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ST. JOHNS UNIVERSITY TO HOST THE 40th ANNUAL MACUB CONFERENCE SATURDAY, OCTOBER 20, 2007

PAUL FISHER AND SAM RHINE TO PRESENT KEYNOTE ADDRESSES

Paul Fisher

St. Johns University will host the 40th Annual Fall MACUB Conference on Saturday, October 20, 2007. The conference will feature keynote addresses by Paul B. Fisher and Sam Rhine.

Dr. Fisher is a Professor of Clinical Pathology and a Chernow Research Scientist in the Departments of Pathology and Oncology, and Neuro-Oncology Program Director at Columbia-Presbyterian Medical Center. Dr. Fisher recently discovered six novel genes that could change human melanoma cells in culture, and other human tumor cell lines, into cells that lose cancerous properties. His other undertakings include making monoclonal antibodies (MAb) specific for cancer antigens through surface epitope masking; upregulating tumor surface antigens for improved immune recognition and enhanced MAb-tumor targeting through targeted antigen modulation; and developing better approaches to identify, isolate, and clone new cancer genes, including tumor suppressors and genes involved in making cancer cells more invasive and metastatic.

Sam Rhine

Sam Rhine has attended Indiana University, Indiana School of Medicine, and Harvard Medical School. He has devoted himself to genetics education for 20+ years. He is a gifted speaker with a passion to teach the world the applicability of genetics to daily living. Sam specializes in the most current information in such fields as Human Genetics, Biotechnology, Cloning, Stem Cells, Reproductive Biology, Prevention of Birth Defects and the Prevention of AIDS. He will present the latest technologies and information available on stem cell research.

For the past several years, Sam has concentrated on presenting Genetic Update Conferences. These one-day conferences for biology teachers and students are designed to teach the latest in genetic advances, hot research areas, and career opportunities. Sam takes biology from the textbook to the heart by posing ethical dilemmas we each will face as technological advances continue.

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Color Vision and Oxygen Uptake in *Fundulus heteroclitus*

by
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Introduction

Most fishes are thought to have color vision[1]. Few studies have been performed that examine the effect of background color of holding tanks on the respiration of fishes[2]. In one study the behavior of Nile tilapia, *Oreochromis niloticus,* was examined when exposed to black, blue, green, yellow, red, and white colors. Here the highest respiratory frequency of fish occurred in a white background. This observation was made by counting opercular movements. In a study[3], employing the same six colors, the VO_2 (mls O_2/ gram of fish/per hour) uptake of white perch, *Morone americana,* denied access to surface waters was determined. The lowest VO_2 occurred in black vessels, with fish in green vessels having the highest VO_2.

The purpose of this study was to determine the VO_2 in the killifish, *Fundulus heteroclitus,* exposed to the colors previously mentioned.

Materials and Methods

Fish were obtained from North Pond, a fresh water pond located on Sandy Hook, New Jersey, during August and September, 2006. Fish were maintained in a university laboratory for two weeks prior to testing, in green colored tanks, at 22°C in aged tap water. Twenty-four tests, each of which included the six colors, were performed using 144 fish. Males and females were tested separately, using a single fish in each vessel. Fish were weighed (Ohaus model B300) at the conclusion of each test. The test vessels used, dissolved oxygen readings, and VO2 determinations were made as described elsewhere[3].

To determine if background color resulted in differences in VO_2, analysis of variance (alpha = 0.05) was performed for males and for females. If significant differences occurred Tukey's test of the means was performed. The same sequence of analyses were performed for males and for females, for their weights. Illumination above the test vessels and beneath the acetate cover within the test vessels was determined with a foot candle light meter (Ex Tech Inst. #401027, Waltham Mass).

Results

The VO_2 for both males and female fish in black vessels were significantly lower (alpha = 0.05) than for those fish in red vessels. The VO_2 was lower for both sexes in black vessels than for fish in blue, green, white and yellow vessels, but not significantly different (Table 1). There was no significant difference in the weight of fish tested in the different colored vessels for either males or females (alpha = 0.05) (Table 2).

For the fishes tested, males weighed approximately 20 per cent less than the females, but had a 13 percent higher VO2 than females (Tables 2, 3). Foot candles (FC) above the vessel covers was 25. Within the vessels FC ranged from 0.7 to 4.3 (Table 3).

Discussion

Fish in black containers of either sex had lower VO_2s than fish exposed in other colors. There was no apparent relationship to the FC. Red had the second lowest FC after black. In a study[3] fish exposed in black vessels also had the lowest VO_2. However, the highest VO_2s in this study occurred in red vessels whereas the highest VO_2s in the other study occurred in green vessels[3]. The other study utilized white perch. This suggests that different cone arrangements are operating, and that color reception varies in different species. For example, the goldfish retina contains rods and four cone types in juveniles, and three cone types in adults[4].

In another study using the same colors, but with a different type of apparatus it was observed that respiratory frequency was lowest in fish in a red background and significantly higher in fish in a white background[2]. In that study opercular movements were counted to determine respiratory activity. That method is not however a quantitative measurement such as the examination of actual O_2 uptake employed in this study since the depth of respiration may result in greater O_2 uptake. For example, in one study[3], reference is made to a yawning movement that occurs three to four times

Table 1. VO_2/gram/hour for male, and for female *Fundulus heteroclitus* in different colored vessels.						
	Black	**Blue**	**Red**	**Green**	**White**	**Yellow**
VO_2, Males	0.18	0.22	0.24	0.22	0.21	0.20
VO_2, Females	0.16	0.18	0.22	0.18	0.19	0.19

Table 2. Weight (grams) comparisons for male, and for female *Fundulus heteroclitus* in different colored vessels.						
	Black	**Blue**	**Red**	**Green**	**White**	**Yellow**
Weight, Males	5.28	4.68	4.64	4.65	4.57	4.52
Weight, Females	5.95	6.45	4.64	6.18	5.66	5.06

Table 3. Foot candles in the test vessels beneath the transparent covers.						
	Black	**Blue**	**Green**	**Yellow**	**Red**	**White**
Ft Candles	0.7	3.7	1.5	5.1	1.1	4.3

each hour. That movement was observed for fish in all colors except for those in a black background. This movement affects the respiration rate and its resulting VO_2.

The smaller (average weight) males, except in red vessels where the average weight of males and females were the same, had higher VO_2s than females. When small and large adults of a species are compared, the total metabolism of the larger animals is higher, but the metabolic rate of the small exceeds that of the large[5].

There was no correlation between foot candles within the vessels and VO_2 for either males or females.

Further tests of small, shallow water fish species are planned. Of interest is if the VO_2 in fish exposed to black backgrounds is consistently lower in comparison to fish exposed in other colors, and if colors other than red and green result in highest VO_2s, using this system of measurement.

Cone ratios and types, and their arrangement in the retina vary in species. Even though fishes may live in the same general habitat (e.g., shallow water) they can have different absorbance values, depending on microhabitat, niche, and other factors[6]. These factors probably affect visual responses and concomitant physiological responses, including VO_2.

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A Remnant Stand of *Pinus taeda* L. the Bigwood Stand, Hertford County, North Carolina

by

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ABSTRACT

A forest containing over mature loblolly pine, *Pinus taeda* L., the Bigwood Stand, Hertford County, North Carolina, was sampled with twenty 10x 10 meter quadrats. All trees with a dbh of 7.6 cm or greater within these quadrats were sampled. *Liriodendron tulipifera* ranks first in relative dominance with a RDo of 28.3 at this site. *Pinus taeda* ranks second in relative dominance (RDo value of 21.3). The average age of the surviving loblolly pine at the Bigwood Stand was 175 years. Though *Pinus taeda* is declining, it is still a conspicuous component of the Bigwood Stand, Hertford County, North Carolina. The loblolly pine sere may persist for a longer period of time on lowland sites than predicted in current ecological texts.

Introduction

Students of ecology have long appreciated the concept of succession, an orderly process of community development that is generally directional and therefore predictable[1,2,3]. Secondary succession occurs on sites of previous plant habitation[3]. Secondary succession also occurs when natural vegetation is disturbed or destroyed by fire, lumbering, windthrow or logging[1].

Billings[4,5] studied secondary succession on the Piedmont of North Carolina near Durham, North Carolina. His dissertation at Duke University[4] was published in Ecological Monographs[5]. Billings noted that *Digitaria sanguinalis* (crab grass) was the first annual to dominate abandoned fields. By late summer after the first year of abandonment, seeds of *Conyza canadensis* (Horseweed) were carried to the field; these germinated and by early winter produced conspicuous rosettes. Horseweed was abundant in early summer in two year abandoned fields. *Ambrosia artemisiifolia* (ragweed) was also common and was joined in late summer by perennial aster, *Aster ericoides* (white aster). *Andropogon virginicus* (broom sedge) was the dominant species during the third growing season. Pines, especially *Pinus echinata* (short leaf pine) may get established in Peidmont fields following three years of abandonment, and by ten years was the most conspicuous member of the old-field community with a density of over 7,500 individuals per hectare. Hardwoods, especially *Quercus spp*. (Oaks) become more numerous than pines by the 50th year but may not become dominant until the developing community is 100 years or older.

Many ecology texts, namely Oosting[6] Odum[2] and Smith and Smith[3] note the decline of pine in aging communities. Smith and Smith[3] recorded that *P. echinata* declined to near zero individuals after 110 years in old fields of the North Carolina Piedmont. Yet there are several papers that indicate that the pines sere persists for a far longer time[7,8,9].

Pinus taeda L., loblolly pine, is the most commercially valuable pine in the southeastern United States. *Pinus taeda* is also known as oldfield pine, North Carolina pine and Arkansas pine. Loblolly pine's natural range is from southern New Jersey south to central Florida and from Florida, west to eastern Texas. It is the most commercially important species in the coastal plain and Piedmont of North Carolina and comprises over half of the standing pine volume in the southern United States[8].

Pinus taeda is "medium lived" pine[8]. Trees older than 200 years are rare though several investigators Stalter[7] Baker and Landon[8], and Pederson *et al.*[9] have reported individual trees over 200 yrs. old. Stalter[7] reported a giant Loblolly in the Congaree Swamp, South Carolina, with a dbh of 5' to be approximately 250 yrs. old in 1968. This tree survived Hurricane Hugo, September, 1989, and is still alive as this paper goes to press. Baker and Langdon[8] reported a 245 yr. Loblolly pine in North Carolina, one of 20 individual trees in a stand of *P. taeda* with an average age of 240 yrs.

Hurricane and hurricane spawned tornadoes are responsible for extensive damage in southeastern forests. Hurricane Hugo, one of the most powerful storms to strike South Carolina during the 20th century, ravaged the Congaree Swamp with hurricane force wind gusts and tornadoes September 21 and 22, 1989 destroying many of the mature *P. taeda*.

Students of ecology have long-noted the role of pine in community development in the southeastern United States[1,2,4,6,10]. The pines are gradually replaced by hardwoods especially oak (*Quercus spp*.) and

hickory (*Carya spp*.). Many ecology texts[2,6] include this information in chapters on "community ecology/community development/plant succession." According to Oosting[6], a climax forests of oak-hickory is attained on abandoned farmland in the Piedmont of North Carolina after 150 yrs.

There are exceptions to the time it takes hardwoods to assume dominance in burned, abandoned, wind-throw or cut-over land in the Piedmont of the Carolinas. Stalter[7] described a mature stand of *Pinus taeda* in the Congaree Swamp, South Carolina where most of the pine were 100-175 yrs old. *Pinus taeda* was the dominant tree in the portion of the Conagree Swamp sampled by Stalter[7] with a relative dominance (percent basal area) value of 48.

Jones et al[11] described an old-growth forest stand of the Boiling Springs National Area, South Carolina. Diameter of the largest and oldest *P. taeda* here ranged from 90 to 110 cm. Two 107 cm *P. taeda* were cored and found to be 123 and 150 years old respectively. Jones *et al*[11] reported that 150 yr. old Loblolly Pine approached the maximum age attained for Loblolly Pine in the Piedmont of South Carolina. Jones *et al*[11] cited Batson et al's 1955 study at Boiling Spring[12] where there were score or more *P. taeda* with trunk diameters ranging to an excess of 1.5m. Twenty five years later, in 1980, the number of large *Pinus taeda* at Boiling Spring had been reduced to ten individuals; over half of the large *P. taeda* reported by Batson *et al*[12] died.

Pederson *et al.*[9] discussed the age structure and possible origins of old *Pinus taeda* stands in the Congaree Swamp, South Carolina. Two of the stands in the Pederson *et al*[9] study, stand six and seven, contained large old *P. taeda*. The average age of the *P. taeda* cored in stand six was 137 years with a range of 122-156 years while average
age in the stand seven was 157.5 yrs, with a range of 124-227 yrs.

Pederson *et al.*[9] reported the there were two or more age classes of *Pinus taeda* in the Congaree Swamp, South Carolina. Pederson *et al*[9] claimed that Swails *et al*[13] and Stalter[7] reported even aged stands of *Pinus taeda* in the Congaree Swamp. Yet the second line of the abstract of Stalter's[7] Congaree Swamp paper states, "Annual ring counts indicated that most trees (*Pinus taeda*) are 100-175 years old." Stalter[7] also reported in the first sentence, second paragraph, of the results section, "Most of the *P. taeda* in the stand are from 60 to 98 cm in diameter. Fifty borings and the annual ring counts on the stumps of three cut pine indicate that most individual *P. taeda* are 100-175 years old; a few trees are older." In spite of the aforementioned information, Pederson *et al*[9] claimed that Stalter[7] described, "an even aged stand," of *Pinus taeda* in the Congaree Swamp.

Methods

Trees in the Bigwood Stand with a dbh 7.6 cm or greater were sampled in twenty 10x 10m quadrants (Table 1). An additional stand of *Pinus taeda*, a 110 yr stand located within Duke Forest, Durham, North Carolina was also sampled by the twenty 10 by 10m quadrats. Relative dominance data, RDo, for the trees found within the "Duke Forest" is presented in Table 2. Density and frequency data for *Pinus taeda* stands varying in age from 9 yrs to 180 yrs are presented in Table 3. Nomenclature follows that of Radford *et al*[14].

Results and Discussion

Acer rubrum, the most abundant tree at the study site, had the highest importance value, 46.50, *Liriodendron tulipifera* attained the highest relative dominance (RDo) value followed by *Pinus taeda* (Table 1). Other trees listed in order of decreasing relative dominance are *Liquidamber styraciflua, Quercus falcata* and *Carya spp*. *Acer rubrum* ranks first in relative density followed by *Carya sp., Quercus falcata, Oxydendron arboreum* and *Liquidamber styraciflua. Pinus taeda* ranks 8th in relative density.

Living *P. taeda* were observed in four quadrats (Table 3). Dead loblolly pine, either standing or fallen were observed in six quadrats, including five quadrats where no living Loblolly pine were observed. One dead standing *P. taeda* with dbh of 117cm was the largest loblolly pine in North Carolina when it was alive.

Most of the living *P. taeda* in the stand ranged from 35 to 93cm in diameter. Annual ring counts provided by the International Paper Co. on living *P. taeda* in 1995 indicated that most *P. taeda* were over 175 years old. Although the living loblolly pine appeared to be healthy, there were more dead standing or recently fallen *P. taeda* at the site than living trees.

Data presented in Table 2 shows the relative importance of *P. taeda* to hardwoods in old field succession on lowland sites. The data for stands 15 to 90 years old were transposed from counts made by Oosting[1]. Data for the 110 yrs stand are from the Mud Creek site, Duke Forest,

Table 1. Density, (D) Relative Density (RD) Frequency (F) Relative Frequency (RF) Basal Area (BA) Relative Dominance (RDo) and Importance Value for trees in the Bigwood Stand, Hertford County, North Carolina.

Species	D	RD	F	RF	BA	RDo	IV
Acer rubrum	1.05	21.8	65	21	358.96	3.7	46.5
Liriodendron tulipifera	0.3	6.3	25	8.1	2746.53	28.4	42.8
Liquidambar styraciflua	0.5	10.4	30	9.7	1832.34	18.9	39
Quercus falcata	0.6	12	40	12.9	1302.98	13.5	38.9
Pinus taeda	0.2	12.5	20	6.5	2058.53	21.3	32
Carya spp.	0.7	4.2	32	11.3	560.79	5.8	31.7
Oxydendron arboreum	0.6	1406	45	14.5	342.42	3.5	30.5
Quercus alba	0.45	12.5	20	6.5	111.52	1.2	17.1
Cornus florida	0.1	9.4	5	1.6	14.14	0.1	3.8
Carpinus caroliniana	0.1	2.1	5	1.6	62.84	0.7	4.4
Ilex opaca	0.005	2.1	5	1.6	98.18	1	3.6
Fraxinus spp.	0.005	1	5	1.6	63.62	0.7	3.3
Quercus michauxii	0.005	1	5	1.6	50.27	0.5	3.1
Quercus phellos	0.005	1	5	1.6	78.54	0.8	3.2
			99.9	100.1	9881.66	100.1	

Table 2: The relative importance of *Pinus taeda* and hardwoods in old field succession on lowland sites.

Age of Stand (Years)	Relative Density (%)	Relative Dominance (%)
15	92	99
18	89	99
34	30	88
45	22	64
90	15	76
110	23	76
180	4.2	21.3

The data for all stands except the 110 and 180 year old stands were transposed from the counts made by Oosting (1942).

Table 3. Density and Frequency of *Pinus taeda* in a successional series of seven bottom land pine stands.

Age of Stand (Years)	Density	Frequency
15	20.3	100
18	19.3	100
34	7.9	100
45	4.9	100
90	3.0	90
110	1.1	95
180	0.2	20

Durham North Carolina measured by Stalter and Dial in 1997. Pine comprised almost all the percent basal area (RDo) in 15 and 18 yrs stands, and approximately 75 percent of the relative dominance of stands of 100 yrs old. Although the density and frequency of *P. taeda* declines from 15 to 110 yrs, relative dominance declined very slowly (Tables 2 and 3). At sites over 150 yrs, *P. taeda* has low density and frequency values, (Table 3) but because of its large trunk diameter (dhb) *P. taeda* is still and important component of the forest (Table 2).

Pomeroy (1968 personal communication) stated that *P. taeda* in the Bigwood Stand, Hertford County, North Carolina averaged 145 yrs when cored in 1950. While the Bigwood Stand was thriving at the sites in 1968[7], *P. taeda* has declined rapidly in the past 30 years[7]. There were more dead standing trees, and fallen *P. taeda* present at the site in 2002 that living Loblolly Pine. The data in this study suggest that *Pinus taeda* may remain dominant on lowland sites in the southern United States, for 150 years or longer if it can escape logging, wind throw, insect infestation and if it can escape cutting[7,9,12,15].

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The Effect of Grape Seed Extract on Hematopoietic Stem Cells in the Umbilical Cord Blood

by
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ABSTRACT

The human umbilical cord blood is known to possess more progenitor and hematopoietic cells than peripheral blood and is a very good candidate for studying the effect of natural health substances (NHSs) on monoucleocytes (MNC) subsets. Cells capable of initiating the human cell engraftment (Severe-Combined-Immunodeficiency-Repopulating cells) are contained in the $CD34^+$ cell fraction, which was the focus of the current study in the light of the effect of grape seed extract on hematopoietic stem cells in the human umbilical cord blood. The target cells were from the human erythroleukemia cell line K562 cells. Cell line K562 CLL 220 is the model karyotype of the human leukemia cell line, K562 (ATCC CCL 243).

Introduction

A well planned systemic approach for treating patients with cancer should aim at the multiple physiological and biochemical pathways, which involve tumor evolution. Such an approach should also control and restrict any damages to normal tissues. Angiogenesis is a basic course in the enhancement of cancer cells. Various natural health substances are reported not only to decrease angiogenesis but also described to have the potential of other anticancer properties. Herbalist have provided hundreds of years of remarkable information on the anticancer pursuit of numerous natural health substances. Natural health substances (NHSs), such as Resveratrol and Proanthocyanidin (grape seed extract, GSPE), not only are reported to have a high degree of antiangiogenic activity but also other numerous relationships that can hinder tumor series and decrease the risk of metastasis. NHSs target various molecular pathways other than angiogenesis, including vascular endothelial growth factor (VEGF)[1], epidermal growth factor receptor (EGFR), the HER-2/neu gene, the protein kinases, coagulation pathways and others. Laboratory studies are as well strengthening these relationships, which are already recorded in the customary texts. The following NHSs are routinely used for anticancer therapy and are antiangiogenic for the sake of multiple co-dependent courses: Quercetin dihydrate, Astaxanthin, Ginsenoside Compund-K (CK-1), *Cordyceps Sinensis* (TCS-W), Caffeic acid, Bilobalide, Caffeic acid, Eugenol, Rutin hydrate, wheat grass extracts, resveratrol, GSPE, and *Ganoderma lucidum*[2,3]. Grape

seed extract containing GSPE and other antioxidants are being used as nutritional supplements by many health conscious individuals. The advantegeous effects of GSPE have been reported, however, still little is known about their mechanism(s) of action. One of the beneficial effects of GSPE is chemoprevention of cellular damage. The exact mechanism by which GSPE mediates chemoprevention is not yet understood. Exposure to GSPE results in a significant reduction in apoptosis[4]. Conversely, VEGF is believed to be the most prevalent, efficacious, and long-term signal that is known to stimulate angiogenesis in wounds. The latter is rich in oxidants such as hydrogen peroxide contributed by neutrophils and macrophages. GSPE facilitates oxidant-induced VEGF-expression in keratinocytes. In fact, GSPE is a group of biologically active polyphenolic bioflavonoids that are synthesized by many plants. GSPE contain 5,000 ppm Resveratrol. GSPE can as well up-regulate hydrogen peroxide[1].

We investigated the phenomena of hematopietic stems cells in the human umblical cord blood cells, which were pretreated with the extract of GSPE. Up to 78% to 100% of the human umbilical cord blood cells express the hematopoietic progenitor-cell-surface- marker CD34, which can consistently engraft, develop, and proliferate in the hematopoietic tissues of sublethally irradiated severe-combined-immunodeficiency (S*CID)* mice[5,6,7,13]. Data suggest that cells capable of initiating the human cell engraftment, namely, SCID-repopulating cells are contained in the $CD34^+$ cell fraction, which was the focus of the current study. CD34 not only can be found on the hematopoietic stem cells; while conversely, CD45 can be identified on all nucleated hematopoietic cells.

Materials and Methods

This study was approved by the Internal Review Board of the Hospital, where babies were delivered. The study as well conformed to the Declaration of Helsinki. Each of the six individuals who provided umbilical cord blood gave written consent. The umbilical cord blood (UCB) was taken under aseptic technique from the umbilical vein after the full term baby was delivered and before the placenta was separated from the uterus.

Technique of Magnetic-bead Cell Separation

CD34CD45+hematopoietic stem cells separated from the UCB mononucleocytes (MNCs) were augmented with the procedure of a positive magnetic-bead cell separation (MACS, *Miltenyi Biotec, US).* We adapted the technique of Gritzapis, *et al.*[11]. The magnetically tagged cells were placed into two MACSy RS1 separation columns (Miltenyi Biotec), which had been equilibrated with the buffer solution in the magnetic field of the Vario MACSy separator (Miltenyi Biotec, US). The olumns were then prepared by rinsing with buffer solution. Retained cells were extracted from the column to ascertain that they were away from the magnetic field. Aliquots of the selected cells were then stained by the PC5/ FTIC labelled anti-$CD34CD45^+$ monoclonal antibodies (Coulter Immunotech, US) to examine the purity of $CD34CD45^+$ stem cells. This purity was ascertaind by flow cytometry analysis.

After enrichment of $CD34^+$ hematopoietic stem cells by FACS BD (Fluorescence Activated Cell Sortying) to isolate/identify cells expressing with a certain surface marker, the hematopietic stem cells were incubated for 7 days with six different concentrations of GSPE ranging from 10 to 100 μg/mL. The size of effector cells was assessed using Centricon (cut-off: 30 kDa, Amicon Millipore Co. US).

The Preparation of Monoclonal Antibodies

All monoclonal antibodies to surface antigens such as CD34, CD 45, CD26, CD19, CD14, and CD3 (FITC; Serotec, US) were obtained from Coulter Immunotech, US. We chose the following for this study: $CD34^+$-Enriched Cells originated from the umbilical cord blood[5], $CD45^+$ lymphocytes and granulocytes, CD14-a monocyte/macrophage differentiation marker[8], and CD26- a cell surface protease, expressed on many cells of

the immune system including some $CD4^+$ T-cells and macrophages[9].

The Activation of Effector Cells

Highly augmented $CD34CD45^+$ stems cell suspensions were cultured in a medium supplemented with RPMI-1640 for 24 hours (37^oC, 10% CO_2). Six different concentrations of GSPE and of positive controls respectively, ranging from 10 μg/mL to 100 μg/mL by serial dilution, were added to cell suspensions for preincubation treatment prior to the subsequent cytotoxicity test. The control group was treated with phosphate buffered saline (PBS). After 7 days of incubation, the cultures were washed with PBS and then re-suspended in a medium containing 20% fetal bovine serum (FBS), at which point they were ready for the cytotoxicity assay.

The Expression of Stem-Cell Mediated Cytotoxicity

The results of the effect of GSPE on hematopoietic stem cell mediated cytotoxicity were expressed as ratios of survival of K562 cells of GSPE treated groups versus the controls (K562 cells).

The Preperation of Target Cells

We used the human erythroleukemia cell line K562 (CCL-243, American Type Culture Collection (ATCC) as an MNCs-sensitive target for cytotoxicity assays. The percentage of lyses against target cells was measured and reported as the ratio of sample % vs. control % of cell gated in flow cytometry. Scores of 1.50 or higher were considered significant.

To detect target cell survival, we used Alamar Blue, a colorimetric indicator, which after uptake by living cells changes from a oxidized, non-fluorescent, blue state to a reduced, fluorescent, red state.

Flow Cytometric Attainment

Flow cytometry was performed with a FACSclibur cytometer (Becton Dickinson). The instrument was set for two-colour analysis using FACScomp software and was calibrated using Calibrite beads (Beckton Dickinson) with a threshold of 200 on Foward Scatter (FSC) to exclude debris. Data were collected in listed mode and analyses were performed using CellQuest software version 3.1f (Becton Dickinson) and Win MIDI version 2.8 software. At least 10,000 target cells were collected and analyzed. During the procedure, CD34, CD19, CD14, and CD3 were used against lymphocyte, and their surfaces were marked. Fluorescent markers, PC5-labeled methods and Fluorescein 5-isothiocyanate (FITC) labeled methods were used (Figs. 1, 3). For the cytotoxicity analysis, the tests were done at the different effector/target cells ratios of 5:1, 20:1 and 80:1. A two tailed p value < 0.005 indicates a statistically significant difference.

Results

After treating the mononuclear cells (MNCs) of the human umbilical cord blood (hUCB) with crude extract of NHSs, we observed the ratio change of sample % vs. control % gated in flow cytometry. Flow cytometric analysis of four different CDs (CD 34, CD 19, CD14, and CD 3) revealed that among CD34 hematopoietic stem cells, the ratio of samples % vs. controls % after the treatment with GSPE was 2.18, which was the highest among all the eleven NHSs. By comparison, the ratio after treatment with the extract of wheat grass was 0.61, which was the lowest, (Fig. 1).

Among the 11 NHS, there were 52 measures having the ratio of sample % vs. control % of cell gated in flow cytometry less than 1.50, while three greater than 1.50, (Fig. 2). The t test for independent samples was done. It revealed that the difference between these two groups was statistically significant (two tailed $p < 0.005$).

The purity of $CD34CD45^+$ stem cells was ascertained by flow cytometry analysis and scored up to 95%. The cytotoxicity at an effector/target (E/T) ratio of 5:1 was not significant, compared to that of the controls. Conversely, when E/T ratios were as high as 80:1, no high cytotoxicity effect was observed.

Such a phenomena was likely due to the over-saturation of cell numbers (data not shown). The highest level of cytotoxicity was noted at an E/T ratio of 20:1 when the effector cells were pre-incubated with 100 μg/ml GSPE, but not those at an E/T ratio of 5:1, and 80:1, respectively.

It is noted that there was individual variance of different fractions of stem cell size. Stem cell cytotoxicity increased by 1.57 fold while the negative control was 2.13 ($p<0.05$) for the fraction of cells sized > 30 kDa, whereas 1.07 fold ($p<0.05$) for those sized < 10 kDa after pre-treatment with 100 μ/mL of the GSPE extract. Note that, when compared with the untreated control, the negative control was 2.13. This ratio was significant for the former, > 30 KDa fraction of stem cells (1.57), while not significant for the latter, <10 Kda fraction (1.075), according to Nociari et al's method of separation[12] (Fig. 3).

By comparison, cells after enrichment of $CD14CD26^{+}$ MNCs/Macrophages revealed that their cytotoxicity increased significantly by the aforementioned E/T ratio of 1.51, and 1.613 ($p<0.01$) in pre-incubation with 1 μM and 10 μM, respectively of TPA (*a priori* tumor promoter 12-O-tetradecanoyl phorbol-13 acetate), which had served as positive controls (Data not shown).

Discussion

The human umbilical cord blood is known to possess more progenitor cells than peripheral blood, and is an excellent candidate for studying the effect of NHSs on MNC subsets. The UCB samples from six different individuals after treatments with GSPE exhibited changes in the ratio of sample % vs. control % gated in flow cytometry, predominantly for the hematopoietic stem cells with CD34CD45 staining in the current study. Recognizing the repopulating characteristics of the human hematopoietic stem/progenitor cell fractions is vital for predicting their

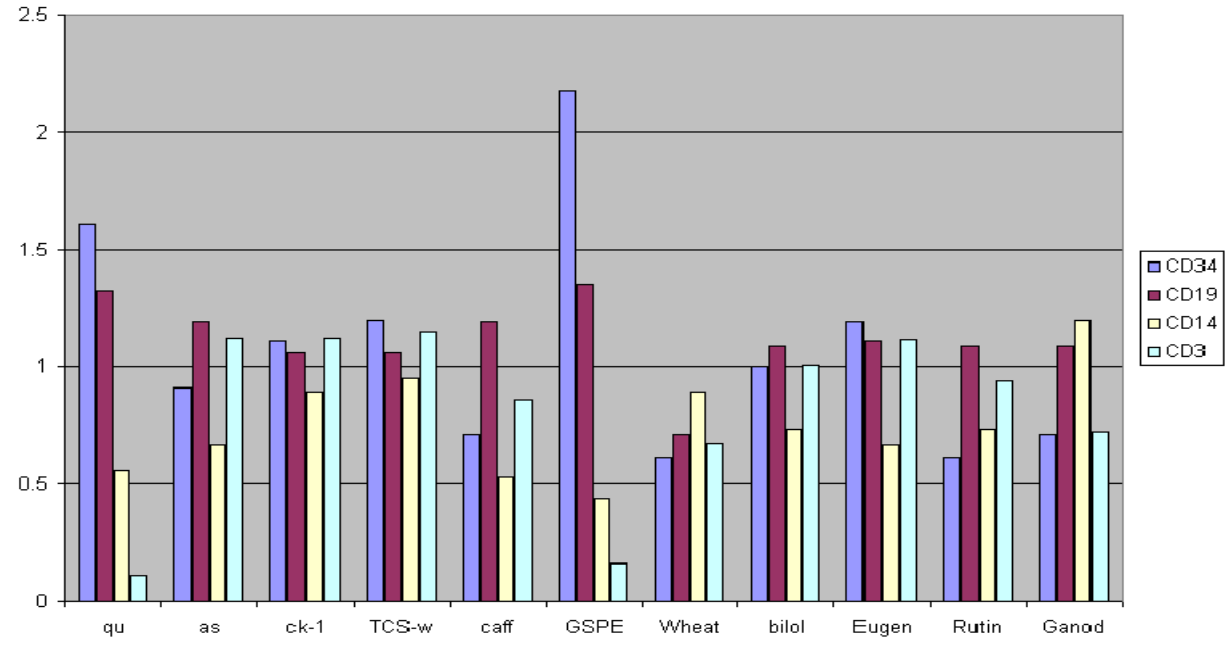

Fig. 1. Flow cytometric analysis of four different CDs (CD 34, CD 19, CD14, and CD 3) revealed that among CD34 hematopoietic stem cells, the ratio of samples % vs. controls % after the treatment with GSPE was 2.18 (See Y- Axis), which was the highest among the eleven NHSs (See X-Axis). By comparison, the ratio after treatment with the extract of wheat grass was 0.61, which was the lowest. CD34 can be found on cematopoietic stem cells; while CD45 can be identified on all nucleated hematopoietic cells.

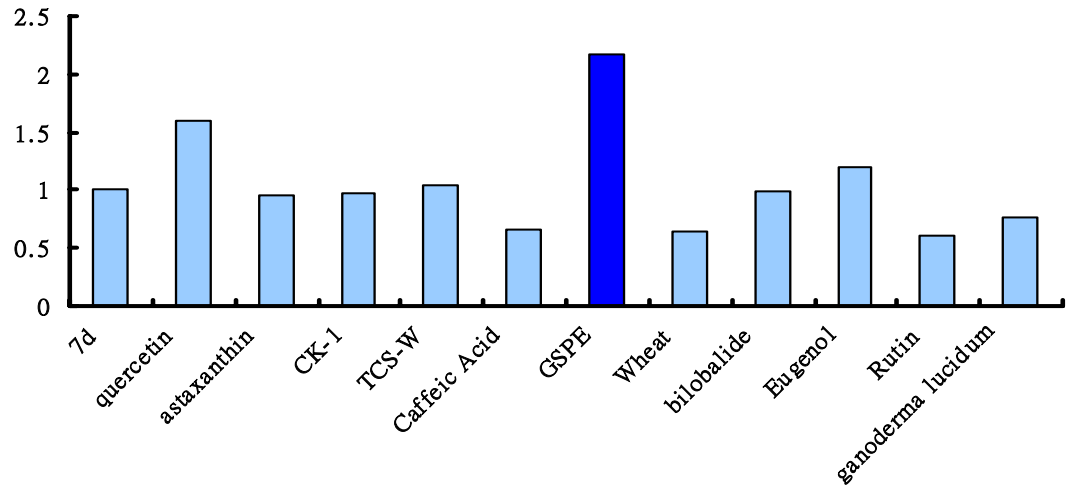

Fig. 2. Among 11 natural health substances (See X-Axis), there were 52 measures having the ratio of sample % vs. control % of cell gated in flow cytometry less than 1.50, while the remaining three greater than 1.50.

performance, for instance, after transplant into high-risk patients following high-dose of radiation therapy. Nevertheless, our preliminary investigation did have some limitation, which may temper our conclusion and should be addressed in future study. For instance, technically, the long diagonal lane of events in the flow cytometry seems to include the sheer quantity of some dead cells, which may in turn affect the counting of precisely real CD34-stained cells. Hence, some experimental aspects must be more profoundly assessed for future evaluation.

Conclusion

On balance, with new developments[14], the future identification and purification of the molecule(s) of the crude extracts of GSPE, which are active for augmenting development and proliferation of the hematopietic stem cells in the human umbilical cord blood, may open a new avenue of managing angiogenesis, and conversely, may as well potentially promote the oxidant-induced VEGF-expression.

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Bioaccumulation and Tissue Distribution of Arsenic, Cadmium, Copper and Zinc in *Crassostrea virginica* Grown at Two Different Depths in Jamaica Bay, New York

by

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ABSTRACT

Historically, Jamaica Bay was a site of extensive oyster beds and shellfish culture leases that supported a significant oyster fishery in the New York area. The industrial and urban expansion of the early 1900's led to over-harvesting and a deterioration in water and bay sediment quality that coincided with shellfish decline and the ultimate disappearance of oysters from the bay. Over the past 50 years, efforts to arrest and reverse the pollution problems of Jamaica Bay have been undertaken but the area still contains metals and other pollutants at levels higher than NYS Water Quality Standards. Previous we showed that *Crassostrea virginica* seed transplanted to the bay had excellent growth and survival despite the bay's pollution problems. In this study we measured the one-year bioaccumulation and tissue distribution of four metals in *C. virginica* seed that were transplanted to the bay at two different depths: one foot from the surface and one foot above the sediment. Tissues of *C. virginica* were dissected, dried and digested in nitric acid. Arsenic, cadmium, copper and zinc levels were measured using electrothermal vaporization with deuterium lamp background correction in an atomic absorption spectrophotometer fitted with a THGA graphite furnace. Metals were distributed in the various tissues in µg/g dry weight amounts, which correlate well with published values for whole oysters grown in other polluted areas. Metal distributions were not homogeneous throughout the animals and in most of the tissues tested, oysters grown near the surface accumulated more metal than those positioned near bay sediment.

Introduction

Jamaica Bay, a 26 square mile estuarial embayment situated between southern Brooklyn and Queens, NY and a major inlet opening to the Atlantic Ocean, lies just east of the entrance to NY Harbor and the mouth of the Hudson River. It is home to the Jamaica Bay Wildlife Refuge, which encompasses 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. 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[1].

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 Americans and early European colonists[2]. Historically, *C. virginica,* flourished in Jamaica Bay and the NY/NJ Harbor area as either self-sustaining or farmed populations[3]. Jamaica Bay's oyster industry observed a steady decline in production after its peak in the early 1900's[4]. 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 rapid urbanization and local industrialization of the area at the turn of the century was followed by years of unregulated

industrial, urban and residential dumping which contaminated bay sediment, causing adverse effects on benthic organisms and bioaccumulation further up the food chain. 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. The shellfish problem was not unique to Jamaica Bay for around the same time declines in estuarine shellfish populations had occurred throughout the entire east coast of the United States and other important oyster fisheries, like that of Chesapeake Bay and Virginia's James River, had also started to collapsed[5,6,7]. 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.

Over the last 50 years, major efforts have been undertaken by federal, state and local authorities to arrest and reverse the pollution problem and while natural stocks of *C. virginica* have not returned to the bay, there has been a resurgence of many other marine organisms. Despite improvements, Jamaica Bay still exhibits poor water quality[8] and studies indicate the presence of various metal pollutants, including arsenic, cadmium, chromium, copper, iron, lead, mercury, nickel and zinc, in bay sediment[9,10,11,12,13,14,15]. Heavy metals have been found to inhibit growth in a variety of mollusc species including oysters[16,17,18]. In 2002, our lab did a study to determine the growth and survival *of C. virginica* seed transplanted to Jamaica Bay in protected containers at two ecologically different locations, at two different depths: one foot below the surface and one foot off the bottom. Despite bay conditions, the oysters at both locations and both depths had excellent growth and survival rates[19]. The results of this project generated many new questions such as: to what extent are oysters acquiring bay pollutants in their tissues, how are they able to adapt to this stress, what are the physiological effects on their various organ systems, and what are the consequences of the polluted environment on the long term survival and reproductive success in Jamaica Bay.

Bivalves are particularly good accumulators of heavy metals[20,21,22,23] and being sessile, tend to reflect local contaminant concentrations more accurately than crustaceans and free-swimming finfish. The EPA considers *C. virginica*, as one of six target bivalve species recommended for contaminant monitoring in Mid-Atlantic coastal waters including Jamaica Bay[24]. Target metal analytes on the EPA Fish Contaminant Workgroup list include arsenic, cadmium, mercury, selenium and tributyltin[25]. The Mussel Watch Project of the National Oceanic and Atmospheric Administration (NOAA) monitors copper, lead, nickel, and zinc, in addition to the analytes on the EPA's target list[26]. Numerous other reports have been made on the bioaccumulation of these and other heavy metals in oyster species of in the US and around the globe[27,28,29,30,31,32].

In this study we measured the one year bioaccumulation and tissue distribution of four metal pollutants (arsenic, cadmium, copper and zinc) in *C. virginica* seed, that had been transplanted to the bay in 2002 at two different depths: in floats, one foot below the surface, and in hanging nets, one foot off the sediment. Since Jamaica Bay sediment is a major reservoir of metal pollutants, it was hypothesized that metal accumulations might be greater in the tissues of bottom positioned oysters, since they were grown nearer to a more concentrated source of metal pollutants.

Materials and Methods

In June 2002, four modified Taylor Floats[33] 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 in Jamaica Bay at Fort Tilden at Gateway National Park Marine Station (GNPMS), and at Kingsborough Community College Marina (KBCCM) in Brooklyn's Sheepshead Bay, a large cove of Jamaica Bay (NY/NJ Baykeeper license #LG P00 583). The two other floats that were designed to sink and position the oyster seed 1 foot off the bay bottom, were placed at the same two sites. Both surface and bottom floats were inspected and cleaned of any fouling, biweekly in the summer and monthly in the winter. After 2 months, the bottom floats were deemed clumsy/difficult to work with and were replaced with commercially constructed hanging nets, suspended one foot off the bottom, for the rest of the experiment.

Seed survival and growth, as well as various parameters of bay water quality including temperature, pH, dissolved O_2, chlorophyll a, salinity, and turbidity were monitored throughout the one year experimental period. In July 2003, samplings of one-year old oysters were removed from each site and depth to determine the bioaccumulations of arsenic, cadmium, copper and zinc in various tissues.

Prior to tissue isolation, all glassware was acid-washed in dilute (5%, wt/wt) nitric acid in deionized water to remove any bound metals. Washing was followed by a thorough rinse with deionized water to remove any remaining acid. All acids used were Fisher trace-metal grade. Representative oysters were cleaned, shucked, and their tissues dissected to determine metal bioaccumulations. The mantle, gill, palps, posterior adductor muscle and stomach were removed with stainless steel instruments. The tissues were blotted and placed in pre-weighed Pyrex flasks to determine wet weights. A 1-2 g sampling of each animal's shell was also removed, weighed, and processed similarly for metal analysis. All tissues were dried in an oven at 120°C and reweighed to determine dry weights. Dried tissue samples were digested in concentrated nitric acid on hotplates. Digested samples were adjusted to a final volume of 10 ml in dilute (0.2 %, wt/wt) nitric acid. Aliquots of each sample were analyzed for arsenic, cadmium, copper and zinc determinations by electrothermal vaporization with deuterium lamp background correction in a Perkin Elmer AAnalyst 800 Atomic Absorption Spectrophotometer with a THGA Graphite Furnace. Metal levels were recorded as µg/g dry weight. Statistical analysis was determined by a Wilcoxon Matched-Pairs Signed Ranks Test using GraphPad InStat version 3.00.

Results

C. virginica at both locations and depths were deemed to have excellent growth and survival. After one year, oyster seed at both sites and depths had over 75% survival, and growth, as measured by shell height, had increased over 300% with average growth rates for bottom maintained oysters exceeding those maintained near the surface by approximately 20%[19].

Figures 1-4 show the one-year bioaccumulations of arsenic, cadmium, copper and zinc in the mantle, gill, palps, adductor muscle, stomach and shell of top positioned oysters compared to bottom positioned oysters. For each metal, top oysters from both sites (GNPMS and KBCCM) were combined, and bottom oysters from both sites were combined, to generate top/bottom comparisons in each chart of the figure. No significant difference in tissue metal accumulation could be discerned between top position oysters at GNPMS and top positioned oysters at KBCCM, or between bottom position oysters at GNPMS and bottom positioned oysters at KBCCM (data not shown). The various tissues of the one-year old Jamaica Bay oysters readily accumulated arsenic, cadmium, copper and zinc. The metal content of the tissues were not homogeneously distributed and were different for the oysters maintained at the top as compared to the bottom. Soft tissues accumulated metals in the µg/g dwt range,

which is comparable to other published reports for *C. virginica* growing in other areas[34,35]. Of the four metals tested, zinc showed the most bioaccumulation in all oyster tissues including shell.

In soft tissues, zinc accumulations were greater than copper, which were greater than cadmium. Arsenic showed the lowest soft tissue accumulations. For arsenic, cadmium and copper, the gills, mantle and palps accumulated the most metals; while for copper, the stomach accumulated considerable more than the other tissues. With all four metals, the soft tissues of oysters positioned one foot below the surface accumulated more metal than their bottom positioned counterpart, with the exception of stomach which not only showed very high copper accumulations but demonstrated a greater copper accumulation in bottom dwelling oysters.

Shell tissue also accumulated metals but to a lesser extent. With the exception of zinc, shell metal accumulations were 10-100x less than metal accumulations seen in any one type of oyster tissue. Zinc and copper accumulation were in the µg/g dwt range while cadmium and arsenic accumulations were in the ng/g dwt range. Average shell zinc levels (µg/g) were higher in top positioned oysters (top: 123 ± 20, bottom: 47 ± 16); average shell cadmium levels (ng/g) were higher in top positioned oysters (top: 28 ± 9, bottom: 12 ± 4); and average shell arsenic levels (ng/g) were higher in top positioned oysters (top: 213 ± 37, bottom: 92 ± 18). Only copper showed slightly higher average accumulations (µg/g) in bottom positioned oysters (top: 1.033 ± 0.145, bottom: 1.450 ± 0.352.

Discussion

Marine bivalves are filter feeders that take up and accumulate metals and other pollutants from the water column or via ingestion of contaminants adsorbed to phytoplankton, detritus and sediment particles. Because they are sessile, they reflect local contaminant concentrations more accurately than crustaceans and free-swimming finfish. Marine bivalves such as oysters and mussels have been extensively used as model organisms in environmental studies of water quality[36,37]. Trace metals are taken up and accumulated by oysters and many other marine invertebrates to tissue and body concentrations usually much higher on a wet weight basis than concentrations in the surrounding seawater[38].

In this study we measured the one year bioaccumulation and tissue distribution of four metal pollutants (arsenic, cadmium, copper and zinc) in *C. virginica* seed, that had been transplanted to the bay in 2002 at two different depths: one foot below the surface and one foot above the sediment. Past years of unregulated industrial, rural and residential dumping, and previously inadequate wastewater treatment has caused Jamaica Bay sediment to be a major reservoir of metal pollutants. While the content and degree of metal contamination in the sediment can vary greatly depending upon site tested, the presence of many metal pollutants throughout Jamaica Bay sediment is widespread. In a 2002 report to the New York State Department of Environmental Conservation and the NY/NJ Port Authority[13], sediment sampled at 3 different sites in Jamaica Bay indicated that the content of many metals including arsenic, cadmium, copper and zinc were present at levels above the "Effects Range Low Level" (ERL) guideline and at some sites were above the "Probable Effects Level" (PEL) guideline. Arsenic levels ranged from 8.6-11 mg/kg dwt. (ERL and PEL, 8.2 and 41.6, respectively). Cadmium levels ranged from 2.2-5.7 mg/kg dwt. (ERL and PEL, 1.2 and 4.2, respectively). Copper levels ranged from 85-160 mg/kg dwt. (ERL and PEL, 34 and 108.2, respectively). Zinc levels ranged from 45-307 mg/kg dwt. (ERL and PEL, 150 and 271, respectively). A 1991 report on the annual input of heavy metals to Jamaica Bay revealed that sewage effluent carries the largest quantities of zinc, copper and cadmium to the bay[39].

Our results indicate that over a one-year period of growth in Jamaica Bay,

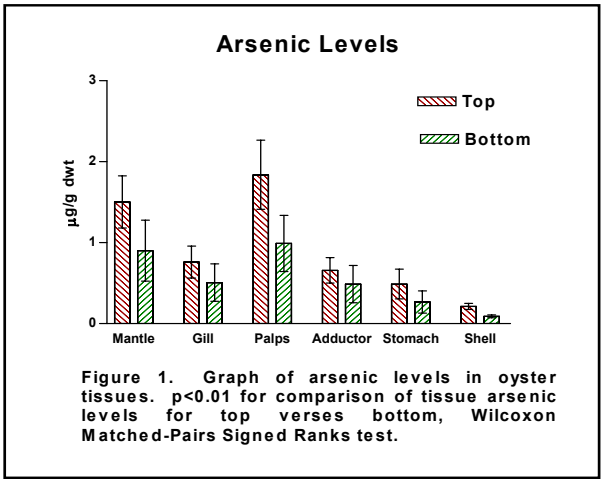

Figure 1. Graph of arsenic levels in oyster tissues. p<0.01 for comparison of tissue arsenic levels for top verses bottom, Wilcoxon Matched-Pairs Signed Ranks test.

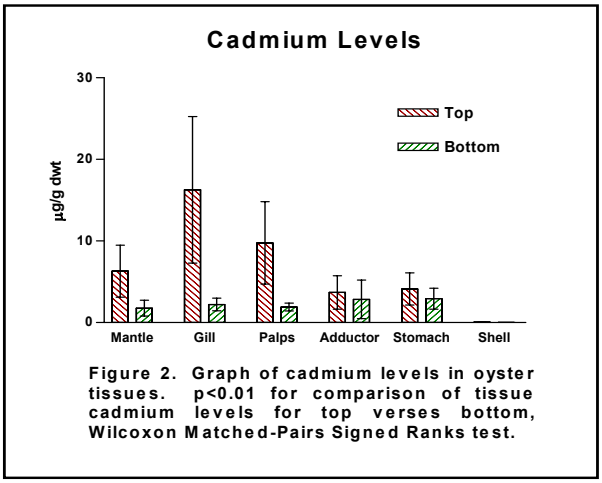

Figure 2. Graph of cadmium levels in oyster tissues. p<0.01 for comparison of tissue cadmium levels for top verses bottom, Wilcoxon Matched-Pairs Signed Ranks test.

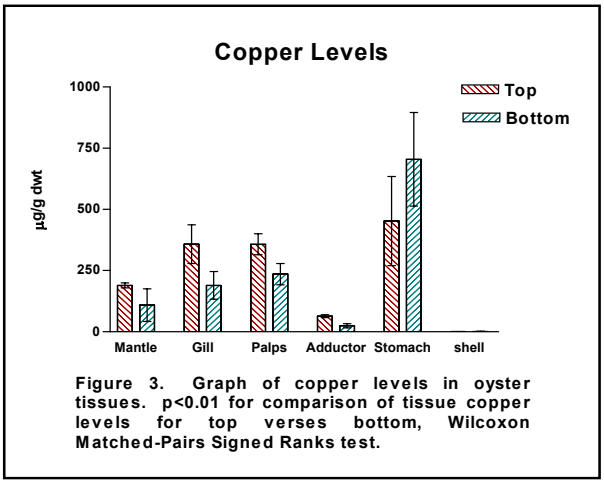

Figure 3. Graph of copper levels in oyster tissues. p<0.01 for comparison of tissue copper levels for top verses bottom, Wilcoxon Matched-Pairs Signed Ranks test.

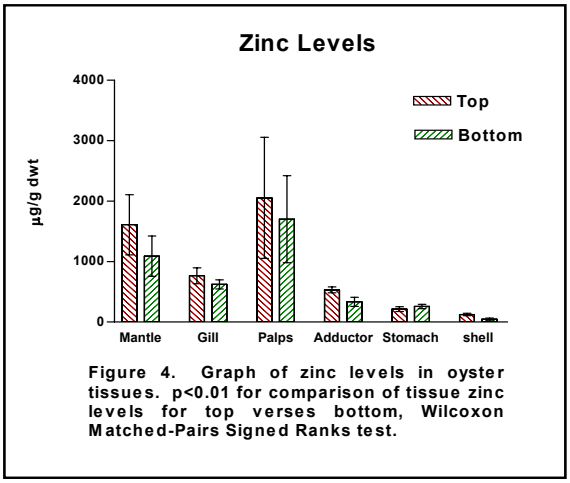

Figure 4. Graph of zinc levels in oyster tissues. p<0.01 for comparison of tissue zinc levels for top verses bottom, Wilcoxon Matched-Pairs Signed Ranks test.

considerable amounts of arsenic, cadmium, copper and zinc can accumulate in the tissues of oyster seed. Arsenic and cadmium are believed to serve no essential role in animal or plant metabolism[40,41]. Copper and zinc function as micronutrients, but when in excess even these essential elements can cause ecotoxicological effects. Therefore, organisms that are exposed to and accumulate metal pollutants require a means of physiological detoxification, typically by binding to high affinity sites in inorganic granules, or to various proteins like apoferritin or cysteine-rich metallothionein[42,43].

Arsenic is the twentieth most abundant element in the earth's crust and sources of aquatic arsenic are both natural as well as anthropogenic. It is released naturally to the atmosphere from volcanic eruptions and forest fires[44] and to water via natural weathering processes[45]. Anthopogenic sources include pollution due to fossil fuel combustion, mining/smelting, effluents from sewage treatment facilities, leaching from hazardous waste disposal sites, and production and use of arsenic compounds as pesticides and as a wood preservative[46]. The toxicity of arsenicals is highly dependent upon the nature of the compound with trivalent arsenic compounds considered more toxic than pentavalent arsenic compounds, and inorganic forms more toxic than organic forms[47,48]. Arsenic and arsenic-containing organic compounds have not been shown to accumulate to any great extent in aquatic organisms[49], with perhaps the exception of *C. virginica*, which in a bioaccumulation test was able to generate a very high bioconcentration

factor (BCF) of 350 after 112 days of exposure to phytoplankton containing arsenic[50]. In addition *C. virginica* may be particularly resistant to the toxic effects of arsenic for data comparing the acute toxicity of inorganic arsenic (III) indicated a very high acute value for *C. virginica* compared to *C. gigas,* a related species (7500 ug/l; 326 ug/l, respectively)[51].

Cadmium, a trace metal widely distributed in soil, air, water, and living things[40], is naturally present in zinc, lead, and copper deposits. It is also released into the environment from several anthropogenic sources including smelting and mining, electroplating, application of phosphate fertilizers, waste disposal operations, and the manufacturing and disposal of paints, alloys, batteries, and plastics[52,53,54,55,56]. Cadmium is a cumulative human toxicant that can cause a variety of adverse health problems including renal dysfunction and degenerative bone disease[57,58]. Cadmium has been found to bioaccumulate in fish and shellfish tissues in estuarine/marine waters[59,60] nationwide and New York has issued advisories for this metal in all of its marine coastal waters.

Copper is plentiful in the environment and is an essential micronutrient for the normal growth and metabolism of all living organisms[61,62,63]. The United States is the major world producer and consumer of copper and its compounds. Copper releases to the global biosphere—which may approach 1.8 million metric tons per year[64] - come mostly from anthropogenic activities[65]. Copper is among the most toxic of the heavy metals in freshwater and marine biota[61,66]. Excess copper can cause cellular damage by generating oxygen free radicals and inactivating biological thiols into disulfides[62]. Inputs of copper into aquatic ecosystems have increased sharply during the past century due to a number of reasons including atmospheric fallout from industrial activities, waste and industrial discharges, and leaching of antifouling marine paints and wood preservatives[67,68]. Copper is elevated in sediments of many marinas, probably as a result of copper containing antifouling paints used on boats housed in these marinas[69]. Bioavailability and toxicity of copper to aquatic organisms depends on the total concentration of copper and its speciation[70]. In marine ecosystems, the high copper levels measured in heavily contaminated coastal areas sometimes approach the incipient lethal concentrations for some organisms[71]. Among marine organisms, the highest accumulations are generally found in molluscan tissues and soft parts, especially those of cephalopods and oysters[65]. Bioconcentration factors for copper are highest for American oysters after exposure for 140 days (20,700-28,200), and lowest for bay scallops (*Argopecten irradians*) after exposure for 112 days (3,310) and for softshell clams after exposure for 35 days (3,300)[72].

Zinc is ubiquitous in the tissues of plants and animals[73] and functions as a cofactor for many enzymes in both aquatic and terrestrial organisms[74]. Like many other trace metals, zinc can be toxic if present at high concentrations in aquatic ecosystems[75,76,77]. Most of the zinc introduced into aquatic environments eventually is partitioned into the sediments. Anthropogenic sources include domestic and industrial sewage, combustion of fossil fuels and solid wastes, corrosion of zinc alloys and galvanized surfaces, road surface runoff, smelting and mining operations, and erosion of agricultural soils[78,79,80,81,82]. In seawater zinc exists in a dissolved state, as a solid precipitate, or adsorbed to particle surfaces. Zinc in molluscs is usually associated with high molecular weight proteins, with diet (as opposed to ambient water zinc concentrations), from collection locales with elevated sediment zinc burdens, and with particulate matter from dredging and storm perturbations[83,84]. Molluscan life processes do not seem to be affected by excess zinc accumulations and zinc is frequently accumulated far in excess of the organism's immediate needs[83].

In this study *C. virginica* seed, transplant to Jamaica Bay, readily accumulated arsenic, cadmium, copper and zinc and the distribution of these metals were not homogeneous throughout oyster

tissues. The unequal distribution of all 4 metals among the tested oyster tissue is not likely a random event. Copper values were particularly high in stomach tissue and are more likely a reflection of the copper-laden microalgea and detritus the animal ingested rather than actual tissue accumulations. For the other tissues, the cellular concentration of metallothionein and other metal binding organic or inorganic compounds probably varies depending upon physiological parameters and each tissue's role in metal detoxification. In the case of copper and zinc, which are micronutrients, the unequal distribution and known extensive accumulations possible in the American oyster may correlate with some physiological function for these two metals. Both of these nutrients are exclusively sequestered in oyster amebocytes, a cell type credited with many indispensable responsibilities for oyster survival, including antimicrobial activities for defense and nutrition[85]. The fact that copper and zinc accumulations, which were most abundant in the gills, mantal and palps, were not homogeneous throughout oyster tissue may be more associated with amebocyte function and distribution than a detoxified storage of these metals.

A number of studies have reported on metal accumulations in the shell of *C. virg*inica from various sites[86,87,88,89]. Our results are in accordance with previous findings with shell tissue accumulating metals but to a lesser extent. Copper and zinc concentrations were in the ug/g dwt range, while cadmium and arsenic concentrations were in the ng/g range. Even though metal accumulations in shell were much lower than that found in oyster soft tissue, if one considers that the shell of the animal typically makes up over 75% of the animal's weight, then the overall amount of metal present in the shell of the whole animal could be significant[87]. Therefore measurements of metal accumulations in oyster shell are important for they can represent a terrestrial reservoir of aquatic pollutants that can be adsorbed or released into the environment depending upon the nature of the metal and ambient concentrations. In addition, metals and other aquatic pollutants may influence the normal mineralization and metal composition of oyster shells, possibly decreasing shell strength and stability. Frazier[90] believed that shell thinning was linked to effects of copper and cadmium on shell calcification enzymes. If competent shell structure depends on metal composition, then deviations in metal composition may adversely affect shell quality, compromising the long-term health and survival of the animal.

Comparing metal accumulations in *C. virginica* seed grown at different depths in the bay, our results indicate that for most of the tissues studied, top-maintained oysters accumulated more metal pollutants than oysters maintained near the sediment. Differences in general water quality parameters such as pH, temperature, dissolved O_2, salinity, and turbidity could not account for our findings because these parameters, which were monitored throughout the experimental period, indicated no statistical variation at either site or at either depth[19]. Our results also indicate that wide variations in metal accumulations were possible even among individuals positioned at the same site and same depth. This is commonly reported by monitoring agencies for bioindicator organisms and can be due to many causes including age, seasonal factors and individual variability within the population[34]. While our oysters were all of the same age and reproductive status (subadult), differences in metal accumulations could still exist due to feeding patterns, extent of glycogen stores, adaptability to acute stresses, or overall health of the organism[91,92,93]. Even sex can be a factor for variations in metal accumulations for in the bivalve *Donax trunculus* L., females were shown to accumulate higher concentrations of zinc than males[94]. Differences in feeding patterns or overall health may also be an explanation for the disparity in metal accumulations between surface and bottom dwelling oysters. Bivalves accumulate pollutants not only from the water column and

metal-laden detritus, but also via ingestion of metal contaminated phytoplankton. While our measurements of chlorophyll-a in surface/ bottom water showed no consistent variation[19], the extent of feeding and the species and metal content of the microalgae available to the oysters near the surface might have been different from that available to oysters growing near the sediment. It also is possible that the increase in metal accumulations seen in top maintained oysters may have been due to a difference in top/ bottom overall health or early infection rates with known oyster pathogens such as *Haplosporidium nelsoni* (MSX disease) or *Perkinsus marinus* (Dermo disease). 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[95,96]. Other studies have shown a positive correlation between tissue burden of certain metals, especially copper and zinc, and immuno-defense related characteristic of oysters[85,97,98]. Also to consider is the fact that top but not bottom dwelling oysters may have been exposed to additional bay contaminants, including floatables and organic pollutants, that either enhanced metal accumulations or compromised oyster health and therefore soft tissue growth. Differences in soft tissue weights can significantly affect trace metal concentrations by simply diluting or concentrating the animal's total metal body burden[99,100,101,102,103,104,105]. It was a surprise that oysters positioned near the sediment not only had lower overall metal accumulations, but also had better growth rates when compared to top grown oysters[19]. It is currently unknown whether this pattern of greater growth and lesser metal accumulations in Jamaica Bay is site specific or if metal accumulation would have been greater and growth worse in bottom dwelling oysters if a more polluted site were available for our study. The fact that the bottom placed oysters accumulated less tissue arsenic, cadmium, copper and zinc than their top placed counterpart is interesting and may itself be an explanation for the faster bottom growth rate. However, only 5 tissues and these 4 metal pollutants were studied in this report. More work needs to be done here and at additional sites in Jamaica Bay to determine if other metal pollutants and other oyster tissues show similar patterns of metal accumulations.

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Eustrongylides sp. (Nematoda: Dioctophymatoidea) Occurrence in the Fourspine Stickleback, *Apeltes quadracus*

by

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Larvae of *Eustrongylides* have been reported in 14 orders of fish worldwide, including Gasterosteiformes[1], but not in the fourspine stickleback[2,3]. This fish occurs from Labrador[4] to Virginia[5]. It is abundant in salt water, but sometimes enters fresh waters[4]. A collection of 143 stickleback (114 males, 29 females) were collected from a 575 meter X 75 meter fresh water pond with a mud bottom (Fig. 1) located on Sandy Hook, New Jersey. The pond is surrounded by Phragmites (Fig. 2). Fish were collected both with a 20 foot X six foot 3/8 inch mesh seine and with long-handled dip nets with ¼ inch mesh on several occasions in September and October, 2006. Fish averaging 38 mm total length (range 32-52 mm) were sacrificed and their viscera removed and examined (Fig. 3). Five males (4%) and one female (3%) contained at least one nematode (Fig. 4). One fish contained two nematodes. The average length of the worms was 40 mm. One other fish species, *Fundulus heteroclitus,* the mummichog, occurs in the pond. Examination of the viscera of 44 mummichog (25 males, 19 females) yielded a nematode infection rate of 56% for males and 47% for females. Infected fish of this species contained one to 15 worms (the average length of the worms was 90 mm). The pond is utilized by several bird species that are hosts for adult *Eustrongylides* sp. The nematode eggs, passed by bird feces deposited in the pond, are eaten and develop in tubificed oligochaetes, the first intermediate hosts[6]. The infected oligochaetes are then eaten by some of the fishes in the pond, the second intermediate hosts of *Eustrongylides* sp.

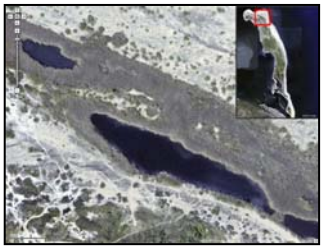

Figure 1. Northpond, a freshwater pond located at Sandy Hook, New Jersey. Insert in upper right, is an aerial view of Sandy Hook. The area outlined in red shows the location of Northpond.

Figure 2. Collecting site at Northpond.

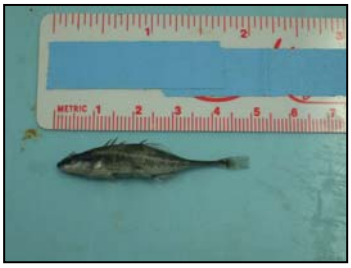

Figure 3. Fourspine Stickleback (female) collected from Northpond.

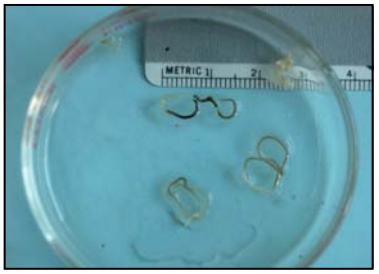

Figure 4. Nematodes, *Eustrongylides* sp. removed from the Fourspine Stickleback.

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The **Treasurer** of the Association is responsible for the preparation of an annual fiscal report, processing of dues, preparing regular financial reports for the Executive Board meetings, income tax reports, and other duties usually pertaining to this office.

The **Members-at Large** shall chair committees (Articulation, Exhibition, etc.) and handle other assignments as directed by the Executive Board.

Normally, each candidate for Vice-President, Recording Secretary and Treasurer should have been a Member-at-Large for at least one term and each candidate for Member-at-Large should have attended at least one Annual Conference.

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