MACUB

IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Fall, 2005 Volume 27, Issue 1

38th Annual Fall MACUB Conference
Saturday, October 29, 2005
Monmouth University, West Long Branch, NJ
Conference Theme - *Global Infectious Disease*

Keynote Addresses

Dr. Rita Colwell - *Climate, Infectious Disease, and Human Health: The Cholera Paradigm*

Dr. Colwell is the Chairperson of Canon US Life Sciences, Inc and Distinguished Professor at the University of Maryland at College Park and at the John Hopkins University Bloomberg School of Public Health. She also was the Director of the National Science Foundation from 1998—2004.

Dr. Henry - *Molecular Therapies for HIV Prevention*

Dr. Henry is a molecular geneticist in Bethesda, Maryland. He is currently a post-doctoral fellow in National Cancer Institute at the National Institutes of Health. His area of interest is HIV/ AIDS research and awareness. His current research project at the NIH focuses on the development of live and topical HIV microbicides in the laboratory of his colleague, Dr. Dean Hamer.

Monmouth University is a private, coeducational, comprehensive University founded in 1933 with over 50 undergraduate and graduate degree programs within five schools. With a total student headcount of approximately 6,000, the average class size is 22 students, with a student-faculty ratio of 18:1. Monmouth has a diverse student body, with 18.4% of the undergraduate full-time student body in Fall 2003 representing minority groups, and students coming from 21 counties in New Jersey, 24 states, and internationally. The 155-acre campus is located in Central New Jersey. The campus includes 53 buildings, including Woodrow Wilson Hall, registered as a National Historic Landmark.

The Biology Department is comprised of 10 full-time faculty members. The curriculum gives students a basic diversified background in the life sciences and prepares them for graduate work, professional school, laboratory work in government and industry, and careers in teaching. Undergraduate degree concentrations include Biology, Biology with Molecular Cell Physiology, Biology with Marine and Environmental, and Biology with Education (elementary or secondary). New in 2005 is a major in Marine and Environmental Biology and Policy. Degree concentrations have research requirement or elective options in areas of cell biology, marine and environmental biology, microbiology, molecular biology, neuroscience and reproductive biology.

The Department has averaged 36 graduates per year over the past five years. 15% were accepted into medical, dental, or other professional school, 15% were directly accepted into a graduate program in the sciences, and ~50% are confirmed to have found employment in their field. Monmouth also has a Medical Scholars Program, which permits acceptance into medical school at Drexel University following completion of requirements at Monmouth. About 10% of the biology majors graduate with a double major in education and gain positions teaching grades K-12. The Biology Department maintains high-quality students, with ~25% graduating with high honors (GPA> 3.5).

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Instructions for Authors

IN VIVO is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

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.

In This Issue:

MACUB 2005 Executive Board 2

Instructions for Authors 2

MACUB Web Site 3

Call for Poster Judges 3

A Microarray Analysis of Gene Regulation in Radiation-induced Plant Tumor and *Arabidopsis thaliana* Tissues by James J. Campanella, Quinn Vega, Chunguang Du, William Graff and Olumbusola Gomes 4

Growth and Survival of the American Oyster *Crassostrea virginica* in Jamaica Bay, New York by Gary Sarinsky, Margaret A. Carroll, Ebere Nduka and Edward J. Catapane 15

Call for Manuscripts/Reviewers 27

Affiliate Members 27

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Call For Poster Judges

If you would like to be a poster judge at the conference, please call or email:
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Dr. Sarwar Jahangir, 718-368-5743, e-mail sjahangir@kbcc.cuny.edu

A Microarray Analysis of Gene Regulation in Radiation-induced Plant Tumor and *Arabidopsis thaliana* Tissues

by

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ABSTRACT

In animals, studies of tumor formation have led to an understanding of cellular regulatory mechanisms at the genetic and hormonal levels. Using Microarray technology, we have examined the over- and underexpression of genes in a radiation-induced tumor from the model plant *Arabidopsis thaliana* to determine what factors may act in regulating plant cell division. Many potential proto-oncogenes were found overexpressed in the tumor. The cell wall cross-link reducing enzyme Xylosidase was determined to be overexpressed, potentially leading to faster growing tumor cells. Four putative proto-oncogenic cytosolic protein kinases were found at high levels in the tumor tissue. Additionally, two protein kinase receptor homologues and two transcription factors (homologues of the R2R3-MYB and ethylene response plant transcription families) also were found to be overexpressed. We also detected a series of underexpressed genes, but most have no obvious potential as tumor supressors in plants. One of the underexpressors is a DNA damage repair gene that could account for increased levels of mutation in the tumor and an overall greater likelihood of tumorigenesis. Research will continue to determine which of these factors may be most directly responsible for the tumorigenesis in the tumor tissue.

INTRODUCTION

Multicellular organisms are dependent on correctly functioning growth induction pathways for their normal morphological development. Loss of cellular growth control can lead to tumor formation in both plants and animals. In animals, studies of these abnormalities have led to an understanding of cellular regulatory mechanisms at the genetic and hormonal levels[1,2]. The isolation and characterization of oncogenes and tumor suppressor genes in animal cells has revealed the careful balance that is needed to maintain enough cellular growth for replacement of damaged or aging cells while simultaneously keeping rigid control mechanisms in place to suppress over-growth.

Plants presumptively have growth controls homologous to animals. It has been proposed that plant tumors naturally arise when growth control mechanisms become uncoupled[3]. These plant tumors may arise spontaneously from environmental factors such as radiation or mutagenic chemicals and derive from endogenous changes in the plant genome. Studies of such tumors might offer a rich source of information on growth control.

Analyses have been performed on radiation-induced tumors to investigate growth control in the model plant *Arabidopsis thaliana*[3,4,5,6]. One goal of

these tumor-isolation studies was to aid in the search for plant oncogenes whose altered expression could lead to uncontrolled growth and hormone autonomy in plants.

Persinger and Town[5] exposed *Arabidopsis* seed to ^{60}Co γ-ray radiation, grew the seedlings aseptically from these treated seeds, and subsequently isolated seven tumors from hypocotyl and apical regions. The tumors were studied for morphology and growth habit. All shared the trait of hormone autonomy, requiring neither auxins nor cytokinins to grow on media in tissue culture.

Some of the tumors were also examined physiologically and genetically to ascertain how they differed from normal plant tissues[3,6]. Town *et al*.[3] employed subtraction hybridization libraries using these tumors to identify a number of cDNAs whose expression is enhanced in the tumor versus normal hormone-dependent tissue. They identified several of these cDNAs using GenBank homology searches, including a membrane channel protein, a lipid transfer protein, and hydroxyproline- and glycine-rich proteins.

In addition to the seven original hormone-autonomous tumors isolated, two additional tumors were isolated as well[3]. These additional tumors were progressive forms of neoplasmic growth. Progressed tumors have additional mutations that further set them apart morphologically and physiologically from the parent tumor. One of the progressed tumors that was never genetically characterized was "2.10A". The unprogressed parent of 2.10A was 2.10, a light-green, hard, slow-growing and undifferentiated tumor. When 2.10A progressed, it changed considerably to become dark-green, soft, and fast-growing[3,5]. The progressed tumor remained undifferentiated and hormone-autonomous. Nucleic acids from the 2.10A tumor were not initially isolated for genetic analyses because the tumor's endogenous nucleases interfere with DNA and RNA extraction (Christopher Town, personal communication).

Our goals in this present study were to employ the newest techniques of nucleic acid extraction to obtain the mRNA from the 2.10A tumor and use cDNA derived from those transcripts to perform heterologous microarray analysis with normal tissue. We were interested in addressing several major questions. First, we wanted to determine whether the overexpressed and underexpressed cDNAs in 2.10A were the same as those characterized in other radiation-induced tumors[3,6]. Second, with the complete sequencing of the *Arabidopsis* genome[7], we wanted to gather further clues on the process of tumorigenesis and plant oncogenes. Finally, we wanted to discover which over- or underexpressed gene products were candidates to be manipulated in future genetic experiments examining plant tumor induction and regulatory control of growth.

MATERIALS and METHODS

Plants and Plant Growth

A. thaliana seeds for the Landsberg erecta ecotype were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, Ohio, USA). The 2.10A tumor tissue was kindly supplied by Dr. Christopher Town, The Institute for Genomic Research, Rockville, Maryland.

The *Arabidopsis* seeds were surface sterilized for 10 min with 500-1000 μl of 30% Clorox bleach/0.1% Triton X-100, agitated every 2 min during incubation, washed three-times in 1 ml

sterile, distilled water, and re-suspended in 1 ml distilled water. Seeds were then plated on to Murashige-Skoog agar medium (Sigma Co., Catalog #M-5519), cold-treated 1 wk in the dark at 4°C, then incubated at 23°C under constant light (cool white, fluorescent, ~100 µmol/s/m^2) in a plant growth chamber (Percival Scientific, Model E-30B) for 10 days after germination.

The 2.10A tumor tissue was plated and grown on Murashige-Skoog agar medium (Sigma Co., Catalog #M-5519) aseptically under constant light (cool white, fluorescent, ~100 µmol/s/m^2) in a plant growth chamber for 10 days.

RNA Extraction

Total RNA was extracted from ~200 mg of *A. thaliana* Landsberg erecta ecotype and 2.10A tumor tissue (10 days old) using the RNeasy RNA extraction kit (Qiagen Corporation, Valencia, California). Before extraction, micropestles and all microfuge tubes were treated with an 8% solution of RNA Secure (Ambion Corporation, Austin, Texas) for 10 min at 65°C. RNA samples were stored as aliquots until analysis.

cDNA Labeling

cDNA production and Cy3/Cy5 labeling were completed using the 3DNA EX expression array detection system microarray (Genisphere, Hatfield, Pennsylvania) following the manufacturer's instructions. Specifically, 2.5 µg of total RNA (tumor and wild type) was mixed with 5 pmol of the appropriate primer in a 10 µl reaction. The samples were heated to 80°C for 10 min followed by the addition of 4 µl 5x RT buffer, 1 µl 10 mM dNTP mix, 4 µl RNAse free H_2O and 200 Units of reverse transcriptase. The samples were then incubated at 42°C for 2 hr. The reaction was stopped with the addition of 3.5 µl of 0.5 M NaOH/50 mM EDTA, pH 8.0, the DNA/RNA hybrids denatured by heating at 65°C for 10 min followed by neutralization with 5 ml 1M Tris-Hcl, pH 7.5. For precipitation, the samples were combined and mixed with 3 µl of 5 mg/ml linear acrylamide, 250 µl 3 M ammonium acetate, pH 5.2 and 875 µl of 100% ethanol. The samples were incubated for 20 min at -20 °C and centrifuged for 15 min at 14,000 g. The supernatant was removed, and the pellet washed with 300 µl of 70% ethanol. The cDNA pellet was allowed to air dry, and the pellet resuspended in 5 µl H_2O.

Chip Hybridization.Chip Hybridization

The resuspended cDNA was mixed with 5 µl of capture reagent (Cy3 and Cy5 label) along with 10 µl of hybridization buffer (40% formamide, 4X SSC, 1% SDS, 2X Denhardt's solution). The samples were incubated for 15 min at 55°C to prehybridize the cDNA to the dyes. The probes were then added to the *Arabidopsis* microarray (Ellen Wisman, Michigan State University), covered and incubated overnight in a dark, humidified chamber at 60°C. Following overnight incubation, samples were washed for 10 min at 55°C with 2X SSC, 0.2% SDS, 10 min at room temperature with 2X SSC and, finally, for 10 min at room temperature with 0.2X SSC. Samples were air-dried and scanned using ArrayWoRx microarray scanner (Applied Precision, Issaquah, Washington).

The microarray chips used in this analysis were spotted with a total of 11,174 Expressed Sequence Tags (ESTs) from 37,000 available *Arabidopsis* ESTs.

Microarray Image Processing

ScanAlyze[8,9] obtained the signal intensities of each microarray spot. The arrays had 22 columns and 22 rows. The spot to spot spacing was 198 μm. Spot width and length were 130 μm. Flaws on the microarray, such as dust specks, cover slip movement, and blotches of unwashed dye, were visually flagged to be disregarded by the analysis software. ScanAlyze calculated spot intensities, background, ratios, and correlations between the two dyes.

The mean intensity was calculated for each spot and the mean background intensities subtracted from each. Data was exported to SAS (Statistical Analysis Software, SAS Institute Inc., North Carolina) and normalized for physical biases between samples. The intensities of each spot were recalculated using a Log2 transformation to ensure approximately normal distribution within each gene and improve the statistical quality for the analysis of variance.

ANOVA

The spot intensity data was imported into SAS for further statistical analysis. Analysis of variance of microarray data was performed by first log-transforming the raw fluorescence data on the base-2 scale[10], allowing each unit to correspond to a two-fold difference in expression.

The arrays and dye channels within each array were normalized with respect to one another by computing the fluorescence intensities for each clone relative to the sample mean. The data was then fit to a gene-specific significance model. A Proc Mixed algorithm was employed to fit each of the fluorescence intensity and spot size parameters into the model simultaneously using maximum likelihood[10].

Clustering Analysis

The Cluster program[8] was employed to search for groups of genes with similar properties and visualize the results. The Hierarchical clustering method[11] which employs a least-squares method of analysis was used to analyze our data. The Treeview program[12] was used to visualize trees that were generated by Cluster.

RESULTS and DISCUSSION

Up-Regulated Clustered Genes

Our analysis of the chip arrays brought to light ten genes that had greater than a two-fold increase in expression. A volcano plot distinguished all the genes on the arrays and their levels of expression (Fig. 1). Each spot in the volcano plot represents an mRNA transcript over- or underexpressed in the tumor tissue relative the “normal” *Arabidopsis* tissue. The genes on the plot farthest to the left are those that are underexpressed and those farthest to the right are those that are overexpressed. Candidate genes of interest were all those transcripts on the volcano graph that were more than two-fold over- or underexpressed on the X-axis.

The GenBank accession codes for the overexpressed genes that were of most interest were: AA597386, H76821, T41925, BE520366, T21658, R30336, AA404849, AA720202, R65020, and AA395645. The sequences for all these putatively overexpressed genes were retrieved from GenBank and checked for function using The Institute for Genomic Research’s (TIGR) *Arabidopsis* library search engine None of these genes demonstrated any homology to any other gene filed in GenBank, nor did TIGR

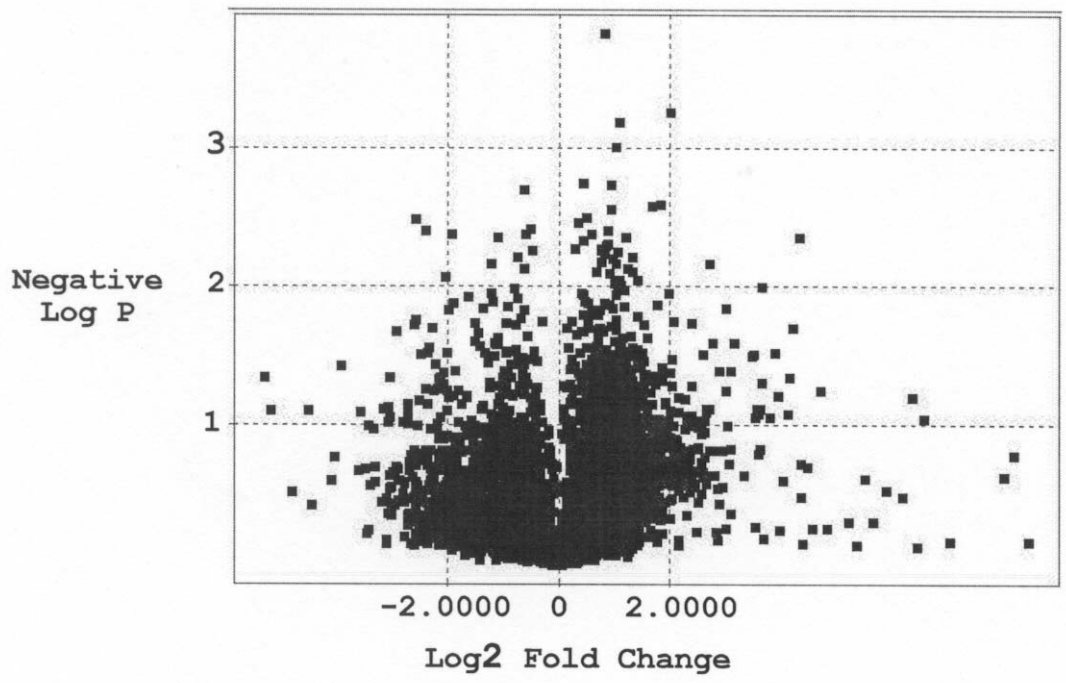

Figure 1. Volcano plot of overexpressed and underexpressed genes in the 2.10A tumor tissue. The Volcano plot was generated using the SAS application.

BLAST searches reveal any functions. Since no functionality was evident to aid in our investigation, a clustering analysis was performed on the putative genes of interest to statistically determine with what other overexpressed genes these initial genes might be associated. Five (T41925, BE520366, R30336, AA404849, and AA720202) of the overexpressors did not cluster with any other genes. The other five overexpressors were all clustered in groups ranging from groupings of three to over 100 genes.

Most of the clustered genes seem to be uncharacterized and of unknown function. TIGR BLAST searches were performed on all clustered sequences to identify homologues to known genes in the sequence database. All putative up-regulated genes with similarity scores of greater than 1000 were recorded (Table 1).

Many of the overexpressed genes in 2.10A probably are not directly involved with induction of the neoplastic state of the tissues. A quick scan of the overexpressed products (Table 1) suggests that many of these genes (alcohol dehydrogenase, coatomer protein, beta-tubulin, porin, kinesin, etc.) are house-keeping proteins and not directly involved in growth stimulation or control. However, there are other genes that may be associated with the tumor morphology of 2.10A or directly involved in tumorigenesis and hormone autonomy.

One of the more intriguing of the overexpressed products is xylosidase (W43153) (Table 1). Xyloglucan is a cell wall component that is thought to be part of the load-bearing component of the cell, inducing cross-linking, a major factor in the rate of cell expansion[13,14]. Xylosidase is thought to play a major role in control of

Table 1. Up-regulated Gene Clusters. (Bold = oncogenic potential)

Genes	Function/Homology	Genes	Function/Homology
Genes clustered with AA395645		Genes clustered with H76821	
H76173	ATP-binding protein	AA597318	myosin-like protein
AA586152	**Protein kinase**	T43396	cysteine proteinase AALP
R64871	**Cyclin-dependent protein kinase inhibitor**	T44093	tyrosine phosphatase
AA713066	nod-like gene	H36798	hevein-like protein
BE522210	formin-like	N37341	NADH dehydrogenase
T13648	**Protein kinase receptor**	BE522874	S-adenosylmethionine decarboxylase
		N65267	brassinosteroid-6-oxidase
Genes clustered with AA597386		R90372	ubiquitin-specific protease
W43153	**Xylosidase**	AA394381	ADP-ribosylation factor-like protein
T41825	nop56-like protein	BE522037	H+-transporting ATPase
N65694	heat shock protein	T04716	seed maturation-like protein
		BE524760	CTP synthase
Genes clustered with BE520752		**T04369**	**ethylene response transcription factor**
W43132	α-crystallin family	AA721923	xylose isomerase
		AA721864	regulatory subunit of protein phosphatase 2A
Genes clustered with R65020		AA597405	Serine/arginine-rich protein
R90584	H+ transporting ATPase	R90288	Dim1 homologue
T76228	**Calpain**	H76807	ribosomal protein L34
R30029	RNA binding domain protein	BE522973	transporter protein
AA712969	proteomsome regulatory subunit	**T45415**	**leucine-zipper DNA binding protein**
N65168	Vicilin-like	**T45845**	**Protein kinase receptor**
N38328	Transmembrane channel protein (aquaporin)	AA712299	helix protein
		T21564	**protein kinase receptor**
T22497	Ribonucleoprotein F	**T21568**	**cysteine proteinase inhibitor**
AA597488	ADP ribosylation factor	AA713080	mitochondrial uncoupling protein
T21564	**Protein kinase**	R84043	Lhcb3 chlorophyll a/b binding protein
		H36107	glucosyltransferase
Genes clustered with T21658		AA605379	Iron/Ascorbate oxidoreductase
BE522338	potassium channel protein	T43219	glycerol kinase
AA395382	ubiquitin chain binding protein	**H37474**	**calcium-dependent protein kinase**
T20920	spermidine synthetase		
N38463	α-expansin precursor		
T46113	**protein kinase**		
R30493	aconitase		
N38539	arogenate dehydrogenase		
AA713124	hypersensitive related protein		
AA597945	**R2R3 MYB transcription factor**		
T21047	seed imbibition protein		

cell expansion and growth[14]. The enzyme is able to reduce the cross-linking chains of xyloglucan and allow the cells to grow more rapidly. We hypothesize that xylosidase overexpression may be one of the factors that allows the progressed 2.10A to grow at a faster rate than its parent tumor 2.10. Xylosidase is usually closely regulated[14], but unregulated expression could allow much faster levels of cell expansion and growth than normal.

A series of cytosolic protein kinases (AA586152, T21564, T46113, H37474) are overexpressed genes in 2.10A (Table 1). Protein kinases (PK's) are known to be major signal transduction components of growth cascades[15,16]. Several cytosolic PK's in animals have

proto-oncogenes, including c-src, BRAF, c-fps and c-abl[17,18]. It may be that these *Arabidopsis* PK's are not themselves oncogenes but are being overexpressed as a result of upstream signaling of the true oncogene. Alternatively, any one of these uncharacterized PK's may itself be an oncogene causing increased signalling activity in downstream PK's and inducing oncogenic growth. This is a difficult result to dissect because, as with the results of earlier studies[3,6], it is difficult to distinguish cause from effect. In addition to the cytosolic PK's, two protein kinase receptors (T13648, T45845) are also overexpressed in 2.10A (Table 1). Again, these genes are potential oncogenes. Mutated or overexpressed protein kinase receptors are the largest group of known oncogenes and have been shown to cause many growth abnormalities in animal cells[19,20]. These two receptors may have mitogenic activity as in animal cells. There is evidence in plant cells that protein kinase receptors (receptor-like PK's, calcium dependent PK's, and histidine PK's) act in various ways to control growth[21,22]. Histidine kinases have been shown to act in signaling pathways in plants[21,22] and as receptors for the plant hormone ethylene. Another histidine kinase in *Arabidopsis* acts as a receptor for the growth-stimulating phytohormone cytokinin[22]. Given that these PK receptors can be stimulated to signal growth by phytohormones, they have the potential to become oncogenic.

MYB proteins are a diverse family of DNA-binding transcription proteins that are found in both animals and plants[23]. One of the major plant transcription factors from this family, R2R3-MYB (AA597945), was found up-regulated in 2.10A (Table 1). The R2R3-MYB family is the largest characterized plant transcription factor family, with at least 125 distinct members[23]. The function of this plant transcription factor varies with each member of the family, but all participate in plant responses to environmental factors and in mediating phytohormone actions. Knockouts of the MYB factors do not show any strong phenotypes[23]. However, overexpression studies have been done with MYB demonstrating that under a strong constitutive promoter, the gene induces the ectopic formation of embryos and trichomes in otherwise normal plant tissues[24]. This would suggest that MYB has a strong growth promotion activity and a great potential for oncogenic induction under the correct conditions.

Two additional transcription factors were found overexpressed in the tumor (Table 1): a leucine zipper DNA binding protein (T45415) and a transcription factor for an ethylene response element (T04369). Since both of these factors stimulate growth by directly binding to DNA, they have the potential to become mutated and overexpressed, inducing uncontrolled growth in cells.

The cysteine protease calpain (T76228) is also overexpressed in 2.10A (Table 1). Calpain has been shown to be related to oncogenicity in animals. Src-induced transformation of chicken fibroblasts is accompanied by calpain-mediated proteolytic cleavage of the focal adhesion kinase (FAK) and disassembly of the focal adhesion complex[25]. It is unclear whether calpain also has the ability to induce such activity in plants, but antisense experiments inhibiting calpain expression in the 2.10A tumor might shed some light on its regulatory activity.

The final up-regulated gene of primary interest is cysteine proteinase inhibitor (CPI) (Table 1). Cysteine proteases in plants are able to induce apoptosis, and

Table 2. Down-regulated Gene Clusters (Bold=oncogenic potential)

Genes	Function/Homology	Genes	Function/Homology
Genes clustered with N65739		Genes clustered with T04566	
BE520752	Translation factor EF-1 alpha-like protein	T21564	protein kinase
W43132	Heat Shock Protein 20	AA597488	ADP-ribosylation factor-like protein
		AA713080	mitochondrial uncoupling protein
Genes clustered with T43740		H36107	glucosyltransferase
N96590	**DNA damage repair enzyme**	AA404831	kinesin-like protein
		T22497	ribonucleoprotein F
Genes clustered with N38397		N38328	transmembrane channel protein
N65693	receptor protein kinase	R30029	RNA-binding domain protein
AA605524	vacuolar sorting protein	AA712299	one helix protein
AA651580	disease resistance proteins	T45845	receptor protein kinase
Genes clustered with T45007			
N38604	casein kinase protein		
R83963	nucleosome assembly protein		
AA395695	cysteine endopeptidase protein		
Genes	**Function/Homology**		

the CPI is able to inhibit apoptosis[26]. Apoptosis in animals has been shown to be a regulatory function in which cells with irreparably damaged DNA undergo programmed cell death. An overall somatic decrease in apoptosis would potentially lead to increased oncogenesis. It may be that the up-regulation of the *Arabidopsis* CPI in 2.10A is involved in oncogenesis by inhibiting normal levels of apoptosis.

Down-Regulated Clustered Genes

Ten genes were found to be down-regulated with at least a two-fold decrease in expression: N65739, AA395604, T43740, N38397, T45007, T04566, N65510, AA395461, BE520787, and T22675. The sequences for all of these underexpressed genes were retrieved from GenBank and checked for function using The Institute for Genomic Research's (TIGR) *Arabidopsis* library search engine (None of these genes showed any homology to any other gene on GenBank, nor did TIGR BLAST searches reveal any known homologous functions.

We performed a cluster analysis of the underexpressors and found that half of these genes (AA395604, N65510, AA395461, BE520787, and T22675) did not cluster with any other genes. The other five underexpressors clustered with three to twenty-three other underexpressed genes. Most of the clustered genes shared no homology with any characterized *Arabidopsis* genes, but we took note of all the linked genes that were functionally characterized (Table 2).

The underexpressed genes (Table 2) are more difficult to categorize for oncogenic potential than the overexpressed genes (Table 1). There are fewer down-regulated genes and most seem to be house-keeping genes. Three underexpressors seem to be signal transduction proteins (two protein kinase receptors and a cytosolic protein kinase); however, because the role of these kinases in plant cell division is not known,

It is unclear how down-regulation of these genes might lead to oncogenesis.

The only underexpressed gene that may be directly related to oncogenesis is a DNA repair enzyme (N96590) (Table 2). It has been demonstrated in animal systems[27] that down-regulation of DNA repair enzymes can lead to increased levels of mutation concomitant with increased potential for oncogenesis. In human cells, for example, the down-regulation of the mismatch DNA repair gene leads to microsatellite instability and carcinogenesis, including colorectal cancer[28]. The mutation of this *Arabidopsis* DNA repair enzyme may have directly induced the oncogenic progression of 2.10A. It seems more likely, though, that the repair enzyme down-regulation may have been more of a contributing factor to initial oncogenesis than an on-going influence to continuing oncogenesis. If this is true, the overexpression of this repair enzyme in 2.10A would probably not revert the tissues to a non-neoplastic state, since the mutagenic damage has already been done to the genome.

CONCLUSIONS

Overexpressed genes in the 1.2A tumor previously characterized[3,6] do not overlap exactly with those found in the 2.10A tumor. There is only one general trend of protein types commonly overexpressed in both 1.2A and 2.10A, the membrane channel proteins. Overexpression of membrane channel proteins could result in increased nutrient uptake or transport, allowing growth of the tumorous tissue in the absence of normal hormonal stimuli. This type of increased nutrient uptake is seen in plant tumor cells infected by the soil bacterium *Agrobacterium tumefaciens*[6,29], so this common element between 2.10A and 1.2A may have some tumorigenic basis.

We have detected many characterized and uncharacterized genes that are both underexpressed and overexpressed in the 2.10A tumor. The uncharacterized genes remain a puzzle that future analysis and research may answer.

As in the previous *Arabidopsis* tumor investigations by Campell and Town[6] and Town et al.[4], there are limitations to this present analysis. The biggest concern is the difficulty in separating cause from effect. It is unclear whether overexpressed/underexpressed genes are a result of tumorigenesis or themselves inducing tumorigenesis. Consequently, there is an inherent difficulty in determining explicitly which genes are most important in tumor physiology.

We look at this study as a guide, pointing in the direction of future research in understanding plant tumorigenesis. It must be made clear that the conclusions of this study are conjecture and so far have no experimentally supported results. However, several genes of interest have been identified here and further study of these genes in radiation-induced tumors should aid in the understanding of hormone-autonomous tissue growth and tumorigenesis.

ACKNOWLEDGMENTS

The authors wish to gratefully acknowledge A. Malcolm Campbell of the Genome Consortium for Active Teaching (GCAT) for technical support and chip reading, as well as Ellen Wisman (Michigan State University) for providing the *Arabidopsis* microarray chips. Finally, we thank Lisa Campanella for her help in editing this article. This work was also

we thank Lisa Campanella for her help in editing this article. This work was also supported by a Sokol grant for undergraduate research from Montclair State University and NSF grant #MLB987S793.

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Growth and Survival of the American Oyster *Crassostrea virginica* in Jamaica Bay, New York

by

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ABSTRACT

Jamaica Bay is a major inlet opening to the Atlantic Ocean. It was abundant with oysters until early 1900's. Over-harvesting, pressure from predators, parasitic invasion and declining water quality often are cited as causes. Despite actions to arrest and reverse the pollution, oysters are not reestablished. We are studying factors relating to the rehabitation of *Crassostrea virginica* in Jamaica Bay to determine if the water quality and environmental conditions are suitable for their survival. Oysters placed in Jamaica Bay grew well when housed in protective containers and growth was influenced by placement near the sediment as compared to the surface. Oysters placed 1 foot above the sediment grew larger that those suspended 1 foot below the surface. Water temperature, pH, turbidity, salinity, conductivity, chlorophyll-a and dissolved O_2 were taken to compare water quality at each site. To study growth and survival in a more natural condition, oyster seed and adults were placed just off the bottom in unprotected containers and photographed. After 1 year they are growing and surviving well and there has been evidence of reproduction. Thus far there are no serious signs of predation by crabs or starfish. The study shows that Jamaica Bay water quality is suitable for oyster growth under the various conditions of our experiments.

INTRODUCTION

Jamaica Bay is a 26 square mile estuarial embayment situated between southern Brooklyn and Queens that communicates with Lower New York Bay and the Atlantic Ocean via Rockaway Inlet. Much of the bay is incorporated within the Jamaica Bay Unit of Gateway National Recreation Area, a unit of the National Park Service (NPS). The bay is home to the Jamaica Bay Wildlife Refuge, the only wildlife refuge managed by the NPS, encompassing 9,155 acres of diverse habitats including salt marsh, upland field and woods, fresh and brackish water ponds and an open expanse of bay and islands. Described as “an oasis of nature surrounded by urban development,” Jamaica Bay provides a sanctuary for the protection of wildlife and other natural resources.[1] The bay is also a critical component of a larger watershed that drains naturally or via storm sewers, on the seaward-sloping outwash plain south of the harbor hill terminal moraine[2], and most of the uplands around the bay, including much of the Rockaway barrier beach, are dominated by urban, residential, commercial, and industrial development. Over the years, dredging, filling, and some major developments like the construction of Floyd Bennett Field and John F. Kennedy Airport have

disturbed the bay. About 12,000 of the original 16,000 acres of wetlands have been filled in, mostly around the perimeter of the bay and extensive areas have been dredged for navigation channels or to provide fill for the airports and other construction projects[2].

At one time, wild stocks of the Eastern Oyster, *Crassostrea virginica,* also known as the American Oyster, were found all along the Atlantic and Gulf coasts of North America and for centuries, supported subsistence fishing by Native American and early European colonists[3]. Historically, *C. virginica,* flourished in Jamaica Bay and the NY/NJ Harbor area as either self-sustaining or farmed populations[4]. Jamaica Bay's oyster industry observed a steady decline in production after its peak in the early 1900's. Lack of adequate supply of seed oysters, over-harvesting by commercial fishermen, increased pressure from natural predators, parasitic invasion, changing hydrographic patterns, siltation, and a decline in water quality are all cited as possible causes for the decline. The growing urbanization and local industrialization of the area caused severe pollution problems for the bay. Discharges of inadequately treated sewage were poisoning oysters, clams and ultimately people, and by 1921 the U.S. Department of Agriculture had closed shellfish lands in Jamaica Bay altogether. Jamaica Bay was not the only area that suffered a serious loss of oyster beds. During the same time there were declines in estuarine shellfish populations along the entire east coast of the United States and other important oyster fisheries, including that of Chesapeake Bay, also started to collapsed[5]. Today, very few if any wild oysters are found in Jamaica Bay, and the dramatic loss of this historic oyster bed has permanently altered the structure and function of the bay's benthic ecosystem.

Since the 1970's, there have been some major government initiatives aimed at protecting and improving the health of Jamaica Bay. In 1972 federal legislation incorporated much of the bay within Gateway National Recreation Area, creating the Jamaica Bay National Wildlife Refuge, and establishing that management of the bay would be guided by NPS policies regarding both resources and use of the area[1]. In 1992, the New York State Department of State designated Jamaica Bay as a "significant coastal habitat"[6]. In 1993, the New York City Department of City Planning designated Jamaica Bay as "one of three special natural waterfront areas"[7] and the New York City Department of Environmental Protection completed a comprehensive watershed management plan for the bay to better protect and restore its habitats and improve its water quality[8]. Other efforts included the construction of sewage treatment plants with improved treatment procedures, and better enforcement of environmental regulations to help deter further pollution. As a result of these initiatives, many marine organisms that had been in decline are now thriving in the bay. However, the once abundant *C. virginica* has yet to show any signs of reestablishing itself to Jamaica Bay.

According to a Reconnaissance Study by the U.S. Army Corps of Engineers[9], Jamaica Bay still exhibits poor water quality. There are contaminated sediments in the area causing adverse effects on benthic organisms and bioaccumulation further up the food chain. Loadings of nutrients and organic matter into the bay from sewage treatment plants and runoff have resulted in phytoplankton blooms and high suspended solid

concentrations which, in turn, have increased water turbidity and decreased bottom dissolved oxygen concentrations[2]. The mean depth of the bay has been increased by dredging from 3 to 13 feet, with some areas dredged deeper than 60 feet, further contributing to hypoxic and anoxic conditions in these poorly flushed areas[9]. A 2004 report by the New York City Department of Environmental Protection states that throughout the Harbor, Jamaica Bay has suffered the most significant decline in water clarity with high levels of algae growth in specific regions of Jamaica Bay largely responsible for this decline[10].

Oyster beds are important to an estuary ecosystem. They provide an environment for a variety of invertebrates, fish and benthic algae; increase habitat structure and faunal diversity; and may reduce turbidity and hypoxia by reducing suspended silt and phytoplankton populations[11]. The filtering action of oysters can significantly alter the phytoplankton assemblage in an embayment[12,13] and dense populations of suspension-feeding shellfish have been shown to have a significant positive impact on basin-wide water quality and phytoplankton dynamics[14, 15, 16, 17].

Since efforts to arrest and reverse the pollution problems in the bay has not resulted in an improvement in water quality nor the return of *C. virginica* to its natural habitat, studies aimed at determining the growth and survival of transplanted oysters, may be the first important step in assessing the feasibility of a future oyster restoration program in Jamaica Bay. In July 2001 our lab initiated a one-year study to monitor the growth and survival of *C. virginica* seed transplanted in protected surface floats to two ecologically different locations in Jamaica Bay. After just a six-week exposure in the bay, both sites showed substantial oyster growth (up to 110%) and excellent seed survival (over 85%)[18]. By the end of the one-year period, these oysters had attained a nearly 400% average increase in shell height along the anterio-posterior axis and survival remained over 80%[19]. While our lab has since used many of the original 2001 seed for various other physiology/biochemical studies, a sampling of 150 of these adults are being maintained in protected floats to follow their long term growth and survival.

To further test the growth and survival of oyster seed under more natural but perhaps stressed conditions, this study was designed to (1) compare the growth and survival of *C. virginica* when oyster seed was positioned in protected containers one foot off the sediment versus protected floats suspended one foot below the surface in Jamaica Bay; and (2) monitor the growth and survival of oyster seed and adults when placed in an undisturbed and unprotected container at the bottom of the bay.

MATERIALS AND METHODS

In June 2002, four modified Taylor Floats[20] of approximately 3' x 4' were constructed using PVC tubing and 1/4" mesh nylon screening. Each float was designed to hold up to three 1/8" mesh nylon boxes in which oyster seed were to be placed. Each float had 1/4" nylon mesh lids to keep out predators. Oyster seed of approximately 20 mm anterio-posterial (height) shell lengths and 5 mm shell hinge width were obtained from Frank M. Flower & Sons, Inc. Oyster Nursery in Oyster Bay, NY. 150 oyster seeds were distributed among the 3 nylon boxes in each float. Two floats were positioned 1 foot below the water surface at two

ecologically different sites in Jamaica Bay, the Gateway National Park Marine Station (GNPMS) at Fort Tilden, and the Kingsborough Community College Marina (KBCCM) site in Brooklyn's Sheepshead Bay, a large cove of Jamaica Bay. The two other floats that were designed to sink and position the oyster seed 1 foot off the bay bottom were each placed at the same sites. Over the next 13 months, water quality was monitored and oyster seed growth and survival were measured under each condition. Surface and bottom bay water was collected using a Van Dorn Bottle. Salinity, pH, dissolved oxygen, specific gravity, and temperature were measured with a Horiba Water Quality Testing Instrument. Chlorophyll-a levels were measured spectrophotmetrically using an Aquamate Spectrophotometer and according to the trichromic method of the American Public Health Association[21]. Both surface and bottom floats were inspected and cleaned of any fouling, biweekly in the summer and monthly in the winter. At those times, the shell lengths (height) and hinge width of each oyster were measured with calipers. After 2 months, the bottom floats were deemed clumsy/difficult to work and were replaced with commercially constructed hanging nets, suspended one foot off the bottom, for the rest of the experiment.

In July 2004, a second set of 300 oyster seed (20 mm average height) and two dozen adult oysters (75 mm average height) were placed directly in an uncovered sunken float and left undisturbed at the bay bottom at KBCCM. Oyster growth and survival were monitored through the summer 2004 and again in summer 2005 using an Atlantis AUW 5600 underwater color camera connected to a monitor. Pictures were captured on a HP 3660 PDA with a Fly Jacket Video Capture card.

RESULTS

Growth of *C. virginica* to harvestable size (about 75mm) can take from one to three years, depending on temperature, water salinity and food supply[22]. Our results indicate that top and bottom *C. virginica* seed at both locations grew at rates comparable to or better than what has been reported for comparable clean sites. Figure 1 shows the growth of shell height along the anterio-posterial axis of top and bottom positioned oysters over 2 growing seasons (July 2002 to September 2003) at GNPMS and KBCCM. At both sites bottom positioned oysters demonstrated faster growth rates than top positioned oysters. This difference in height growth rates between bottom and top was more significant at the GNPMS site. After 3 months, the rates of bottom seed height growth compared to top seed, were 50% greater at the GNPMS site, but only 18% greater at the KBCCM site. By the end of the 13 month period, bottom seed height growth had exceeded top seed height growth by 60% at the GNPMS site but by only 9% at the KBCCM site. A similar pattern was found when oyster seed hinge width was measured. Figure 2 shows that by the end of the second growing season, width growth rates of bottom oysters at GMPMS and KBCCM were 20% and 7% faster respectively, than their top oyster counterparts. Overall, bottom-positioned oysters at the GNPMS, which grew an average of 420% in height and 410% in hinge width over the 13 month period of the study, showed the best growth rate. Figure 3 shows very good oyster seed survival for top and bottom positioned oysters at both the 3 and 13 month periods. Under the protected conditions of our experiment, there were no significant differences in survival rates for top or bottom position oysters at either location.

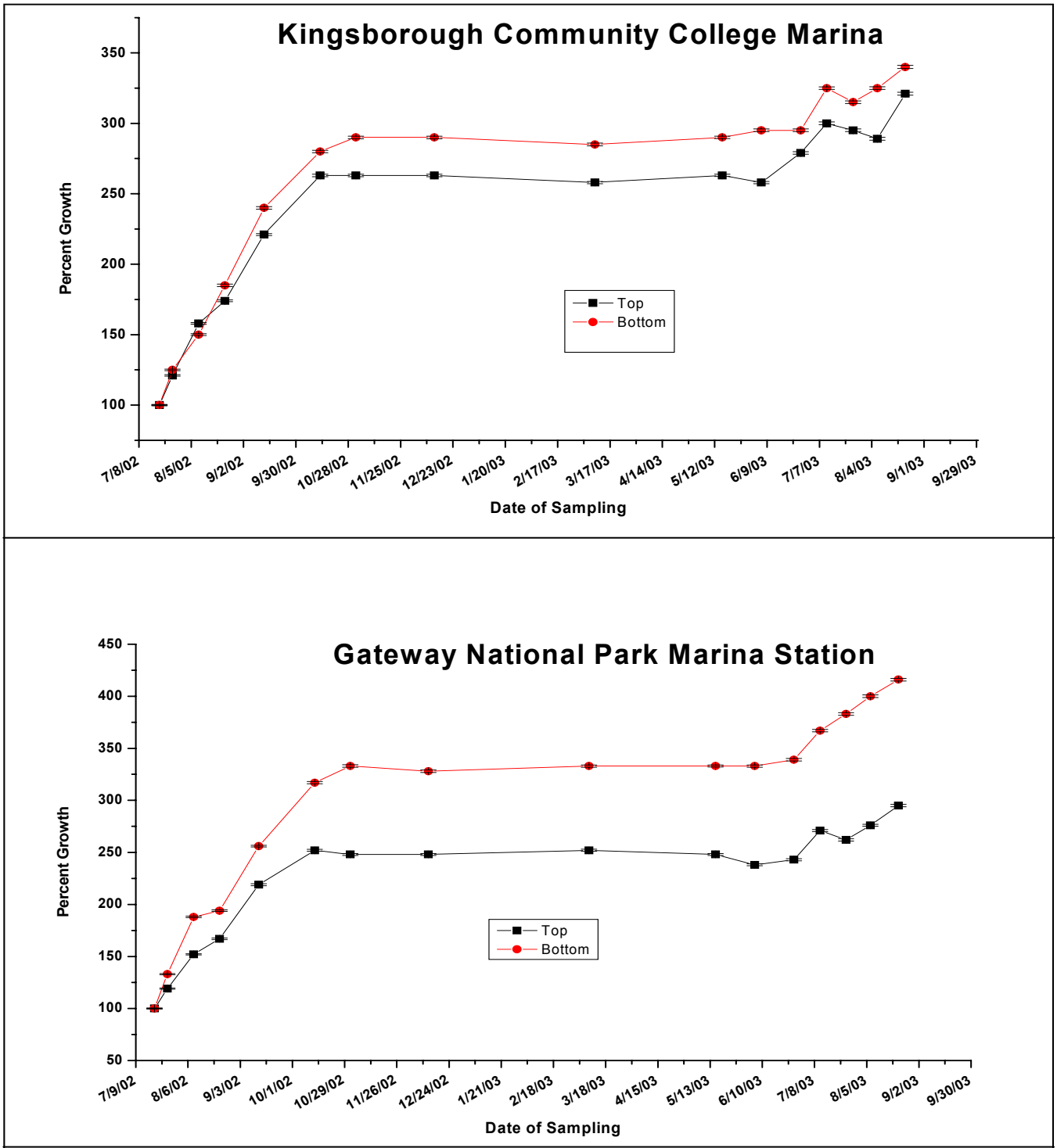

Figure 1a,b: 150 oyster seed were place in nylon containers and positioned in Taylor Floats either floating at the surface or just off the bottom. Their height were measure as indicated. The results of the bottom oysters are significantly different from those at the top.

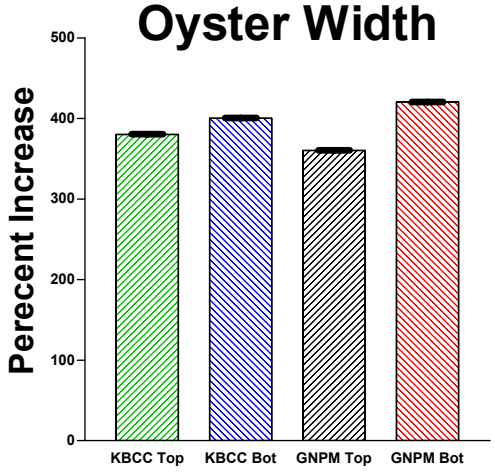

Figure 2: Measurements of oyster widths at the two sites. N = 150 oysters at each site. The differences in widths between the top and bottom oysters are significantly different.

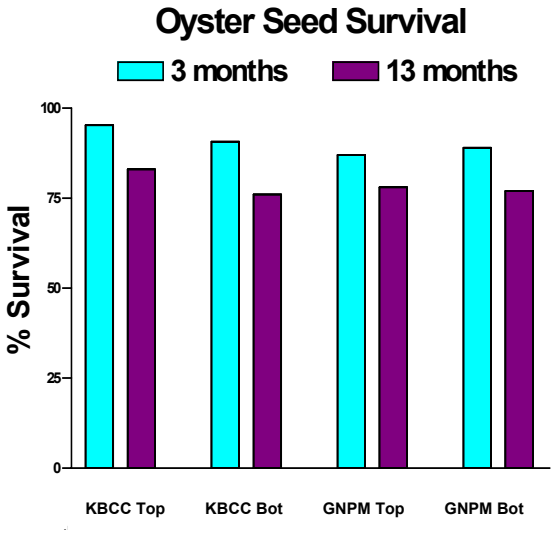

Figure 3: Measurement of oyster survival. N = 150 oysters at each site. There were no significant differences between survival rates at either site or between the tops and bottoms.

At 3 months survival was at least 87%, and by the end of the 13 month period oyster seed survival was at least 75%. Figure 4 shows an analysis of the top and bottom water quality at each site between July 2002 and August 2003. There were insignificant differences among the parameters monitored between the two sites or between the top and bottom. The only parameter that correlated significantly with oyster seed growth was water temperature with higher growth rates occurring during warmer periods. No growth correlations were seen with the other parameters measured.

For Figure 5 an underwater camera was used to film oyster seed and adults that were placed in an uncovered sunken float, left undisturbed at the bay bottom at KBCCM. Photographs indicate minimal siltation or fouling and no evidence of crab or starfish predation. After 1 year, seed grew and the filmed oysters appeared to be in good shape with no signs of mass predation or mortality. While performing maintenance on the floats containing the remaining cohort of the original 2001 oysters, we fortuitously found eight new seed oysters adhering to the inside of a nylon oyster bag, along with 2 seed clams (Figure 6). Two of the seed oysters were alive, 1 had recently died and the others had died at least a few days or weeks earlier based upon tissue degradation.

DISCUSSION

This study shows that oyster seed survived and grew very well under the conditions of our experiments. Top and bottom positioned oyster seed, which were protected from predators but still subject to pollutants in the sediment and waters of Jamaica Bay, had very good survival and excellent growth rates. At both sites, oysters positioned one foot above the bottom grew at a statistically significant greater rate than top-positioned

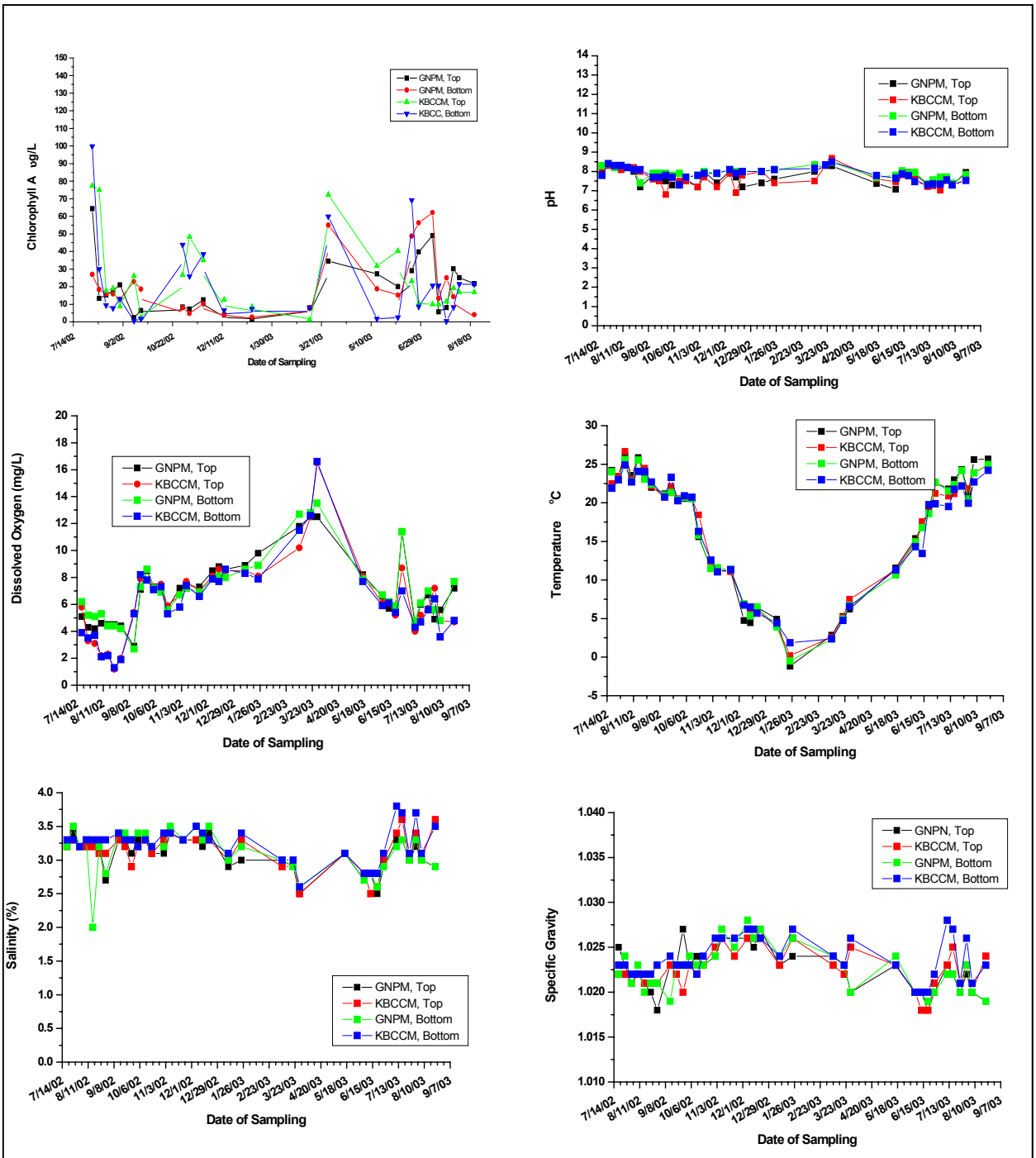

Figure 4: Analysis of water samples taken at the top or bottom at the Kingsborough Marina and Gateway National Park Marina.

oysters. Considering the high turbidity, contaminated sediment, and overall poorly judged benthic environment of Jamaica Bay, these findings were unexpected. Furthermore our results tend to contradict current aquaculture recommendations that stress the use of water column suspension techniques, a method that has become an integral part of the *C. virginica* industry. Water column suspension technique, also referred to as "off-bottom" culture is the major method used to cultivate oysters all around the world and was designed to minimize bottom predators, provide easier access for harvesting oysters, and maximize the use of a three-dimensional space for cultivation[23]. In Japan, one of the largest oyster producers, cultivation methods include rafts, lantern nets, and longlines. In Australia, another large oyster-producer, sticks and trays are used. In France and England, oysters are cultivated on off-bottom posts, in mesh nets, and on longlines. In 1962, Shaw reported that seed oysters suspended from log rafts in the waters of Chatham, Massachusetts grew almost twice as fast and had six times greater survival than bottom grown oysters[24]. Ruesink *et al*.[25] showed that bottom-placed *C. gigas* has a slower growth rate than those grown on PVC poles in the water column. In the water column, silt loading is reduced and presumably algal concentration is increased and these reasons are attributed to the observed faster growth in oysters in suspended culture rather than those kept on the bottom.

Many explanations can account for the differences seen in the top/bottom growth rates of our experiments. Oysters do best in areas where salinities are from 10 to 30 parts per thousand (ppt), water flow is adequate to bring food, sediment does not smother oysters, and oxygen concentrations remain greater than 3 ppm[22]. Our analysis of water quality parameters correlates well with what has been reported by others. Measurements taken by the United States Fish and Wildlife Service[2] in Jamaica Bay indicate average yearly ranges for temperature of 1 to 26°C, salinity of 20.5 to 26 ppt (2.05 to 2.6 %) dissolved oxygen of 3.5 to 18.5 milligrams/liter, and pH of 6.8 to 9. Algae growth is reported to be on the increase throughout the New York/New Jersey Harbor, possibly due to an increase in nutrients, especially nitrogen, into the Harbor and this trend is most noted as a major problem in Jamaica Bay. Through the late 1980s Jamaica Bay had summer chlorophyll-a averages in the 10-20 µg/l range; since 1992, values have ranged from 30-50 µg/l[10]. While our salinity values were higher than what has been reported by the USFWS, this can be explained by the fact that both sites at located near Rockaway Inlet and would therefore be more expected to have salinities closer to that of ocean water (35 ppt). While standard water quality parameters showed no significant difference between the GNPMS and the KBCCM sites or between top and bottom (fig. 4), correlating oyster growth to water quality is complicated and not straightforward.

The fact that our bottom-positioned oysters had faster growth rates might be site specific. Debris and organic pollutants floating on the bay surface may be a factor, as might the more constant stress of surface wave movements at both marina sites. While measurements of chlorophyll-a in surface/bottom water showed no consistent variation, the type and quality of microalgae available to the two groups of oysters might be different. Alternatively, our bottom-dwelling oysters may not have done as well if they weren't

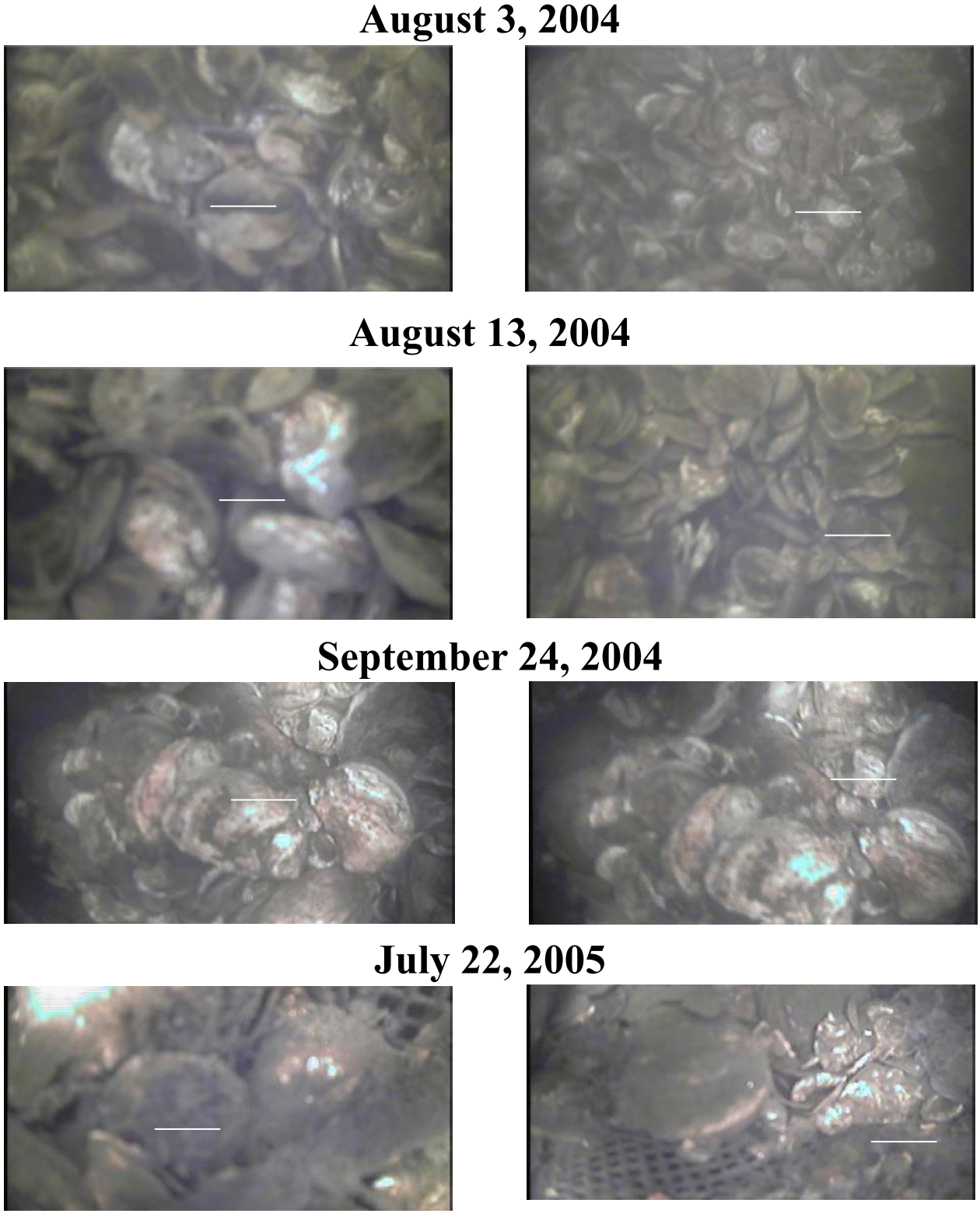

Figure 5: Underwater photos of seed (right) and adult (left) oysters placed unprotected on the bottom of the Kingsborough Marina site. Markers = 25 mm.

Figure 6: Picture of oysters which reproduced in the wild along with 2 clams.

periodically cleaned of silt and other fouling, or if they had been positioned directly and left undisturbed on the soft sediment of the bay. These are some of the factors that will be considered in our future studies of oyster growth.

Oysters provide a habitat for both commensal and competing organisms. The boring sponge, barnacles, tunicates, clam worms, and several species of algae were found living on both top and bottom positioned oysters with no major detrimental effects. Occasional small mud crabs were found with our protected oysters, and they were removed during float/cage maintenance. When not cultivated in protected environments, oysters are also subject to a variety of natural large predators. Depending upon salinity, oyster drills, rock crabs, knobbed whelks, channeled whelks and starfish can all prey on oysters, but none of these predators were found with our protected oysters. Photos of the oysters, which were positioned at the bottom in sunken floats without protection from large predators, also appeared to be surviving and growing well without signs of serious predation.

The severity of impacts by disease and parasites on oyster populations is thought to be related to water quality; higher salinity, high temperatures, and nutrient loading appear to make oysters more susceptible to disease. Protistan parasites, in particular *Perkinsus marinus* (Dermo disease) and *Haplosporidium nelsoni* (MSX disease) are currently considered to be major threats on the longterm survival of *C. virginica*[26]. While neither organism is harmful to humans, nor a health threat to humans who ingest infected shellfish, these parasites can chronically weaken and eventually kill *C. virginica* over a period of years[26, 27]. Both parasites thrive in salinities above 15 ppt and exhibit lowered virulence at lower salinities[28]. This may become a major problem for our transplanted oysters since both sites have higher than average bay salinity. We will be monitoring the infection rates of both these organisms as part of our long-term study on growth and survival of *C. virginica* in Jamaica Bay.

Historically, Jamaica Bay was a site of extensive oyster beds and shellfish culture leases that supported a significant oyster fishery in New York dating back to the 1800's. The Canarsie area in the northwest portion of the bay was a center of commercial and recreational fishing with a substantial shellfish industry for mainly oysters and hard clams that reached its peak in the early 20th century[29]. The decline in wild *C. virginica* stocks throughout the eastern seaboard of the United States[3] has led to concerted efforts focused on oyster restoration[30] in many areas including a major effort in Chesapeake Bay as well other efforts in Connecticut, Virginia, Massachusetts, Florida, and North and South Carolina. Locally, the NY/NJ BAYKEEPER® (Baykeeper) has been working since 1997 to restore oyster habitat at 3 sites in the Hudson-Raritan Estuary including Liberty

Flats in the Upper New York Bay, Keyport Harbor in Raritan Bay, and the Oyster Point on the Navesink River[31]. However, no such restoration attempts have yet been made in Jamaica Bay.

Jamaica Bay is one of the most valuable natural resources within the New York City area. Its ecological significance has been recognized by city, state and federal agencies. However, the bay continues to be threatened by poor water quality, loss of upland and wetland buffer, and disturbance of habitat areas[2]. Attempts to reestablish oyster beds to Jamaica Bay may be one way to help preserve/improve the bay's ecosystem. While modern sewage technology and regulatory efforts to arrest the further polluting of the bay are important, the resurgence of oysters will provide an added and natural means of improving Jamaica Bay water quality.

ACKNOWLEDGMENTS

This work was supported by grants 0516051091 of the New York State Education Department, 1R25GM62003 of NIGMS, 0516041071 of the New York State Education Department, the CUNY Groundworks Program, and 66288-0035 of PSC-CUNY to G. Sarinsky, M.A. Carroll, E. Nduka and E.J. Catapane. We thank Frank M. Flower & Sons, Inc., Oyster Bay, NY for supplying oysters. We thank the following students for their dedicated work on the project: J. Attia, W. Barreiro, S. Edwards, J. Espinoza, A. Greene, L. Gumenik, C. King, K. Lett, J. Luxama, S. Persaud, I. Reid, R. Rodriguiz, M. Stewart-Walker, M. Tejeda and O. Urdanigo.

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