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RITA COLWELL

TO PRESENT KEYNOTE ADDRESS AT 38th ANNUAL MACUB CONFERENCE

MONMOUTH UNIVERSITY TO HOST MACUB CONFERENCE SATURDAY, OCTOBER 29, 2005

Monmouth University will host the 38th Annual Fall MACUB Conference on Saturday, October 29, 2005 The conference will feature a keynote address by Dr. Rita Colwell.

Dr. Rita Colwell is currently Chairman of Canon US Life Sciences, Inc. and Distinguished University Professor both at the University of Maryland at College Park and at Johns Hopkins University Bloomberg School of Public Health. Her interests are focused on global infectious diseases, water, and health, and she is currently developing an international network to address emerging infectious diseases and water issues, including safe drinking water for both the developed and developing world. She is widely regarded as an expert in cholera and bacterial research.

Rita Colwell

Dr. Colwell was the first woman to be named Director of the National Science Foundation, where she served with distinction from 1998 to 2004, a time of tremendous growth in the Foundation. In her capacity as NSF Director, she served as Co-chair of the Committee on Science of the National Science and Technology Council. One of her major interests include K-12 science and mathematics education, graduate science and engineering education and the increased participation of women and minorities in science and engineering.

Dr. Colwell has held many advisory positions in the U.S. Government, nonprofit science policy organizations, and private foundations, as well as in the international scientific research community. She is a nationally-respected scientist and educator, and has authored or co-authored 16 books and more than 700 scientific publications. She produced the award-winning film, Invisible Seas, and has served on editorial boards of numerous scientific journals.

Dr. Colwell has been awarded 45 honorary degrees from institutions of higher education, including Purdue University. She is a member of the National Academy of Sciences, and an honorary member of the microbiological societies of the United Kingdom, France, Israel, Bangladesh, and the United States. She has previously served as Chairman of the Board of Governors of the American Academy of Microbiology, and as President of the American Association for the Advancement of Science, the Washington Academy of Sciences, the American Society for Microbiology, the Sigma Xi National Science Honorary Society, and the International Union of Microbiological Societies.

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Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

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Characterization of Potential Transplantation Sites for Eelgrass (*Zostera marina* L.) in Jamaica Bay, New York and Eelgrass Growth in a Laboratory Microcosm Mimicking Field Conditions

by

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Abstract

The eelgrass community exists in complex, but measurable physical and chemical conditions of water and sediment. It also presents complex symbiotic associations. This research was conducted to determine the feasibility of transplanting eelgrass, *Zostera marina* L., to Jamaica Bay, New York from Long Island coastal sites, based on three experimental studies. The first study characterized and compared water and sediment at several potential transplantation sites in Jamaica Bay with Long Island sites where eelgrass is growing. The second study evaluated and compared heavy metal concentrations in plants growing in potential Jamaica Bay transplantation sites with eelgrass from the Long Island sites. The third study reproduced the best potential Jamaica Bay transplant site's physical and chemical water/ sediment conditions in a laboratory microcosm to observe whether such conditions can support eelgrass growth. These studies characterized selected parameters of water, sediments and plants at Jamaica Bay and Long Island field sites and demonstrate the short-term ability of Long Island eelgrass to grow successfully under laboratory-reproduced Jamaica Bay field conditions.

INTRODUCTION

Zostera marina L., family Zosteraceae, order Potamogetonales, or common eelgrass, is a submerged, coastal, marine seagrass found in temperate waters of the North Atlantic and Eastern Pacific Oceans. It is a perennial, rhizomatous, aquatic angiosperm having a single, short stem and 4-6 ribbon shaped leaves that grow 30.5-182.9 cm long. *Z. marina* uses a large proportion of its resources for maintenance of roots and rhizomes. Both vegetative propagation (rhizome elongation) and sexual propagation (seeds) maintain beds and allow colonization of new areas[1].

An inhabitant of sandy and muddy bottoms in tidal and subtidal bays and estuaries, eelgrass is an indicator of a healthy coastal-marine environment[2]. This primary producer is a direct food source for Canada geese, sea brants and black ducks, a shelter and nursery ground for a number of juvenile marine organisms: the soft shell clam, *Mya arenaria*, the Atlantic bay scallop, *Argyropecten irradians*, and the blue mussels, *Mytilus edulis*, and serves as a substrate for epiphytic attachment of many organisms such as algae, *Enteromorpha*, trumpet-stalked jellyfish, *Haliclystus* and colonial sea squirts. In addition the matted rhizomes trap nutrient–rich silt, improve water clarity, stabilize sediment and protect coastlines from erosion. Eelgrass also helps to attenuate wave action, and may also improve water quality through toxicant absorption from interstitial water and surface sediments[2,3,4].

In the early 20th century Z. *marina* had a wide distribution in North Atlantic temperate waters. It grew so dense, that it became a nuisance to motorboats, clogging propellers. In the 1930's North Atlantic coastal *Z. marina* suffered a devastating decline in the U.S. and Europe, resulting in losses of approximately 90%[3]. This decline was presumably due to a number of factors: a fungal wasting disease caused by

Labyrinthula, eutrophication, and physical environmental disturbances including channeling, storm erosion and human developmental factors[3]. Recovery has occurred in many places, but eelgrass is still absent in local areas, including the western portion of the Great South Bay and Jamaica Bay (JB) which is adjacent to the Kingsborough Community College campus. Eelgrass beds are abundant elsewhere on the Great South Bay and Peconic Bay[5].

Eelgrass communities are second only to coral reefs in richness and productivity. Their restoration is one way to improve damaged coastal ecosystems caused by oil spills and dredging, for example. Improved water quality, sediment stabilization, and sheltered habitat for juvenile fish and invertebrates are among the many benefits to be gained both locally and globally through restoration efforts. In May 1994, the National Marine Fisheries Service recommended the pursuit of programs concerned with the re-establishment of eelgrass beds in areas where it is now absent, but previously had flourished. New York Sea Grant as well as other local agencies have encouraged coastal restoration efforts[3,6,7,8,9,10,11].

Methods and Materials

With initially identified and characterized two potential transplantation sites in JB for selected field and laboratory water parameters, and compared them with two Long Island (LI) sites where eelgrass is growing. We then identified a third JB field site, Dead Horse Bay. This site's water field parameters were comparable to those measured at the other JB sites and became part of our study. The concentration of selected heavy metals in sediments and plants growing were sampled and compared at all three JB sites and the two LI sites. Next, the field conditions of the best JB site, Dead Horse Bay, were mimicked in the laboratory with transplanted LI eelgrass, creating a microcosm where eelgrass growth and survival ability prior to actual field transplantation were studied.

Two potential transplantation sites were initially identified in JB: Black Wall Marsh (BWM) and Ruffle Bar (RB). Eelgrass is absent at these JB sites, but *Spartina* species grow abundantly. Two sites were indentified on the LI coast where eelgrass as well as *Spartina* grow abundantly and could be utilized as a source for transplantation: Goose Creek in Wantagh (W), and Smith Point Park in Suffolk County (SP). In *Study 1,* these four sites were sampled for selected water parameters. Another was later identified as a JB potential transplantation site, Dead Horse Bay (DHB), bringing the total number of sampling sites to five. As a continuation of study one, all five sites were sampled and tested for the concentration of selected heavy metals in their sediments. In *Study 2,* plants from all five sites were sampled for selected heavy metal content. Only *Spartina* was sampled from the three JB sites because eelgrass does not grow there and both eelgrass *and* Spartina were sampled from the two LI sites. In *Study 3,* water and sediment from the best JB site (DHB) were transported to laboratory tanks, eelgrass from Smith Point was transplanted into these tanks creating a microcosm, and the plants were monitored for growth and vigor. All data were compiled and analyzed through the SPSS statistical program package for descriptives, analysis of variance, and multiple comparisons. Standard sampling techniques and materials were utilized[12], including teflon storage containers for water and sediments analyzed for heavy metals and cores stored frozen and sealed prior to processing.

***Study 1a*: On-site Water Parameters**

Dissolved oxygen, pH, conductivity, salinity, temperature, and turbidity, were measured in the field at two sites in JB (BWM and RB) and at the two LI sites with a Davis Instruments Water Quality Checker with 10 meter cable, Model U-10 (362153-10). Water current speed and direction were measured with the Thomas Scientific Flow Probe #984-WO3. Water depth was determined with the Thomas Scientific Water Level Indicator Model WLZ, or meter stick in shallow areas. Light intensity was measured using an IL 1700 Research Radiometer light intensity meter (International Light, Inc). All measurements reported represent an average of 5-9 independent values measured over a period of 2 years.

***Study 1b:* Laboratory-measured Water Parameters**

Water samples from the same four sites were collected in Wheaton glass bottles and/or teflon bottles, packed in ice, transported to the laboratory, stored at 40° C and analyzed for ammonia nitrogen, chromium (hexavalent), copper, iron, nitrate-N, nitrite-N, phosphate (PO4) and silica, either immediately or within 24 hours of collection using a LaMotte Colorimeter Outfit Model DCL-05 (EPA-Accepted).13 All measurements reported represent an average of 5-9 independent laboratory values measured over a period of 2 years.

***Study 1c:* Laboratory-measured Sediment Heavy Metals**

Sediment samples from all five sites were collected with a Wildco Model 2404-A14-core sampler modified with a #10 rubber stopper replacing the standard plastic "seat" to hold in the cores during pullout. The Lexan cores were packed in ice, transported to the laboratory, and frozen at -6° C. Most frozen-solid cores were utilized for analysis within 2

weeks of collection and were cut using a circular saw into sections representing the following depths in the core: "top": 0-7.62 cm (0-3"), "middle": 7.62-20.32 cm (3-8"), and "bottom": 20.32-35.56 cm (8-14"). Each core section was dried in a 59° C oven for 96 hours, ground for 1-2 minutes to a powder with a Cole-Parmer analytical grinding mill and stored for future analysis at -6° C in screw-top Teflon containers wrapped with Para-film. Two independent 500 milligram aliquots of the powdered core samples from each section of the cores from all sites were digested in a Hach Digesdhal apparatus utilizing trace-metal grade concentrated sulfuric acid followed by electronic clean-room grade 30% hydrogen peroxide[14]. Filtered and diluted to constant 100 ml volume, digestates were stored in teflon containers at 40° C prior to atomic absorption spectrometer analyses. Expression of concentration of heavy metals in sediments and plants follows standard reporting methods[15]. Analyses for chromium, copper, zinc, iron, lead and nickel were carried out with a Perkin-Elmer 3300 with HGA-600 atomic absorption spectrometer. All atomic absorption data represent the average of 6 sample runs for each of the two independent 500 mg aliquots.

Study 2: Laboratory-measured Plant Heavy Metals

All plants collected from the three JB and two LI collection sites were packed on ice, transported to the laboratory, sink-washed of heavy mud and shell material, subdivided with scissors into "root and rhizome" and "leaf and stem" sections, washed further in double-distilled, Millipore-deionized water, dried in a 59° C drying oven for 96 hours, ground for 1-2 minutes to a powder with a Cole-Parmer analytical grinding mill, and stored for future analysis at -6° C in screw-top teflon containers, wrapped with para-film. Two independent 500 milligram aliquots of the subdivided, powdered plant samples from all sites were digested in a Hach Digesdhal apparatus utilizing trace-metal grade concentrated sulfuric acid followed by electronic clean-room grade 30% hydrogen peroxide[14]. Filtered and diluted to constant 100 ml volume,digestates were stored in teflon containers at 40° C prior to atomic absorption spectrometer analyses. Analyses for chromium, copper, zinc, iron, lead and nickel were carried out with a Perkin-Elmer 3300 (with HGA-600) atomic absorption spectrometer. All atomic absorption data represent the average of 6 sample runs for each of the two independent 500 mg aliquots.

Study 3: The Laboratory Microcosm

Three identical sets of aquarium microenvironments were established to carry out the eelgrass transplantation studies. Sediment and water were collected from SP, LI (40°44'N, 72°51'W), and DHB, JB, NY (40°38'N, 73°51'W). The DHB site from JB was chosen as the most promising site for successful transplantation, which was supported by assessment of sediment and plant parameters in studies 1c and 2. Although water parameters were not measured in DHB during the same time frame as for the other JB sites, field water values were within the same range as for the other JB sites. Three identical arrays of four 10-gallon aquarium tanks were set up in fiberglass tubs in the laboratory, in which *Z. marina* plants were cultivated under conditions simulating field parameters of sediment, water, light and temperature. Metal halide lights with 250 Watt bulbs on a timer were used to provide adequate light. An external chilled tub using an Aqua Logic Water Chiller in series with a ½ hp 115V 3750 rpm Dolphin Aqua Sea 4500 pump, functioned as a heat sink to maintain individual tank temperature. Aquaria were maintained under 12-hour light /12-hour dark cycles, at 15-16° C and were monitored for temperature stability and stable light intensity (38-110 uE/m2/sec).

For observations of extensive leaf, shoot and biomass development of eelgrass in the field, plants, sediment and water were collected from SP, LI, NY, and water and sediment from the DHB region of JB, NY. Eelgrass measurements taken prior to aquarium transplantation included rhizome length, number of nodes, wet weight, number of mature leaves, number of shoots, and leaf length. Then the SP eelgrass plants were placed in the aquarium tanks containing combinations of sediment and water from JB, a site where eelgrass is absent, and SP, the LI site where eelgrass grows well.

Aquaria were set up with the following water/ sediment combinations: JB water with JB sediment (JB/ JB); SP water with JB sediment (SP/JB); JB water with SP sediment (JB/SP); SP water with SP sediment (SP/ SP). This cross-matched control design enabled a determination of the effect on eelgrass growth of water, sediment, or a combination of both.

Three single rhizomes were given a unique identifier, and then planted in the sediment leaving a small portion of the top exposed, mimicking its natural position. Aquarium sediment depth was 6.5 centimeters. Air was pumped into each aquarium through a ¼" hose connected to a Sweetwater Regenerative Blower. This pumped air oxygenated the water and prevented stagnant conditions by causing water movement. Each month selected environmental parameters were measured. Water measurements included pH, conductivity, turbidity, dissolved oxygen, temperature and salinity. Light measurements were taken at the source, above the aquarium glass, underneath the plexiglass, and at the level of the sediment. Water in tubs and aquaria was replenished as necessary to maintain tank volume. At the conclusion of the experiment plants were carefully

removed from their tanks and leaf sheaths, rhizome and biomass were re-measured after sediment was rinsed off with distilled water and the plants blotted with paper towels. Vigor of eelgrass specimens was ascertained by morphological measurement of leaf sheaths, comparison of the number of nodes on the rhizome with the number prior to transplantation, and biomass comparisons between the beginning and the conclusion of the experimental period. Morphological measurements included recording rhizome length, number of nodes, wet weight, number of mature leaves, number of shoots, and leaf length. Plants and sediments from the laboratory tanks were then analyzed as in studies 1 and 2 for heavy metal content.

RESULTS

Study 1a: On-site Water Parameters

On-site, field measurements of water pH, dissolved oxygen, conductivity, temperature, turbidity and salinity, measured periodically at JB and LI sites over a 2-year period, are presented in Table 1. Table 1 reveals statistically significant differences in turbidity, conductivity, and salinity between the sites. The most significant differences in turbidity were observed at the SP site compared with the others (Figure 1). The SP site also has the most dense eelgrass population and a bayside geographical location, indirect tidal flushing compared with a direct tidal flushing, oceanic location, of the other sites. Eelgrass also grows at Goose Creek, Wantagh (W), but not as densely as at SP and not with as great an increase in water turbidity. This is consistent with the W site's geographically direct access to tidal flushing. The JB sites are devoid of eelgrass and also have the least water turbidity. Figure 2 show that the two JB sites have essentially the same values for salinity, but they were significantly different from the LI sites. In addition, the LI sites were highly significantly different from each other. The W site's salinity was closest to that of open oceanic water and is consistent with its geographical location. The field values for pH, DO, and salinity match the range of values for these parameters in the NY Bight[16].

Study 1b: Laboratory-measured Water Parameters

The average values for laboratory measurements of ammonia nitrogen, copper, iron, nitrate-N, nitrite-N, phosphate (PO_4) and silica, measured periodically at JB and LI sites over a 2-year period, are presented in Table 2. Table 2 reveals no statistically significant differences in these parameters across all sites except for silica. As with the on-site measurements in Study 1a, the general similarity in most parameters compared across all sites is attributed to the twice daily tidal flushing of all these sites with the same Atlantic waters. The greater silica concentration at SP (Figure 3) may relate to its finer silt sediment quality combined with the frequency of recreational boating dispersing the silt into the water, the density of the eelgrass population with its richer arrays of symbiotic microorganisms and also, as with the case of turbidity, a bayside geographical location with indirect tidal flushing compared with the more direct oceanic locations with direct tidal flushing of the other sites. The laboratory measured values for Cu, Fe, and nitrate match the range of values for these parameters in the NY Bight[16].

Study 1c: Sediment Heavy Metals

The mean values for chromium, copper, zinc, iron, lead and nickel in sediments from each JB and LI site, are presented in figure 4 and 5. Sediment cores were collected from each site and analyzed for heavy metal content at three levels: TOP 0-7.62 cm, MID 7.62-20.32 and BOT 20.32-35.56 cm, but presented for the top and middle core regions only because eelgrass root systems generally grow only in these two upper layers. Iron levels are presented separately in Figure 5 due to its order of magnitude greater concentration in the sediments. Significant differences in concentration are observed when comparing metals between core levels at any one site, and when comparing metals at different sites.

Table 3 shows the comparison of the heavy metal concentration differences between the top and middle levels of the cores at each individual site. DHB is revealed to be the most homogeneous site with only Zn being significantly different between the top and middle core levels (p=.016). The other two JB sites, BWM and RB, are both homogeneous in lead distribution ($p = 0.677$ and 0.8, respectively), but display significant differences in other metal concentrations comparing top and middle core levels ($p < 0.05$). It is clear that BWM and RB differ from each other in which heavy metals are partitioned significantly between upper and middle core levels. The two LI sites, W and SP, were homogeneous in Pb and Cu concentrations ($p = 0.89$ and 0.875 for Pb and 0.829 and 0.356 for Cu) and both were significantly different in Fe concentration. They displayed differences in other metals between top and middle core regions. None of the five sites were perfect matches for each other in metal distribution between the top and middle levels of the cores.

The p values reveal highly significant differences between metals at all sites in all regions of the cores. The groupings of sites are based on statistical multiple comparisons between all sites by region in the cores: e.g., for chromium in core tops (0-3”), W, SP and DHB are alike in Cr concentration (p varying from 0.075 to 1.00 between these three sites) and are significantly different from BWM and RB, these latter two sites being alike in Cr concentration ($p = 0.145$).

Grouping the sites on the basis of similarities and significant differences in concentrations for all the other metals tested, with the top and middle levels of the cores considered separately shows that SP and DHB frequently group together in terms of heavy metal concentrations. BWM and RB are similar in metal concentrations even more frequently than SP and DHB, but they are both from JB and neither area grows eelgrass. It is remarkable that DHB, part of JB, and SP, on the LI coast, display such similarities in sediment metal concentrations.

Study 1c suggests DHB in JB, to be the location most like the SP, LI site with respect to sediment heavy metal concentrations and therefore the best potential JB transplantation site of the ones we studied.

***Study 2*: Plant Heavy Metals**

Figures 6 and 7 present the mean values for the chromium, copper, zinc, iron, lead and nickel content of leaf-stem (LS) and root-rhizome (RR) subdivisions of *Z. marina* from the LI sites, and *Spartina* from JB and LI sites. Iron is presented in Figure 7 due to its order of magnitude higher concentration than the other metals. Analysis of these data show following general observations: iron, chromium, lead and nickel occur in 1-3 orders of magnitude higher concentration than copper and zinc within the tissues of both *Zostera* and *Spartina*. Also, highly significant concentration differences are found for all metals except lead when LS with RR subdivisions for both *Zostera* and *Spartina* are compared. Specifically, the RR subdivision of most *Spartina* and all *Zostera* has higher concentrations of all metals assayed than the LS subdivision at all three JB sites and at one LI site, SP. The other LI site, W, followed this pattern for *Zostera,* but not for *Spartina*, which displayed a generally homogeneous distribution of metals. Three other exceptions were found: zinc at BWM is higher in the *Spartina* LS subdivision ($p = 0.009$), lead at SP is higher in the *Zostera* LS subdivision ($p = 0.007$), and lead is evenly distributed throughout *Spartina* at all sites.

Spartina roots and rhizomes were similar at all sites in their concentrations of chromium, iron, lead, copper, and zinc, except for significant differences in Pb and Zn at W, and Cu at RB. *Spartina* leaves and stems were similar at all sites in their concentrations of copper, iron, and chromium, except for significant differences in Fe at W and SP, and Cr at W. All *Zostera* tissues were similar at both LI sites in their concentration of iron, and significantly different regarding chromium and zinc. Only the *Zostera* LS subdivision was similar at both LI sites in its concentration of copper and nickel. Only the *Zostera* RR subdivision was similar at both LI sites in its concentration of lead.

Table 3 presents ratios of leaf-stem/root-rhizome heavy metal concentration values. Thirty-two of the ratios are seen to be below 1, with only 7 at or above 1. Ratios below 1 indicate greater accumulation of heavy metals in roots and rhizomes. Ratios above 1 indicate greater accumulations of heavy metals in leaves and stems. These data support the conclusion that there was a generally greater accumulation of heavy metals in the roots and rhizomes of both *Spartina* and *Zostera*, compared with their leaves and stems. Table 4 presents statistical multiple comparisons of the *Spartina* data, by field site, and reveals that SP is more like the JB sites than W, and that SP is most like BWM when comparing *Spartina* heavy metal concentrations. Study 2 suggests BWM in JB to be the location most like the SP, LI site with regard to plant heavy metal concentration patterns, and therefore, the best potential JB transplantation site. However, DHB was a close second in this regard, and DHB was also the most suitable site on the basis of Study One. Several other factors were considered in the decision to consider DHB the overall best potential transplantation site and to utilize its water and sediment in Study Three. These include easy land access, physical protection of the site by a natural sand bar, and recommendation of the National Park Service. One other factor is sediment physical quality: BWM sediment is fine and loose; overall it is extremely soupy; it would be very difficult to anchor transplanted *Zostera* in such sediment. By comparison, DHB sediment is very similar to SP sediment: it is granular and compacted. Two other factors are important: BWM is only accessible by boat and has a large mosquito population. DHB is easily accessible from land and has a lower population of biting insects. On these bases, DHB was chosen as the overall best potential JB transplant site of those we assayed and its water and sediment were utilized in Study Three.

***Study 3*: The Laboratory Microcosm**

Table 5 demonstrates comparable survival of transplants in all experimental aquaria environments. The average rhizome length, number of nodes and weight all increased for the eelgrass plants in each of the four water/sediment conditions. The greatest increase in rhizome length occurred under SP/SP simulated (control) conditions (from 9.7 to 36.6 cm average). The SP/JB, JB/SP and SP/SP environments all produced comparable increases with respect to rhizome length. The greatest increase in the number of nodes was again seen in the SP/SP microcosm (from an average of 6.7 to 31.3 nodes), with the other three simulated environments showing comparable increases. Although all four environments demonstrated an increase in average plant weight, the greatest increases were seen in SP/SP and JB/SP plants (from 5.1 to 10.9 gm average, and from 5.2 to

10.8 gm average, respectively). Eelgrass growing in tanks with JB sediment (JB/JB and SP/JB) did not grow as well.

Results for the number of mature leaves, number of shoots and leaf length varied among the plants. In the JB/JB microcosm the number of mature leaves increased from 2.1 to 3.0. In the JB/SP environment there was no change (2.0, average), and in the SP/JB and SP/SP tanks these values decreased from 1.9 to 1.3 and from 2.1 to 1.0, respectively. The average number of shoots decreased in all but the SP/JB environments, where an increase was observed from 2.7 to 2.9. Average leaf length varied, with JB/JB showing an increase from 53.0 to 60.8 cm, and SP/JB, JB/SP and SP/SP all showing decreases (Table 5). Despite the variation in number of mature leaves, number of shoots and leaf length, overall plant weight increased, perhaps due to the increases in rhizome length and node number. The varied results seen in the number of mature leaves, number of shoots and leaf length may partially be attributed to natural growth patterns in eelgrass. For example, in the SP/SP simulated environment the number of mature leaves, number of shoots and leaf length all decreased, whereas the weights increased. This increase corresponds to an increase in rhizome length and node number.

Most of the environmental parameters remained relatively constant over the experimental period. The pH ranged from 7.94 to 8.37 across aquaria and time. Likewise, aquaria water temperature varied less than 1°C, ranging from 15.6°C – 16.5°C, as did salinity, which ranged from 2.53% to 3.49%, and conductivity, which ranged from 40.2 to 53.1 mS/cm. Dissolved oxygen decreased from a range of 7.5-9.7 mg/l to 4.87-6.25 mg/l. This may have been due to lack of tidal changes and natural currents despite the artificially supplied airflow. Turbidity increased in some of the aquaria from 4 to as high as 38 units in the SP/SP aquaria, reminiscent of field conditions (Figure 1) and decreased in others, e.g., from 6-7 to 3-4 units in two of the three JB/JB aquaria. Despite these variations in turbidity, the plants grew in all aquaria. Upon completion of the plant comparative growth experiments the plants were allowed to continue to grow for another year before both plants and sediments were analyzed for heavy metal content.

DISCUSSION

It is not surprising that eelgrass and other coastal seagrass meadows have diminished worldwide during the past century. Coastal industrial and recreational developmental projects, with attendant physical habitat disruption[17,18,19,20] and coastal chemical water pollution from many sources[21,22,23,24,25,26] including eutrophication[27,28,29] have reduced eelgrass acreage. In addition, changing local microenvironmental conditions have contributed to eelgrass' increased susceptibility to parasitic infection[30]. Since the 1600's, JB's history has included agriculture, crops and livestock, fishing (both sports and commercial), industrial development, sewage treatment, shipping, excursion boats, and cargo carriers. Significant topographical changes have occurred within JB in the twentieth century: many channels were filled, others were dredged, and marshes and meadows within the bay were eliminated. Dredging destroys eelgrass by dislodging its root system and destroying the substrate in which it roots[31]. Over-fishing and nutrient pollution have been suggested as causes of coastal ecosystem degradation beginning in the 18th century[32,33,34]. Currently, JB is still subject to periodic dredging and influx of sewage from regions of southern Brooklyn, although much of it is treated.

Transplantation of eelgrass to JB is important even given these circumstances. It is known that *Z. marina* and other salt marsh plants, e.g., *Spartina,* readily absorb heavy metals and that the metals do not appear to interfere with plant growth and viability at typically measured estuarine levels[35,36]. Our results are consistent with these observations.

Table 6 compares "normal" concentrations of metals in sediments from the NY Bight unaffected by dumping, with metal concentrations at NY Bight dumping sites containing dumped dredge spoils, construction rubble, sewage sludge, and chemical discharges from local industry and sediment and plant metal concentrations from this study[15]. We observe that the average metal concentrations in sediments from JB and LI coastal sites are within the range, or exceed the upper limit of the range, of NY Bight dump-site levels for Cr, Cu, Zn, Pb, and Ni, with one exception, LI sediment Cu levels were in range for clean areas of the NY Bight. We also observe that growing and thriving *Spartina* and *Zostera* accumulate, and in some cases concentrate above the sediment level, all metals to varying extents, particularly in their roots and rhizomes (Tables 3, 8, Figures 6, 7). Microcosm studies were consistent with these results for lead (Table 7), and variable for the other heavy metals. The implication is clear that eelgrass and other salt marsh plants may help to clean-up bays and estuaries through absorption and accumulation of the heavy metals both from sediments in which they are growing and in water through leaf absorption in which they are immersed. Even though it is also clear that these absorbed heavy metals may pass through to the food chain as the plants are utilized by other organisms as food sources[37], the higher concentrations of the metals appear to be generally found in the roots and rhizomes which remain buried, and generally out of the food chain, even as the leaves age and break away to float out to sea or onto the shoreline.

Eelgrass re-growth in JB could potentially aid in reestablishing the overall health of this challenged ecosystem for the reasons just discussed and for the many reasons given in the introduction. The local fishing and recreation industries could benefit from the improvement in the bay's water quality and subsequent increase in the quantity and diversity of bay fish and other estuarine organisms. In the past twenty years water quality improvements in JB have occurred (Tanacredi, personal communications), but eelgrass has not returned on its own. Recovery, mostly vegetative of submerged aquatic vegetation has been recorded in other areas including Chesapeake Bay, MD, North Carolina, British Columbia, and the Florida Keys[31,38,39]. Our initial results have encouraging implications for field restoration projects in JB and proximal areas affected by such human perturbations as waste dumping, dredging and oil spills, as well as such natural disturbances as storms and hurricanes. With our continued attempts to cultivate eelgrass in our laboratory microcosms, such plants could have advantages over harvested and re-planted specimens including reduced damage to donor beds, stock availability, stock suitability for the transplantation environment based on breeding of disease and stress-resistance. Genetic variations are often found in disturbed vs. undisturbed habitats[40,41] and potential cost reduction, *e.g.*: transport[31].

We believe that DHB in JB may provide the proper conditions and the sheltered environment for transplanted eelgrass to grow. Our microcosm studies indicate that such eelgrass growth, including production of seeds, is possible in DHB conditions. The importance of vegetative propagation of transplanted mature *Zostera* will be especially important in JB due to high sediment concentrations of heavy metals, which may not inhibit growth of adult plants, but which may inhibit seedling germination[42]. *Z. marina*'s normal growth shows seasonal variation[43] and vegetative propagation is believed to be dominant over growth through seed dispersal in subtidal areas[44,45,46]. Environmental conditions of nutrient availability in water and sediment, water and air temperature, adequate light and calm water are important factors in determining eelgrass growth in a particular location and in maintaining its resistance to disease. Environmental stresses leave eelgrass open to invasion by parasites[45,46]. Spread of eelgrass also depends on sediment suitability, water depth and protection from rough seas. It grows best in sand or mud in shallow subtidal sheltered coastal waters. Sediment type is based on grain size[45,47,48,49]. Z*. marina* grows optimally at a depth of 182.9 cm. Its root zone is found up to 20 cm down from the surface into the sediment. Oxygen, carbon, nitrogen and phosphorus are essential for eelgrass to absorb from seawater for growth, and these substances vary seasonally. Small leaf size, as observed in *Z. marina* in winter, is a consequence of limited availability of nutrients. Eelgrass leaf growth was found to increase in response to sediment inorganic nitrogen levels[50] with ammonium appearing to be a more important nitrogen source than nitrate[45,50,51]. Temperature is the dominant physical growth factor and *Zostera marina* has a wide temperature tolerance. It is dormant below 10°C, vegetative between 10°C and 15°C, and flowers at about 15°C. The amount of seed set also depends on water temperature. On LI vegetative growth is optimal March-May, and sexual reproduction occurs in May-June[2,6,45,51]. Decreased light availability[27,28,29] and increased nutrient levels, in particular sediment sulfide[38], have been associated with the decline of eelgrass. Turbid water limits illumination for photosynthesis that supports eelgrass rhizomes and roots[52]. The maximum depth to which *Z. marina* can grow depends on water transparency. Microorganisms may also influence sediment and water properties including turbidity and levels of nitrogen, carbon, phosphorus, sulfur and oxygen[3,8,53,54].

Over the course of this research, all eelgrass specimens survived and propagated in the laboratory microcosm. Laboratory cultivated specimens mimicked field specimens with respect to life cycle changes in the first months after transplantation to microcosms. New growth and dormancy-associated changes were parallel. Seasonal changes in light cycles and water temperature were absent in our study; future studies should include this parameter, as the growth we observed during the first year paralleling the field conditions was not seen during the second. Increased water temperature and light availability are associated with flower formation. In all aquaria, containing varied sediment and water combinations, eelgrass rhizome length, node number and weights increased. We obtained variable results in number of mature leaves, number of shoots and leaf length although these variations may be normal for eelgrass since they were displayed by our controls (SP/SP) as well as the plants under experimental conditions. These results suggest that neither sediment nor water composition was a significant short-term negative influence on eelgrass growth over the experimental period even as the plants apparently accumulated heavy metals. The microcosms did not provide natural sediment flushing conditions, temperature changes, or natural light cycles. These limitations must have contributed to, if not caused, the long term changes we observed. Ambient conditions in the laboratory were not altered during this experiment. Our experimental results suggest that over the short term, eelgrass can grow well under laboratory simulated DHB field conditions.

Eelgrass communities are species-rich and productive, and include numerous crustaceans, small fish, mollusks, worms and epiphytic algae. Associated microbes and protozoans feed on dead and decaying

leaves. Ecosystems disturbed by human activity are more vulnerable to damage[55,56]. Plant ecosystem diversity increases productivity, which helps to sustain or restore its functioning[57,58]. Whether a diverse ecosystem is helpful in fending off invader species has been debated without conclusion[59]. It has been found that an increased number of species and communities in marine ecosystems have concomitant productivity increases[58]. Charles Darwin identified increased productivity in species-rich terrestrial communities, an observation supported by others in subsequent studies[60]. Habitat restoration efforts are ongoing in the Hudson Estuary, NY/NJ Harbor Estuary, the Great South Bay (LI, NY) and Peconic Bay (LI, NY). Marsh edge erosion on the South Shore of LI has resulted in a depletion of natural filtration. The South Shore Estuary Council, working with the Army Corps of Engineers and the New York State Department of Conservation, is addressing this issue. Moratoriums on waterfront development projects have been imposed in Southold, NY, which is proximal to the Peconic Estuary[61,62], described a role for deadwood in estuaries where it's use as a substrate by fungi, algae and invertebrates could attract bivalves, promoting establishment of a secondary community. Eelgrass habitat restoration would also benefit horseshoe crabs, *Limulus polyphemus*, which may also occupy a significant place in the ecological chain. Migratory birds consume horseshoe crab eggs, and their observed decline coincides with the dwindling numbers of these birds recently recorded[63]. A role for eelgrass either as, or in association with, a keystone species, appears probable in this type of marine ecosystem. Our transplantation and restoration success should also help to preserve and protect remaining communities. Our experimental plants have demonstrated the ability to survive and grow in DHB water and sediment for up to two years; the next phase of experimentation will include field studies. We will next transplant laboratory specimens which demonstrated adaptation to the JB environment to experimental plots and regularly monitor growth, density, short and long-term survival and colonization. Additional plant studies are planned including propagation studies, seed projects and macrophyte and microorganism-association studies. These can provide useful additional information for improving transplantation and remediation success.

Table 1: Field-Collected Water Quality Parameters (mean ± sem)

	Black Wall Marsh (n = 9)	Ruffle Bar (n = 9)	Wantagh (n = 7)	Smith Point (n = 5)	F*	p*
pH	8.24 ± 0.21	8.34 ± 0.19	8.15 ± 0.05	8.22 ± 0.08	0.206	0.892
DO (mg/ml)	7.76 ± 0.61	9.12 ± 0.68	8.63 ± 0.54	7.81 ± 1.06	0.935	0.439
Conductivity (ms/cm)	43.04 ± 0.56	43.07 ± 0.60	47.44 ± 0.61	39.36 ± 1.96	12.192	0.0001
Temp °C	20.24 ± 1.23	20.48 ± 1.29	22.47 ± 1.93	23.66 ± 3.06	0.806	0.502
Turbidity (uE/m2/sec	6.5 ± 1.32	9.3 ± 2.14	11.0 ± 3.08	22.1 ± 5.20	5.12	0.006
Salinity (%)	2.79 ± 0.04	2.78 ± 0.04	3.08 ± 0.04	2.51 ± 0.14	11.955	0.0001

*These values indicate comparisons of means across the four sites.

Table 2: Laboratory-Determined Water Quality Parameters (ppm, mean ± sem)

	Black Wall Marsh (n = 9)	Ruffle Bar (n = 9)	Wantagh (n = 9)	Smith Point (n = 5)	F*	p*
NH3-N	0.076 ± 0.040	0.050 ± 0.030	0	0	1.545	0.226
Cu	0.006 ± 0.003	0.001 ± 0.001	0.004 ± 0.003	0.009 ± 0.008	0.894	0.457
Fe	0.050 ± 0.013	0.048 ± 0.009	0.076 ± 0.032	0.050 ± 0.015	0.521	0.672
NO3N	0.006 ± 0.006	0	0.044 ± 0.030	0.016 ± 0.016	1.648	0.203
NO2-N	0.100 ± 0.017	0.078 ± 0.015	0.013 ± 0.003	0.163 ± 0.157	1.121	0.359
PO4	0.207 ± 0.042	0.232 ± 0.036	0.349 ± 0.240	0.006 ± 0.006	1.132	0.354
Silica	0.660 ± 0.203	0.598 ± 0.148	0.600 ± 0.124	2.254 ± 0.438	10.67	0.0001

*These values represent comparisons of means across the four sites.

Table 3: Plant Heavy Metal LS/RR* Ratios							
	W-S**	SP-S	DHB-S	BWM-S	RB-S	W-Z	SP-Z
Cr RATIO	0.85	0.05	0.22	0.04	0.18	0.20	0.74
Cu RATIO	1.00	0.30	0.09	0.14	0.09	-	1.00
Zn RATIO	3.60	0.53	0.06	1.30	0.04	7.70	0.74
Fe RATIO	0.65	0.04	0.43	0.18	0.20	0.63	0.37
Pb RATIO	1.33	-	0.87	0.91	0.75	0.77	1.09
Ni RATIO	0.81	0.23	0.24	0.42	0.31	-	0.58

A ratio >1 indicates higher concentration in leaves and stems.
A ratio <1 indicates higher concentration in roots and rhizomes.
Of 39 ratios, 32 are <1, and 7 are =1.

*LS/RR = Leaf-Stem/Root-Rhizome
**Abbreviations: W-S = Wantagh-Spartina, SP-S = Smith Point Spartina, DHB-S = Dead Horse Bay Spartina, BWM-S = Black Wall Marsh Spartina, RB-S = Ruffle Bar Spartina, W-Z = Wantagh Zostera, SP-Z = Smith Point Zostera

Table 4: Multiple Comparisons (p values*) of Field Sites for Similarities in Spartina Heavy Metal Concentrations

Sites Compared	Plant Region	Cr	Cu	Zn	Fe	Pb	# Metals Similar	Total # Similarities
DHB and SP	LS	0.616*	0.176*	0.0001	0.453*	-	3	DHB & SP = 7
	RR	0.96*	0.993*	0.003	0.702*	0.52*	4	
BWM and SP	LS	1.00*	0.931*	0.999*	0.822*	-	4	BWM & SP = 8
	RR	0.079*	0.538*	0.001	1.00*	1.00*	4	
RB and SP	LS	0.992*	0.931*	0.002	0.736*	-	3	RB & SP = 6
	RR	0.214*	0.009	0.272*	1.00*	0.039	3	
DHB and W	LS	0.012	1.00*	0.602*	0.873*	-	3	DHB & W = 6
	RR	0.633*	0.06*	0.0001	1.00*	0.0001	3	
BWM and W	LS	0.0001	0.931*	0.034	0.524*	-	2	BWM & W = 5
	RR	0.998*	0.003	0.088*	0.643*	0.002	3	
RB and W	LS	0.001	0.931*	0.893*	0.626*	-	3	RB & W = 5
	RR	1.00*	0.0001	0.0001	0.75*	0.0001	2	

Smith Point is more similar to Jamaica Bay than Wantagh, and Black Wall Marsh is most similar to Smith Point in heavy metal concentrations.
*p > 0.05 and indicate similarity in metal concentration between the sites and plant region being compared.

Table 5: Eelgrass Growth Results in the Laboratory Microcosm under Cross-Matched Water/Sediment Conditions (n = 9, mean ± sem)								
	JB/JB #1*	**JB/JB #2**	**JB/SP #1**	**JB/SP #2**	**SP/JB #1**	**SP/JB #2**	**SP/SP #1**	**SP/SP #2**
Rhizome Length (cm)	9.2 ±-0.90	16.3 ± 2.70	7.1 ± 0.93	14.9 ± 2.17	10.3 ± 1.40	19.0 ± 1.8	9.7 ± 1.33	36.6 ± 8.73
Number of Nodes	6.8 ±-0.43	15.0 ±1.80	6.3 ± 0.87	15.1 ± 1.37	7.7 ± 0.77	19.9 ± 1.2	6.7 ± 0.30	31.1 ± 7.47
Weight (gm)	4.8 ± 0.47	7.3 ± 0.87	5.2 ±1.13	10.8 ± 1.70	6.3 ± 0.67	7.7 ± 0.77	5.1 ± 1.20	10.9 ± 3.13
Number Mature Leaves	2.1 ± 0.07	3.0 ±-0.47	2.0 ± 0.17	2.0 ± 0.23	1.9 ± 0.10	1.3 ± 0.17	2.1 ± 0.07	1.0 ± 0.3
Number of Shoots	3.2 ±-0.27	2.7 ±0.37	2.6 ± 0.17	2.2 ± 0.20	2.7 ± 0.27	2.9 ± 0.30	2.9 ± 0.3	2.3 ± 0.77
Leaf Length (cm)	53.0 ±-3.80	60.8 ± 7.50	44.2 ± 4.80	42.9 ± 6.30	44.3 ± 3.43	29.7 ± 2.4	55.1 ± 5.67	31.9 ± 11.3

#1 and #2 in this column refer to trials. Each experimental condition was run twice.

*JB/JB = Jamaica Bay water and JB sediment; JB/SP = Jamaica Bay water and Smith Point sediment in the tanks; etc.

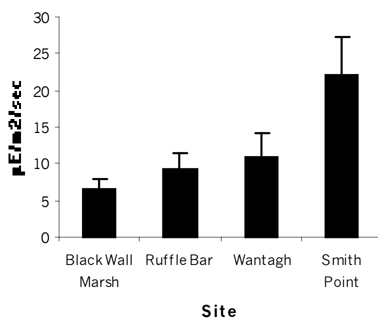
Table 6: Comparison of Metal Concentrations in Sediments and Plants (ppm)							
	Clean Sediments of NY Bight*	**Dumping Areas of NY Bight***	**JB Sediment**	**JB *Spartina* (LS/RR)**	**LI Sediment**	**LI *Spartina***	**LI Zostera**
Cr	6	2 -310	383.9	432.5	644.5	611.2	238.8
Cu	9	8 - 390	34.9	27.7	5.1	11.5	42.0
Zn	19	26 - 1500	157.2	87.3	28.0	83.3	58.4
Pb	13	25 -370	50.3	406.0	142.1	137.7	316.0
Ni	5.5	3 - 37	201.1	472.0	259.0	370.9	131.1

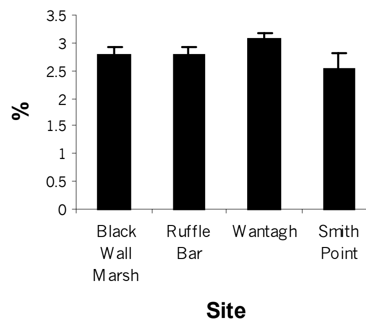
*Carmody, 1973

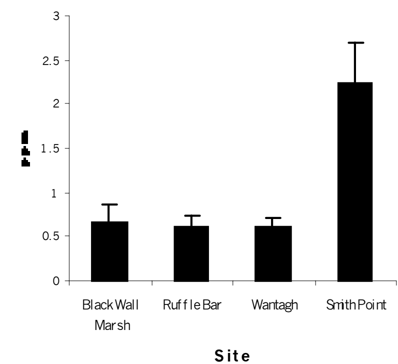
Table 7: Lead Concentrations in Sediments and *Zostera* Growing in Microcosm Tanks (ppm, mean ± sem)								
	SP/SPSed	**SP/SPZos**	**SP/JBSed**	**SP/JBZos**	**JB/SPSed**	**JB/SPZos**	**JB/JBSed**	**JB/JBZos**
Pb	390.0 ± 13.8	445.1 ± 44.4	322.5 ± 21.1	345.6 ± 12.3	303.2 ± 13.3	389.6 ± 19.6	355.6 ±14.0	412.0 ±14.5

Table 8: Ratio of Plant Root/Rhizome Heavy Metal concentrations and the Ave of 0-3" and 3-8" Sediment Regions at all Sites*
(ratio >1 = metal concentrated in root system, ratio <1 = metal concentrated in sediment)

	W-S	SP-S	DHB-S	BWM-S	RB-S	W-Z	SP-Z
Cr	1.28	1.51	1.76	7.52	1.97	0.21	0.52
Cu	0.69	6.61	2.81	0.6	4.86	-	1.91
Zn	0.19	22.75	3.53	0.24	3.06	0.3	7.42
Fe	0.2	1.59	1.01	0.42	0.24	0.22	1.1
Pb	1.74	2.64	6.68	5.12	57.9	2.52	2.12
Ni	1.42	2.5	2.84	2.63	9.02	-	1.08







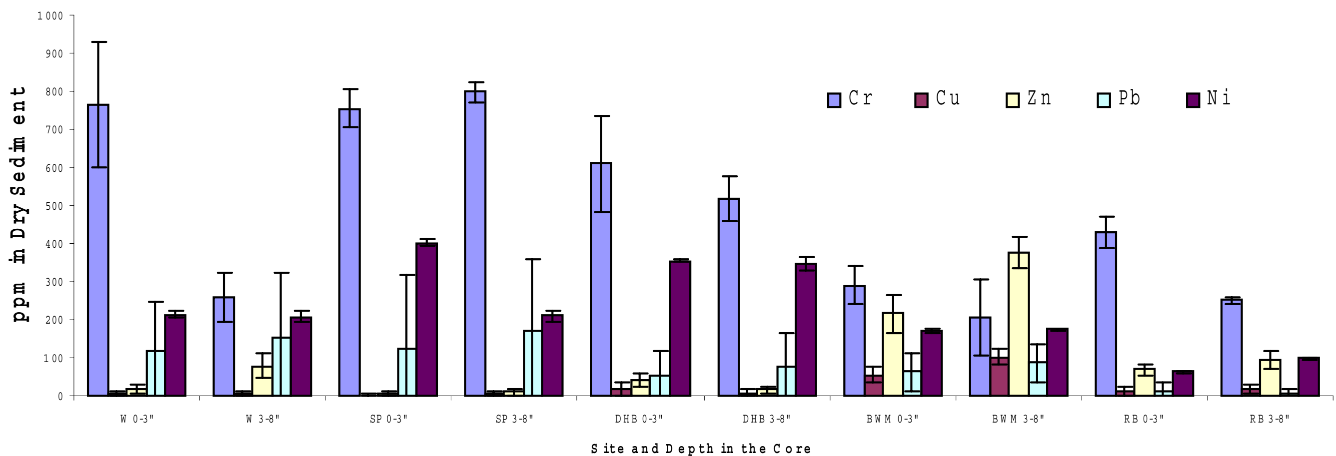


Figure 5: Sediment Core Mean Iron Values by Site and Depth in the Core (mean ± sem)

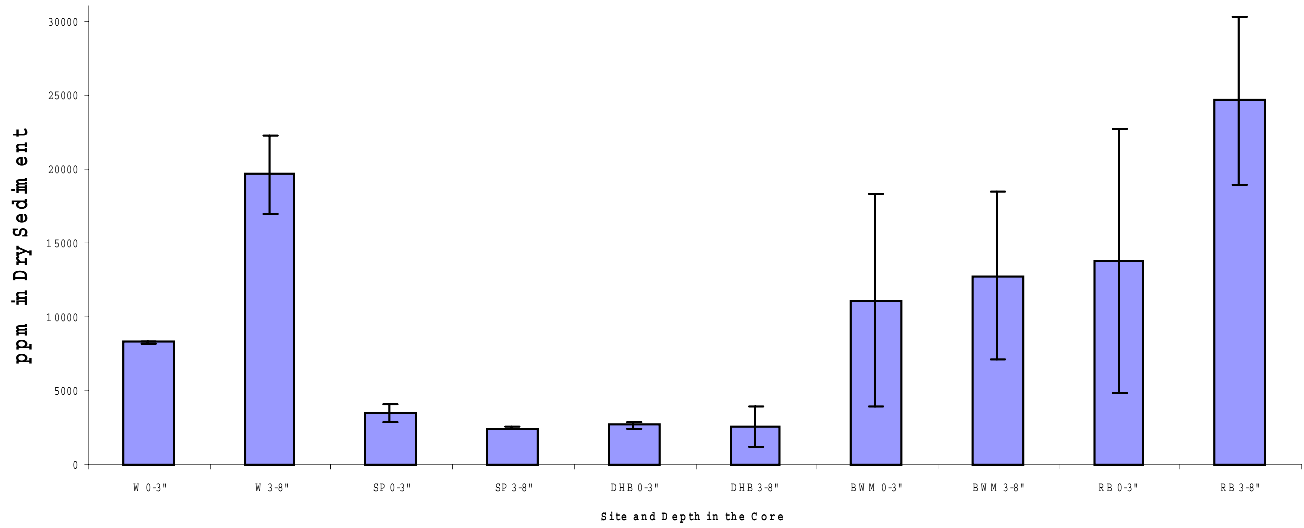


Figure 6: Plant Heavy Metal Concentrations by Site and LS/RR Partion (mean ± sem)

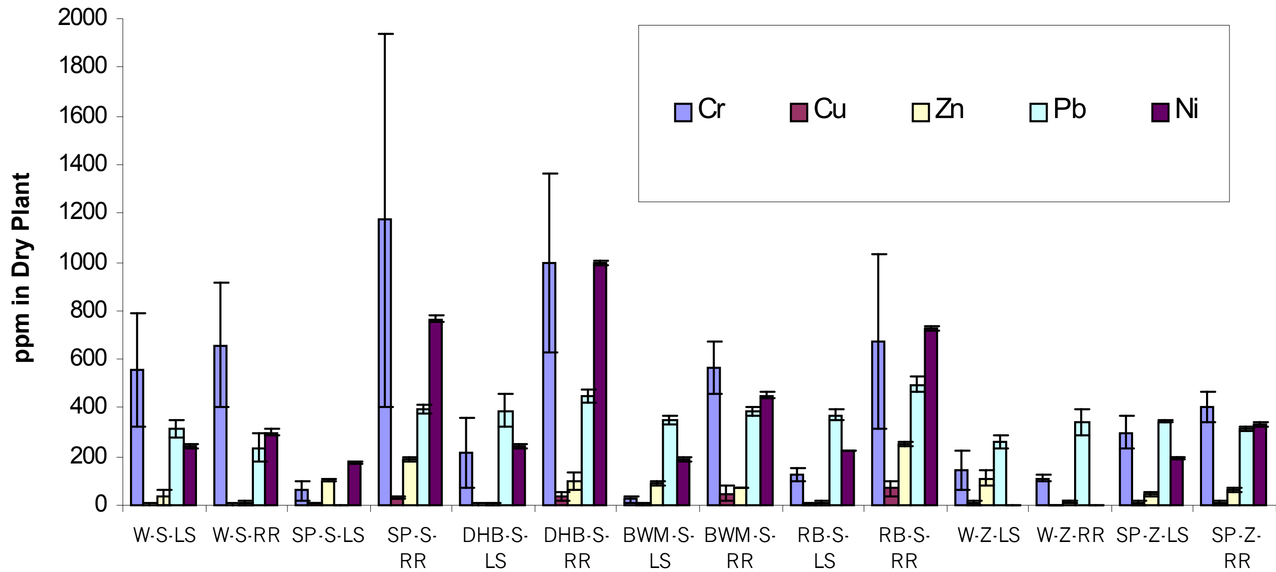
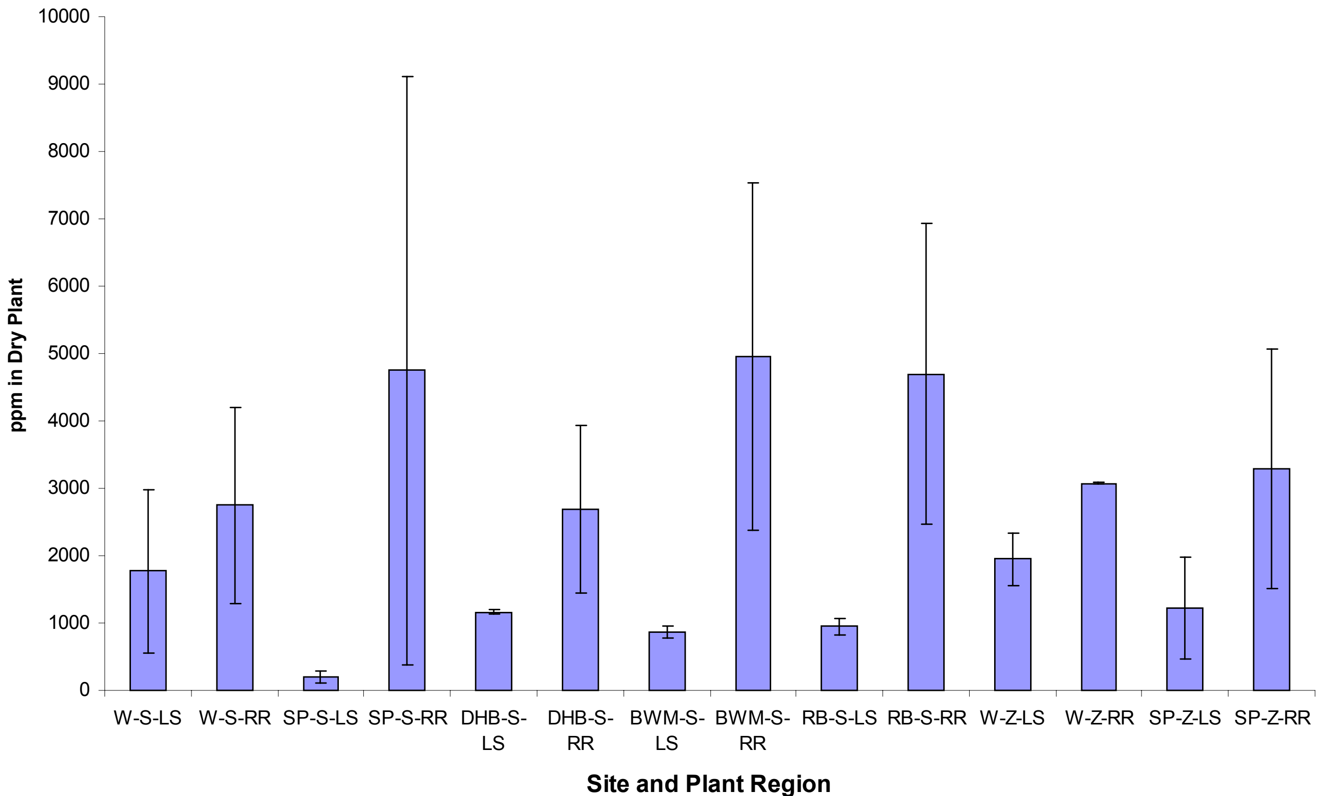


Figure 7: Plant Iron Concentrations by Field Site and LS/RR Partion (mean ± sem)



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A Method Utilizing Buccal Swabbing for Collection and Extraction of High-quality, Newt (*Notophthalmus*) DNA for Use in Phylogenetic Analyses

by

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Abstract

The acquisition of high-quality DNA for use in phylogenetic and molecular population genetic studies is a primary concern for evolutionary and genetic researchers. While such DNA is easily obtained, it often requires the sacrifice of the subjects in question. Many non-destructive DNA sampling methods have been developed and are used with a variety of taxa in applications ranging from genetic stock assessment to molecular forensics. We have developed a field sampling method for obtaining high-quality DNA from newts (*Notophthalmus*) which employs a variation on the buccal swab method and results in the collection of DNA suitable for PCR amplification and polymorphism analysis. The ease and benefits of this method should make it applicable to field-oriented population and conservation genetic studies involving a wide range of amphibians.

Introduction

The acquisition of high-quality DNA for use in phylogenetic and molecular population genetic studies is a primary concern for genetic and evolutionary researchers. While such DNA is easily obtained from studied organisms, it often requires the sacrifice of the subjects in question. Such destructive or lethal sampling has the potential to seriously impact the genetic makeup of populations under investigation and should be avoided whenever possible.

Many non-destructive DNA sampling methods have been developed and are used with a variety of taxa in applications ranging from genetic stock assessment to molecular forensics. Toe clips have been used as sources of DNA for population genetic studies of the Great Plains toad, *Bufo cognatus*[1]. DNA suitable for PCR amplification and analysis of microsatellites in honey bees (*Apis melliera*) has been obtained from wing clips[2]. The molecular phylogeny of the family *Chinchillidae* has been investigated using DNA from hair, blood, feces, and ear tissue[3]. In fish, sources of DNA available for non-lethal sampling include: fin clips, scales, barbels, muscle, blood and sperm[4,5]. DNA suitable for microsatellite analysis and genotyping has even been obtained from chimpanzee (*Pan troglodytes*) feces[6] and sperm whale (*Physeter macrocephalus*) teeth and scrimshaw[7].

A standard method of collecting DNA with minimal invasiveness from humans involves buccal swabbing to dislodge epithelial cells from which the DNA can then be extracted[8,9]. Among the advantages of this method are rapidity and simplicity[10]. These characteristics make buccal swabbing adaptable to a wide variety of situations and particularly amenable to large sample sizes.

We have developed a field sampling method for obtaining high quality DNA from newts (*Notophthalmus*) which employs a variation on the buccal swab method and results in the collection of DNA suitable for PCR amplification and polymorphism analysis. The benefits of this method include its scalability to include large sample sizes, its ambient temperature of field storage and preservation, and its simplicity of sample transport. The ease of our method should make it readily applicable to field-oriented population and conservation genetic studies involving a wide range of amphibians.

Materials and Methods

Red-spotted newts (*Notophthalmus viridescens*) were caught in situ using standard seining methods. The newts originated from Lake Wapalanne in Northwestern New Jersey on the grounds of the New Jersey School of Conservation. Once caught, the newts were held temporarily (20 min - 1.5 hr) in shallow buckets of lake water.

When ~40 newts were captured, buccal smears were taken from each newt by sterilely swabbing their mouths using the wooden ends of sterile cotton-tipped applicators (Moore Medical Corp, New Britain, Connecticut). Cheek cells from the applicator ends were fixed and preserved on site by re-suspension into 100 µL of 100% ethanol in 1.5 mL microfuge tubes. After taking buccal smears, newts were returned to their lake habitat. In the laboratory, the fixed tissue samples were stored at 4°C for 24-96 hours before extraction.

For DNA extraction, the ethanol fixative was first dried from the tissue samples for 10-20 min in a Savant Speedvac vacuum dryer (GMI Inc, Albertville, Minnesota) set at the lowest drying temperature. Tissue samples were then resuspended in 50 µL of TE and RNase (10 mM Tris-Cl, 1 mM EDTA, pH 8.0, 1 unit RNase per 50 µL aliquot). Tissues were lysed by 5 min incubation at 95°C, cooled on ice for an additional 5 min incubation, and centrifuged briefly to collect water condensed on the side of the microcentrifuge tube. The DNA concentration averaged 0.5-1 ng/µL based on electrophoretic analysis and comparison to known molecular weight standards. DNA was stored frozen at -20°C until later PCR amplification.

To test the quality of the extracted DNA, PCR was performed employing amplification primers for detecting microsatellite polymorphisms in red-spotted newts[11]. The PCR amplification conditions principally followed the directions of Vander Zwan et al.[12]. Microsatellites were amplified in 20 µL reactions containing: 1-1.5 ng newt DNA, 10% ThermoPol buffer (New England Biolabs, Inc., Beverly, Massachusetts), 5 pmoles of each primer, 200 µM dNTPs (New England Biolabs Inc., Beverly, Massachusetts), and 1.0 unit Taq polymerase. All amplification was performed in a Mastercycler Gradient Thermocycler (Eppendorf Inc., Germany). The PCR products were subjected to electrophoresis on a 2% agarose gel in 1X sodium borate buffer[13]. The products in the agarose gels were stained with ethidium bromide and imaged using an Ultralum gel documentation system (Ultralum, Inc., Claremont, California) and Scion Image computer software (Scion, Inc., Frederick, Maryland).

Results and Discussion

The quality of the isolated newt DNA is high enough to allow PCR amplification of simple sequence length polymorphisms without further purification. There is some background visible along with the polymorphic DNA bands, but the bands themselves are clearly visible for each individual newt (Figure 1). Microsatellite regions have been successfully amplified at all available newt loci (Nvi2, Nvi7, Nvi11, Nvi14, Nvi18, Nvi19, and Nvi24), although data from only the Nvi14 locus (Forward primer: 5'AAGGTCATCTAACAAAAGAGT 3', Reverse primer: 5' ACAGCATGGCACAGTAT

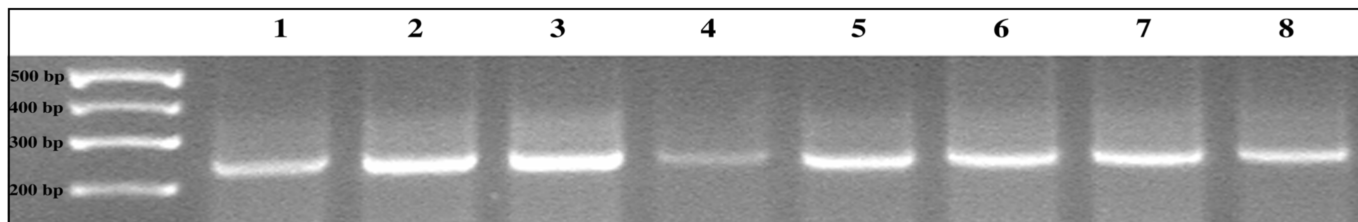

Figure 1. PCR products of red-spotted newt DNA (Lake Wapalanne), employing Nvi14 primers to amplify microsatellite regions. Lane 1: molecular weight markers (Hi-Lo Marker, Minnesota Molecular), size indicated in basepairs. Lanes 2-9: newt Nvi14 microsatellite sequences. 2% agarose gel stained with ethidium bromide.

3') is presented here (Figure 1). Moreover, we have amplified larger polymorphic microsatellite alleles of 300 basepairs using the marker Nvi14 (data not shown). High-resolution agarose gel electrophoretic analyses using comparison to known concentrations of HiLo molecular weight marker (Minnesota Molecular, Madison, Minnesota) were employed to determine the size ranges of unamplified genomic DNA. We found molecular weights ranged from 1000 to 7000 basepairs.

To our knowledge this is the first application of buccal swabbing in newts for purposes of DNA extraction. Variations on this collection method have been commonly used in mammals for many years, but it is possible that characteristics of the epithelial tissues of amphibians made researchers less apt to consider employing this method for sample acquisition. Now that this method has been demonstrated to work efficiently in newts, we hope that it will be used in both field and laboratory work involving a wide variety of amphibians.

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Additional Abstract from the 2004 Fall Conference

The Adhesion Protein, the Platelet F11R (a.k.a JAM1/JAM-A) Expressed in Insect Cells Inhibits the Aggregation of Human Platelets: Role in Inflammatory Thrombosis. **Debra Cortes[1], Anna Babinska[2], Mamdouh H. Kedees[2], Yigal H. Erlich[3], Elizabeth Kornecki[2]. [1]Kingsborough Community College , CUNY, Brooklyn, NY, USA, [2]State University of New York, Downstate Medical Center at Brooklyn, Brooklyn, NY, USA, [3]CUNY, College of Staten Island, Staten Island, NY.**

The F11 receptor (F11R/JAM) is a cell adhesion molecule expressed on the surface of human platelets. The F11R gene is located at position 1q21.2-21.3 on chromosome 1. F11R is also known by the name Junctional Adhesion Molecule 1(JAM-1/JAM-A) when present on edothelial and epithelial cells. Structural characterization of the F11R has categorized this protein as a member of the immunoglobulin superfamily. F11R is a crucial protein involved in the initiation of cardiovascular diseases, in particular thrombosis and atherosclerosis induced by inflammatory agents. F11R plays a role in disease. It is involved in the ability of human platelets to adhere to cytokine-inflamed endothelial cells (EC), thus enabling the initiation of plaque formation and atherosclerosis. To further examine the function of the F11R, we prepared a recombinant F11R protein in insect cells utilizing a baculovirus transfection system. The F11R recombinant protein was collected from transfected insect cells and purified using immunoaffinity column chromatography. The purified F11R recombinant protein was detected by immunoblotting procedures utilizing the monoclonal antibody M.Ab.F11. M.Ab.F11 detects active conformation-dependent epitopes expressed on the F11R molecule, and thus our results demonstrate that the recombinant F11R protein generated in this study by insect cells retains the active conformation of the native F11R that is present on the surface of human platelets. The addition of this recombinant protein to human platelets resulted in a dose-dependent inhibition of platelet aggregation. These results indicate that specific F11R drugs should be effective for the treatment and prevention of thrombosis, atherosclerosis, heart attacks and stroke.

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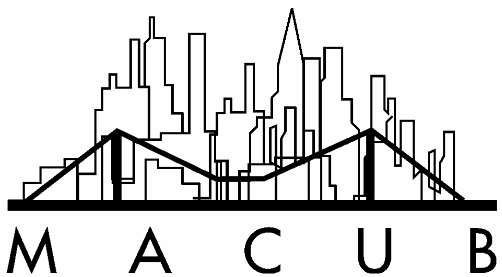

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