



# IN VIVO

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**The 40th Annual MACUB Fall Conference will be held at  
St. John's University at their Queens Campus  
on Saturday October 20th  
This year marks MACUB's 40th Anniversary**

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The Benjamin Cummings/MACUB Student Research Grants provides support for undergraduate students working under the supervision of faculty who are current members of MACUB. Four grants of \$500 each are provided annually by Benjamin Cummings along with registration fees for the annual Fall MACUB Conference, with membership, provided by MACUB. Awardees are required to present the results of research supported by this award at the MACUB Annual Fall Conference following the year of the award.

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Ms. Islam Aly, St. Francis College, Faculty Mentor: Dr. Kathleen Nolan. *The Effects of Native Versus Invasive Grasses on Shrimp Populations in Jamaica Bay and the Hudson River*

Mr. Kwesi W. Blackman, Kingsborough Community College, Faculty Mentor: Dr. Thomas Onorato. *DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) Affects Spermatogenesis in Drosophila melanogaster*

Ms. Anita Zhu, Polytechnic University, Faculty Mentor: Dr. Jin Kim Montclare. *Incorporation of P-Fluorophenylalanine Into Histone Acetyltransferases*

Ms. Michelle Zook, Monmouth University, Faculty Mentor: Dr. Dennis E. Rhoads. *Altered Expression of Receptor Proteins in Adolescent Alcohol Dependency*

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Articles can be submitted electronically to [invivo@mec.cuny.edu](mailto: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. - <sup>1</sup>Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32**: 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

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## **Making Connections: Biology Majors Learning and Study Strategies Inventory**

by

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### **Abstract**

Numerous studies have concluded that the first year college experience is predictive of student success at college. Medgar Evers College is aware of this and has implemented several intervention strategies through the Freshman Year Program to enhance students' success. This study has provided information that can be used to improve the Program's effectiveness and efficiency. It highlights several deficits in our students learning and study strategies, with particular regards to *Anxiety, Concentration, Self Test, Study Aids, and Time Management*. The research will continue in order to determine how wide and deep these deficits are, and what interventions will be most appropriate.

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### **Introduction**

The Biology Department of Medgar Evers College is very diverse in terms of its rich curriculum, faculty and students. The students are mainly of African American ancestry, many of whom are from the Caribbean and most are first generation college students. The majority of students who complete a Baccalaureate degree in Biology are very successful academically and continue their education in medical, graduate and allied health schools. As a faculty member in the Freshman Year Program in the Department of Student Affairs since 1993, I have helped to prepare the fertile ground for a large number of Biology majors in the Freshman Seminar courses which I teach every semester. An objective of these courses is to build students' learning and study skills. Since I am engaged with students pursuing different majors in my courses, I have the opportunity to observe and assess these

skills among students of all majors. My long term goal is to assess new students' awareness about and use of learning and study strategies related to skill, will and self-regulation. Students can use this information to improve their learning, studying and test-taking strategies. Because the information is individualized, counselors and advisors may use it as one of their tools to counsel and advise students. Each student who participated in the study received a copy of their own skills inventory. I started with Biology majors because of the general high level of success by the students, even though they tend to begin college with deficits and because of the department's interest in the learning and study strategies of their majors.

### **Materials and Methods**

The study used the Learning and Study Strategies Inventory (LASSI) survey developed by Dr. Claire Weinstein

of the Department of Educational Psychology, University of Texas at Austin and Dr. David R. Palmer<sup>1</sup>, Texas Health and Human Services Commission. The participating students were first year students enrolled in four Freshman Seminar courses during the Spring 2005 semester.

### **Description of the LASSI**

The Learning and Study Strategies Inventory (LASSI) is designed to provide value insight to students and counselors/advisors. It is both diagnostic and prescriptive. The focus is on both covert and overt thoughts, behaviors, attitudes, motivations and beliefs that relate to successful learning in post-secondary educational and training settings. Among other uses, it can help identify areas in which students could benefit most from educational interventions and therefore is an appropriate instrument for this study.

The LASSI is a 10-scale, 80-item assessment of students' awareness about and use of learning and study strategies related to skill, will and self regulation components of strategic learning. These scales are *Information Processing, Selecting Main Ideas, Test Strategies, Anxiety, Attitude, Motivation, Concentration, Self-Testing, Study Aids, and Time Management*. There are eight items on each of the ten scales. The minimum score possible on each scale is 8 and the maximum score possible is 40.

The *Information Processing, Selecting Main Ideas, and Test Strategies Scales* examines skills. The *Information Processing Scale* assesses how well students can use imagery, verbal elaboration, organization strategies and reasoning skills as learning strategies to help learn new information and skills, and to build bridges between what they

already know and what they are trying to learn and remember. Students who score low on this scale need to acquire methods that they can use to help add meaning and organization to what they are trying to learn. The *Selecting Main Idea Scale* assesses students' skills at identifying important information from less important information and supporting details. Students who score low on this measure need to learn more about how to identify important information so that they can focus their attention and information processing strategies on appropriate materials. The *Test Strategies Scale* assesses students' use of both test preparation and test taking strategies. Students who score low on this measure need to learn how to prepare for and take different types of tests.

The *Anxiety, Attitude, and Motivation Scales* are related to the will component of strategic learning. The *Anxiety Scale* assesses the degree to which students worry about school and their academic performance. Students who score low on this measure need to learn techniques for coping with anxiety. The *Attitude Scale* assesses students' attitude and interests in college and achieving academic success. Students who score low on this measure need to work on goal setting including school and life goals. The *Motivation Scale* assesses students' diligence, self-discipline and willingness to exert the effort necessary to successfully complete academic requirements. Students who score low on this measure need to work on goal setting and individual tasks and assignments.

The *Concentration, Self-Testing, Study Aids, and Time Management Scales* are related to the self-regulation component of strategic learning. The *Concentration Scale* assesses students' ability to direct and maintain their

attention on academic task. Students who score low on this scale need to learn techniques for focusing attention on priorities related to school and their other responsibilities. The *Self Testing Scale* assesses students use of reviewing and comprehension monitoring techniques to determine their level of understanding of the information or task to be learned. Students who score low on this scale need to learn more about methods for reviewing school material and monitoring their comprehension. The *Study Aids Scale* assesses students use of support techniques, materials or resources to help them learn and remember new information. Students who score low on this scale need to learn more about the types of study aids provided in educational materials available to them and how to create their own aids. The *Time Management Scale* assesses students use of time management principles for academic tasks. Students who score low on this measure need to learn more about how to create and stick to schedule, and how to deal with distractions, competing goals and procrastination.

In addition to the 80-items, four variables (age, gender, major, and GPA) were included to provide general statistics.

### **Sampling and Methodology**

The sample consisted of 134 first semester students randomly enrolled in Freshman Seminar I courses. The study was conducted at the end of the Spring 2005 Semester. The survey was completed in class by students based on their self assessment and experience. The responses were scored in accordance with the LASSI techniques and instructions.

## **Results**

### **General Statistics**

Biology students in the sample were younger than the other students in the sample. More than 80 percent of biology majors in the sample fell within the 16 to 25 age group while just over 68 percent of the non-Biology students fell into that age group (Fig. 1).

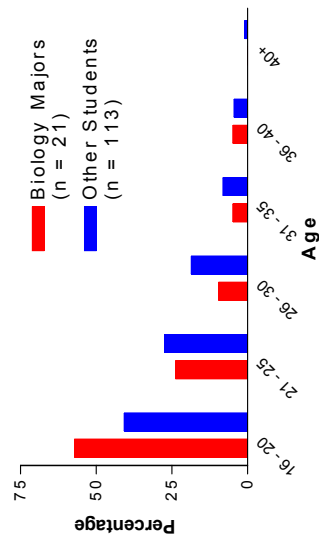
With respect to gender the percentage of male and female students in both the Biology and non-Biology groups was almost the same, with the number of females outnumbering the males by approximately three to one, 76 to 23 percent for biology majors and 73 to 27 percent for the students (Fig. 2).

With respect to grade point average (GPA) the Biology majors had a slightly higher score than the other students,  $2.6 \pm 0.61$  gpa as compared to  $2.4 \pm 0.75$ , respectively but lower dispersion among the grades, 0.61 to 0.75 (Fig. 3).

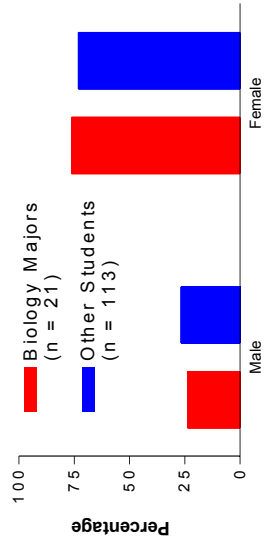
In Figure 4 the mean score and standard deviation for each scale was determined for Biology and non-Biology majors. In both groups students score fairly high on the *Attitude, Information Processing, Motivation, Selecting Main Ideas and Test Strategies Scales*. Conversely, both groups scored low on the *Anxiety, Concentration and Self Testing, Study Aids and Time Management Scales*.

In Figure 5, the mean score and standard deviation of Biology majors are compared with national data provided by the LASSI. In this analysis our Biology majors matched the high national mean scores in the *Attitude and Motivation Scales* and exceeded the mean national average in the *Information Processing, Selecting Main Ideas and Test Strategies Scales*. Both groups scored lower on the other measures.

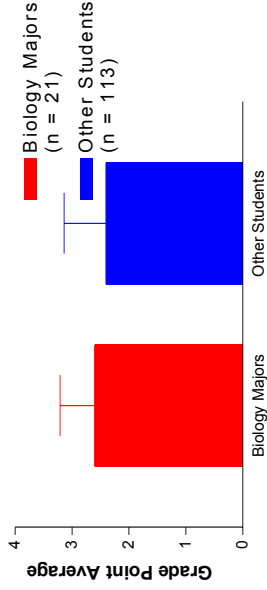
**Figure 1: Comparison of Age**



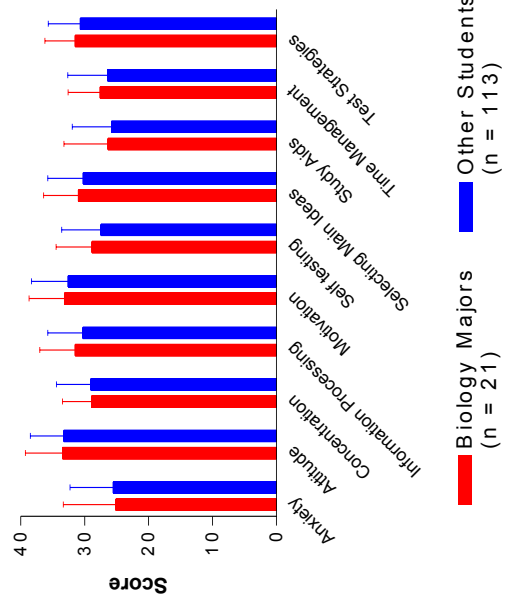
**Figure 2: Gender Comparison**



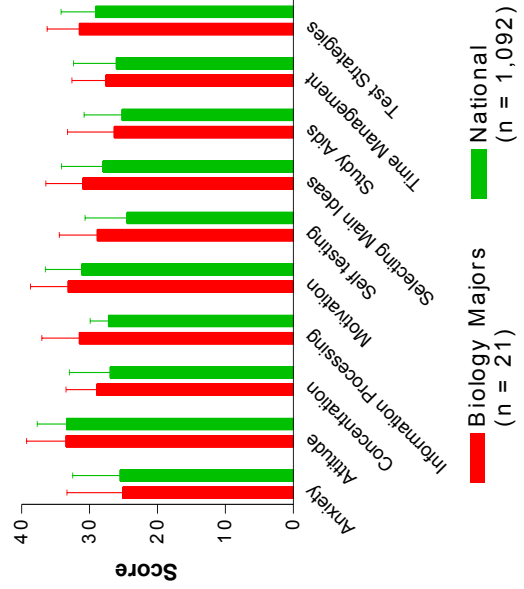
**Figure 3: Grade Point Average**



**Figure 4: Individual Scale Statistics**



**Figure 5: National Comparison**



## Conclusion and Discussion

Based on the study, the areas that appear to need the most urgent attention for Medgar Evers students and perhaps freshman students in general, include *Anxiety, Concentration, Self Test, Study Aids, and Time Management*. *Anxiety* can have a debilitating effect on students' ability to demonstrate their true level of knowledge and skills. Students' inability to concentrate can have a negative impact on their ability to focus their attention on immediate tasks. The ability to *self-test* is fundamental to the acquisition of knowledge. Students who have a significant deficit in this skill will demonstrate low performance. Students who learn to use and create their own *study aids* improve both their learning effectiveness and efficiency. The *Time Management* skill is crucial to self regulation. Students need to have the type of knowledge and self awareness that will help them create realistic schedules.

The sample size is too small to ascribe any general evaluative prescriptions. However, the study provides some insights into the type of assistance that new Biology students and new students in general may need to enhance the chances of success at Medgar Evers College. It can be an important guide to students as they try to improve their academic performance. It also can assist counselors and advisors to customize recommendation for student specific needs.

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<sup>1</sup>Weinstein, C. E., and D. R. Palmer, 2002. *Learning and Study Strategies Inventory*, H & H Publishing Company.



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# Considerations Regarding Biopsychosocially-Derived Pelvic Pain In Women

by

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## Introduction

Some mental illnesses, especially those with significant childhood abuse connections, are also associated with disabling genitoanal and pelvic pain<sup>1</sup>. This is understandable considering that many of the patients with such pain are victims of childhood sexual abuse. In fact, adult-onset vulvodynia has been associated with being sexually abused as a child<sup>2</sup>. The term vulvodynia is reported as being pain around the opening of the vagina which may persist for many months or years. Activities such as riding a bike, tampon insertion or sexual intercourse can exacerbate this pain and discomfort which ranges from burning sensations to only mild irritation<sup>3</sup>. In addition, a significant association between sexual victimization prior to the age fifteen years and late onset chronic pelvic pain has been well established<sup>4</sup>.

## Diagnosis

Of relevance to chronic pelvic pain are biological, psychological, and social factors<sup>5</sup>. The biopsychosocial angle involving a person who suffers from such pain, incorporates the three factors simultaneously. Social and psychological factors are surely key contributors, despite being less tangible than those in the biological realm. The development of this type of pelvic pain, as well as the persistence of associated symptoms and limitations in activities, all are influenced

by such factors. Yet, the biopsychosocial approach still relies on a very concrete biological foundation as one of its requisites. All three components potentiate each other and are entangled as opposed to functioning independently. When discussing the usage of this approach historically, the biopsychosocial model has not been widely used for a task such as selecting patients for back pain surgery<sup>6</sup>, as back pain is so common and there is no established and considerable link with sexual and psychological victimization. As for chronic pelvic pain in women, it is purported that the biopsychosocial approach is promising, for when there is an undeniable history of abuse, memories of the traumatic experiences may exacerbate the pain<sup>7</sup>. Thus, this model can benefit the patient by offering a more comprehensive and multidisciplinary approach towards the management of such pain. This is in contrast to the pure biomedical model which deals solely with tissue damage of which pain has only one component, a pure sensory input. While concrete statistics comparing both models to each other have yet to be established, the National Pain Foundation supports the effectiveness of the biopsychosocial approach for not only the treatment, but also the rehabilitation from pelvic pain as well<sup>8</sup>. The difficulty in studying pelvic pain is the sheer quantity of culprits that the condition umbrellas, however overall treatment statistics have emerged.

Overall, chronic female pelvic pain, of all types, has been estimated to affect upwards of 15% of American women aged 18-50. Pelvic pain has been given specific criteria, via the International Pelvic Pain Society, in order for it to qualify as being chronic<sup>9</sup>:

- It has been present for at least six months.
- Conventional treatments yield little relief.
- The degree of pain perceived seems out of proportion to the degree of tissue damage detected by conventional means.
- Physical appearance of depression is present, such as sleep problems, poor appetite, constipation, and slowed body movements.
- Physical activity is extremely limited.
- Emotional roles in the family are altered, and the patient is displaced from her accustomed role, such as wife, mother, or employee.

## Discussion

Studies have suggested that the majority of chronic pelvic pain sufferers will not find relief. Traditional therapies which are considered as muscle relaxants and anti-inflammatories alone, were shown to lack any efficacy when compared to placebo according to National Institute of Health sponsored randomized trials<sup>10</sup>. Observational cohort studies also help shed the light. One year after evaluation in a chronic pelvic pain specialty clinic, 46% of women who had undergone surgical or nonsurgical treatment reported modest improvement in pain and only 32% found some alleviation of their compounding depression<sup>11</sup>. Of course due to the multifactorial nature of pelvic pain, surgical procedures ranged from hysterectomy and oophorectomy (resection

of the uterus and ovaries, respectively) to vulvar repair. Medical treatments combined pharmacotherapy and psychotherapy to take into account psychosocial contributors to pain. The net result was as follows. The medically treated group and the surgically treated group both had equal odds of improvement and the minority of patients that did experience improvement showed similar measures of pain alleviation.

This at the very least may endorse the notion that more conservative measures which combine pharmacology and psychological treatment can be just as effective as surgery in some chronic pelvic pain sufferers. This is of course dependent upon the multitude of factors at play. On the other hand, pelvic pain due to a mass effect of an ovarian tumor for example, will obviously not stimulate one to think of psychotherapy as a first-line treatment choice.

This article also seeks to ask a very important question about whether the decision to undergo certain treatment types can backfire in certain cases. It will also provide an overview of potential treatment options which may be offered to a very unique and unfortunate set of patients; a set of patients with sexual victimization playing a very prominent role in the etiology of their pain. We are at a time where physiotherapy's role in the treatment of female sexual pain disorders is considered to be a relatively recent advancement<sup>12</sup>. Regarding chronic pelvic pain, the use of nerve blocks, biofeedback, muscle rehabilitation, and even botulinum toxin type "A" are suggested to be of therapeutic value<sup>13</sup>. The fact that pelvic pain sufferers belonging to this distinct patient group are already under excessive stress and have been sexually or physically abused previously, provides for a dilemma. The dilemma stems from the biopsychosocial approach towards their pain. Whether the

invasive nature of the above mentioned treatments will exacerbate or alleviate their chronic pelvic pain should be taken into account. As noted, the three components of the approach potentiate each other and are tightly entangled. For instance, sexually victimized individuals can understandably suffer from a direct disturbance to their physiological systems, particularly the genitourinary system. When such physiological systems are negatively affected or disturbed, the biopsychosocial principle offers the resultant alteration of the psychological and behavioral systems. This in turn facilitates the production of further vicious cycles of escalating physical pain and dysfunction as well as taking its toll on a psychological level. An altered sense of self, depressed states, and self esteem issues, can all cause damage in social and occupational functioning. Coping modalities, rumination (constant reflection and turning a matter over and over in the mind) and emotionally painful memory retrieval are all intertwined within these cyclic processes<sup>14</sup>.

An example would be electromyography, abbreviated as EMG. It is the measurement of the electrical activity in muscles. EMG is used in the biofeedback treatment for painful spasms, spasms of which chronic pelvic pain is strongly derived from. An EMG sensor is actually positioned in the anal canal or vagina which measures and then displays the electrical activity of the muscles being recorded. With the visual feedback provided by the instrument, the healthcare professional will be able to offer step by step instructions in an effort to guide the patient towards deescalating such abnormal muscle activity and thus reducing painful spasms.

## Conclusion

However, again it must be reiterated that the invasive nature of such techniques and the resultant and plausible rekindling effects have yet to be studied in depth. The genitoanal invasive nature of inserting the recorder can understandably be viewed as a stress exacerbating experience in of itself, especially for a patient with a history of being sexual abused.

The move towards future potential research, in regards to whether a procedure such as EMG guided biofeedback should be utilized as one of the therapies for pelvic pain disorders in sexually abused victims, may be warranted. Whether or not there is risk of actually worsening the pain is in question.

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## Epiphytic Bacteria of the Surface of Marine Macroalgae Collected From the NY/NJ Coast, USA

by

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### Abstract

Marine seaweeds represent a favourable substrate for symbiosis with epiphytic bacteria and many marine bacteria are thought to exist associated with seaweeds or invertebrates as epiphytes. In this study, samples from seaweed collected from the New Jersey/New York coast of USA were studied for the presence of marine epiphytic bacteria. Four types of common seaweeds, *Fucus vesiculosus*, *Ectocarpus*, *Enteromorpha intestinalis* and *Ceramium* were washed and prepared for scanning electron microscopy using standard techniques. In addition, cultures were made on marine agar; isolates were gram stained and light microscope photographs were taken. Some isolates were sent for fatty acid and 16S RNA sequencing analysis. The micrographs showed colonization by the bacteria and the presence of many diatoms, indicating the production of microenvironments in the presence of the seaweed. Comparison between the algal samples indicates there is much variation in the type and number of bacteria present. This is confirmed by culture, gram stain and genetic analysis. Micrographs also indicate that the bacteria attach to the seaweed blade by digesting a hole in the surface. These results suggest that seaweed-associated bacteria may be useful for ecological interaction studies and the production of anti-fouling properties. They also produce agarase which allows them to digest the surface of the seaweed for attachment.

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### Introduction

All surfaces that are submerged in seawater will develop biofilms that cover those surfaces. This leads to the development of microbial communities that attract larger organisms. Seaweeds release large amounts of organic carbon into the surrounding environment and provide surfaces for bacteria to colonize<sup>1</sup>. As such they should support an abundant microbial community, but some may produce substances which limit the growth of biofilms<sup>2</sup>. Marine bacteria isolated from living surfaces produced more anti-fouling traits than strains isolated from non living surfaces<sup>3</sup>.

Booth and Hoppe<sup>4</sup> and Danielsson<sup>5</sup> indicated most bacteria do not live freely in marine environments – they live attached to lipid films, or to the surfaces of living or dead marine macrophytes. Booth and Hoppe<sup>4</sup> also demonstrated that the surfaces of large marine algae provide perfect growth conditions for heterotrophic bacteria. Norrans and Sörensson<sup>6</sup> showed that marine bacteria inhabit lipid films, with some species being more abundant than others. Morphological patterns of bacterial samples in offshore Western Mediterranean waters showed that attached bacteria are often large and present in higher concentration than free living bacteria in water<sup>7</sup>. Lorch and Ottow<sup>8</sup>

showed that the diversity and density of epiphytic bacteria increase with the age of the macrophyte and pollution levels of organics in the water surrounding the macrophyte that were present. Factors such as chemical composition of the surrounding water, interactions between the bacteria and their substrate and water turbulence may also affect microbial population and colonization patterns.

Epiphytic marine bacteria interact with their living substrates. Riquelme *et al.*<sup>9</sup> stated that marine bacteria can induce the development and metamorphosis of marine invertebrate larvae, as well as cause disease, encourage the colonization of other fouling organisms and produce growth factors. Bacteria may benefit from substances produced by their epiphytic host, while the host itself may also benefit from microbially synthesized vitamins and mineral nutrients. The bacteria may be parasitic to the host or may be commensal, benefiting from the host and causing no immediate harm unless bacterial populations become too high. Grossart<sup>10</sup> showed that when marine bacteria and diatoms were grown in culture together with adequate nutrients, both species' growth rates increased, but when grown together without vitamins and trace metals, the diatom growth was inhibited or completely negative.

Corre and Prieur<sup>11</sup> found that three forms of bacteria, bacilli, cocci and filamentous forms were present on the kelp *Laminaria digitata*, with rod-shaped bacteria dominant. The densities and relative number of marine epiphytic bacteria change with the season, with the fluctuations seeming highly correlated with the temperature of the seawater. Bacterial numbers were much greater on aged, deteriorating algae than on younger, healthy algal fronds<sup>8,11</sup>. They did

not show the seasonal fluctuation noted in other instances and kelp was shown to produce antibiotic compounds that had an impact in keeping the bacterial populations lower on the healthy algal surfaces. Maximilien *et al.*<sup>12</sup> showed this inhibitory activity by the red algae *Delisea pulchra* to marine epiphytes as well.

Previous studies have shown that there is a symbiotic relationship between seaweeds and bacteria associated with them. Scanning electron microscopy showed that the surfaces of several seaweeds in Jiazhou Bay, China demonstrated a variety of bacterial species including *Vibrio*, *Micrococcus*, *Flavobacterium*, and *Bacillus* to be present<sup>13</sup>. A variety of bacteria are found on the surface of seaweeds, including cocci, rods, and stalked forms. They attach by cell wall stickiness, flagella, fimbriae or stalks. They also secrete high molecular weight polysaccharides to attach<sup>14,15</sup>. Attachment material is found in the form of strands, pads, sheets or capsules<sup>14,16</sup>.

There has been limited work with regard to the presence of these novel bacteria as to their location on the seaweed frond, and the number and types of bacteria present. Culturing and identification of the bacteria has been very difficult to accomplish and identification by traditional means may not be possible. Since many of the bacteria are non-culturable, scanning electron microscopy was used to explore these questions in this study. The objective of this study was to use scanning electron microscopy to explore the diversity of microbial biofilms present on the surface of seaweeds isolated from the New York/New Jersey coast, USA. Isolation, cultivation and gram staining of selected organisms were also undertaken.

## Methods

### Collection

Samples of seaweeds: *Fucus vesiculosus* (Phaeophyta), *Ectocarpus* sp. (Phaeophyta), *Enteromorpha intestinalis* (Chlorophyta), *Ceramium* sp. (Rhodophyta) were obtained from waters off Sandy Hook, New Jersey and Southampton, New York, USA. Specimens were rinsed with seawater, bagged and transported to the lab in coolers with ice. Collection occurred April – October.

### Scanning Electron Microscopy

Specimens were washed twice with a 0.2M Sodium Cacodylate Buffer containing 2.5% Glutaraldehyde (pH 7). Specimens were immersed in each of the following acetone concentrations for 10 minutes each: 30%, 50%, 70%, 80%, 90%, 95% and 100%. They were then left to air-dry overnight. Specimens were sputter-coated with a 100Å thick mixture of gold and palladium (Denton sputter coater). Subjects were mounted on stubs and viewed/photographed with a Hitachi 2460-N SEM (Hitachi High Electronics Corporation, Tokyo, Japan) coupled with a Princeton Gamma Tech (Princeton Gamma Tech, Inc., Princeton, NJ USA) Spirit EDX system, (Version 1.05) for digital imaging.

### Culturing Methods

In order to maximize the chance of culturing the epiphytic bacteria, two separate methods were used. In the first method, 2cm pieces of each of the seaweed species were removed from the collected specimens using sterile technique and rinsed with filtered

seawater. Seaweed pieces were gently placed onto the minimal marine agar (MMA) and grown at room temperature. In the second method, seaweed specimens were swabbed with sterile swabs and were streaked on MMA and grown at room temperature. MMA is made of filtered seawater and 2% agar. Upon successful growth of complex cultures, colonies were isolated and pure cultures were grown on MMA. Slides were prepared of the bacteria, gram stained and viewed under oil immersion at 1000X, using a Zeiss AxioCam. Isolates were gram stained and light microscope photographs were taken with a Zeiss AxioCam. Selected isolates were sent to Microbial ID, Inc. (Newark, Delaware) for fatty acid analysis, using MIDI Sherlock<sup>®</sup> Microbial Identification System and for 16SRNA sequencing.

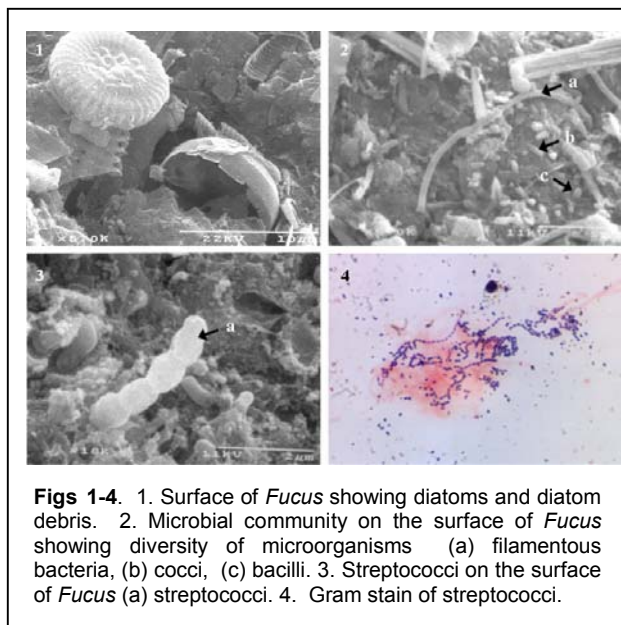
## Results

Four species of marine macroalgae were collected from Sandy Hook, NJ and Southampton, NY. They were studied using SEM and light microscopy for the presence of epiphytic microorganisms. The four seaweeds were: *Fucus vesiculosus* (Phaeophyta), *Ectocarpus* (Phaeophyta), *Enteromorpha intestinalis* (Chlorophyta) and *Ceramium* sp. (Rhodophyta).

### *Fucus vesiculosus*

*Fucus vesiculosus* is commonly known as rockweed or bladderweed and is abundant in northern Atlantic marine environments where it grows attached to the surfaces of rocks. Figures 1 and 2 are micrographs taken from *Fucus vesiculosus* demonstrating an abundant and diverse microbial community. The presence of numerous diatoms and bacteria indicates the rich environment for microbial growth.

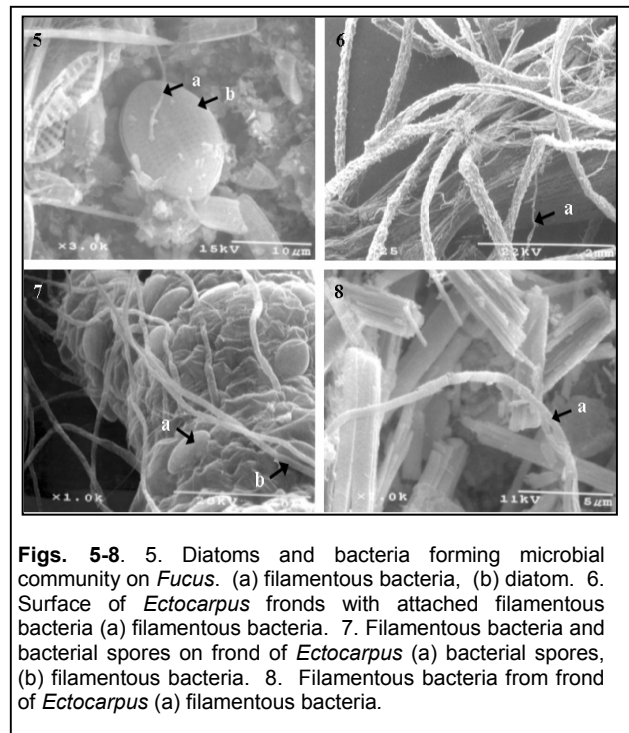
There are both intact and fragmented diatoms on the surface of *Fucus* and much diatom debris. In Figure 2, many bacteria can be seen, cocci, short rods and long filaments. The cocci can be seen in chains in Figures 3 and 4, with Figure 4 showing the gram stain of these gram positive cocci. Figure 5 shows the attachment of one of the filaments to the surface of a diatom, indicating that they are found not only on the surface of the frond, but also on the diatoms as well.



### ***Ectocarpus***

*Ectocarpus* is a filamentous brown algae which can be free floating or attached to surfaces of plants and other macroalgae. Figure 6 shows the fronds with filamentous bacteria closely associated with the seaweed. In Figure 7, the surface of the filament can be seen to be covered with long filamentous bacteria which are numerous on the frond surface. The short, round structures are spores found with the vegetative cells. This was determined by gram and spore stain. In Figure 8, one of the filaments can be seen at greater enlargement. Some other

bacteria are also present on *Ectocarpus*, but the filamentous form predominates. The gram stain (Figure 9) shows gram negative long filaments morphologically similar to those seen in the SEM micrograph. Numerous spores are present.



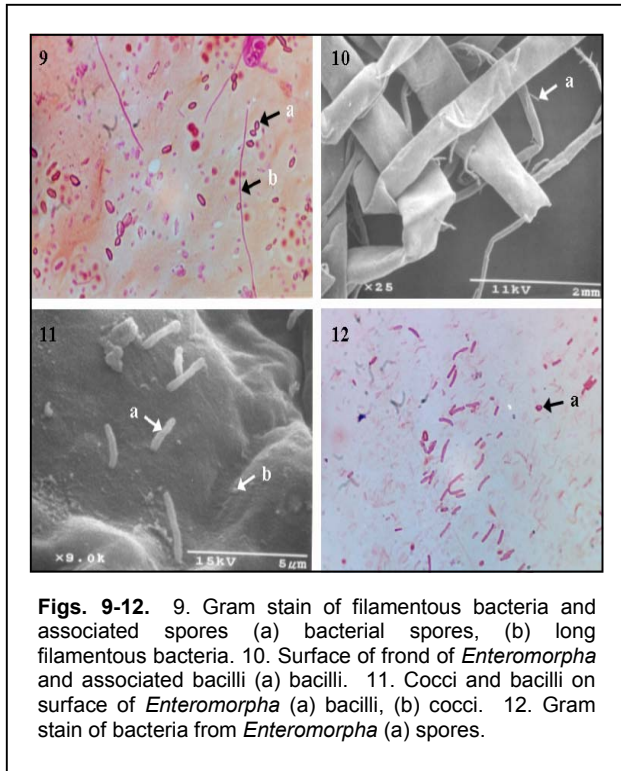
### ***Enteromorpha intestinalis***

*Enteromorpha intestinalis* is a filamentous green algae which grows on rocky surfaces or gravel beds with long filaments, like shoots. SEM micrographs of the surface indicate far fewer bacteria than seen with *Fucus* or *Enteromorpha*. In Figure 10, the long filaments of the seaweed contain few epiphytic bacteria. It can be seen that the surface contains a few rod shaped organisms and some cocci, but are much less numerous than in the previous seaweed samples (Figure 11). Much of the surface is devoid of bacterial epiphytes and the filamentous form is not present. The gram stains of



these bacteria show gram negative rods, with darker staining ends and spore formation. Separate spores may also be seen (Figure 12). The bacteria adhere to the surface of the frond of *Enteromorpha* and can attach so that they are perpendicular to the seaweed surface (Figure 13) and extend out from it.

In Figures 14 and 15, it can be seen that the bacteria attach by digesting



**Figs. 9-12.** 9. Gram stain of filamentous bacteria and associated spores (a) bacterial spores, (b) long filamentous bacteria. 10. Surface of frond of *Enteromorpha* and associated bacilli (a) bacilli. 11. Cocci and bacilli on surface of *Enteromorpha* (a) bacilli, (b) cocci. 12. Gram stain of bacteria from *Enteromorpha* (a) spores.

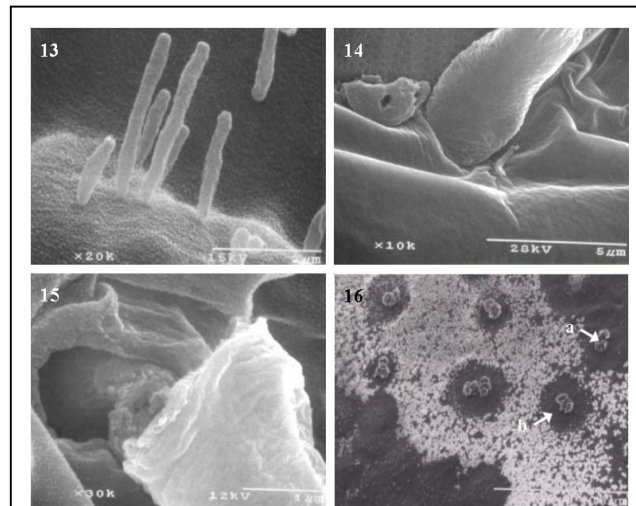
the outer surface of the frond. In culture the bacteria produce agarase which digest the agar present in the medium. Figure 16, is a photograph of bacterial colonies isolated from *Enteromorpha*, showing the digestion of the media surrounding the colonies. The colonies lie within a depression that they have created in the agar. A sample of the bacteria was sent to Microbial I.D. Inc (Newark, Del). Identification through 16S r-RNA and fatty acid analysis has shown that this organism to be *Pseudoalteromonas*, with the confidence level to genus only.

### ***Ceramium***

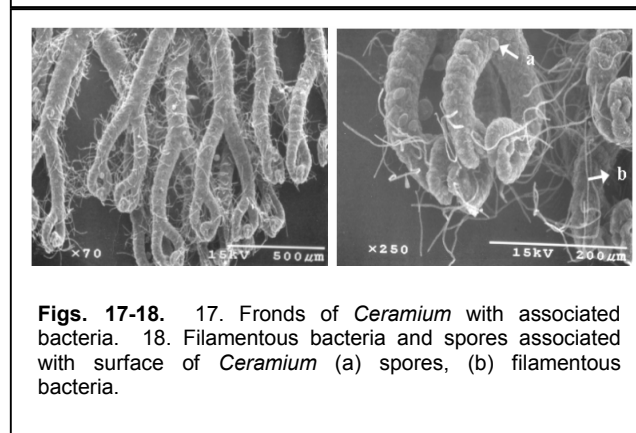
*Ceramium* is a widely distributed red algae found subtidally and intertidally growing on rocks, other algae or animal surfaces. In Figures 17 and 18 the growing tips of the algae can be seen. Long filaments of bacteria can be seen growing in close association and wrapping around the fronds. They extend beyond the seaweed and appear to form roothair-like structures (Figure 17). Spores of the filamentous bacteria can be seen on the surface of the frond.

### **Discussion**

SEM micrographs of the surfaces of the isolated seaweeds show a diversity



**Figs. 13-16.** 13. Bacilli attached to surface of *Enteromorpha*. 14. Attachment structure of bacilli into surface of *Enteromorpha*. 15. Attachment of bacillus on *Enteromorpha*. 16. Colonies isolated from *Enteromorpha* displaying digestion of agar (a) bacterial colonies, (b) zone of agar digestion by agarase.



**Figs. 17-18.** 17. Fronds of *Ceramium* with associated bacteria. 18. Filamentous bacteria and spores associated with surface of *Ceramium* (a) spores, (b) filamentous bacteria.

of microbial life. Micrographs and light microscope photographs of the brown algae *Fucus* and *Ectocarpus*, the green algae *Enteromorpha*, and the red algae *Ceramium* show different types of microbial growth on their surfaces. The bacteria present include rods, cocci and filaments. Previous studies by Corre and Prieur<sup>11</sup> of the morphology and densities of marine bacteria on the kelp species *Laminaria digitata* found that three forms – bacilli, cocci and filamentous forms were present, although the rod shaped bacteria were the dominant forms. While we found all three types of bacteria present, our results also indicate an abundance of rod shaped and filamentous bacteria.

Diatoms join in establishing the microbial community on the seaweed surface. Grossart<sup>10</sup> showed that bacteria and diatoms both benefit from the relationship under optimum conditions, but diatom growth is inhibited with reduced nutrients.

The relationship between the bacteria and their hosts may serve the purpose of reducing biofouling and limiting the number of organisms which can grow on the surface. Antifouling chemicals may be produced by either the algal cells or by the epiphytic microorganisms. The green alga *Ulva reticulata* is often free from biofouling<sup>17</sup>. It is thought that the antifouling mechanism is due to material from the macroalga and the epibiotic bacteria on the surface. It is possible that the bacteria found on *Enteromorpha* play an important role in preventing biofouling. Lemos<sup>18</sup> found that *Enteromorpha intestinalis* yielded the highest number of bacteria with antibiotic properties. We found that the surface of *Enteromorpha* was sparsely inhabited by bacteria and those which are present were limited in diversity. This was also

true of *Ceramium* which appeared to be limited to one type of filamentous bacteria.

Epiphytic bacteria found on the surface of marine macroalgae have been shown to produce agarase which plays a role in adhesion of the bacteria to the surface of the seaweeds. Vera *et al.*<sup>19</sup> isolated and characterized an agarase from *Pseudoalteromonas* as did Holmstrom and Kjelleberg<sup>20</sup>. The organism we isolated from *Enteromorpha* was identified as *Pseudoalteromonas* and was shown to produce an agarase. Holmstrom *et al.*<sup>21</sup> has determined that *Pseudoalteromonas tunicata* demonstrates antimicrobial activity and produces both a yellow and a purple pigment.

Marine bacteria are extremely difficult to culture and identify with most species being uncultivable. Of samples sent for analysis to Microbial ID, Inc. there was considerable difficulty in identification of these organisms. Identification of four of the strains isolated indicated that they most closely matched *Pseudoalteromonas* species. The results of the fatty acid analysis were not the same as for the 16S RNA of the same cultures and the level of confidence in the identification was not high. This indicates that most of these organisms may not have previously been identified.

In conclusion, the surface of marine macroalgae can provide an environment in which microbial communities can form. These communities consist of a variety of bacteria and diatoms which vary in the number and type of bacteria depending on the species of seaweeds. Interaction between host and bacteria may be a factor in reducing biofouling. The production of these bioactive substances should be explored in the future. These

results suggest that seaweed-associated bacteria may be useful for ecological interaction studies and the production of anti-fouling substances.

### Acknowledgments

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**The Effect of Tank Color on VO<sub>2</sub> Uptake in White Perch**  
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**ABSTRACT**

VO<sub>2</sub> was determined for young-of-the-year white perch held for two hours in different colored tanks. The tanks were provided with color filters, and did not allow fish access to an air-water interface. The average VO<sub>2</sub> for fish in black tanks was 0.1627/gm/hr. This was significantly different ( $\alpha = 0.05$ ), by 23 to 31 percent from fish in yellow, red, white, blue, and green tanks. Foot candles within the tanks ranged from 0.0 (black) to 5.4 (yellow). There was no consistent relation between FC and VO<sub>2</sub>.

Key words: Tank color, dissolved oxygen, VO<sub>2</sub>/gm/hour

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**Introduction**

Some species of fish are known to perceive colors. It has been suggested that color vision is present in the teleosts<sup>1</sup>.

Few studies have been devoted to understanding the effects of background color on fish biology<sup>2</sup>. Reduced growth rates have been reported in dim conditions<sup>3,4,5</sup>. In dim light fish were unable to see clearly<sup>3,6</sup>. White or light gray favored survival and growth of *Perca fluviatilis*<sup>7</sup>. Light walls also improved food perception in other fish species<sup>8,9</sup>, whereas tanks with dark walls improved the prey perception and consumption in larvae of three fish species<sup>10,11,12</sup>. Tanks with black walls were found preferable for marine fish larviculture<sup>13</sup>. The behavior, including the respiratory frequency (opercular movements), of *Oreochromis niloticus* (tilapia) in aquaria with six different background colors was examined and it was found that respiration rates were lowest in red aquaria and highest in white aquaria<sup>14</sup>.

The purpose of this study was to determine if the color of tank walls have an effect on respiration (VO<sub>2</sub>) in white perch, *Morone americana*.

**Materials and Methods**

Young-of-the-year white perch were collected from Deal Lake, New Jersey. This is a fresh water lake that empties into the Atlantic Ocean through a flume. The flume allows entry of adult white perch, where they breed and spawn. Ocean waters can enter the lake during extreme high tides. Fish were returned to the laboratory and maintained in aged tap water at 24° C for one day prior to use. Thirteen tests were performed by placing two perch into each of six colored plastic vessels containing two liters of aged tap water. The colors included black, yellow, red, white, blue, and green. Each vessel was then covered with a donut-shaped Styrofoam cover. The cover circle was one inch wide, with its center removed. Pasted to the bottom of each cover was a colored acetate sheet (Colored Acetate sheets #6672092 Avcorn Systems, Inc. Cutchogue, NY) that corresponded in its color to the color of the vessel it covered. The cover was placed on the top of the test vessel so as to prevent an air-water interface. A plastic tube (inside diameter 5 mm) extended through the cover half-way into the vessel (65 mm), and to the outside of the vessel (Fig. 1). At the conclusion of each two hour test a siphon

was started and the water in the test vessel filled a BOD bottle. The dissolved oxygen in this water sample was determined by the Winkler Method<sup>15</sup>. Each of the thirteen test runs employed the six colors concurrently. Test temperatures ranged from 23.25 to 24.00°C. Hardness of the test water was 120 ppm, with a pH 7.5. Fish were weighed at the conclusion of each test. Fish were not fed prior to or during the test.

To determine the  $VO_2$  for the two fish in each vessel in mls/gm/hr the following formula was used:

D.O. at the start of the test minus the D.O. at the end of the test times the mls in the test vessel minus the weight of the two fish (one gm = one ml), divided by the time in the test vessel, divided by the weight of the two fish, times 0.7 to convert to mls of  $O_2$ .

ANOVA<sup>16</sup> was performed to determine if:

1. the  $VO_2$  means for the six colors and
2. the means for fish weights differed. If the F values were significant ( $\alpha = 0.05$ ) Tukey's test of the means<sup>16</sup> was performed to determine which groups were significantly different.

The amount of light, measured in foot candles (FC), and also reported in lux, was determined 1. at the covered surface of the test vessels and 2. beneath the acetate cover within the test vessels (Ex Tech Inst. # 401027 Foot Candle Light Meter, Waltham, Mass.).

## Results

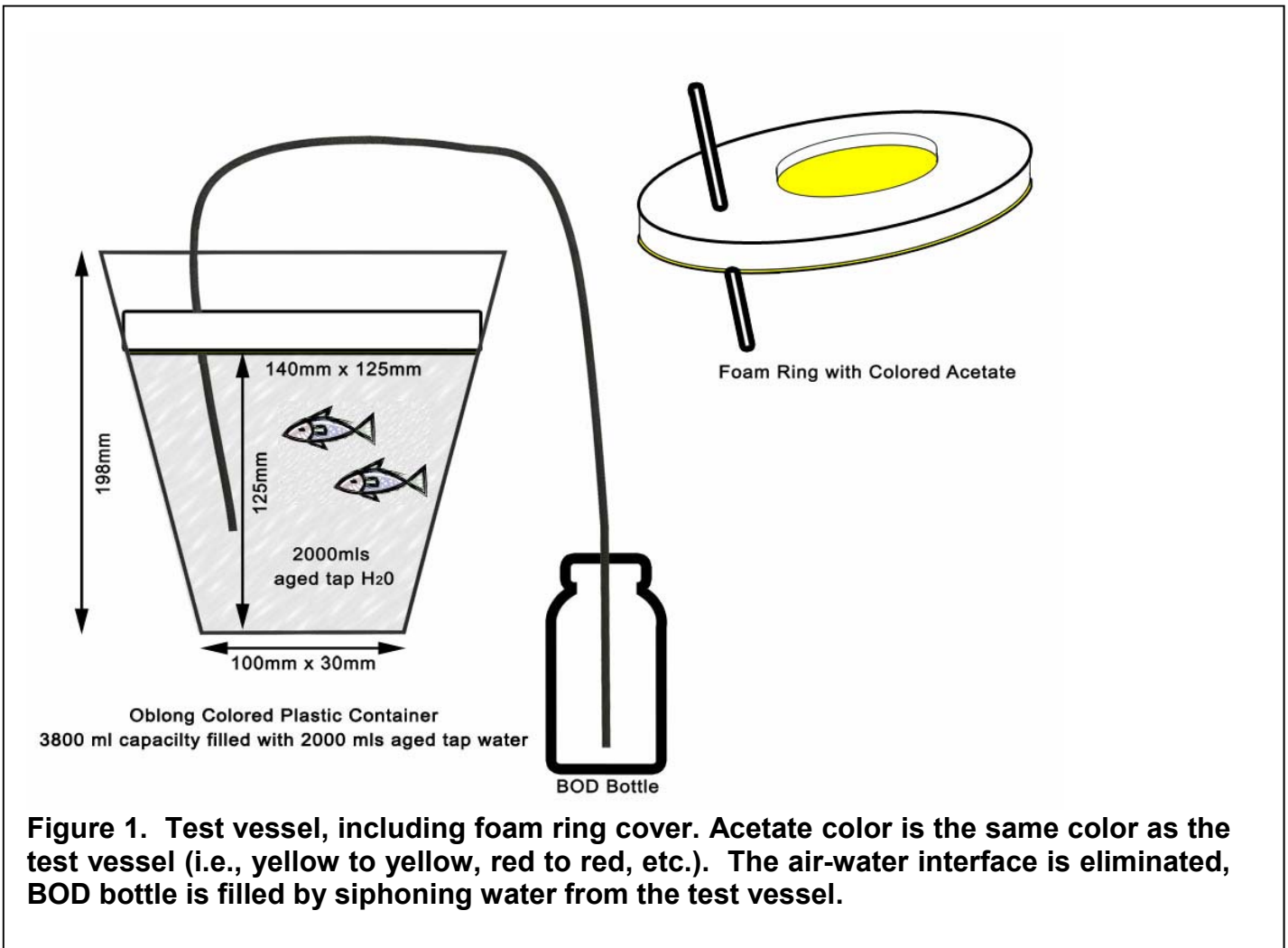
There was a significant difference in the means of the  $VO_2$  between fishes in the different color vessels. Tukey's procedure indicated that the fish in the black container used significantly less  $VO_2$ /gram/hr than those fish in the other colors (Table 1). Fish in the green vessel utilized the greatest amount of oxygen. The F value for the weights was not significant ( $\alpha = 0.05$ ). The foot candles at the top of the vessels ranged from 25 - 30. The FC within the covered vessels ranged from 0.0 to 5.4 (Table 2).

Dissolved oxygen levels at the conclusion of the tests in all colors never fell below 50 percent saturation.

## Discussion

White perch in black tanks used 23 to 31 percent less oxygen than fish maintained in other color tanks. The highest average  $VO_2$  occurred in green and blue tanks. They had an average  $VO_2$  of 0.2375 and 0.2298 respectively. It was observed in a study with tilapia maintained in six different colored aquaria that the lowest and highest respiratory rates occurred in red and white aquaria respectively<sup>14</sup>. The respiratory rates in that study, determined by opercular counts, may not adequately reflect the amount of  $O_2$  utilized by the fish. The higher  $VO_2$  in the blue and green colored tanks in this study may not be due to stress, but rather to improved welfare of the fish. In a study with tilapia, increased aggression was observed in a blue background<sup>14</sup>. Increased aggression was also observed with the fish *matrinxa* in a blue background, but these fish grew three times as much in this color<sup>17</sup>. The researcher indicated that the increased aggressiveness may imply that these fish were unstressed, and were acting naturally<sup>17</sup>. The visual environment of the fishes are blue, green or near infrared<sup>18</sup>. The young-of-the-year white perch used in this study were collected from a lake that has frequent plant blooms. This may result in the green pigment of the cone cells being more effective than blue and the other colors examined. In the black tank only rods would be involved, resulting in diminished activity and the lowest  $VO_2$ . Fish in tanks with the highest FCs, yellow and white, had an average  $VO_2$  4-11 percent less than those in the green and blue tanks.

Based upon the results of this study, transportation of fish in black tanks would be more appropriate for fish survival. However, since dark tanks may reduce active behavior (feeding), green and blue tanks would be more appropriate for growth, but at a cost of increased  $VO_2$  consumption.



**Table 1. Average VO<sub>2</sub>/gram/hour uptake in white perch, *Morone americana*, maintained for two hours in tanks of different colors, and their average weight (grams)**

Color	Black	Yellow	Red	White	Blue	Green
VO <sub>2</sub> /gm/hr	0.1627	0.2120	0.2199	0.2206	0.2298	0.2375*
Wt(gm)	8.09	8.74	8.33	8.66	8.45	7.58*

**Table 2. Foot candles (FC), also presented as Lux, at the top of the test tanks, and beneath the tank covers with the filters in place**

**Foot Candles at the top of the covered test tanks = 25-30 FC (= 269-323 Lux)**

Color	Black	Yellow	Red	White	Blue	Green
FC	0.0	5.4	1.3	4.8	1.2	0.2
Lux	0.00	58.10	13.99	51.65	12.91	2.15

## Acknowledgments

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## The Adverse Effects of Deletion of the Ozone Layer

by

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### Introduction

The ozone layer protects the earth's environment from the ultraviolet B (UVB, 290 to 320 Nanometers) cancer-causing radiation emitted by sun. There is great concern that the ozone layer depletion may cause more UVB from the sun reaching the lands and waters. This increase may cause an escalation in the respective prevalence of cataract and skin cancers for some people. Additionally, such an increase of UVB may also damage crops and animals. However, it is not only the threat to people, crops, and animals that necessitates calls for serious damage-control policies, but there are as well other reasons prompting concerns. Among such reasons are changes in the stratosphere, which may create abnormal weather patterns, shifting wind and rainfall belts and also may alter the global temperature. These changes may certainly affect our natural lives<sup>1</sup>.

Chlorofluorocarbons (CFCs) are nontoxic, nonflammable chemicals containing carbon, chlorine and fluorine. They are used in the manufacturing of aerosol sprays, blowing agents for foams and packing materials. They have been used not only as solvents, but also as refrigerants. CFCs interact with the ozone layer by depleting it from the stratosphere. A single chlorine atom from a disintegrating CFC can destroy 100,000 ozone molecules. Due to the rapid exchange between atoms of CFCs and nature's inability to replenish them, the ozone layer may thus become depleted. CFCs released from products used by consumers may take between ten to fifteen years to reach the stratosphere. Unfortunately, due to

the slow speed of this process, even if people today stopped using ozone depleting products - such as those found in refrigerators and freezers, air conditioners, automobile air conditioners, cleaning products, bleach, scouring powder, pest controls, fire extinguishers, etc, it may take a very long time to eventually replenish the ozone layer<sup>2</sup>.

The severity of this problem is further compounded because hitherto, we only addressed CFCs and not the volatile organic compounds (VOCs). VOCs such as aerosol cooking sprays, automotive cleaners, hair products, insecticides, and other products all may damage the atmospheric ozone<sup>3,4</sup>.

### Background

Data from NASA satellites revealed that the ozone levels dropped significantly from 1978 to 1991 over the mid-latitude areas, including the continents of North and South America, Africa, Asia and Australia where the greatest part of the world population is concentrated<sup>5</sup>. Analysis of the satellite data contradicted a widely held theory which suggested that ozone weakened in the mid-latitudes merely because ozone-depleted air flowed in from the poles. The satellite data further suggested that ozone is being destroyed directly over the mid-latitudes.

As early as in April of 1995, it was suggested that the atmospheric changes resulting in ozone layer depletion were caused by human activity regarding the use of CFCs, which was observed from geostationary satellites<sup>6</sup>. Another study, half a year earlier, reported observations of ozone

layer depletion that provided further evidence of the harmful effect of CFCs on the earth. The author as well noted that volcanic gases were not the primary cause of the ozone layer depletion as suggested by other scientists<sup>7</sup>.

It is very important that we not use ozone-depleting chemical compounds containing molecules of chlorine, fluorine or bromine. The most harmful chemicals are those categorized as "Classical" by the United States Clean Air Act Amendment of 1990, which are CFCs, halons, carbon tetrachloride, and 1,1,1-trichloroethane (methyl chloroform). These chemicals account for almost 80 percent of our ozone layer depletion<sup>4</sup>.

It is true that some steps have been taken to protect the ozone layer from depletion. The U. S. Federal Trade Commission established guidelines for the use of "ozone friendly" and "ozone safe" products. These terms identify products that do not deplete the ozone layer. Any product labeled as "ozone friendly" or "ozone safe" should not contain CFCs or any other chemicals harmful to the ozone layer<sup>8</sup>.

The Vienna Convention of 1985 gave serious impetus to the protection of the ozone layer. The convention sponsored by the United Nations Environment Program (UNEP) set up an international structure to address the damage to the environment. It committed the signatory countries to prevent further damages to both humans and the environment due to ozone layer depletion. The services of UNEP include scientific, technical and legal cooperation, as well as the free communication of important information that impacts on the environment<sup>9</sup>.

As early as September of 1987, the Montreal Protocol in Canada, initially signed by 23 nations, had a mandate to reduce CFCs and other chemicals by 50% of their levels at that time. Unfortunately, some industries refused to follow through on the Montreal Protocol. Notwithstanding, Du Pont Company was persuaded to use substitutes for its CFCs chemicals. In contrast to the mandate of the Montreal Protocol, China has a greatly increasing CFC production and

could have a serious effect to the earth preservation. There was some disagreement in the world community to phase out CFCs as of 1990. The European Community, however, was more forceful. They wanted a 50% cut in CFCs production between 1991 and 1992, an 85% cut in 1995-1996, and to end production by 1997 and 2000. In reality, upon the end of 2000, it was not surprising that such an attempt was unsuccessful. The United States latter established an international fund to help poor nations in dealing with the chemicals that deplete the ozone layer<sup>9</sup>. Unfortunately, the outcome has been far beyond satisfactory.

### **Protecting Ourselves from the UVB Rays Coming Through the Hole in the Ozone Layer**

While the United States is not presently in the worst area of UVB radiation danger, due to the 4 to 8% decrease in the ozone layer over the last 15 years, UVB radiation has made an unhealthy environment that we must protect ourselves from. The damage is usually created after repeated exposure to UVB over long periods of time. Skin cancer or cataracts can be avoided or significantly reduced by keeping the sun rays from our eyes and skin with following steps:

- Develop the habit of wearing clothing that shields us from the sun rays, such as fabrics with tight weave and a wide-brimmed hat that keeps UVB Radiation away from your face, ears, and neck.
- During the summer months, whether at the beach or strolling in the streets or park, use a sunscreen that has a skin protection factor (SPF) of at least 15 on exposed skin.
- Try to stay in shaded areas or indoors during the hours between 10 a.m. and 3 p.m.
- Protect your eyes from the sun rays by wearing sunglasses that absorb UVB radiation outdoors<sup>10</sup>.

## **Difficulty in Measuring Loss of the Ozone Layer**

Monitoring at the South Pole has shown that the ozone hole has started developing more often since 1991. Data from balloon-borne sensors used to measure ozone values revealed that ozone values were low during the second week of September, a week earlier than the previous years<sup>11</sup>. The ozone hole normally opens in September when the sunlight returns to the extreme south, energizing reactions in which CFCs and chlorine pollution breaks down ozone molecules in the stratosphere. The depletion continues until the early part of October, when the warm winds appear in the same region, stopping the cycle of CFCs and other chemical destruction.

Although CFCs appear over both the North and South Poles, conditions at the North Pole are very different from the South, and somehow, even more complex. There is a great difference in the distribution of land and water between the North and South Poles. Most land masses lie in the Northern Hemisphere. This mass of land and water is the reason for intense atmospheric disturbances in the Arctic. The flow of air over large mountains in the lower atmosphere forces air in the stratosphere to wander. Due to these disturbances, an Arctic whirling mass wanders and lurches chaotically. The instability of the Arctic vortex (a whirling mass of wind and water) is good news for the ozone layer, since it keeps more clouds from forming in the stratosphere over the North Pole. Often before the sun arrives in the spring, temperatures have risen, the clouds have evaporated and nitrogen oxides have already shut off ozone layer depletion. These conditions make predicting losses of the ozone layer very difficult. At the South Pole the amount of air that goes into the vortex is limited during the spring. This allows atmospheric scientists to easily calculate yearly ozone losses as compared to the unpredictable situation in Arctic. Ozone loss is measured by comparing the spring and early winter levels. In the more dynamic Arctic,

ozone-rich air pours into this region, makes it harder to measure the amount of ozone layer destroyed in the spring<sup>12</sup>.

## **Adverse Effects on the Climate**

Because of the complexity of the climate system, atmospheric scientists can only speculate about the real effects of CFCs and other chemicals on the ozone layer. However, if chlorine levels stay high, together with the warming of the earth's surface (the "greenhouse effect"), more water will evaporate from the oceans. This water vapor could contribute to the formation of more polar stratospheric clouds that further deplete the ozone layer.

*"The truth is that we have chlorine going up into the atmosphere from all kinds of sources. It's not just CFCs. It's chlorine from sewage treatment plants, water treatment plants, backyard swimming pools, and even household chlorine bleach. We were told that these molecules were too heavy; that they couldn't get into the stratosphere, but it isn't true. Atmospheric scientists have known this for years--it is lunch table conversation."<sup>13</sup>*

## **The Prediction of Further Damage**

Conversely, increases in terrestrial solar UVB radiation forecast for the earlier 21 century may affect the stability of DNA of plants, and consequently effects our own environment. However, it is not only the damage to skin as cancer formation<sup>14-17</sup>, the threat to people, crops, plants<sup>18-26</sup>, rearrangement of DNA of plants<sup>27-36</sup> and animals that calls for the need of serious damage-control policies, but also that the changes in the stratosphere may create abnormal weather patterns such as shifting wind and rainfall belts, and also may alter the global temperature and cause DNA damage in plants due to depletion of stratospheric ozone and acid rain, and the mechanism of photorepairing for the damage<sup>37</sup>. We can easily observe the harmful effects on climate by smoke as well understand the harmful effect of a nuclear winter<sup>38-40</sup> and the

injection of soot<sup>41</sup>. Soot, also known as lampblack or carbon black, is a dark powdery deposit of unburned fuel residues, usually composed mainly of amorphous carbon that accumulates in chimneys, automobile mufflers, the insides of smoke saunas and other surfaces exposed to smoke, especially from the combustion of carbon-rich organic fuels in the lack of sufficient oxygen. The combustion is thus incomplete. Lampblack is sometimes used only to refer to carbon deposited from incomplete burning of liquid hydrocarbons, while 'carbon black' may be used to refer to carbon deposited from incomplete burning or pyrolysis (decomposition or transformation of a compound caused by heat) of gaseous hydrocarbons such as natural gas (Wikipedia).

## Conclusion

With such a scary scenario regarding the ozone layer hole above Antarctica, 2006 is one of largest on record, covering an area more than three times the size of Australia. Each year an ozone hole forms over the Antarctica and then closes; and in 2006 the hole is the fourth largest on record. Conversely, in the year of 2005, in Antarctica, the total area of ice-melting phenomena was the largest in the past 30 years. Its ice-melting size is about as much as the area of California. Changing global temperatures and ozone-depleting chemicals in the atmosphere have caused the increasing size of the hole. Notwithstanding the impact of chemicals on the ozone layer, it is not getting worse, but unfortunately is not improving either. These chemicals reached a peak in the late 1990s, and we have seen a steady decline since then. However, it has not been enough of a decline to have a measurable positive impact on stratospheric ozone over Antarctica.

We all have a responsibility to prevent further destruction of the ozone layer. In order to fulfill such a responsibility, we ought to strictly follow the principles of environmental ethics by establishing and enforcing regulations; hopefully we may then be able to

prevent the air we breathe from being further contaminated. Therefore, obviously, much more rigid damage-control policies are really and urgently indicated.

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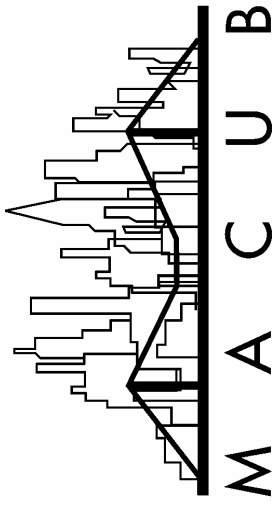
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