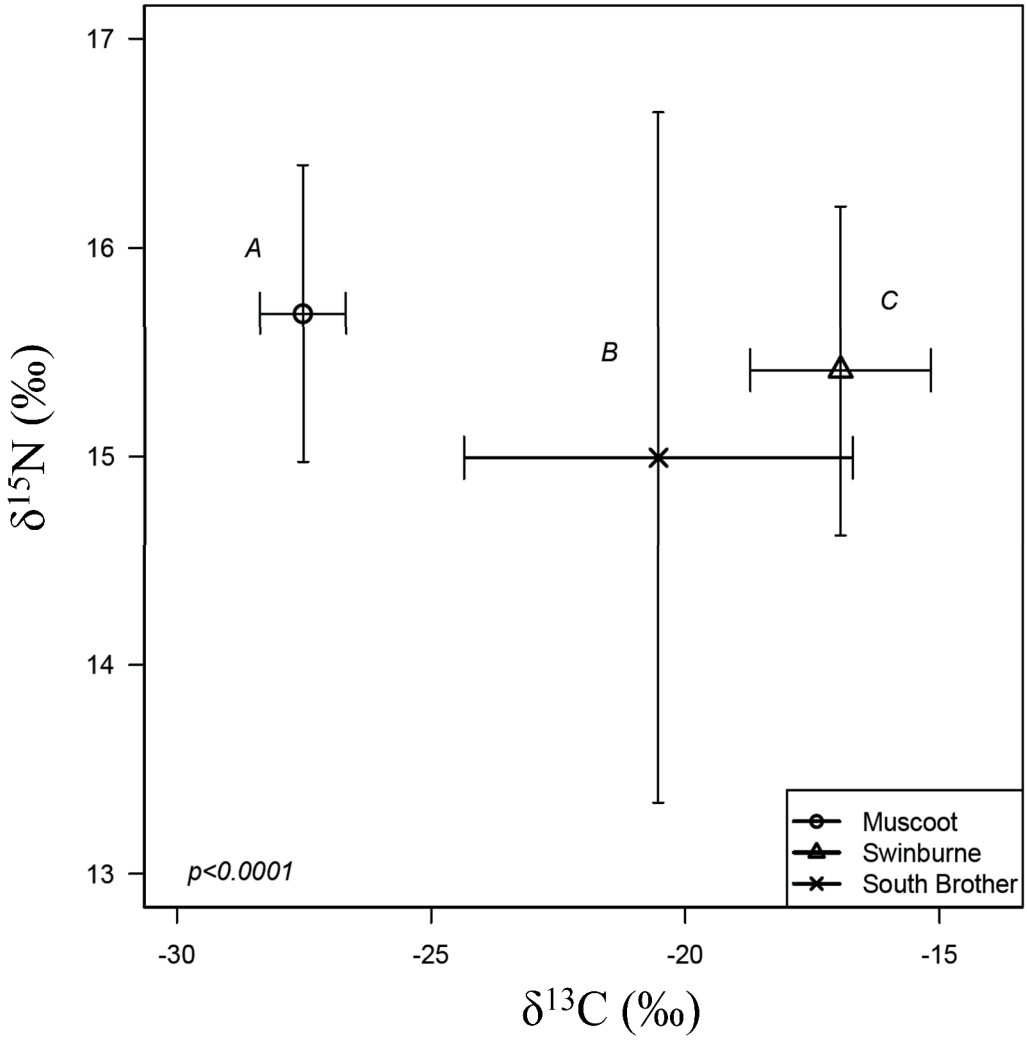


Figure 2. Cormorants across the New York-New Jersey Harbor Estuary. Mean ± SD $\delta^{13}C$ versus $\delta^{15}N$ among breeding colonies of cormorants in 2010 (Muscoot Reservoir Island, Swinburne Island, and South Brother Island). $\delta^{13}C$ varied significantly over time ($p<0.0001$). Different letters signify statistically significant differences among colonies according to Tukey-Kramer HSD.

Long-legged Wading Birds

No significant differences in isotopic signatures were observed among colonies of Great Egrets or Black-crowned Night-Herons (the only long-legged wading birds

sampled at multiple locations). However, wading birds nesting on South Brother Island tended to have greater ranges in $\delta^{13}C$ than conspecifics nesting on Hoffman Island. Great Egret $\delta^{13}C$ values had a range of 11.66 ‰ on South Brother Island in comparison to a range of 5.75 ‰ on Hoffman Island. Black-crowned Night-Herons exhibit smaller differences in $\delta^{13}C$ range: 8.67 ‰ on South Brother Island and 6.32 ‰ on Hoffman Island.

Inter-specific Variation

To determine inter-specific variation in foraging behavior at a single nesting colony, variation in $\delta^{13}C$ and $\delta^{15}N$ was analyzed among species nesting on Hoffman and Swinburne islands combined, as these two nesting islands exist in very close proximity and therefore have the same available surrounding foraging habitat. Significant variation in both $\delta^{13}C$ and $\delta^{15}N$ was observed using a MANOVA with species as the single factor ($p<0.0001$; Fig. 3).

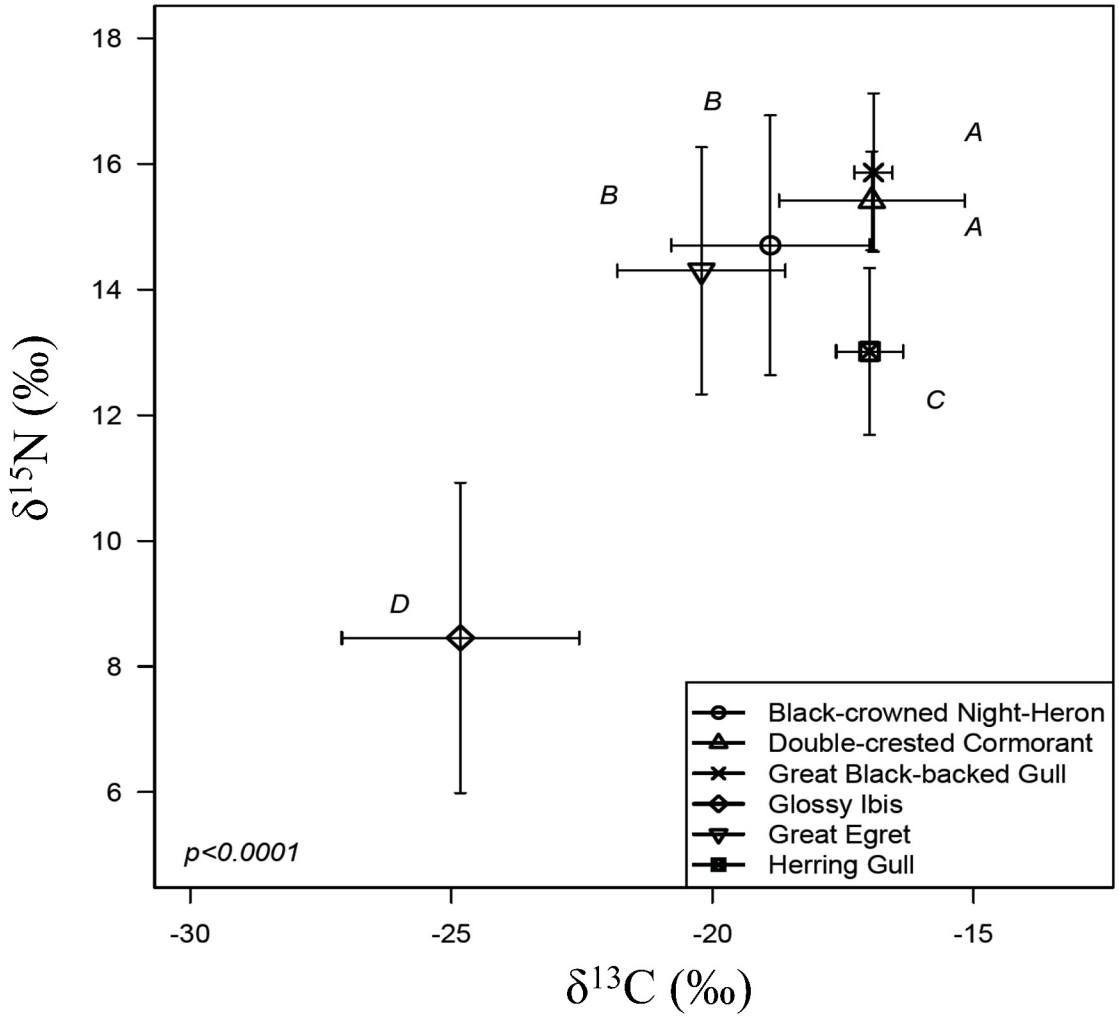


Figure 3. Colonial waterbird species on Hoffman & Swinburne islands. Mean ± SD $\delta^{13}C$ versus $\delta^{15}N$ among species on Hoffman & Swinburne islands combined. Both $\delta^{13}C$ and $\delta^{15}N$ varied significantly among species ($p<0.0001$). Different letters signify statistically significant differences among species according to Tukey-Kramer HSD.

Great Egret and Black-crowned Night-Heron isotopic signatures were not statistically different from one another. Double-crested Cormorants and Great Black-backed Gulls also were not statistically different from one another. Isotopic signatures of both Herring Gulls and Glossy Ibis were individually distinct from all other species nesting on Hoffman and Swinburne Islands in 2010.

DISCUSSION

The results of this study elucidate foraging behaviors of a suite of colonial waterbird species nesting within New York Harbor. These data allow us to identify key habitat types and forage base that are particularly important to individual populations. Both intra- and inter-specific variation in foraging behavior were examined in order to make observations of foraging behaviors on both the population and breeding colony scales.

Intra-specific Variation Among Colonies

Double-crested Cormorants exhibited significant variation in isotopic signatures across the breeding colonies at which they were sampled ($p<0.0001$; Fig. 2). As predicted, the predominant foraging habitats represented in the isotopic signatures reflected the available habitat types in the vicinity of each colony. Cormorants from Muscoot Reservoir Island had isotopic signatures indicative of a predominantly freshwater diet (relatively low $\delta^{13}C$ values). Birds nesting at this colony were most likely foraging at Muscoot Reservoir and the surrounding reservoir and river system. Cormorants from Swinburne Island had isotopic signatures indicative of a predominantly marine diet (relatively high $\delta^{13}C$ values), and were most likely foraging within Lower New York Harbor and the surrounding coastal Atlantic Ocean. Cormorants from South Brother Island exhibited a greater range in $\delta^{13}C$ signatures than observed at the other two colony locations (nearly twice the range observed in Swinburne Island cormorants, and nearly six times the range observed in Muscoot Reservoir Island cormorants), indicating that a wider range of foraging habitats contributed to the diet of these birds. This

observation reflects the diverse nature of foraging habitat availability near South Brother Island, located in the tidal East River surrounded by its freshwater tributaries. The area surrounding South Brother Island is also highly developed, and cormorants nesting at this location may forage at greater distances from the island to meet their metabolic needs. The resulting increased foraging distance would also explain the greater range of foraging habitats exhibited in the isotopic signatures of cormorants from the South Brother Island colony. These data provide a valuable perspective on intra-specific variation in foraging behavior of cormorants within the New York-New Jersey Harbor Estuary system.

Interestingly, no significant intra-specific differences were observed in isotopic signatures of Great Egrets or Black-crowned Night-Herons (both nesting on Hoffman and South Brother islands). As long-legged wading birds, the foraging strategies of these two species may also require them to forage at greater distances from their colony sites, exposing them to a larger range of potential foraging habitat types. While long-legged wading bird species appear to be foraging in similar habitat types regardless of nesting colony location, the range in $\delta^{13}C$ signatures was greater in birds from South Brother Island than from Hoffman Island, as observed in cormorants. These results indicate that long-legged wading birds, as well as cormorants, nesting at South Brother Island may be foraging in a wider range of habitats, and potentially at a greater foraging distance, than conspecifics nesting on Hoffman Island.

Inter-specific Variation

As anticipated, a significant inter-specific variation in foraging behavior of species nesting on Hoffman Island was observed ($p<0.0001$; Fig. 3). The species sampled

on this island represent a wide range of foraging strategies and the significant variation in both $\delta^{13}C$ and $\delta^{15}N$ reflect those differences.

Glossy Ibis tend to forage by probing for invertebrates in shallow water, fields, and marshes (Davis and Kricher 2000). Based on the significantly lower $\delta^{13}C$ values observed in this species, it can be concluded that freshwater foraging habitat is the most important habitat type for this population during chick rearing. Glossy Ibis also exhibited significantly lower average $\delta^{15}N$ values, which may indicate a difference in source nitrogen in ibis foraging habitats, but most likely also indicates that ibis were foraging at a lower relative trophic position than other species nesting on Hoffman Island. As ibis primarily consume low trophic position prey, this observation supports the general understanding of ibis foraging ecology.

Great Egrets and Black-crowned Night-Herons exhibited no significant differences in $\delta^{13}C$ and $\delta^{15}N$ values. These species may therefore share similar forage base and range of foraging habitats within the New York-New Jersey Harbor Estuary. Based on average $\delta^{13}C$ values observed for both of these species, these long-legged wading birds are expected to forage in a mix of both freshwater and marine environments.

Double-crested Cormorants are diving birds that prey on a wide variety of fish. Cormorants at Swinburne Island exhibited relatively high $\delta^{13}C$ values indicating a large proportion of marine resource use at this location. Interestingly, the Great Black-backed Gulls observed on adjacent Hoffman Island (an island on which cormorants also nest) exhibited $\delta^{13}C$ and $\delta^{15}N$ values very similar to those observed in cormorants. The general understanding of the foraging strategies of these two species does not explain this

phenomenon; gulls tend to be more generalist scavengers and cormorants are piscivorous, diving predators. Great Black-backed Gulls, however, have been known to exhibit predatory behavior (Good 1998), and were observed during this study predating cormorant nests when cormorant colonies were disturbed. However, if cormorant nest contents (nestlings or eggs) were the main component of Great Black-backed Gull diet, higher $\delta^{15}N$ values would be expected in gulls than in cormorants. As this is not the case, the similarity between cormorant and Great Black-backed Gull isotopic signatures is hypothesized to be due to a combination of gulls predating early-stage cormorant nests, and scavenging for food scraps in the understory beneath the cormorant colony. Regardless of the specific scavenging or predatory behavior, the data suggest that cormorant-related diet items may be the most important food source for Great Black-backed Gulls nesting on Hoffman Island.

Herring Gulls also exhibited relatively high $\delta^{13}C$ values, although significantly lower $\delta^{15}N$ values than observed in the Great Black-backed Gulls. Herring Gull isotopic signatures did not overlap with cormorant signatures as observed in the Great Black-backed Gull, although Herring Gulls have also been observed predating cormorant nests. Anthropogenic resource use, including scavenging of human food waste, could lead to high $\delta^{13}C$ values due to the consumption of C4 photosynthetic plant materials such as corn- and sugar-based foods. Such resource use would also lead to lower $\delta^{15}N$ values due to the low relative trophic position of these diet items. Herring Gull isotopic signatures, therefore, may be explained by either the scavenging of lower trophic position fish from the marine environment, or anthropogenic resource use. Further analysis of these feathers using EA-IRMS to measure $\delta^{34}S$ would determine which interpretation is most likely.

Sulfur isotopic ratios in feathers are primarily indicative of the type of aquatic habitat (freshwater versus marine) in which the bird has recently foraged. While $\delta^{13}C$ can also indicate freshwater or marine resource use, several other factors independent of salinity can influence these signatures. $\delta^{34}S$ could serve as a more straightforward indicator of freshwater versus marine resource use in this case.

Implications for Conservation

Government action to conserve New York Harbor colonial waterbirds has generally focused on protecting critical breeding habitats (Elbin and Tsipoura 2010). The results of this study indicate that, even within a single mixed-species breeding colony, different colonial waterbird species encounter and rely upon very different foraging habitats while provisioning their young. Management efforts must consider population-specific foraging behavior to effectively conserve New York Harbor's colonial waterbirds.

Waterbirds have high metabolic needs and are top-level consumers in the food web. As top predators in aquatic systems, colonial waterbirds have the potential to encounter biomagnified concentrations of contaminants in their diet, and birds foraging at higher trophic positions may be at relatively greater risk (Burger and Gochfeld 2004). Double-crested Cormorants, Great Black-backed Gulls, Great Egrets and Black-crowned Night-Herons exhibited the highest average $\delta^{15}N$ values in the New York Harbor colonial waterbird community, potentially indicating higher relative trophic position. These birds may therefore be at greater risk concerning exposure to environmental contaminants.

An understanding of foraging behavior, including the knowledge of which areas and resources are most important to species of conservation concern, is critical to the

conservation of colonial waterbirds in the New York-New Jersey Harbor Estuary. Stable isotope analysis of feathers is a powerful and non-invasive tool for studying foraging ecology of colonial waterbirds in urban systems. Combining results from this study with foraging behavior observations and diet studies will improve the understanding of the foraging landscape used by different waterbird species in the harbor, and will help guide resource managers in the protection of important foraging habitat for these charismatic flagship species.

Future Directions

To complement these findings of the important foraging habitat types used by colonial waterbirds in New York Harbor, the next step in this line of research will be to determine the relative importance of specific diet items to the overall diet of birds at different focal colonies. Stable isotope analyses (comparable to those presented in this study) will be conducted on common diet items found in cormorant regurgitant, collected from Swinburne and South Brother islands in collaboration with a study conducted by Grubel and Waldman (2009). Using a stable isotope mixing model (including novel information on tissue fractionation rates in cormorants; Craig et al. In preparation), the relative importance of these diet items in the overall diet of cormorants at these nesting colonies will be determined (Bond and Jones 2009). These analyses will add a greater level of detail to the understanding of the foraging landscape used by colonial waterbirds in this system.

ACKNOWLEDGEMENTS

We would like to thank the National Park Service, the New York City Department of Parks and Recreation, and the New York City Department of Environmental Protection for access to protected colonial waterbird nesting islands throughout New York Harbor. Additional support has been provided by the Berryman Institute, the Cornell University Biogeochemistry and Environmental Biocomplexity Program, the Garden Club of America, the Leon Levy Foundation, the Morris Animal Foundation, the National Oceanic and Atmospheric Administration, and the United States Department of Agriculture.

LITERATURE CITED

Barrett, R., K. Camphuysen, T. Anker-Nilssen, J. Chardine, R. Furness, S. Garthe, O. Hüppop, M. Leopold, W. Montevecchi, and R. Veit. 2007. Diet studies of seabirds: a review and recommendations. ICES Journal of Marine Science: Journal du Conseil 64:1675.

Bearhop, S., D.R. Thompson, S. Waldron, I.C. Russell, G. Alexander, and R.W. Furness. 1999. Stable isotopes indicate the extent of freshwater feeding by cormorants *Phalacrocorax carbo* shot at inland fisheries in England. Journal of Applied Ecology 36:75-84.

Bond, A.L. and I. Jones. 2009. A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. Marine Ornithology 37:183-188.

Burger, J. and M. Gochfeld. 2004. Marine birds as sentinels of environmental pollution. EcoHealth 1:263-274.

Cherel, Y., K.A. Hobson, and H. Weimerskirch. 2000. Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. Oecologia 122:155-162.

Craig, E. 2010. New York City Audubon's Harbor Herons Project: 2010 Nesting Survey – 25th Annual Report. New York City Audubon, New York, NY.

Craig, E.C., B. Dorr, and P.D. Curtis. In preparation. Tissue-specific fractionation rates in the Double-crested Cormorant (*Phalacrocorax auritus*).

Davis, W.E. and J. Kricher. 2000. Glossy Ibis (*Plegadis falcinellus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/545

Elbin, S.B. and N.K. Tsipoura (Editors), Harbor Herons Subcommittee. 2010. Harbor Herons Conservation Plan- New York- New Jersey Harbor Region. New York-New Jersey Harbor Estuary Program.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40:503-537.

Good, T.P. 1998. Great Black-backed Gull (*Larus marinus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/330

Grubel, C. and J.R. Waldman. 2009. Feeding habits and the effects of prey morphology on pellet production in Double-crested Cormorants, *Phalacrocorax auritus*. Section VIII:1-28 pp. *in* S.H. Fernald, D. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2008. Hudson River Foundation.

Hatch, J.J. and D.V.C. Weseloh. 1999. Double-crested Cormorant (*Phalacrocorax auritus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/441

Hebert, C.E., D.V.C. Weseloh, A. Idrissi, M.T. Arts, and E. Roseman. 2009. Diets of aquatic birds reflect changes in the Lake Huron ecosystem. Aquatic Ecosystem Health & Management 12:37-44.

Hobson, K.A. and R.G. Clark. 1992a. Assessing avian diets using stable isotopes I: Turnover of 13C in tissues. The Condor 94:181-188.

Hobson, K.A. and R.G. Clark. 1992b. Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. Condor 94:189-197.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314-326.

Hothem, R.L., B.E. Brussee and W.E. Davis, 2010. Black-crowned Night-Heron (*Nycticorax nycticorax*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/074

McCrimmon, D.A., J.C. Ogden and G.T. Bancroft. 2001. Great Egret (*Ardea alba*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/570

Mizutani, H., M. Fukuda, Y. Kabaya, and E. Wada. 1990. Carbon isotope ratio of feathers reveals feeding behavior of cormorants. The Auk 107:400-403.

Pierotti, R.J. and T.P. Good. 1994. Herring Gull (*Larus argentatus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/124

Post, D.M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703-718.

Seitz, S. and S. Miller. 1996. The Other Islands of New York City : A History and Guide. Second edition. The Countryman Press, Woodstock, VT.

Tsipoura, N., K. Ruskin, K. Mendillo, K. Mylecraine, T. Smith, S. Elbin, and J. Rowden. 2010. Abundance and distribution of foraging colonial waterbirds in the New York/New Jersey Harbor: A collaborative citizen science approach. Poster Presentation. Bird Conservation Conference in the Northeast. Oct. 19-21. Plymouth, MA.

Special thanks to Quang Huynh for his assistance in formatting documents.