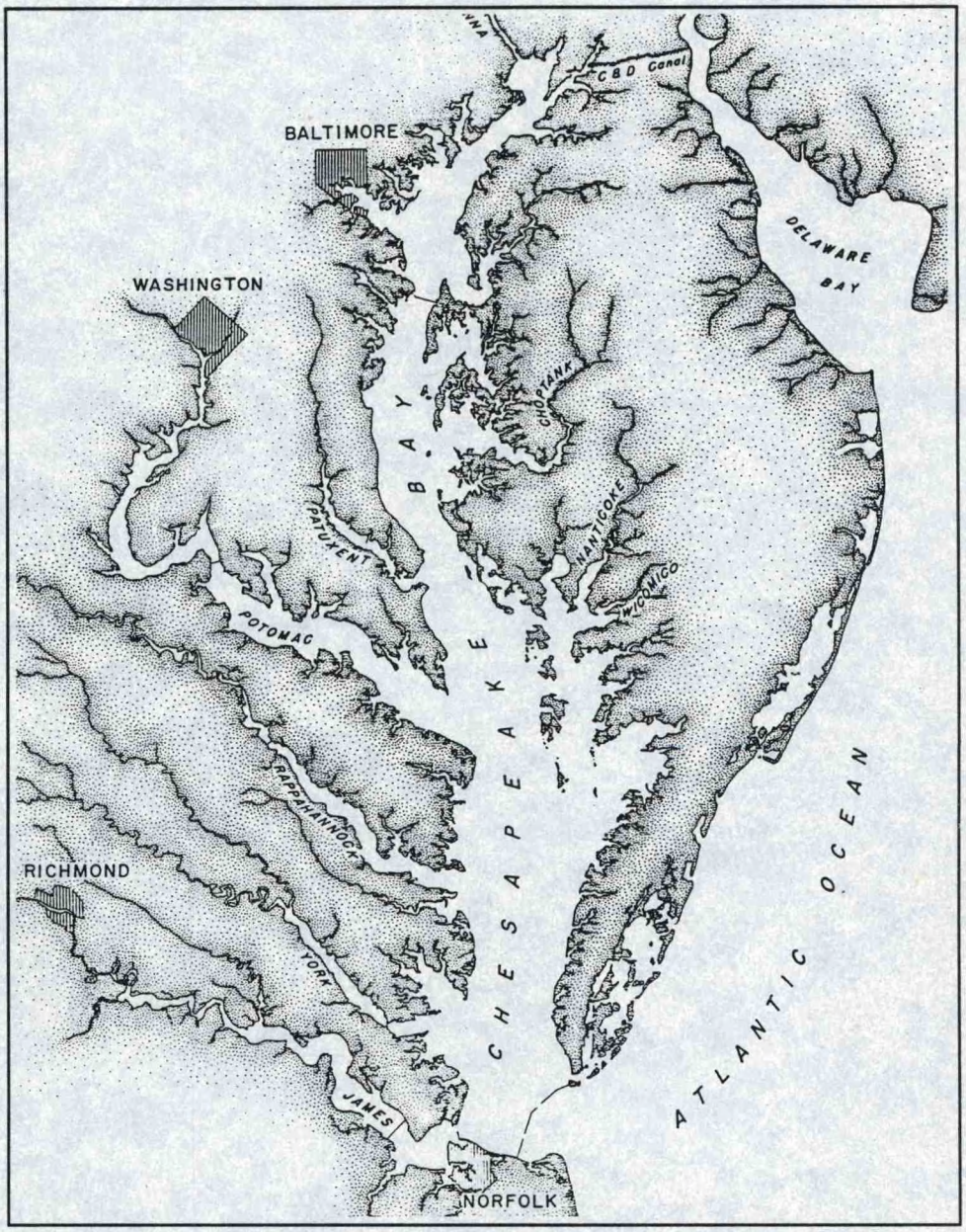
Maryland and Virginia Sea Grant College Programs

Environmental Effects Research on Chesapeake Bay

Toxics Research Program



Maryland Sea Grant

Virginia Sea Grant College Program

Submitted to the
National Sea Grant College Program
National Oceanic and Atmospheric Administration

April 1993

July 1, 1993

Proposal to: The National Sea Grant College Program
NOAA, U. S. Department of Commerce

Submitted by: VIRGINIA GRADUATE MARINE SCIENCE CONSORTIUM
Virginia Sea Grant College Program
MADISON HOUSE - 170 Rugby Road
University of Virginia
Charlottesville, VA 22903

Title: Chesapeake Bay Toxics Research Program: Virginia Portion for 1994

Amount requested from Sea Grant:	$ 413,393
Matching funds proposed:	$ -000-
Duration of proposed activity:	twelve months
Proposed starting date:	1 January 1994

We, the undersigned, certify that, in the event this proposal is accepted, in whole or in part, our signatures on this proposal constitute acceptance of and compliance with statutes and regulations of the U. S. Government and the U. S. Department of Commerce as detailed in Part Three, "The National Sea Grant Program, Background and Suggestions for Proposals", dated March 1, 1972, and that pages 63 to 107 of that publication are incorporated by references as part of this proposal.

Principal Investigator

_____
Signature

William L. Rickards, Director
Virginia Sea Grant College Program

221-28-3691

Virginia Graduate Marine Science Consortium
MADISON HOUSE - 170 Rubgy Road
University of Virginia
Charlottesville, Virginia 22903

Institutional Representative

_____
Signature

D. Wayne Jennings
Director of Sponsored Programs

223-42-1185

Office of Sponsored Programs
P. O. Box 9003
Carruthers Hall
University of Virginia
Charlottesville, Virginia 22903

U. S. Department of Commerce
National Oceanic & Atmospheric Administration

ASSURANCE OF COMPLIANCE WITH THE NONDISCRIMINATION CLAUSE
Applicable to:

Title: Chesapeake Bay Toxics Research Program:
Virginia Portion for 1994

VIRGINIA GRADUATE MARINE SCIENCE CONSORTIUM
Virginia Sea Grant College Program
MADISON HOUSE - 170 Rugby Road
University of Virginia,
Charlottesville, VA 22903

(1) hereby warrants, convents, agrees, and assures that it will conduct the program/project described by the above identified application/proposal, as it may be revised or modified prior to any grant award, in compliance with all requirements of the recipient imposed by or pursuant to the Nondiscrimination clause appended hereto, which clause shall also be incorporated into any grant awarded on the basis of such proposal, and

(2) agrees and acknowledges that this assurance of compliance is a prerequisite condition to approval of the proposed or any grant or grant modification or amendment extending any Federal financial assistance which may be in reliance on the representative made by this assurance and that the United States shall have the right to seek Judicial enforcement thereof, and that this assurance of compliance shall be binding upon it, its successors assignees, and transferees.

_____
Date

_____
Signature

D. Wayne Jennings
Director of Sponsored Programs
Office of Sponsored Programs
P. O. Box 9003
Carruthers Hall
University of Virginia
Charlottesville, Virginia 22903

Title Page

Date of this Proposal:	June 18, 1993
A Proposal to:	The Office of Sea Grant/NOAA U.S. Department of Commerce
Name and Address of Submitting Institution:	University of Maryland College Park, Maryland 20742
Title:	Environmental Effects Research on Chesapeake Bay: Toxics Research Program
Total Amount Requested From Sea Grant:	$413,445 (Virginia) $366,607 (Maryland) (Maryland/Virginia)
Duration of Proposed Activity:	Twelve months
Proposed Starting Date:	January 1, 1994
Year of Activity:	Nine
Previous Grant Amount, if Renewal:	1991: $679,106 1992: $557,207
Proposed Match:	$7,300
Principal Investigator:	

Signature: Christopher F. D'Elia
Director, Maryland Sea Grant
Social Security Number:
040-40-7366

Signature: Christopher F. D'Elia
Director, Maryland Sea Grant
Social Security Number:
040-40-7366

Signature: Richard F. Neville
Acting Provost, Maryland
Biotechnology Institute
Social Security Number:
074-24-5156

For administrative details, contact Jane Rice, Assistant to the Associate Director for Administrative and Financial Affairs, Maryland Biotechnology Institute, College Park, Maryland 20742. Phone: 403-4691.

U.S. Department of Commerce
National Oceanic and
Atmospheric Administration

ASSURANCE OF COMPLIANCE WITH THE NONDISCRIMINATION CLAUSE
Applicable to:

SEA GRANT COLLEGE PROGRAM

UNIVERSITY OF MARYLAND
COLLEGE PARK, MARYLAND

(1)hereby warrants, covenants, agrees and assures that it will conduct the program/project described by the above-identified application/proposal, or as it may be revised or modified prior to any grant award or subsequent to any grant award, in compliance with all requirements of the "recipient" imposed by or pursuant to the nondiscrimination clause appended hereto, which clause shall also be incorporated into any grant awarded on the basis of such proposal, and

(2)agrees and acknowledges that this assurance of compliance is a prerequisite condition to approval of the proposal or any grant or grant modification or amendment extending and Federal financial assistance which may be extended to it by the U.S. Department of Commerce will be in reliance on the representation made by this assurance and that the United States shall have the right to seek judicial enforcement thereof, and that this assurance of compliance shall be binding upon it, its successors, assignees, and transferees.

_____
Date

_____
Signature of offical authorized to sign.

Dr. Richard F. Neville, Acting Provost
Maryland Biotechnology Institute

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NOAA FORM 90-4 (ADAPTED) NOAA, U.S. DEPT OF COMMERCE

SEA GRANT BUDGET

GRANTEE: Virginia Graduate Marine Science Consortium

GRANT/PRO. NO: CBT-TRP

PRINCIPAL INVESTIGATORS: W.L. Rickards, VGMSC

DURATION: 1/1-12/31/94

A. Salaries and Wages 1. Senior Personnel	No.	Man-Mo.	Sea Grant Funds	Grantee Funds
a. Prin. Investigator	7	8.80	36078	
b. Associates:			0	0
Sub Total:			36078	0
2. Other Personnel				
a. Professionals	1	2.00	5004	
b. Research Assoc.				
c. RA Grad. Stud.	6		78800	
d. Prof. School Stud.				
e. Pre-Bac. Stud.				
f. Secret./Clerical				
g. Technical/Shop	1		7666	
h. Hourly Labor	2		8928	0
Total Salaries and Wages			136476	0
B. Fringe Benefits			13954	0
Total Sal. Wages & Fringe Benefits			150430	0
C. Permanent Equipment			21500	0
D. Expendable Supplies			26988	0
E. Travel				
1. Domestic - US & Possessions		1.	10701	
2. International		2.	0	0
Total Travel			10701	0
F. Pub. and Documentation Costs			4525	0
G. Other Costs				
1. vessel rental			11220	
2. tuition			4200	
3. gas cylinder rental			1644	
4. waste disposal, computer			2874	
5. photocopying, graphic arts			1341	
6. instrument service contracts			4725	
7. workshops, conferences			7126	
8. telephone, postage			1000	
9. CBEEC staff assistance			25000	
10. two PD revised projects			100000	
Total Other Costs			159130	0
TOTAL DIRECT COSTS (A through G)			373274	0
Indirect Costs: On Campus:			40119	
Off Campus:				
Total Indirect Costs			40119	0
TOTAL COSTS			413393	0

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO:
University of Maryland, Maryland Sea Grant College
PRINCIPAL INVESTIGATORS: Christopher F. D'Elia

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	11.9	1.5	53,312	5,000
b. Associates	2.2	1.0	6,490	
Sub Total			59,802	5,000
2. OTHER PERSONNEL				
a. Professionals	20.0		45,330	
b. Research Associates	18.0		24,040	
c. Res. Asst. Grad. Std	1.0		12,328	
d. Prof. School Student				
e. Pre-Bac Students				
f. Secretarial-Clerical	1.2		2,000	
g. Technical-Shop				
Total Salaries/ Wages	40.2		143,500	5,000
B. FRINGE BENEFITS			32,231	0
Total Salaries,Wages and Fringe Benefits			175,731	5,000
C. PERMANENT EQUIPMENT			6,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT			32,300	
E. TRAVEL				
1. Domestic	5,300			
2. International	0			
Total Travel			5,300	
F. PUBLICATIONS AND DOCUMENTATION COSTS			2,600	
G. OTHER COSTS				
1. Computer costs			1,200	
2. Copying,Library and Communication			1,700	
3. Analytical and Shop Services			12,000	
4. Fuel,Boat Time and Vehicle Usage			3,250	
5. Equipment Use and Maintenence			1,300	
6. Subcontracts				
7. Service Contracts			4,047	
8. Waste Disposal			500	
9. Other			2,500	
Total Other Costs			26,497	
TOTAL DIRECT COSTS			248,428	5,000
INDIRECT COSTS				
(On Campus) : % of			118,179	2,300
(Off Campus) : % of			0	0
Total Indirect Costs			118,179	2,300
TOTAL COSTS			366,607	7,300

VIRGINIA SEA GRANT COLLEGE PROGRAM

CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES

TOXICS RESEARCH PROGRAM - 1994

ACTIVITY BUDGET

	NOAA FUNDS	MATCHING FUNDS
MARINE RESOURCES DEVELOPMENT		
Pollution - Toxics	$ 215,931	$ 000
PROGRAM MANAGEMENT AND DEVELOP.		
Program Development	$ 197,462	$ 000
TOTAL	$ 413,393	$ 000

MARYLAND SEA GRANT COLLEGE

Activity Budget Sheet for 1993

	NOAA FUNDS	MATCHING FUNDS
Marine Resources Development Biological Oceanography	$366,607	$7,300

INTRODUCTION

CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES: TOXICS RESEARCH PROGRAM

Joint Maryland and
Virginia Sea Grant College Programs

Christopher D'Elia, Director
Maryland Sea Grant College Program

William Rickards, Director
Virginia Sea Grant College Program

BACKGROUND

In order to restore the productivity and ecological health of the Chesapeake Bay, the federal/state Chesapeake Bay restoration program has set as a goal the reduction of nutrients and of toxic substances into the estuary, Implementation of effective management strategies is basic to improvement of habitat for ecologically and economically important species, and to the many benefits associated with a properly functioning ecosystem. Such actions require a sound scientific basis, however, and many questions need to be addressed as to the impact of anthropogenic materials in the estuary.

To fill this information need, the National Oceanic and Atmospheric Administration (NOAA), through the Maryland and Virginia Sea Grant College Programs, began a major effort to address the issue of environmental effects due to low dissolved oxygen in the Bay. This study, initiated in September 1985, has shed light on both the causes and consequences of hypoxia, and results of this research are now being used by state and federal management and regulatory agencies. Funding levels from NOAA averaged about $400,000 annually, transferred within NOAA's Estuarine Programs Office to be administered by the National Sea Grant Office (NSGO). In 1987, the Chesapeake Bay Environmental Effects Committee (CBEEC) was established by NOAA to oversee this research effort and to provide direction for future programs addressing other critical environmental problems. The committee includes representation from the Maryland, Pennsylvania and Virginia scientific and management communities.

NOAA was reorganized in 1989 and the EPO disbanded, with many of its functions being folded into the new Coastal Oceans Program (COP). Currently, funding from NOAA is transferred directly from National Marine Fisheries Service to NSGO, and thence to the state Sea Grant Programs. In 1990, the Environmental Protection

Agency's (EPA) Chesapeake Bay Liaison Office, with approval from the Chesapeake Bay Toxics Subcommittee, joined the Environmental Effects research proram by augmenting the fiscal support with the stipulation that the Program be redirected from hypoxia to studies of toxic contaminants in Chesapeake Bay, an area where considerable information is needed to support planned management actions.

CBEEC recognized the need to address the issue of toxics and accepted the task of developing a cooperative long-range, multidisciplinary, multi-institutional research effort patterned after the Hypoxia Research Program. In so agreeing, the Committee noted that there were several continuing hypoxia projects needing a final year's support. CBEEC further recommended that the initial focus of toxics research be on the ecological effects of contaminants, which complemented previous studies of hypoxia. This focus reflected an awareness that the total funding level in 1990, $783,000, was inadequate to support the type of broad-range toxics research program envisioned by the Bay Program's Research Planning Council (RPC 1989). CBEEC, RPC, and others have recommended that increased resources be directed to the toxics issue and that the research areas be broadened as the funding permits.

The present proposal covers the fourth year in which emphasis is placed upon the ecological effects of toxics in the Bay. Several of the projects (R/CBT-18, 19 and 20) are presented in very brief form since they are renewals of projects previously approved for subsequent funding as long as initial progress was judged acceptable by CBEEC. The balance of the projects (R/CBT-22, 23, 24 and 25) in this proposal are new to the Program.

HYPOXIA RESEARCH PROGRAM

The hypoxia research program began in 1985, and was funded annually at the level of approximately $400,000. The program, which was conceived as an ecologically oriented study focussing on system-level effects has greatly refined the understanding of the complex relationships between nutrients, production of organic material, carbon cycling and the development of hypoxia. In addition, the impact of low dissolved oxygen on the functioning and productivity of key species and communities was found to be significant.

The results of this research program have become widely recognized in the scientific community, and are receiving attention from managers because of their applicability to similar problems throughout the world. Special sessions devoted to this research were convened at the January and December 1988 American Geophysical Union meetings and at the October 1989 Estuarine Research Federation meeting in Baltimore. In addition, a

contributed sessions focussed on the effects of hypoxia was included in the December 1990 Chesapeake Research Consortium conference on Bay research.

A recently published book, Oxygen Dynamics in Chesapeake Bay, summarizes the current level of understanding of the interaction of physical, chemical and biological processes that create hypoxic conditions, was edited by the Virginia and Maryland Sea Grant programs. A workshop convened in late 1991 to achieve agreement among the researchers involved with the hypoxia program resulted in the document Dissolved Oxygen in the Chesapeake Bay: A Scientific Concensus. This report highlights the history of hypoxia in the Bay, the primary causes and what would be the predicted response of the system to nutrient reductions.

TOXICS RESEARCH PROGRAM

As indicated above, the hypoxia program had an ecological focus on system-level environmental effects. In conceiving the toxics research program, CBEEC felt that it was important to keep, as much as possible, the ecological focus of the predecessor program on hypoxia and to begin a smooth transition to the study of toxics. The idea of developing a well-integrated program with continuity to previous research was particularly attractive to CBEEC, given the limited funding available, and given the inherently great expense of conducting research in a subject area as broad as toxics. However, in deciding on the ecological focus of the initial phases of the toxics research program, CBEEC clearly recognized that as the program builds and out-year funding increases, proportionally more resources will have to be invested in projects dealing with organismal, cellular, molecular and physiological processes, and in the promising and burgeoning area of biomarkers.

The following sections review the goals of the early phases of the toxics research program, describe the processes leading up to the selection of the successful proposals, and provide a brief precis of those proposals.

Initial GOALS of the CBEEC Toxics Research Program

* To understand how Chesapeake Bay ecosystem processes influence the transport, fate and effects of toxicants; and

* To understand the effects that representative toxicants have upon ecological processes, including trophic dynamics, in the Bay.

These goals are to be accomplished through the establishment of a multi-year, interdisciplinary and inter-institutional research program, supported initially by funding from NOAA and EPA, and administered jointly by the Maryland and Virginia Sea Grant College Programs.

Priorities

Long-term objectives for the toxics research program were based on the Chesapeake Bay Program Research Planning Committee's "Toxic Research Prioritizations" document, and focus on increasing the understanding of the source, transport, fate and effects of toxicants in support of the development of ecological risk assessments for the Chesapeake Bay. The Chesapeake Bay Environmental Effects Committee prepared an RFP based on these needs, emphasizing an ecosystem approach to the issue of toxics in the Chesapeake Bay. Copies of the RFP are available, upon request, from the Virginia Sea Grant College Program.

Principal research needs were identified and noted in the Request for Proposals:

1. Understanding of the factors controlling the distribution of toxics in various parts of the Bay ecosystem, including a quantitative assessment of the dynamics of these processes which can later serve as input to models.

2. Determining the existence of potential biological effects of anthropogenic substances, especially those affecting ecosystem processes such as trophic relationships, reproductive success, and productivity. This information can later be used to evaluate the effects upon populations and biotic communities, a major need for ecological risk assessment.

Proposal Review and Selection Process

The sequence of events for the proposal selection and review follows:

* The Request for Proposals was drafted by CBEEC, reviewed by the Toxics Subcommittee , revised and distributed throughout the Bay Region.

* 13 new project proposals plus 3 renewals were received by CBEEC;

* All CBEEC members (and Toxics Subcommittee liaisons) received all proposals for review;

* Full proposals were peer-reviewed through the independent National Sea Grant review process: for each proposal, up to 5 mail-in-review comments were solicited; in addition two members of an ad hoc review panel convened by National Sea Grant critiqued each new project proposal; CBEEC representatives observed this panel discussion;

* CBEEC met with NOSG to discuss summaries of the reviews for each proposal, to discuss their merits relative to the objectives of the Toxics Research Program, and to make final funding recommendations based on programmatic criteria.

Final selections were made using two basic criteria: excellence of the science as determined by the review process and relevance to programmatic goals. The committee recognized that resources, as well as the pool of proposals, would not allow complete coverage of all relevant issues, but the final list of projects constitutes an excellent fourth-year program that builds on earlier efforts. In future years, continued focusing of the RFP and additional funding resources will assist in filling gaps in needed research. From an ecosystem perspective, CBEEC feels that the program will produce information on a number of important processes affecting the behavior of toxicants in the Bay.
A conceptual diagram of these processes (Fig. 1) compared to the selected proposals indicates good coverage of major pathways. The projects will also address many of the specific recommendations in the most recent RPW **1990 Research Priorities** document (May, 1990).

Projects to be funded in 1994

* Dickhut, R.M. "Determination of the volatile/absorptive exchange of hyrophobic organic contaminants across the air/water interface of lower Chesapeake Bay." (Continuing). This research seeks to quantify the gaseous transfer of toxic organic chemicals from the atmosphere to aquatic systems and the role of the air/water surface microlayer, i.e. whether resistant to this exchange or a separate, potentially controlling phase in vapor transfer at the air/water interface. This information will be used to determine the net flux of organic contaminants between the atmosphere and the Bay.

* Weiner, R. and M. Walch. "Interaction of copper and cadmium with microbial benthos biofilm and effects on oyster larval set." (Continuing). This project will examine the bioconcentration of toxic trace elements within microbial biofilms and the impact of this phenomenon on the set and survival of oyster spat, which are preferentially attracted to such films prior to metamorphosis. This information will be useful for oyster management, culture and restoration programs.

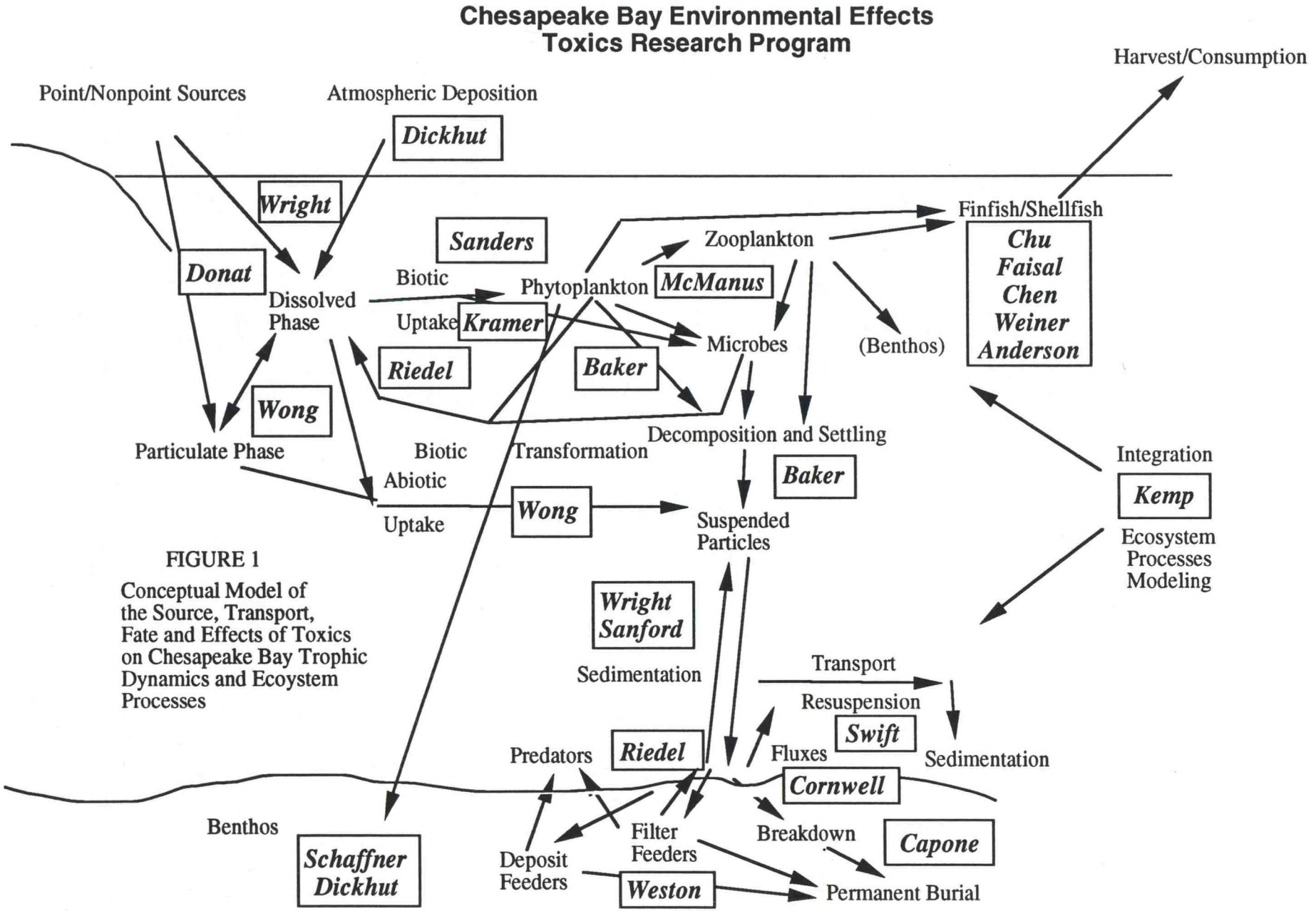

FIGURE 1

Conceptual Model of the Source, Transport, Fate and Effects of Toxics on Chesapeake Bay Trophic Dynamics and Ecosystem Processes

* Riedel, G.F., J.G. Sanders and C.C. Gilmour. "Contaminant flux from sediments: impact on Chesapeake Bay food webs." (Continuing). This study is utilizing microcosms to measure the availability and rate of uptake of toxic trace elements from sediments by natural estuarine communities, and the impact of the materials on lower trophic levels (phytoplankton and zooplankton) in Chesapeake Bay.

* Anderson, R.S. and E.M. Burreson. "The effects of environmental contaminants on the progression of Perkinsus marinus infection in the Eastern oyster." (New). This project will attempt to elucidate the role which toxic substances may play in the cell-mediated changes that occur in oysters which become infected with the disease Perkinsus marinus. Immune functions in oysters exposed to toxic substances appear to be mediated so that the disease organisms more readily infect the host. The results will be of use in oyster management, culture and restoration programs.

* Kramer, J.G. "Metallothionein in marine coccoid cyanobacteria: cloning, transcriptional analysis and application to the assessment of metal stress in natural communities of pico-plankton in Chesapeake Bay." (New). This research will employ cloned genes as probes to determine the relative effects of metal exposure upon the expression of metallothioneins in cyanobacteria from the lower Bay and its tributaries. The results will assist managers in assessing the impact of heavy metals upon the microbial community in the Bay.

* Wright, D.A. and R. Dawson. "A risk assessment for Dimilin use in the northern Chesapeake Bay: a model study for nonpoint-source runoff." (New). This project will characterize the role of Dimilin in the Bay, and it will form the basis of a hazard assessment for this compound's mode of action. Chemical application, run-off and toxicity data will be combined to generate a model risk assessment for non-point source pesticide run-off.

* Dickhut, R.M. and L.C. Schaffner. "Organic contaminant metabolite production, elimination, and bioavailability in benthic macrofauna of lower Chesapeake Bay." (New). This research will further examine the role of benthic organisms in the flux of toxic substances from the sediments and the transformations of such substances which occur as a result of ingestion, digestion and excretion by the organisms. The results will find application in modeling the mass balance of toxic contaminants in the Bay

while providing insight into the role of benthic organisms in the bioavailability of such substances via trophic transfer to demersal consumers.

The complete project proposals and budgets appear elsewhere in this proposal package.

PROGRAM MANAGEMENT

Funds are requested by discrete projects in order to clarify program activities and to facilitate program review and evaluation. The administration of funds will be through the Sea Grant Program of the individual investigator's institution. A series of meetings devoted specifically to data exchange and analysis will be held at appropriate times during the study. Data management, exchange and analysis will be assisted by the Maryland Sea Grant Program through the Sea Grant Computer Facility. General assessment of these efforts is provided by the Environmental Effects Committee, an advisory group which offers guidance to NOAA Chesapeake Bay research efforts. Grants management and programmatic oversight are provided by the Office of Sea Grant, NOAA.

REFERENCE

Chesapeake Bay Program Research Planning Committee 1989. Toxics Research Prioritizing. EPA Chesapeake Bay Liaison Office, Annapolis, MD.

SEA GRANT PROJECT SUMMARY

SD-SID-No.:_____
Specialist:_____
SG Class:_____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Determination of the volatile/absorptive exchange of hydrophobic organic contaminants across the air/water interface of lower Chesapeake Bay
PROJECT NUMBER: R/CBT-18
REVISION DATE: 06/15/93
PROJECT STATUS: 2
INITIATION DATE: 01/01/93
SUB PROGRAM: Toxics/CBEEC
COMPLETION DATE: 12/31/94

PRINCIPAL INVESTIGATOR: Rebecca M. Dickhut
EFFORT: 0.6 mo.
AFFILIATION: Virginia Institute of Marine Science
AFFILIATION CODE: 5101

S. G. FUNDS: $ 0
STATE MATCHING FUNDS: $ 0
LAST YEAR'S SG FUNDS: $ 0
LAST YEAR'S MATCH FUNDS: $ 0
PASS-THROUGH FUNDS: $ 87,963
LAST YEAR'S PASS-THROUGH: $ 95,824
RELATED PROJECTS: all R/CBT projects
PARENT PROJECTS: R/CBT-1
SEA GRANT CLASSIFICATION: Pollution - other - Toxics (45)
KEYWORDS: toxic, organics, kinetic, environment, behavior

OBJECTIVES: To quantify the volatile/absorptive exchange of HOCs across the air/water interface of Lower Chesapeake Bay. We propose to experimentally determine HOC mass transfer coefficients and uptake rates by surface microlayer material to assess whether this region acts as an inert layer of resistance to HOC gaseous exchange or a separate, active potentially controlling phase in HOC vapor transfer at the air/water interface.

METHODOLOGY: Our approach will be to directly measure HOC fugacities in surface waters using a floating sparger apparatus, collect surface microlayer samples using a rotating drum apparatus and measure HOC fugacities using a shipboard sparger system, and measure HOC vapor phase concentrations, and hence, fugacities using a high volume air sampler. Using a gas saturation technique and open tubular column liquid chromatography, we will determine HOC fugacity capacities, uptake rates, and diffusivities in collected microlayer material. A comparison will be made between the estimated HOC mass transfer coefficients in the surface microlayer to the rate of uptake of HOCs by particulate material in this region to deduce if the surface film can be viewed as an inert layer of resistance to gaseous exchange or an independent potentially controlling phase for HOC vapor transfer at the air/water interface. A two-film model, or modifications thereof, will be used to assess the volatile/absorptive flux of selected HOCs between the atmosphere and surface waters of lower Chesapeake Bay.

RATIONALE: To accurately assess atmospheric inputs HOC toxicants to Chesapeake Bay it is necessary to consider all of the major air/water exchange processes. Preliminary assessments show that vapor transfer fluxes of selected HOCs are likely of the same order of magnitude as wet depositional fluxes in the southern Chesapeake Bay region. The volatile/absorptive (gaseous) transfer of toxic organic chemicals between the atmosphere and aquatic systems is driven by concentration or fugacity gradients between the compartment and the physical-chemical properties of the contaminants. With the data collected in this study and those we are currently assembling as part of the Chesapeake Bay Atmospheric Deposition (CBAD) study, we propose to examine the models for determining the net flux of HOCs between the atmosphere and waters of the lower portion of Chesapeake Bay.

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO: R/CBT-18

PROJECT TITLE:
Determnation of the volatile/absorptive exchange of hydrophobic organic contaminants across the air/water interface of lower Chesapeake Bay

PROJECT STATUS: 2

PRINCIPAL INVESTIGATORS:
R. M. Dickhut

DURATION: 1/1/94 - 12/31/94

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	1	0.60	2135	0
b. Associates:	0	0.00	0	0
Sub Total:			2135	0
2. Other Personnel				
a. Professionals			0	0
b. Research Assoc.			0	0
c. RA Grad. Stud.	2	12.0	26400	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop			0	0
h. Hourly Labor	1	6.0	6048	0
Total Salaries and Wages			34583	0
B. FRINGE BENEFITS			1039	0
Total Sal. Wages & Fringe Benefits (A+B)			35622	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			10500	0
E. TRAVEL				
1. Domestic - US & Possessions	1. 3053		3053	0
2. International	2.		0	0
Total Travel			3053	0
F. PUBLICATION AND DOCUMENTATION COSTS			525	0
G. OTHER COSTS				
1. Vessel rental			10720	
2. Tuition			2800	
3. Insurance/leases/rentals/waste disp.			2520	
4. Contractual Services (instrument service)			4725	
5. Communications/copy card			315	
6. APRC/Graphic arts			476	
7. Computer costs			1404	
8.				
9.				
10.				
Total Other Costs			22960	0
TOTAL DIRECT COSTS (A through G)			72660	0
INDIRECT COSTS: On Campus: (5% of A2c;			1320	0
45% of A1a-b,A2a,b,d-h,B,D,E,F,G3-5)			13983	
TOTAL INDIRECT COSTS			15303	0
TOTAL COSTS			87963	0

DETERMINATION OF THE VOLATILE/ABSORPTIVE EXCHANGE OF HYDROPHOBIC ORGANIC CONTAMINANTS ACROSS THE AIR/WATER INTERFACE OF LOWER CHESAPEAKE BAY

Rebecca M. Dickhut, Ph.D.
Assistant Professor - Dept. of Physical Sciences
School of Marine Science
College of William and Mary
Virginia Institute of Marine Science
Gloucester Point, VA 23062

INTRODUCTION

Chemical transfer across the air/water interface is one of the dominant processes that controls concentrations and residence times of toxic organic chemicals in aquatic ecosystems. Air/water exchange mechanisms include: wet and dry particle deposition, vapor washout, volatilization/absorption at the water surface (vapor transfer), bubble stripping and bubble bursting. Semivolatile hydrophobic organic chemical (HOC) pollutants exist in both the vapor and particle-associated phases in the atmosphere, and thus, are subject to both particulate and gaseous transport pathways. Consequently, to determine the net atmospheric input of HOCs into a water body such as Chesapeake Bay, both depositional and volatile/absorptive exchange processes need to be considered.

Recent studies have demonstrated the relative importance of the diffusive exchange of HOCs via volatilization/absorption at the air/water interface in determining the overall flux of semivolatile HOCs between the atmosphere and a water body (Mackay *et al.*, 1986; Baker and Eisenreich, 1990). Mackay *et al.* (1986) developed a model describing the transfer of organic chemicals between the atmosphere and a water body, illustrating that volatilization of HOCs out of a water body can be as large as depositional inputs of HOCs into a water body. Baker and Eisenreich (1990), utilizing this model, indicated that mean volatilization fluxes of polychlorinated biphenyls (PCBs) out of Lake Superior during the summer may be similar to atmospheric deposition inputs to the lake.

In this study, we propose to assess the volatile/absorptive exchange of HOCs across the air/water interface of lower Chesapeake Bay by directly measuring the fugacities, and the spatial and temporal variability of HOC fugacities, for selected HOCs in the atmosphere and surface waters of the southern Chesapeake Bay region. We will evaluate the influence of the surface

microlayer on the diffusive flux of HOCs by assessing whether the microlayer acts as an inert layer of resistance to HOC vapor transfer or as a separate, active region potentially controlling HOC gaseous exchange. With the data collected in this study and those we are currently assembling as part of the Chesapeake Bay Atmospheric Deposition (CBAD) study, we propose to examine the model set forth by Mackay *et al.* (1986) for determining the net flux of HOCs between the atmosphere and lower Chesapeake Bay.

Relevance to the Problem

Semivolatile HOCs have caused particular environmental concern due to their highly hydrophobic nature (low water solubility, large octanol/water partition coefficient), ability to bioconcentrate, and resistance to chemical or microbial degradation. These substances include: combustion-related polycyclic aromatic hydrocarbons (PAHs), industrially derived polychlorinated biphenyls (PCBs), chlorinated naphthalenes used as wood preservatives, and other halogenated aromatic chemicals such as dioxins, furans, terphenyls and organochlorine pesticides. Many of these chemicals are known to be toxic.

In order to compile a legitimate mass balance for HOCs in an aquatic system, it is necessary to consider all of the major air/water exchange processes (Mackay *et al.*, 1986). In the Chesapeake Bay watershed, researchers conducting the Chesapeake Bay Atmospheric Deposition (CBAD) study are determining the wet and dry depositional fluxes of selected HOCs and trace elements to Chesapeake Bay (Baker *et al.*, 1991). Furthermore, through ongoing CBEEC research (Dickhut, 1990), we are measuring the air/water partitioning and kinetic mass transfer properties of HOCs necessary for modeling the air/water exchange of organic contaminants.

To quantify the volatile/absorptive exchange of HOCs, and hence, appropriately determine the net atmospheric input of HOCs to Chesapeake Bay, we propose to measure the fugacity gradients (air/water, air/surface microlayer, surface microlayer/water) for selected HOCs across the air/water interface, evaluate the spatial and temporal variability of HOC fugacities, and assess the influence of the surface microlayer on the air/water diffusive exchange potential. We will experimentally determine HOC mass transfer coefficients and uptake rates by surface microlayer material to assess whether this region acts as an inert layer of resistance to HOC gaseous exchange or a separate, active, and potentially controlling phase in HOC vapor transfer at the air/water interface. This information, in conjunction with the air/water partitioning and kinetic mass transfer properties of HOCs being generated through our recent CBEEC research project (Dickhut, 1990), will allow for reliable assessment of the volatile/absorptive exchange of HOCs at the air/water interface of Chesapeake Bay.

OBJECTIVES

Overall Objectives

The primary objective of the proposed research is to assess the volatile/absorptive exchange of hydrophobic organic contaminants (HOCs) across the air/water interface of southern Chesapeake Bay. We will:

1. directly measure the fugacities of selected HOCs in the atmosphere and surface waters of the southern Chesapeake Bay region,

2. evaluate the influence of the surface microlayer and air/water interfacial conditions (i.e. temperature, wind speed) on the diffusive flux of HOCs,

3. assess the spatial and temporal variability in HOC fugacities in the atmosphere and surface waters of lower Chesapeake Bay.

1993 Objectives

1. Design, fabricate, and validate surface water sparging systems for measuring fugacity.

2. Remodel/repair and evaluate existing rotating drum surface microlayer sampler.

3. Initiate field sampling, laboratory experiments, and sample analyses.

1994 Objectives

1. Conclude field sampling through an annual cycle, laboratory experiments, and sample analyses.

2. Evaluate data, compile modeling results, and prepare manuscripts for publication.

PROJECT CHANGES

No changes in the project objectives or methods, as outlined in the original proposal, are anticipated at this time.

PROGRESS

This project was initiated in January, 1993. The in situ sparging systems, described in detail in the original project proposal, for measuring surface water fugacities are currently being constructed. Rehabilitation of our rotating drum surface microlayer sampler is also currently underway, as is the design of a shipboard sparging system for measurement of surface microlayer fugacities. The high volume air sampler for measurement of gas-phase fugacities has already been purchased and calibrated for operation.

Completion of all sampling equipment is anticipated no later than June, 1993. Subsequently, preliminary evaluation of all of the devices will be conducted near shore on the York River. We anticipate initiating sampling on the Chesapeake Bay by midsummer, 1993; we are currently discussing the logistics of our sampling requirements with the VIMS vessel operations staff. The analytical procedures for both the field and laboratory components of this research have been previously established in our laboratory through related research projects (Dickhut *et al.*, 1990).

REMAINING PROJECT ACTIVITY

The remaining project activities include all field sampling, sample analyses, flux modeling, and laboratory experiments on surface microlayer material as described in detail in the original project proposal. Briefly, we will directly measure fugacities for selected HOCs in surface waters of lower Chesapeake Bay using a floating sparger apparatus. We will also collect surface microlayer samples using a rotating drum apparatus and measure HOC fugacities using a shipboard sparger system. Measurment of HOC vapor phase concentrations, and hence, fugacities will be made using a high volume air sampler. Using a gas saturation technique and open tubular column liquid chromatography, we will determine HOC fugacity capacities, uptake rates, and diffusivities in collected microlayer material. A comparison will be made between the estimated HOC mass transfer coefficients in the surface microlayer to the rate of uptake of HOCs by particulate material in this region to deduce if the surface film can be viewed as an inert layer of resistance to gaseous exchange or an independent, potentially controlling phase for HOC vapor transfer at the air/water interface. A two-film model, or modifications thereof, will be used to assess the volatile/absorptive flux of selected HOCs between the atmosphere and surface waters of lower Chesapeake Bay.

DISSEMINATION OF RESULTS

The results of this work will be distributed to appropriate agencies and individuals as advised by the local Sea Grant office. Results will also be presented at national scientific meetings (e.g. Society of Environmental Chemistry and Toxicology, Estuarine Research Foundation) and at CBEEC Toxics Research Program workshops as requested. We will cooperate with other Toxics Research Program PIs as requested and to the extent allowable via the progress of our research in order to facilitate an improved understanding of the fate and effects of toxic substances in the Chesapeake Bay ecosystem. Manuscripts will also be prepared for publication in appropriate peer-reviewed scientific journals.

PERMANENT EQUIPMENT

Funds requested for development of two floating spargers and a shipboard sparger for measurement of surface water and microlayer fugacities, respectively, and for remodeling/repair of the rotating drum surface microlayer sampler were included in the Year 1 project budget as were funds for a high-volume air sampler and a constant temperature bath/circulator.

LITERATURE CITED

Baker, J.E., T.M. Church, G.A. Cutter, R.M. Dickhut and J.M. Ondov. "Atmospheric deposition of organic contaminants and trace elements to the Chesapeake Bay region: The Chesapeake Bay Atmospheric Deposition Study, 1991-1992". A workplan submitted to the EPA Chesapeake Bay Liason Office.

Baker, J.E. and S.J. Eisenreich. 1990. Concentrations and fluxes of polycyclic aromatic hydrocarbons and polychlorinated biphenyls across the air-water interface of Lake Superior. Environ. Sci. Technol. **24**: 342-352.

Dickhut, R.M. 1990. "Air/water partitioning and mass transfer properties of toxic organic chemicals" Chesapeake Bay Environmental Effects Toxics Research Program, Project No. R/CBT-1.

Dickhut, R.M., J.M. Ondov and G.A. Cutter. 1990. "Quality assurance/quality control plan for the southern Chesapeake Bay Atmospheric Deposition study" submitted to US EPA Region III, Chesapeake Bay Liaison Office, Annapolis, MD 21403.

Mackay, D., S. Paterson and W.H. Schroeder. 1986. Model describing the rates of transfer processes of organic chemicals between the atmosphere and water. Environ. Sci. Technol. **20**: 810-816.

PROJECT NUMBER: R/CBT-18

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1993	1994
Design, fabricate, and validate surface water sparging systems; remodel/repair and evaluate existing rotating drum surface microlayer sampler.	———	
Execute field sampling program through an annual cycle, perform laboratory experiments and sample analyses.	———	———
Evaluate data, compile modeling results, and prepare manuscripts for publication.		———
PROJECTED BUDGET: FEDERAL	95,824	87,963
PROJECTED BUDGET: MATCH		

NOAA FORM 90-2 (ADAPTED) U.S. DEPARTMENT OF COMMERCE

*** SEA GRANT PROJECT SUMMARY 1994***

Title: Interaction of Copper and Cadmium with Microbial Benthos Biofilm and Effects on Oyster Larvae Set

Project Number: R/CBT-19
Grant Number:
Sub Program:

Revision Date: 4/20/93
Initiation Date: 1/1/94
Completion Date: 12/31/95

Principal Investigator: Ron Weiner
Affiliation: Dept. of Microbiology, Univ. of MD, College Park, MD

Man-Months: 1.0

Principal Investigator: Marianne Walch
Affiliation: Center of Marine Biotechnology, Balt., MD

Man-Months: 1.0

Proposed Fed Funds: $39,128
Current Fed Funds: $37,265
Fed Funds to Date: $37,265
Current pass through:

Proposed Match Funds: $7,300
Current Match Funds: $7,300
Match to Date: $7,300

Related Proj:
Parent Proj:
Sea Grant Classification #: 45

Keywords: Biofilms, copper, cadmium, oysters, toxicity

Objectives:

1. Test biomagnification of Cu and Cd by microbial biofilms.
2. Test set of *Crassostrea virginica* and *C. gigas* on biofilms exposed to Cu and Cd.
3. Test toxicity of Cu and Cd for *C. virginica* and *C. gigas*.

Methodology

1. Measurement of Cu and Cd by atomic adsorption spectroscopy.
2. Collection of autochthonous Chesapeake Bay biofilms and single species biofilms of *Shewanella colwelliana* and *Hyphomonas* MHS-3.
3. Purification of exopolysaccharide by physico-chemico, enzymatic and chromatagraphic methodologies.

Rationale:

It is well documented that many marine microbial biofilms are comprised primarily of anionic exopolysaccharide (EPS), that such films cover nearly all marine surfaces, are a significant part of neritic sediments, and that such biofilms sequester numerous cations with varying affinities. It is also accepted that microbial biofilms are beneficial to invertebrate set - i.e. that microfouling precedes macrofouling. Thus, metals of concern such as copper and cadmium may be bioconcentrated on surfaces that are also important for oyster larvae to initiate metamorphosis, a process that has been shown by several laboratories to be extremely sensitive to the presence of heavy metals.

This study will help determine the fates and effects of Cu and Cd so that the resources of the Chesapeake Bay can be more knowledgeably managed.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO: RCBT-19
University of Maryland, College Park, Md.
PRINCIPAL INVESTIGATORS: Ron Weiner
Marianne Walsh

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.0	1.5	3,500	5,000
b. Associates	1.0	1.0		
Sub Total			3,500	5,000
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res.Asst.Grad.Std.	1.0		12,328	
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			15,828	5,000
B. FRINGE BENEFITS			2,572	
Total Salaries,Wages and Fringe Benefits			18,400	5,000
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			6,300	
E. TRAVEL				
1. Domestic	2,100			
2. International				
Total Travel			2,100	
F. PUBLICATIONS AND DOCUMENTATION COSTS				
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication				
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage				
5. Equipment Use and Maintenence				
6. Subcontracts				
7. Service Contracts				
8. Waste Disposal				
9. Other				
Total Other Costs			0	0
TOTAL DIRECT COSTS			26,800	5,000
INDIRECT COSTS				
(On Campus) : 46 % of 26,800/5,000			12,328	2,300
(Off Campus) : % of				
Total Indirect Costs			12,328	2,300
TOTAL COSTS			39,128	7,300

INTERACTION OF COPPER AND CADMIUM WITH MICROBIAL BENTHOS BIOFILM AND EFFECTS ON OYSTER LARVAL SET

Ronald Weiner, Professor of Microbiology, University of Maryland, College Park, MD

I. INTRODUCTION

A. Overview. Anoxia and toxics are two factors that have been strongly implicated in the decline of benthic populations in Chesapeake Bay. A component of the EPA/Sea Grant toxic program research group is examining partitioning and speciation of metal toxics in the sediments. The approach proposed here interfaces with the program by examining the interactions between cadmium (Cd) and copper (Cu) and microbial biofilms. This is important because biofilms are a major component of marine sediments and substrata. Moreover it has already been demonstrated that microbial exopolysaccharide (EPS), which is the matrix of biofilms, concentrates metal cations. These same biofilms have also been shown to be beneficial to oyster set. Taken together, these factors are especially interesting, since they suggest a mechanism to explain why metallic compounds in the water column may be especially toxic to oysters (and probably other invertebrates as well) at very low concentrations; that is, these toxics are bioconcentrated on substrata that can cue set. This translates into exposure to magnified concentrations at a target site during the vulnerable larva to spat transition period. The remainder of this section will briefly discuss these factors (emphasizing just why the examination of metal species/biofilm interactions will contribute to the overall program) as background to the hypothesis to be tested.

B. Marine Microbial Mats. Marine microbial mats, comprised of biofilm (largely EPS) and micro and macro organisms, have been termed the oldest and most common "form of life on earth" (Cohen, 1989). They are an abundant component of aquatic sediments and surfaces. A number of investigations have established a general pattern of periphytic succession for colonization of clean surfaces immersed in seawater. Quickly after submersion, surfaces are coated by organic matter (Loeb and Neihof, 1975), after which bacteria attach and begin to grow, forming microcolonies within several hours (Corpe, 1973; Gerchakov et al., 1976). Subsequently, diatoms, fungi, protozoans, micro-algae and other microorganisms attach to the surface, forming what is termed the primary slime layer (DiSalvo and Daniels, 1975; Cundell and Mitchell, 1977). This primary microbial colonization

appears to be a prerequisite for the final stage of succession in which larger organisms, viz., microalgae and invertebrates, attach and grow on the surface (Corpe, 1973; Gerchakov et al., 1976).

C. Biofilms and Oyster Set. For more than 50 years bacterial films have been observed to provide attractive settlement surfaces for oyster larvae (Cole and Knight-Jones, 1939, 1949), but the molecular mechanism had not been elucidated. We have been studying the settlement and metamorphosis of larvae of the oysters Crassostrea virginica and C. gigas and have demonstrated that films of specific marine bacteria promote oyster set, both in the laboratory and in the field (Bonar et al., 1985; Weiner et al., 1989). Research has focused on films and metabolic products of Shewanella colwelliana which was isolated from an oyster hatchery setting tank (Weiner et al., 1985a). This bacterium produces at least two types of cues which affect oyster larvae: one is a set of soluble metabolites that triggers so-called "search" (or settlement) behavior (Coon, 1990, Weiner, 1990), and the other is a surface-associated biofilm component which offers a conducive substratum upon which behaving larvae attach and irreversibly metamorphose into juvenile oysters (Bonar et al., 1989).

D. Bioconcentration of Metal Species by Biofilms. As noted above, microbial cells attached to surfaces, may grow and produce copious amounts of highly hydrated exopolymers which form an intercellular matrix enclosing a complex and multilayered microbial community (Gerchakov et al., 1976; Costerton and Cheng, 1982). It is well known that these microbial films in nature adsorb, sequester, or otherwise biomagnify a variety of inorganic and organic compounds (Sutherland, 1980; Geesey, 1982; Geesey and Jang, 1989) because the exopolymer matrices act as ion exchange resins which bind a variety of charged molecules (Costerton and Lashen 1983). Such binding of metallic ions by microbial exopolysaccharides is well documented in diverse areas of research (Ferris et al., 1989; McLeon et al., 1990; Norris and Kelly, 1982). Algal polysaccharides, for example, have been implicated in the sequestering of heavy metal pollutants in animal guts (Tanaka et al., 1971). The gelatinous matrix of Zooglea has been found to remove metal ions from solution (Friedman and Dugan, 1968). Complexation of copper, iron, and manganese by biofilm exopolymers has been implicated in corrosion reactions that occur on metal surfaces (Geesey and Mittelman, 1985; Walch, 1986; Black et al., 1987, Jolley et al., 1988). Extracellular polymers produced by marine bacteria also may react chemically with heavy metals (Corpe, 1975; Sutherland, 1980; Horikoshi, el al., 1981). Some bacteria are protected from the toxic effects of metals such as copper and cadmium by their EPS (Bitton and Friehofer, 1978; Gadd and

Griffiths, 1978; Flatare et al., 1985). EPS of iron bacteria bioconcentrate copper to levels 3,890 times that of the suspending medium (Morgan 1961).

There are 2 mechanisms by which EPS can scavenge trace metals from water: weak electrostatic interactions by uncharged polysaccharides, and ionic interactions by acidic EPS. Metal binding by uncharged EPS occurs as a result of coordination between metal cations, and the oxyanion and hydroxyl groups on the sugar molecules. The affinity for metals exhibited by uncharged EPS usually decreases with increasing ionic radius of the metal (Geesey and Jang, 1990). D-glucose molecules bind Zn (II) and Cd (II) by using 2 of their hydroxyl (OH) groups and 4 H_2O, forming a six coordinate metal cation. Hg (II) is bound by the OH groups of 2 sugar molecules and 2 H_2O molecules (Tajmir-Riahi, 1989).

The most important mechanism for trace metal scavenging by EPS is the formation of salt bridges between metal ions and the carboxyl groups on acidic polysaccharides which is the type of EPS synthesized by many marine microorganisms (Sutherland, 1980). In this mechanism, polyvalent metal ions bind to 2 anionic groups on 2 separate polymer chains. Carboxyl groups are found in EPS in the uronic acid species of neutral sugars, or in pyruvate or succinate, which form ketal bonds with sugar subunits. Linear charge density affects metal-EPS interactions, which are stronger when the polymer exists as a gel rather than dissolved in solution; the charge density is higher in the former state. Carboxylated polysaccharides exhibit preferential binding to cations with large ionic radii, and with few exceptions, they have higher selectivity for transition metals over alkaline earth metals (Geesey and Jang, 1990).

Differential metal affinity by different alginic acid EPS produced by Pseudomonas spp. are suggested to be due to variations in the positioning of the hydroxyl groups of the sugar monomer subunits (mannuronic acid, galacturonic acid, and guluronic acid). The spacing of the coordinating oxygens has a major influence on which cations will bind, and on the bond stability (Haug and Smidsrod, 1970). The formation of stable metal complexes involves both electrovalent (nonionic) and coordinate covalent bonding. The more coordinating bonds formed between the metal cation and the polymer, the more stable the complex. Alginic acid polymers, rich in repeating guluronic acid subunits, form a series of 'cavities' which offer a 4 oxygen coordination with Ca, Sr, and Cu (Rees, 1972; Kohn 1975). Cooperative binding with guluronic acid rich regions of other polymer strands is required for cavity formation. Galacturonic acid rich regions form cavities offering only 3 oxygen atoms for coordination, and mannuronic acid forms even shallower cavities. These configurations have lower metal-binding capacity. Cu ions in solution displace Ca ions bound to alginic acid. Metal ions in solution are always fully coordinated by H_2O molecules. When a metal ion becomes complexed by an organic ligand, there is a displacement and reordering of H_2O molecules; the number of water molecules displaced depends on the

dimension and charge of the metal ion and on the size of the coordinating group of the ligand (Geesey and Jang, 1990). It has been proposed that Cu ions have a larger ordering effect on the H_2O molecules than Ca ions, and that the displacement of Ca by Cu on the polymer should be favored by the relatively large positive change in entropy.

The binding of Mo by a rhizosphere Pseudomonas sp. was determined to be carried out by its uronic acid-containing EPS capsule (Tan and Lowtit, 1976). The EPS produced by Pseudomonas aeruginosa was reported to bind Mo and Cu through the glucuronic acid subunit of the polymer (Stojkovski, et al, 1986). The complexation was carried out by the 2 oxygen atoms of the carboxyl group and the oxygens of the hydroxyl groups of C3 and C4 of the uronic acid molecule. The acidic EPS synthesized by the marine pseudomonad P. atlantica contains mannose, glucose, galactose, glucuronic acid, and galacturonic acid, and it selectively binds Mg and Cu over Ca, Zn, Pb, and Ni (Corpe, 1975). Klebsiella aerogenes EPS is composed of rhamnose, mannose, glucose, and glucuronic acid along with acetate and pyruvate groups; it removes metals from solution in the order: Cu > Cd > Co > Mn > Ni (Rudd, et al., 1983).

E. Toxicity of Metals for Oysters. It has been reported that sublethal concentrations of heavy metals such as zinc (Robers and Bonar, 1990), tributyl tin (D. Wright, personal communication), and copper (Phelps, 1983; Coglianese and Martin, 1981) adversely affect invertebrate development, possibly by interfering with ionic regulation by the larvae (Muller, 1973; Pechenik and Heyman, 1985) or the activity of respiratory enzymes (Simkiss, 1984). Robers and Bonar (1986) and Bonar (1990) have found that very low levels of zinc (<0.1 ppm) can completely block the metamorphosis of healthy, competent oyster larvae. These levels are 100 to 1000 times lower than those that are lethal to the larvae. Likewise, cobalt and cadmium proved to be effective inhibitors of metamorphosis at concentrations substantially below those which were found to be toxic to larvae (Robers and Bonar, personal communication).

In a previous study, supported by Sea Grant, we found that tributyl tin (TBT), the active ingredient in many antifouling paints and coatings, clearly is concentrated on biofilms of the estuarine bacterium S. colwelliana. More importantly, we have found that TBT concentrations of 0.01 ppb had no effect on either Crassostrea virginica or C. gigas larvae when biofilms were not present. The larvae remained swimming and viable. In the presence of S. colwelliana biofilms, however, 0.01 ppb TBT blocked set of the oysters and eventually was lethal (Weiner et al., 1992). Oysters are filter feeders and could consequently ingest EPS along with microorganisms. If adsorption to the EPS neutralized the toxicity of TBT, that effect was certainly of less consequence than the strong affinity of EPS for tin. EPS concentrated TBT from the water column >8 fold (Matthias, et al., 1988; Weiner et al. 1992). Experiments, testing the effects of

TBT on L-3,4-dihydroxyphenylalanine (DOPA)-induced search behavior and on epinephrine-induced metamorphosis of oyster larvae, showed that the inhibitory effect occurred during the metamorphosis stage of larval set. Other sublethal effects of TBT on oysters include feeding rate (0.02 ppm; Lawler and Adrich, 1987) and compensation for hypoxia (0.01 ppm; Lawler and Adrich, 1987).

F. Proposal Relevance to the Problem. This introduction has so far discussed: a) that microbial mats are prevalent; b) that the mats are "nourishing" habitats to some benthic animals; c) that mat EPS can sequester metal cations; d) that very low concentrations of some metals have sublethal effects on oysters; e) that Cd and Cu are on the toxics of concern list. Therefore oysters and other benthos could be exposed to concentrations of toxics that may result in reduced settlement, metamorphosis, and survival of juveniles..

II. HYPOTHESIS AND OBJECTIVES

We will test the hypothesis that species of Cu and Cd partition out of the water column onto microbial biofilms where they can interfere with oyster set. The first objective will be to determine bioconcentration factors by biofilms of Hyphomonas MHS_3, and by autochthonous biofilms "harvested" from Chesapeake Bay, using known concentrations of the metals in artificial estuarine water. Secondly, we will do the same series of experiments, but use water taken from five Chesapeake Bay Stations. Thirdly, we will determine the effects of combinations of biofilms, control, metal-spiked, and autochthonous water, on set of competent C. gigas and C. virginica larvae.

Fourthly, we have coordinated an interface with J. Donat, at Old Dominion University on his funded project on "Determination of Chemical Speciation of Dissolved Copper and Cadmium..." This collaborative effort should be synergistic to both projects. We will provide laboratory constructed and well-characterized benthic biofilm exopolysaccharide for his analysis of organic ligand-metal species interaction. In turn, we will utilize his collected, analyzed Chesapeake Bay water from five sample stations. and he can contribute to the analysis of the concentration and speciation of Cu and Cd in biofilms.

In 1993, the focus has been and will continue to be on the known monospecies biofilms, optimizing metal detection technology. We are controlling such variables as biofilm thickness and density.

In 1994, concentration of Cd and Cu by authochthonous films will be determined. In each year the effects of Cu and Cd on both C. gigas and virginica will be examined (See section VI for detail).

III. PROGRESS TO DATE

Thus far we have been funded for approximately four months (17% of the scheduled project duration). During this time we have refined several research protocols (see below: Project Changes), charactrized the known biofilm of Hyphomonas MHS-3 (Task 1), non-film forming negative control (RAD varient), established that Shewanella and Hyphomonas biofilms sequester both Cd and Cu and have shown that PPB concentrations of these metals do not inhibit either the growth or metabolism of the biofilm forming procaryotes. These data both support our hypothesis (see section II) and demonstrate that it can be fully tested within the proposed time frame. Some specific results follow.

We now routinely make biofilms of MHS-3 on glass and teflon. These are high density with confluent cells and biofilm, approximately 0.7 mm thick after 80 hrs growth in Marine Broth. EPS accounts for greater than 50% of the film by weight. It was also just discovered that the EPS has negative charge (binds cationic ferritin and adheres to anionic exchange column at pH 7) which is one mechanism by which it is believed to sequester metals. We have taken numerous electron and phase contrast micrographs, characterizing these films in the past four months.

Also in the opening phase of this project, we determined the effects of Cd directly on the control bacterium. Cultures of planktonic Hyphomonas MHS-3 in Marine Broth were growth inhibited at cadmium concentrations of 1 ppm in which final titers were reduced to 75% of control cultures. Cultures grown in 500ppb cadmium yielded final titers of 90% the cell density of control cultures. No growth inhibition was observed in cultures in the presence of 100ppb or 200ppb cadmium.

The concentrations at which cadmium inhibits growth of planktonic Hyphomonas (biofilms of which would theoretically be even more resistent) are far greater than have been reported in the Chesapeake Bay and are also far greater than those which affect oyster larvae. Since environmental concentrations of Cd neither inhibit the bacteria nor the bacterial products that enhance set, we conclude that cadmium effects oyster larval set either directly or synergistically, as a metal-biofilm complex.

Table 1 (see p. 6A) presents data from a Water Resources related study. It includes baseline results of carefully controlled laboratory experiments using synthetic spiked sea water. The important finding was that 2.2ppb (effective water column concentration) Cu does not affect viability of C. gigas but does reduce search behavior and totally inhibits set on biofilms. It is also interesting that C. gigas' set appears to be more sensitive to Cu than does set of C. virginica.

Table I. Effects of Cu on Viability (**V**), Searching Behavior(**S**), Metamorphosis (**M**) and Set on Biofilm (**B**) of *Crassostrea virginica* (1.9% Salinity) and *C. gigas* (2.8% Salinity).

Oyster		C. gigas				C. virginica			
[1]Conc. (ppb)		0	100	500	1500	0	100	500	1500
[2]Effective Conc. (ppb)		0	0.44	2.2	6.6	0	0.97	4.9	15
Cu	[3]**V**	++	++	++	++	++	++	++	+
	[4]**S**	++	++	+	+				
	[5]**M**	++	++	++	+	++	++	++	0
	[6]**B**	++	+	0	0	++	+	+	0

1 - Absolute concentration in water.
2 - Free ionic species according to metal speciation program (WQ4F) at specific salinity.
3 - Viability in 24, 48, and 96 hours. ++, >90% Vs Control ; +, 25-89% Vs Control ; 0, <24% Vs Control
4 - L-dihydroxyphenylalanine (L-DOPA) induced searching behavior after 96 hours of metal exposure.
5 - Epinephrine (EPI) induced metamorphosis, metals were added with EPI for 4 hours exposure, EPI was removed and metals added.
6 - Biofilms (B) of 96 hours cultures of Hyphomonas MHS-3. Marine broth was replaced with MBL seawater prior to addition of competent larvae.

IV. PROJECT CHANGES AND REMAINING PROJECT ACTIVITY: METHODOLOGY

A. Project changes. There will not be any deviations of consequence. In the three months in which this project has been funded, combined with results from related projects, we have learned which of our proposed approaches work particularly well and will focus on those. Consequently, Hyphomonas MHS-3 will primarily be used to form known biofilms, since we have demonstrated that it induces excellent set, forms consistant biofilms under a wide variety of conditions (including Teflon substrata) and sequesters Cu and Cd. A mutant in our collection Hyphomonas MHS-3 RAD does not produce biofilms and will serve as a negative control.

Secondly, we have aquired the expertise to analyze the compositions and properties of autochthonous films and therefore, needn't rely on the Delmar Navy research biofouling group for that purpose. Thirdly, related work on Cu has been completed, sponsored by a Water Resources Research Grant, so that we will be able to focus more on Cd. Fourthly, our experiments with EDAX technology have shown limited sensitivity in the PPB range rendering it less useful for this work. As proposed, AAS will provide the necessary data. Lastly, we will construct biofilms on teflon lined beakers, in addition to Sigma Cote treated vessels, to further reduce interference from metal contamination and to minimize non-specific binding to non-experimental surfaces.

B. General Approach. Our group has extensive experience and publications on oyster set (Weiner, et al., 1988; 1989; 1990, Tritar, et al., 1991), microfouling and on EPS production, purification and structure (Abu, et al., 1986; 1991; Labare, et al., 1989). Hyphomonas MHS 3, which enhances oyster set and forms biofilms on teflon (used whenever possible to avoid metal contamination), will be grown periphytically in Marine Broth (2216, Difco) to obtain biofilmed microcosms (Weiner, et al., 1990). Films will be monitored under epifluorescent- and electron-microscopy. Autochthonous biofilms will be collected from teflon, mylar and polystyrene surfaces, suspended in the Severn River.

If it becomes important to study EPS binding affinities for Cd, without the influence of other biofilm molecules, EPS will be purified. Since it is possible that pure EPS could have altered hydration, conformation and metal binding characteristics, another approach can also be used. EPS over-producing strains of Hyphomonas will be grown in synthetic minimal media and the EPS gently separated from the cells by shearing and centrifugation. Such exopolymer is relatively free of macromolecular contamination.

Bioconcentration factors of Cu and Cd, will be determined using data from flame and flameless atomic absorption spectroscopy (AAS, sens. 0.1-1.0 ppb) as

currently being done in collaboration with Prof. G. Helz, Chemistry Department UMCP. Additional measurements will be made by J. Donat.

Percent Crassostrea set is obtained by dividing those metamorphosed (cemented, velum loss, shell growth) after 24-48 hrs. by the number of competent larvae (250 μm, eyed, searching, etc.). The collection and handling of estuarine water for analysis will be as described by J. Donat. The specific methodologies and resources to meet the objectives (please see previous section) follow:

C. Biofilms and Exopolysaccharide

1. Bacteria. The bacterium to be used (Hyphomonas sp. MHS3) was selected (a) for its ability to grow in estuarine conditions suited to the oysters, C. virginica and C. gigas (Weiner, et al., 1985b), (b) for its influence on oyster set and development; (c) for its ability to produce large amounts of EPS, which could potentially bind and concentrate toxic metal pollutants (Labare and Weiner, 1991; Weiner, et al., 1992b); (d) because polysaccharide production and larval settlement cue-production mutants have been isolated (Weiner, et al., 1992a) and (e) because we have purified and chemically characterized its EPS. Natural biofilms will be collected by immersing surfaces in Chesapeake Bay water. We have been collecting autochthonous biofilms, some 1-2 mm thick, from Severn River near Annapolis.

2. Production of Model Biofilms. Biofilms will be established on the inside surfaces of 150-ml glass Teflon, or polymer beakers. In some cases the surfaces will be treated with Sigma Cote (Sigma Chem. Co.) to enhance hydrophobicity, which increases efficiency of biofilm formation and reduces the possibility to metal contamination. In all cases, surfaces will be scrupulously cleaned by acid washings, and HPLC grade water will be used. These films can be exposed to known concentrations of Cu or Cd in artificial estuarine water or to Chesapeake Bay water and, when appropriate, competent oyster larvae can then be added to the beakers to test set on the biofilms. We have used this technique previously to test the effects of TBT, metal ions and pesticides on oyster set, and it has proven to be an extremely easy, reliable, and efficient method for obtaining reproducible set and large numbers of replicates.

Other beakers will be set up to assess "bioconcentration factors" (BCF). Films will be exposed to ppb conc. of Cd, diluted in artificial estuarine water (AEW) for various times until saturation points are determined. The AEW will be removed and the amount of Cd remaining in the "dissolved

phase" will be determined. Five ml of unspiked AEW will be used to wash the biofilm from the beakers. Four ml will be used for metal analysis. The other ml will be used for viable plate counts, the phenol sulfuric acid test for reducing sugars, the Bradford assay for protein, and for direct microscopic count (see next section: evaluation of the biofilms).

3. Evaluation of Biofilms. Microbial attachment and growth will be routinely monitored by light and epifluorescence microscopy and by protein/carbohydrate assays. Cell viability will be determined by standard plate count on Marine Agar. Electron microscopic techniques will be used to examine spatial distribution of cells and production of exopolysaccharides in the films. Behavior of oyster larvae on the various treated biofilms will be examined microscopically and videotaped.

a. Epifluorescence Microscopy. To monitor growth and attachment of cells, we will stain cells with acridine orange, a fluorescent dye that binds to cellular DNA (Hobbie et al., 1977). This is done by immersing the surfaces in a buffered 0.1% acridine orange solution for three minutes, rinsing with water, and drying. Stained surfaces are then observed under ultraviolet light, and attached cells appear orange or yellow against a black background.

b. Transmission and Scanning Electron Microscopy. We will use Transmission and Scanning Electron Microscopy to examine the model biofilms and to occasionally visualize the attachment of oyster larvae to specific portions of biofilms. We have already had good results characterizing the films by EM and will continue to monitor them with this technology.

c. Carbohydrate and protein assays. Preliminary studies have shown that monospecies biofilms of S. colwelliana and Hyphomonas MHS-3 are comprised of 70-90% EPS (wet wt.), the remainder being cells and cell associated materials. Carbohydrate measurements provide a reliable estimate of biofilm production, and protein measurements reflect cell concentration, especially when these parameters are considered relative to microscopic observations and viable counts.

Total carbohydrate concentrations precisely quantify pure EPS. The phenol-sulfuric assay was shown to be most accurate (Labare and Weiner, 1991). Briefly, sulphuric acid (10N; 100°C) hydrolyses glycosidic linkages and the subsequent dehydration of the released monosaccharides yield derivatives of furfural which react with phenol, yielding products which are quantitated spectrophotometrically (White and Kennedy, 1986).

Protein will be measured by the Bradford assay (1976), based on the binding of Coomasie Blue to protein. This, now standard, colorimetric assay is read at A_{595}.

D. Purification of Exopolysaccharide. Our laboratory routinely purifies the EPS (Weiner, et al., 1992b). Cultures are grown in a fermentor (New Brunswick, C14 Bioflo) and harvested three hours into the stationary phase of growth. EDTA (10mM final conc.) is added to dissociate bound EPS from the cells which are removed by centrifugation. EPS is precipitated 2x by 2-4 volumes of isopropanol and lyophilized to yield a semi-purified EPS preparation. Increasing purities are obtained by dialysis (12Kd cutoff), by phenol extraction to remove bound protein, DNAase, RNAase and Protease K treatments to remove remaining residues of contaminating polymers, and by additional alchohol precipitations and lyophilizations. Highest purity is obtained using ion exchange chromatagraphy. Purified EPS will be used in lieu of biofilms in some experiments.

E. Metal-Binding Assays. Metal binding by biofilm exopolymers will be evaluated by atomic absorption spectrophotometry (AAS).

1. AAS. Bioconcentration factors of copper and cadmium will be determined using data from flame and flameless AAS (sensitivity 0.1-1.0 ppb). in collaboration with Professor G. Helz, Univ. Md. Chem. Dept. Briefly, prior to AAS analysis, organic matter (e.g. biofilm) is digested, in this case wet digestion using HNO_3 (APHA, 1985) since some organic complexes of Cu and Cd are volatile. Cu and Cd are concentrated by chelation with ammonium pyrolidine dithiocarbomate (APDC) and extracted with methyl isobutyl ketone (MIBK). The sample is aspirated into the flame and atomized. The concentration of a metal is proportional to the amount of energy absorbed at its specific wavelength.

2. We will also provide J. Donat with laboratory constructed, well-characterized biofilms and exopolysaccharides for analysis of organic ligand-metal species interaction. In turn, we will use Chesapeake Bay water that he has collected and analyzed and he can contribute to the analysis of the concentration and speciation of Cu and Cd in biofilms.

F. Assessment of Larval Set on Biofilms. We will conduct oyster setting trials in our College Park laboratory and at the Maryland Department of Natural Resources oyster hatchery at Deal Island. C. virginica larvae are spawned during the summer at Deal Island and at VIMS. Larvae of the Pacific oyster, C. gigas, are shipped to us from approximately March through October by the Coast Oyster Co. in Quilcene, Washington.

Setting experiments will be done in the laboratory using the biofilm and

purified EPS-coated glass beakers described earlier. In these experiments 5-7 day old bacterial films are exposed to varying concentrations of the metals. The metal/seawater solution is removed, and fresh seawater is added containing 2-4 oyster larvae per ml. The beakers are aerated, incubated for 48 hrs, the seawater is poured off, and the number of attached and metamorphosed larvae are counted. In some cases, after counting, the seawater is replaced, food algae added, and subsequent growth and development of the spat are monitored. Larvae will also be added directly to the metal/seawater solution (of selected beakers) as one control for desorption effects.

In hatchery experiments, biofilms will be grown on glass and polystyrene surfaces, as described above, and these surfaces will be placed into closed aquarium tanks that have been filled with aerated seawater or water that has been callaboratively collected from the five sample stations. Precautions will be taken that no toxics are released into hatchery or bay water. We will be interested as well in long-term survival and development of the spat. To evaluate this, surfaces with attached spat will be placed into nylon mesh bags and submerged in the Chesapeake Bay at a location near the hatchery.

We will also evaluate the effects of addition of the metals to seawater and of actual Chesapeake Bay water samples on viability, swimming, DOPA-induced searching behavior, and epinephrine-induced metamorphosis of competent oyster larvae. Assays of settlement "search" behavior (Coon et al., 1985) will be performed in 24-well tissue culture plates. 20 to 40 larvae are placed in each well with 500 uL of filtered, metal-free artificial seawater and varying additions of toxic metals. 10^{-4}M DOPA is added, and the number of larvae swimming or crawling with the foot extended during 30-second periods at 4- to 10-min. intervals, recorded. Metamorphosis inhibition also will be evaluated in 24-well plates as described above, but with 10^{-4} M epinephrine added and the number of metamorphosed larvae (as determined by shell growth) counted after 24 hrs.

V. EXPECTED RESULTS AND DISSEMINATION

The hypothesis is that Cu and Cd will be bound and concentrated by the pure culture and autochthonous biofilms and that existing or potential levels in Chesapeake Bay may disrupt oyster development in benthos biofilms. Results of this study will be included in progress/final reports, workshops, at least one international conference (e.g. Microbial Ecology) and in peer review literature (e.g. J. Shellfish Res.; Appl. Environ. Microbiol). Together with other studies in the Chesapeake Toxics Program, the data would become a basis for management and regulatory decisions.

VI. RELATIONSHIPS TO OTHER WORK

This proposal interfaces with the Chesapeake Toxics Program in the following areas. a) Transition and speciation of metals in dissolved and particulate phases (e.g. with J. Donat); b) Uptake of particulate-associated toxicants (Weston, et al.); c) There are also a number of tangential but potentially important aspects such as 1) comparative examination of toxicity of Cu and Cd on C. virginica and C. gigas 2) potential bioassay system of effects of contaminated water on epinipherine induced metamorphosis.

This proposal is also synergistic with a research proposal to the Water Resources Research Center (WRRSC). That proposal, submitted to supplement this one, has limited funding (11K) for a single graduate student to begin in summer of 1992 to emphasize the development, features and bioconcentration of Zn and Cu by pure culture biofilms. Later, that student would work with more preliminary aspects of modeling the laboratory biofilms and while the student funded in this proposal would work with the autochthonous films, Chesapeake Bay sample-water, set in the hatchery and the sublethal effects of Cu and Cd on oysters.

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PROJECT NUMBER: R/CBT-19

VI. PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	1993 JFMAMJ	1993 JASOND	1994 JFMAMJ	1994 JASOND
1. Optimization of biofilm microcosms	----			
2. Toxicity (on viability, search and set of Cu & Cd to C. gigas and C. virginica	----------	----	----------	----
3. Bioconcentration of Cd and Cu on monospecies biofilms	----------	----------		
4. C. gigas C. virginica set on biofilms, exposed to Cu and cd	----------	----	----------	----------
5. Chesapeake Bay autochthonous biofilms bioconcentration of Cd and Cu		----------	----------	
6. Set of C. gigas and C. virginica on autochthonous biofilms			----------	----------
7. Bioconcentration by biofilms of Cu and Cd in Chesapeake Bay water (5 sample stations)		----	----------	----
8. Set of C. virginica and C. gigas on monospecies and autochthonous biofilms exposed to Chesapeake Bay Water from 5 sample stations			----------	----
9. Reports, seminars and publications		------		-----

PROJECTED BUDGET:				
	FEDERAL			
	MATCH			

NOAA FORM 90-2 (ADAPTED) U.S. DEPARTMENT OF COMMERCE

*** SEA GRANT PROJECT SUMMARY 1994***

Title: Contaminant Flux from Sediments: Impact on Chesapeake Bay Food Webs

Project Number: R/CBT-20
Grant Number:
Sub Program:

Revision Date: 06/18/93
Initiation Date: 01/01/93
Completion Date: 12/31/94

Principal Investigator: Gerhardt F. Riedel
Affiliation: BERL, Academy of Natural Sciences

Man-Months : 2.25

Principal Investigator: James G. Sanders
Affiliation: BERL, Academy of Natural Sciences

Man-Months: 1.5

Principal Investigator: Cynthia C. Gilmour
Affiliation: BERL, Academy of Natural Sciences

Man-Months: 0.7

Proposed Fed Funds: $150,698
Current Fed Funds: $142,066
Fed Fund to Date: $142,066
Current pass through:

Proposed Match Funds:
Current Match Funds:
Match to Date:

Related Proj: R/CBT-13
Parent Proj: R/CBT-7
Sea Grant Classification #: 45

Keywords: sediments, contaminant, chronic effects, anoxia, heavy metals

Objectives: To use microcosm studies to measure the availability and the rate of uptake of toxic elements released from contaminated sediments, by natural estuarine communities and the effect of these trace element on the lower trophic levels.

Methodology: Toxic trace element concentrations and distributions will be measured in 500 L microcosms with treatments consisting of contaminated and clean sediment subjected to anoxia versus contaminated and clean sediments not subjected to anoxia.

Rationale: Large reservoirs of toxic trace elements reside in Chesapeake Bay sediments. Their effect on the Bay depends on the extent to which they are able to leave the sediment. This study proposes to test the hypothesis that sediment contaminants are causing changes in the structure of lower trophic levels.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO: RCBT-20
University of Maryland, The Academy of Natural Sciences
PRINCIPAL INVESTIGATORS: J.G. Sanders
C.C. Gilmour
DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	4.5		22,596	
b. Associates				
Sub Total			22,596	0
2. OTHER PERSONNEL				
a. Professionals	20.0		45,330	
b. Research Associates				
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			67,926	0
B. FRINGE BENEFITS (17.5% of Total Salaries)			11,887	
Total Salaries,Wages and Fringe Benefits			79,813	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			9,000	
E. TRAVEL				
1. Domestic				
2. International				
Total Travel			0	
F. PUBLICATIONS AND DOCUMENTATION COSTS			1,000	
G. OTHER COSTS				
1. Computer costs			700	
2. Copying,Library and Communication			500	
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage			1,750	
5. Equipment Use and Maintenence			800	
6. Subcontracts				
7. Service Contracts				
8. Waste Disposal				
9. Other			500	
Total Other Costs			4,250	0
TOTAL DIRECT COSTS			94,063	0
INDIRECT COSTS				
(On Campus) : 60.21 % of 94,063			56,635	
(Off Campus) : % of Total Indirect Costs			56,635	0
TOTAL COSTS			150,698	0

CONTAMINANT FLUX FROM SEDIMENTS: IMPACT ON CHESAPEAKE BAY FOOD WEBS

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INTRODUCTION

There has long been a presumption by both the public and scientific community that chronic releases of contaminants are responsible for a perceived degradation of the environment. However, there is very little scientific evidence for such a conclusion, since conclusively assigning blame for any trend in living resources is extremely difficult. Given the enormous variability of such resources due to climate, weather, fishing pressure, and other unknowns, along with the lack of suitable control systems for comparison, such perceptions must be treated with at least a little skepticism. Moreover, single species acute and chronic toxicity studies ordinarily suggest that orders of magnitude higher concentrations of contaminants than are found in the environment are necessary to cause significant deleterious effects.

With the implementation of regulations and programs controlling the release of pollutants into surface waters, we have seen and can expect a gradual and continued decrease of many contaminants in our rivers, lakes, bays, and estuaries (Smith et al., 1987). However, as pollutant loading of the water column decreases, the major sink for pollutants--the sediments--may exert an important influence on overlying water quality. The sediments in the Chesapeake Bay, as in many other coastal systems, contain elevated amounts of toxic substances and are an enormous potential repository for continuing inputs of toxic substances (Helz and Huggett, 1987; Sanders and Riedel, 1988). Toxic substances present in sediment are not completely removed from the environment, as they can be returned to the water column by diffusion or the action of benthic invertebrates (Riedel et al., 1987,1989). A crucial question is whether or not

the sediment bound contaminants represent a significant threat to the ecosystem.

Our current study has developed an experimental system which will allow us to examine the sub-acute effects of sediment-bound toxic contaminants on estuarine food webs. The experimental system will allow us to determine the rates at which toxic pollutants flux between the sediment and the water column, to expose natural plankton systems to contaminants released from those sediments, and to determine the effects of those contaminants on the plankton. We will manipulate the system to produce anoxia over the some of the sediments, in order to produce changes in the flux of contaminants, and will determine how this affects the planktonic community. We will also use the resulting planktonic communities to feed populations of zooplankton and other filter feeding animals, and determine how any resulting changes in the phytoplankton community structure and contaminant accumulation affect the growth and reproduction of the herbivores.

We believe that this experimental design will be able to detect subtle changes in the function of the estuarine ecosystem caused by the presence of contaminated sediments. All of the steps linking the presence of contaminated sediments to changes in estuarine ecosystems have been tested in our laboratory over the past several years, but have not been assembled in one experiment with organisms in a natural system. For example, in our recent grant sponsored by CBEEC, we have shown that benthic organisms and anoxia have significant overlapping and interacting effects causing the toxic element arsenic to flux from sediments from Baltimore Harbor. Conversely, copper showed significant flux out of oxic sediment, while anoxia and organisms had a reverse effect on the flux of copper, causing copper to become more highly bound by the sediment. Other projects, (funded by EPA, NOAA, Sea Grant and Maryland DNR), have demonstrated that low concentrations of some toxic elements (for example arsenic and copper) added to estuarine microcosms cause significant changes in the phytoplankton community, shifting dominance away from larger diatoms (considered good food for zooplankton and large filter feeders), to smaller diatoms and flagellates (considered poor food) (Sanders and Cibik, 1985, 1988; Sanders et al., 1991). We have also shown that the changes engendered in the phytoplankton by low concentrations of arsenic, when presented to zooplankton as food, cause a decrease in growth and fecundity (Sanders 1986; Sanders et al., 1988; Sanders et al., 1993), leading to altered fluxes of carbon and overall shifts from predominance by metazoan grazers to increased importance of microbial food chains and degradation pathways.

However, to date, none of these experiments have spanned the entire chain of causality, linking contaminant flux from the sediment and the factors that control flux to the estuarine food chain, which is the goal of our current CBEEC project. We believe that our results will yield an important test of the hypothesis that pollutants from contaminated sediments are a significant factor controlling contaminant uptake by phytoplankton and subsequent changes in species composition within plankton communities. If this link is shown to be a significant one, we can begin to assess the

potential for system impact from chronic loadings.

Because the sediment in our experiments contains a wide variety of contaminants (as indeed most polluted sediments do), it will be very difficult to determine which (if any) compound is responsible for any negative effects that may be observed. Therefore, we are studying as many of the likely candidate substances as possible by examining the fluxes and distributions of several common contaminants in our experimental systems. After the initial review and acceptance of this project in 1992, it was agreed that we would continue to study arsenic, copper and mercury, drop organic compounds and add cadmium and nickel to our elements of interest, based on managers' and reviewers' comments.

OBJECTIVES

Overall Objectives

The overall objective of the research program proposed here is to test the hypothesis that contaminated sediments, and the effects of anoxia on contaminated sediments, can influence the phytoplankton and herbivore communities in Chesapeake Bay. Our research program is broken down into two subprojects of one year duration which will test supporting links of this hypothesis.

1993 Objectives

In the first year (not yet completed) we will test the hypothesis that contaminated sediments in the absence of anoxia can produce changes in the phytoplankton and zooplankton similar to the changes we have observed with phytoplankton microcosms dosed with low concentrations of arsenic or copper. This is an important link in the chain of causality in the proposed hypothesis. We will examine whether microcosms receiving water which has interacted with clean sediment develop a phytoplankton community that differs from systems with either no sediment or contaminated sediment. If changes occur, and we hypothesize that they will, we will seek to determine the controlling factors of the change in the water as a result of passage over the sediment.

1994 Objectives

In the second year of the project (for which we are asking for this renewal) we will test the hypothesis that the occurrence of anoxia over contaminated sediments causes greater deleterious effects (or at least different effects) on estuarine food webs than contaminated sediments exposed to normal surface waters. Experiments will parallel

the first year's design, with the addition of coupled systems using anoxic sediments. This is particularly significant in that the largest changes in trace element flux have been in response to the presence of anoxic water in contact with the sediment.

PROJECT CHANGES

No significant changes in objectives or methods to our study plan have been made after the revisions following its original review and acceptance.

PROGRESS

At present, we are on schedule with this project. At this point in the project, our most significant objective is planning and readying for the experiments to take place this summer. Our experimental work is scheduled for summer, when phytoplankton and benthic organisms are at high abundances and in their most active states, and benthic fluxes are at maximum values due to temperature and microbial activity. Moreover, in the second year, we will be examining the effect of anoxia on planktonic food webs. Anoxia is also a predominantly summer phenomenon in the Bay, when surface waters warm up and contribute to the stratification. Therefore, our experimental work will occur during the summer months when anoxia is most prominent, and when the organisms are most abundant and active.

We are currently analyzing the data from our previous CBEEC funded project, "The role of benthic infauna and fluctuating oxygen concentrations in the flux of toxic trace elements from Chesapeake Bay sediments". In this study we examined the flux of trace elements (arsenic and copper particularly) out of sediments collected from Baltimore Harbor under a set of oxygen and organism treatments. We found that sediments subjected to anoxic water showed a substantial flux of As out of the sediment, while copper disappeared from the overlying water. Sediments with aerobic water overtop showed no flux of arsenic from the sediment, but a substantial outward flux of copper instead. The presence of burrowing organisms appeared to mimic the effect of anoxia to some extent, inducing a flux of arsenic in oxic treatments, and reducing the copper flux. We have also analyzed some of the the samples for other trace elements (cadmium and nickel) to further extend the results, and to integrate the study with the current CBEEC study. Preliminary results suggest that cadmium follows a pattern similar to copper, flux out of sediments under oxic water, and loss to sediment under anoxic water.

REMAINING PROJECT ACTIVITY

The bulk of this year's work remains to be completed. We expect that the microcosm experiment will be started in late June or early July. Chemical and biological measurements will be carried out from that time through the remainder of the grant period.

DISSEMINATION OF RESULTS

Results of our research will be submitted for publication in peer-reviewed journals, as have been our previous studies on the flux of toxics from sediments. We will also present the results at regional, national and international scientific meetings. We anticipate that the results of our first two years will be written up during the upcoming year. Results from the first years' studies were presented at the ERF meeting in San Francisco in November 1991, at the ASLO meeting Sante Fe in February 1992, at the IAWPRC 16'th Biennial Conference in Washington, D.C. in May 1992, and at the joint ERF/ECSA meeting in Plymouth, England, September 1992. Preliminary data were published in Sanders and Riedel, 1992. The data from both years' research will be discussed at the Toxics Research Workshop in Solomons, Maryland on May 5-6, 1993.

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PROJECT NUMBER: R/CBT-20

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	1993	1994
Microcosm experiments comparing estuarine plankton communities with and without clean and contaminated sediments with aerobic water over sediments	XXX	
Microcosm experiments comparing estuarine plankton communities with clean and contaminated sediments with aerobic and anaerobic water over the sediments		XXX
PROJECTED BUDGET:	$142,066	$150,698

SEA GRANT PROJECT RECORD FORM SG-SID-No.:_____
Specialist:_____
SG Class: _____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: The effects of environmental contaminants on the progression of *Perkinsus marinus* infection in the Eastern oyster
PROJECT NUMBER: R/CBT-22
PROJECT STATUS: 1
SUB PROGRAM: Toxics/CBEEC
REVISION DATE: 06/15/93
INITIATION DATE: 01/01/94
COMPLETION DATE: 12/31/95

PRINCIPAL INVESTIGATOR: Robert S. Anderson **EFFORT:** 1.2 mo.
AFFILIATION: Chesapeake Biological Lab., UMCEES
AFFILIATION CODE: 2414
CO-PRINCIPAL INVESTIGATOR: Eugene M. Burreson **EFFORT:** 1.0 mo.
AFFILIATION: Virginia Institute of Marine Science
AFFILIATION CODE: 5101

S.G. FUNDS: 0
LAST YEAR'S SG FUNDS: 0
PASS-THROUGH FUNDS: $ 72,113
STATE MATCHING FUNDS: 0
LAST YEAR'S MATCH FUNDS: 0
LAST YEAR'S PASS-THROUGH: 0
RELATED PROJECTS: All R/CBT projects
PARENT PROJECTS:
SEA GRANT CLASSIFICATION: Pollution-other-toxics (45)
KEYWORDS: oysters, *Perkinsus marinus*, disease progression, pollutant effects

OBJECTIVES: The main objective is to test the hypothesis that pollutant stress may predispose aquatic organisms to infectious diseases or accelerate the progression of the disease. The host-parasite system proposed is *Crassostrea virginica* - *Perkinsus marinus*; the environmental toxicants under study will include a lower MW polycyclic aromatic hydrocarbon (naphthalene) and tributyltin. An attempt will be made to associate pollutant-induced changes in disease progression to changes in cell-mediated immune function.

METHODOLOGY: Progression of the disease in individual oysters will be followed during chronic exposure to the toxicants. Exposure will be via water at environmentally relevant concentrations. Disease diagnosis will be carried out on small hemolymph samples repeatedly taken from individual oysters. Immune status will be measured by quantifying hemocyte phagocytic capacity and chemiluminescence, an indication of cellular antimicrobial activity.

RATIONALE: Environmental chemicals are immunosuppressive and potentiate reduced resistance to infectious diseases in mammals; this relationship needs further evaluation with regard to pollutant impact on economically important aquatic species and the incidence of their diseases. Analysis of oyster cellular immunity, as influenced by the exposure protocols, will provide information on physiological mechanisms related to altered disease susceptibility. Knowledge gained from this project should find practical application in shellfishery management by contributing to understanding the effects of environmental pollutants on the severity and progression of a major disease of oysters.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO: RCBT-22
University of Maryland, Center for Environmental And Estuarine Studies
PRINCIPAL INVESTIGATORS: S. Anderson
E. M. Burreson

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.2		7,833	
b. Associates				
Sub Total			7,833	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates	6.0		12,040	
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			19,873	0
B. FRINGE BENEFITS (1a x 19% , 2b x 32%)			5,341	
Total Salaries,Wages and Fringe Benefits			25,214	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			5,000	
E. TRAVEL				
1. Domestic	1,000			
2. International				
Total Travel			1,000	
F. PUBLICATIONS AND DOCUMENTATION COSTS			500	
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			400	
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage				
5. Equipment Use and Maintenence				
6. Subcontracts				
7. Service Contracts			3,063	
8. Waste Disposal				
9. Other				
Total Other Costs			3,463	0
TOTAL DIRECT COSTS			35,177	0
INDIRECT COSTS				
(On Campus) : 39 % of 35,177			13,719	
(Off Campus) : % of				
Total Indirect Costs			13,719	0
TOTAL COSTS			48,896	0

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO: R/CBT-22B

PROJECT STATUS: 1

PROJECT TITLE:
The effects of environmental contaminants on the progression of Perkinsus marinus infection in the eastern oyster.

PRINCIPAL INVESTIGATORS:
E. M. Burreson

DURATION: 1/1/94 - 12/31/94

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	1	1.00	5943	0
b. Associates:			0	0
Sub Total:			0	0
2. Other Personnel				
a. Professionals	1	2.0	5004	0
b. Research Assoc.			0	0
c. RA Grad. Stud.			0	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop			0	0
h. Hourly Labor			0	0
Total Salaries and Wages			10947	0
B. FRINGE BENEFITS			3065	0
Total Sal. Wages & Fringe Benefits (A+B)			14012	0
C. PERMANENT EQUIPMENT			0	0
D. EXPENDABLE SUPPLIES			1000	0
E. TRAVEL				
1. Domestic - US & Possessions	1. 1000		1000	0
2. International	2.		0	0
Total Travel			1000	0
F. PUBLICATION AND DOCUMENTATION COSTS			0	0
G. OTHER COSTS				
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
Total Other Costs			0	0
TOTAL DIRECT COSTS (A through G)			16012	0
INDIRECT COSTS: On Campus: 45% of A,B,D,E			7205	0
Off Campus:				
TOTAL INDIRECT COSTS			7205	0
TOTAL COSTS			23217	0

EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON THE PROGRESSION OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER

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INTRODUCTION

A fundamental, but as yet unresolved, question related to the consequence of pollutant exposure on estuarine organisms is that of altered susceptibility to infectious diseases. In other words, does chronic, sublethal exposure to toxic chemicals enhance susceptibility to disease, presumably as a result of chemically-mediated suppression of the immune response? In the case of fish and other vertebrates, this question can be answered in the affirmative: the incidence and severity of various diseases are greater in polluted environments (Sindermann, 1993), various forms of immunosuppression have been produced by environmental toxicants under laboratory conditions (Hetrick et al, 1979; Knittel, 1981; Anderson and Brubacher, 1992; Anderson, 1993), and impaired immune capability has been shown in fish collected from chemically stressed sites (Wedemeyer, 1970; Snieszko, 1974; Weeks and Warinner, 1984). The putative effects of

toxicants on disease resistance in bivalves are less well documented, but some suggestive evidence is available and will be discussed briefly. The need for more detailed information on the impact of toxicants on disease progression and immunotoxicological responses in oysters takes on added significance because of the decimation of the native oyster population by parasitic diseases.

Oysters and other bivalve mollusks depend heavily on their circulating blood cells (hemocytes) for defense against pathogenic microorganisms (Cheng, 1981). These phagocytic cells resemble leukocytes of the monocyte/macrophage lineage and have been highly conserved throughout metazoan evolution. Biologically active molecules with agglutinating and/or lytic activities are also present in bivalve blood (hemolymph), but their immunological roles are difficult to prove and their activities are not usually altered by pollutant stress (Anderson, 1987). The only well documented function for humoral molecules in oyster hemolymph is the phagocytosis-augmenting (opsonic) activity of naturally occurring hemagglutinins (Tripp, 1966). However, the titer of this factor is highly variable and is apparently not responsive to modulation by environmental chemicals. Therefore, hemocyte functions were selected for the immunotoxicological studies proposed in this application.

Perkinsus marinus infections pass through several stages of increasing intensity until the hemolymph can contain thousands of the parasites per ml. These organisms exist both free in the serum and within the hemocytes, eventually exhausting any cellular or humoral defense mechanisms available to the oyster. One component of this study will quantify processes central

to the cells' ability to kill microbes and establish resistance to infectious disease: phagocytic capacity and the production of cytotoxic reactive oxygen intermediates (ROIs). Luminol-augmented chemiluminescence (CL) will be used to quantify ROI production by hemocytes, this method has been successfully used to measure killing capacity of leukocytes (Horan et al, 1982) and to screen for immunomodulatory chemicals (Tam and Hinsdill, 1990) in macrophages. Phagocytic activity of the hemocytes will be quantified by measuring the uptake of fluorescently-labeled particles (Hed, 1986). These observations on cellular immune parameters will be carried out to complement and provide mechanistic insight regarding the studies of disease progression. Disease progression itself will be followed by quantifying the numbers of *P. marinus* in the hemolymph of experimentally infected oysters held in the presence or absence of model environmental pollutants.

The CL response of *Crassostrea virginica* blood cells either exposed in vitro or collected from oysters after various periods of in vivo exposure to selected heavy metals, pesticides or other organic compounds, was measured by Larson et al (1989). They found that copper in both in vivo and in vitro studies, was the most effective agent tested with regard to ability to depress the CL response, although most of the compounds appeared to suppress CL, particularly at high exposure levels. Certain compounds, such as cadmium, aluminum, zinc, dieldrin and naphthalene, apparently caused increased CL at low levels, but this effect was usually reversed at higher concentrations.

Fisher et al (1989, 1990) reported that in vitro tributyltin (TBT) treatment of hemocytes from *C. virginica* or *C. gigas* produced slight stimulation at low concentrations (0.4 ppb), followed by dose-dependent suppression of CL at higher concentrations. The suppressive TBT concentrations (40-400 ppb) exceeded those found in most environmental samples; nonetheless, hemocytes of field-exposed oysters are probably exposed to high TBT levels as a result of bioaccumulation.

Work in this laboratory has demonstrated the immunosuppressive effect of cadmium on oyster hemocyte CL (Anderson et al, 1992a). When the cells were exposed in vitro to sublethal cadmium levels, a dose-dependent inhibition of ROI production was consistently recorded. Several CL parameters were measured: resting or basal CL (the activity of phagocytically unstimulated cells), peak CL (the maximal CL response induced by phagocytosis of yeast particles), and the total CL response (obtained by integrating the area under the curve of the induced CL response). Peak and total CL were significantly inhibited by 10 and 2 ppm cadmium, respectively; but the basal CL activity was not affected by any concentration tested (1-50 ppm). The availability of cadmium to the cells was influenced by the composition of the medium, especially the presence of autologous serum which contained metal-binding proteins. Regardless of the nominal cadmium concentration in the medium, the actual CL inhibition experienced was shown to be a function of the intracellular cadmium concentration. An attempt was made to duplicate the in vitro immunosuppressive effects by in vivo exposure of oysters. The oysters were exposed for two weeks to 0-0.25 ppm cadmium; toxicity, as manifested by decreased condition index (Roesijadi and Klerks, 1989) and reduced shell growth (Shuster and

Pringle, 1969), starts to become evidence at ~0.20 ppm. Effects on CL responses of oyster hemocytes collected after exposure to all cadmium concentrations tested ($\leq$0.25 ppm) were variable and showed no significant dose-dependency. This finding was probably explained by the fact that in no case did intrahemocytic cadmium levels recorded in the in vivo study approach those produced in the CL-suppressed cells in vitro (2.2 vs. ~15.0μg Cd/mg protein, respectively).

Similar chemically-induced inhibition of luminol-augmented CL responses of oyster hemocytes has also been produced by exposure to particulate brass, copper, and pentachlorophenol (Anderson et al, 1992b; Roszell and Anderson, 1992; Anderson et al, 1993). Such evidence implicates these xenobiotics as potential immunotoxicants, by extension of the criteria already developed for mammals (Tam and Hinsdill, 1990). It should be noted that the metal (TBT) selected for this current proposed study is orders of magnitude more immunotoxic (at least to mammals) than any of the metals studied previously.

Relevance to the Problem

The effects of important Chesapeake Bay toxicants on the progression of a major parasitic disease of oysters will be quantified. The proposed study is an initial step in determining these effects on oyster populations in the field; it concentrates on laboratory-maintained populations exposed to individual priority pollutants. The contaminants in question are representative of metals and polycyclic aromatic hydrocarbons (PAHs) found in the Bay. Specifically, they are tributyltin and a low molecular weight aromatic (naphthalene) commonly found in aqueous extracts of PAH-

contaminated sediments. Naphthalene and tributyltin (TBT) are recognized as toxicants of immediate or potential threat to the Chesapeake Bay system (EPA, 1991a; 1991b); these compounds have also been implicated as immunomotoxicants of oysters (Larson et al, 1989; Fisher et al, 1990; Rice and Weeks, 1990).

Current studies by Chu and Hale (1992) indicate exposure of oysters to aqueous extracts of PAH-rich Elizabeth River sediments increased their levels of *Perkinsus marinus* infection. Their immunological findings included enhanced hemocyte CL and chemotaxis following in vitro exposure of the cells to the extracts, but CL was either reduced (2-3 wks) or unchanged (5 wks) in cells withdrawn from exposed oysters. Therefore, the available preliminary results suggest that modulation of hemocyte immune functions can be produced by exposure to xenobiotics, and that this may be expressed as reduced resistance to disease. However, more direct evidence is required to demonstrate the phenomenon convincingly and to show that it has real-world significance with regard to intensity of infection.

Our approach is to examine the effects that pollutants might have on the progression of the disease in individual oysters. Effective diagnosis and staging of all but the very earliest stages of *Perkinsus* infections can be carried out on small hemolymph samples that can be periodically withdrawn from the adductor muscle of individual *C. virginica*. This kinetic approach will provide a sensitive technique to measure in vivo immunosuppression by toxicants, and the immunoassays will provide in vitro correlates.

OBJECTIVES AND HYPOTHESIS

Hypothesis to be Tested

Exposure of *C. virginica* to relevant pollutants will produce immunosuppression which will lower their resistance to *P. marinus* infection; this effect will be manifested by more rapid progression of the disease and altered hemocyte-mediated immune responses. The methodology exists to permit disease progression (via hemolymph diagnoses) and immune status (via assays of phagocytosis and microbial killing) to be followed in individual oysters during the course of the experiments. This approach will bring a higher level of precision and sensitivity to bear on the study of immunotoxicology as it relates to disease resistance in aquatic organisms.

Overall Objectives

The major objectives are to determine (1) if chronic exposure of *Crassostrea virginica* to model aquatic pollutants will increase susceptibility to (and/or alter the progression of) *Perkinsus marinus* infection and, (2) if these physiological changes can be correlated to changes in hemocyte immune function.

1994 Objectives

(1) To determine the best experimental procedure for establishing experimental infections with *P. marinus* from continuous cultures, particularly with regard to dosing and subsequent hemolymph sampling schedules. (2) To evaluate the effects of tributyltin exposure on *P.*

marinus progression and oyster immune parameters.

1995 Objectives

(1) To evaluate the effects of naphthalene *P. marinus* progression and oyster immune parameters.

(2) To attempt to establish the dose-dependency of the TBT and PAH responses outlined above.

METHODOLOGY

Experimental infection: Continuous culture of *P. marinus* has recently been perfected (LaPeyre et al, 1993) and these cells will be used to experimentally infect oysters for the proposed experiments. Cultured cells will be introduced to 10 gal. aquaria each containing 15 oysters held at 24°C and 20ppt salinity. An appropriate dose as determined below will be introduced every other day for five days.

The use of cultured *P. marinus* has distinct advantages over natural infection or use of minced, infected oyster tissue. With cultured cells, an unlimited and quantifiable amount of pure *P. marinus* cells can be available on demand. Thus, infections can be produced and experiments conducted without regard to seasonal effects on the natural abundance of *P. marinus*. In addition, pure cultures eliminate other organisms or substances that may affect immune function and that may be present in minced oyster tissue.

Preliminary experiment to determine proper dose and schedule: Eight 10 gal. aquaria will be established, each with 10 uninfected oysters obtained from Mook Sea Farms, Maine. Aquaria

will be maintained at 24°C and 20ppt, and oysters will be fed cultured algae daily. Oysters in the aquaria will be exposed to cultured *P. marinus* cells as follows.

1. 1 x 10^3 cells/oyster every other day for 5 days (replicate tanks).
2. 1 x 10^4 cells/oyster every other day for 5 days (replicate tanks).
3. 1 x 10^5 cells/oyster every other day for 5 days (replicate tanks).
4. 1 x 10^6 cells/oyster every other day for 5 days (replicate tanks).

After the exposure period, a notch will be cut in each oyster adjacent to the adductor muscle and 300 μl of hemolymph will be withdrawn every two weeks for ten weeks from 1 replicate tank, and every 3 weeks for 9 weeks from the other replicate. *Perkinsus marinus* cells in the hemolymph will be quantified using the technique of Gauthier and Fisher, 1990. Intensity will be determined as number of cells per 1.0 ml of hemolymph. The dosing schedule for the experiments will be the lowest dose that produces infections in all of the oysters; the bleeding schedule will be the one that yields the lowest mortality.

Diagnosis of *P. marinus*

A modification of the method described by Gauthier and Fisher (1990) will be used for hemolymph diagnosis. Notches will be cut in oyster shells posterior to the adductor muscle using a lapidary saw. A 300 μl hemolymph subsample from the adductor muscle will be added to 1.0 ml of fluid thioglycollate medium (FTM) fortified with 50 μl penicillin/streptomycin (25 units of each/ml). Cultures will be incubated in the dark at 27°C for 5-7 days. Following incubation the samples will be centrifuged at 403 x g for 10 minutes, the supernatant removed and the pellet resuspended in 1.0 ml of 2M NaOH and incubated at room temperature for 30 minutes. The

samples will then be washed twice with distilled water and finally resuspended in 1.0 ml distilled water with 50 μl Lugol's stain (1:6 dilution). The samples will be gently mixed with a pipette and transferred to 24-well culture plates. A Zeiss inverted microscope will be used to examine the samples; the number of *P. marinus* cells per well will be counted and multiplied by 3.3 to determine cells per ml. If there are over 500 cells per well, doubling dilutions will be made until an accurate count can be made. Differences in mean intensity of *P. marinus* among treatment groups at each sample time will be identified by ANOVA.

At the termination of each experiment, each oyster will be shucked and examined for gross pathology. A small piece of mantle, gill and rectal tissue will be excised from each oyster and cultured in FTM to provide a *P. marinus* tissue infection level for comparison with hemolymph values. Tissue infections will be categorized as light, moderate or heavy per standard procedures (Ray, 1952). A section of tissue that includes gill, mantle and visceral mass will also be preserved in Davidson's AFA and processed for paraffin histology. Sections will be examined for common oyster parasites and histopathological conditions.

Phagocytosis

Hemocytes (1×10^6) are introduced into the wells of a 96-well microtiter plate, suitable for tissue culture. The cells are maintained in a cell support medium: containing antibiotic-antimycotic solution (penicillin G, streptomycin, amphotericin B), 5% fetal calf serum, 1% dextrose in filter-sterilized ambient estuarine water. Fluorescently-labeled phagocytic target particles (heat-killed yeast or *P. marinus*) are added; final particle: hemocyte ratio is ~20:1. The particles are

routinely FITC-labeled in our laboratory, using reagents from Molecular Probes, Oregon. The kinetics of particle uptake are determined using a fluorescence concentration analyzer (Pandex Corp.), which carries out flourometric readings on the 96-well microtiter plate. The principle of the assay is that the fluorescence of the extracellular FITC-labeled particles can be quenched by the addition of trypan blue, leaving only the phagocytosed particles to be quantified by the fluorescence concentration analyzer.

Chemiluminescence

Standardized hemocyte suspensions are introduced into pony scintillation vials in the presence of luminol. All vials and reagents have been dark-adapted for at least 24 hr prior to use and all manipulations are carried out in a dark-room under dim red illumination. The samples are read for chemiluminescence (CL) in a 1900 CA Packard Tri Carb liquid scintillation analyzer with programmed single photon counting capability operated at ambient temperature. The gain setting across the photomultiplier tube is set at 60% and the internal static suppressor is inactivated. The instrument is modified by the addition of a second computer disc drive to permit storage of data for subsequent statistical analysis and graphics preparation, as well as a printer to provide hardcopy.

Hemocyte CL is measured with luminol in the medium to enhance the signal as a result of oxygen radical-luminol interactions (Larson et al, 1989; Anderson and Brubacher, 1991). This gives an accurate measure of CL that can be directly related to cellular pathogen killing capacity. The CL activity of unstimulated hemocytes is recorded for 5-10 min to establish a baseline level

of ROI activity. The respiratory burst is elicited by the addition of opsonized zymosan particles (cell:particle ratio = 1:20) or by the addition of phorbol myristate acetate (PMA). The kinetics of the stimulated CL are followed by continuous counting for at least one hour. Controls with added catalase (500 U/ml) or superoxide dismutase (400-600 U/ml) will also be run; at these concentrations the enzymes produced 60 - 70% and 20 - 30% inhibition of peak CL with mouse macrophages, respectively (Tam and Hinsdill, 1990). The data are plotted and printed using a Sigmaplot software package; parameters of particular interest include: the slope of the initial CL response, the peak CL value, the time required to reach peak CL, and the area under the curve (the total CL response). These values for cells from controls and cells from pollutant exposed animals are compared by appropriate statistical methods and significant differences between treatment groups identified by ANOVA. Where appropriate, Student's t-tests (two-tailed) will be used. In the case of severe nonhomogeneity of variance, data will be analyzed by the Kruskal-Wallace test for main effects and Jonckheere's test to evaluate the concentration response (Hollander and Wolfe, 1975; Schefler, 1980).

Experimental Design

Oysters will be purchased from Mook Sea Farms, Maine, to insure the initial *Perkinsus*-free status of the animals. These oysters have been used by various researchers at VIMS since 1991, they acclimate well to experimental conditions in the laboratory. The oysters will be acclimated for 2 weeks (24°C, 20ppt), appropriate experimental groups exposed to test xenobiotics for an additional 2 weeks, (nominal xenobiotic concentrations will be prepared and continuous exposures will be carried out via weekly static-renewal procedures throughout the course of the

studies), experimental infection with *P. marinus* will be carried out, and hemolymph samples will be collected for diagnosis and immunoassays.

For each xenobiotic (and each concentration tested) there will be four treatment situations: (1) uninfected, no xenobiotic; (2) uninfected, xenobiotic; (3) infected, no xenobiotic; and (4) infected, xenobiotic. Aquaria will be maintained at 24°C, 20ppt and the oysters will be fed cultured algae daily. Animal requirements: 60 for each treatment situation and chemical concentration tested, this will provide N=15 for all parameters tested; at least two replicates for each concentration are planned. In each treatment situation at least 15 individual oysters will be numbered and sampled repetitively to determine disease progression, and 45 oysters will be used to determine immune parameters. Due to the relatively large amount of hemolymph required for the immunoassays, these oysters cannot be sampled more than once. Sample times: disease progression - 2, 4, 6, 8, 10 wks; immunoassays - 2, 6, 10 weeks, or the best times as determined in the above described preliminary experiments to establish optimal dosing and sampling schedules..

Xenobiotic concentrations used for the initial studies will be at the high end of the concentrations reported in local aquatic environments. This a standard toxicological approach designed to screen for chemically-mediated biological effects. Once established as effective at this relatively high dose, the xenobiotic in question will be tested at lower levels to determine the dose dependency of the response (disease progression and/or immunosuppression). Suggested water concentrations for the first studies: 300pptr TBT [Bay water concentrations can be as high as

530pptr, acute *C. virginica* (embryo) toxicity LC_{50} ≅ 0.7 ppb (EPA, 1991b)]; and 2.5ppm naphthalene [Elizabeth River concentrations can be as high as 2.9ppm, acute toxicity for *C. gigas* LC_{50} ≅ 200ppm (EPA, 1991a), no *C. virginica* data available].

EXPECTED RESULTS

Insight will be gained regarding the effect of xenobiotic exposure on the intensity and progression of *Perkinsus marinus* infection in the Eastern oyster, *Crassostrea virginica*. The proposed xenobiotics represent two major classes of environmental pollutants (metals and PAHs), are on the Toxics of Concern List (as defined by the Chesapeake Bay Program), and will be administered at environmentally relevant concentrations. Analysis of oyster cellular immunity, as influenced by the exposure protocols, will provide complementary information on the immunological basis of chemically altered disease progression. Knowledge gained from this project should find practical application in shellfishery management by contributing to understanding the effects of environmental pollutants on the severity of a major disease of oysters.

DISSEMINATION OF RESULTS

In addition to publication of the results in peer-reviewed literature, findings will be presented at scientific meetings via contributed and invited papers. The prospects for dissemination at national and international symposia are very good; the PIs have ongoing records of being invited to participate in and/or organize symposia at meetings such as National Shellfisheries Association, Society for Invertebrate Pathology, Pollutant Responses in Marine Animals,

Modulators of Fish Immune Responses, World Congress on Cell and Tissue Culture, etc. We also are periodically asked to present our data to various state and federal agencies.

RELATIONSHIPS TO OTHER WORK

The proposed study will complement the on-going research of Drs. Chu and Hale already funded by the CBEES Toxic Research Program. They have presented data indicating increased levels of *Perkinsus marinus* infection in oysters exposed to aqueous extracts of PAH-rich sediments. Our research will examine the kinetics of *P. marinus* infection in oysters exposed to a particular low molecular weight, water soluble PAH, and expand the study to include the effect of a metal contaminant on disease progression. In addition to building on Dr. Chu's initial observations on the effects of pollutants on the antimicrobial capacity of oyster hemocytes via measurement of chemiluminescence, we plan to quantify the effects of chemical stressors on hemocytic phagocytosis.

This project "fits" very well with our work under past support from Maryland Sea Grant and the Department of Defense to study other aspects of the effects of environmental contaminants on oyster immunity; it extends these projects by the use of new methodological advances. This proposal is very similar to an application to be submitted to Maryland Sea Grant for 1994-95 funding.

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PROJECT NUMBER: R/CBT-22

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1994	1995
1. Preliminary studies to establish optimal *P.marinus* dosing and sampling times	xxx	
2. Disease progression, TBT effects	xxxxxxxx	
3. Immunoassays, TBT effects	xxxxxxxx	
4. Disease progression, naphthalene effects		xxxxxxxx
5. Immunoassays, naphthalene effects		xxxxxxxx
6. Establish dose-dependency relationship for each xenobiotic	xxx	xxx
PROJECTED BUDGET: FEDERAL	72,113	76,631
MATCH		

NOAA FORM 90-2 (ADAPTED) U.S. DEPARTMENT OF COMMERCE

*** SEA GRANT PROJECT SUMMARY 1994***

Title: Metallothionein in marine coccoid cyanobacteria: Cloning, transcriptional analyses and application to the assessment of metal stress in natural communities of picoplankton in Chesapeake Bay

Project Number: R/CBT-23
Grant Number:
Sub Program:

Revision Date:
Initiation Date: 01/01/94
Ccompletion Date: 12/31/95

Principal Investigator: Jonathan G. Kramer
Affiliation: COMB

Man-Months: 4

Principal Investigator:
Affiliation:

Man-Months:

Proposed Fed Funds: $37,668
Current Fed Funds:
Match to Date:
Current pass through:

Proposed Match Funds: $0
Current Match Funds:

Related Proj:
Parent Proj:
Sea Grant Classification #: 45

Keywords: Toxic metals, metallothionein, *Synechococcus*, molecular biology

Objectives: The objective of this program is to assess the extent and degree that metals impact the picoplankton community in Chesapeake Bay and selected tributaries. Specifically this objective includes the isolation and cloning of the gene encoding metallothionein (MT) from representative cyanobacteria (*Synechococcus* spp.). The cloned genes will be used as probes to examine variations in transcription rates in natural populations isolated from the Lower Bay, Elizabeth River and James River. The goal of the program is to apply the newest molecular biological techniques to the problem of determining how metal stress impacts the Chesapeake Bay ecosystem.

Methodology: The structural gene encoding metallothionein (smtA) will be isolated from marine cyanobacteria by either of two routes: screening pre-existing genomic libraries with a heterologous probe to smtA from the freshwater *Synechococcus* spp. PCC 6301, or by PCR amplification of the coding region. Probes generated from the cloned fragments will be used in quantitative Northern hybridizations to assess variations in transcription patterns of the gene. RNA will be purified from natural populations of *Synechococcus* spp. collected by bulk filtration of size-fractionated water from stations in the Lower Bay and tributaries. The results of hybridization analyses of field samples will be compared and contrasted to patterns observed in laboratory cultures exposed to a suite of metals (Cd, Cu, Cr, Hg, and Zn).

Rationale: Understanding the impact of metals upon the estuarine biota is complicated by the fact that exposure often occurs chronically at sublethal levels. Development of high resolution techniques to examine specific organisms represents a viable means to assess how environmentally relevant concentrations of toxic materials impact a community. The molecular biological methods proposed here represent a novel approach to this problem and exploit specific metal responsive systems in a important member of the autotrophic community of Chesapeake Bay. This program will merge field studies with detailed laboratory development of molecular biological tolls. This will lead to a fundamental understanding of the physiology of metal responses in the target species and their ramifications in the natural environment. Therefore, this study will establish a new basis for understanding how metals affect the microbial community of Chesapeake Bay.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO: RCBT-23
University of Maryland, Center of Marine Biotechnology
PRINCIPAL INVESTIGATORS: Jonathan G. Kramer

DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	4.0		13,333	
b. Associates				
Sub Total			13,333	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates				
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical				
g. Technical-Shop				
Total Salaries/ Wages			13,333	0
B. FRINGE BENEFITS			3,467	
Total Salaries,Wages and Fringe Benefits			16,800	0
C. PERMANENT EQUIPMENT				
D. EXPENDABLE SUPPLIES AND EQUIPMENT			6,000	
E. TRAVEL				
1. Domestic	1,000			
2. International				
Total Travel			1,000	
F. PUBLICATIONS AND DOCUMENTATION COSTS			500	
G. OTHER COSTS				
1. Computer costs			500	
2. Copying,Library and Communication				
3. Analytical and Shop Services				
4. Fuel,Boat Time and Vehicle Usage				
5. Equipment Use and Maintenence			500	
6. Subcontracts				
7. Service Contracts				
8. Waste Disposal			500	
9. Other				
Total Other Costs			1,500	0
TOTAL DIRECT COSTS			25,800	0
INDIRECT COSTS				
(On Campus) : 46 % of 25,800			11,868	
(Off Campus) : % of				
Total Indirect Costs			11,868	0
TOTAL COSTS			37,668	0

METALLOTHIONEIN IN MARINE COCCOID CYANOBACTERIA: CLONING, TRANSCRIPTIONAL ANALYSES AND APPLICATION TO THE ASSESSMENT OF METAL STRESS IN NATURAL COMMUNITIES OF PICOPLANKTON IN CHESAPEAKE BAY

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INTRODUCTION

A number of metals are prominent members of the Chesapeake Bay Program's Toxics of Concern List. These elements are thought to cause a tangible stress to the estuarine ecosystem over a variety of trophic levels. In order to understand the nature of this stress it is essential to determine the fundamental mechanisms that structure the responses of impacted organisms. Ultimately, these responses can be propagated throughout the biota and effect the functioning of the ecosystem as a whole. Because discerning these impacts is often complicated by the fact that metal exposure is chronic rather than acute, sensitive measures of biological responses are needed to accurately assess how and when stress occurs in the estuarine environment (Sanders and Cibik 1988). The focus of this research program will be to describe how the autotrophic picoplankton respond to the stress imposed by low levels of toxic metals. Specifically, I will address this problem by studying key molecular biological characteristics of a target population, the coccoid cyanobacteria, *Synechococcus* spp., in Chesapeake Bay and in laboratory studies.

Many organisms respond to metal exposure by producing a specific class of proteins known as metallothioneins (MT). In general terms the MTs are characterized by a low molecular weight (< 10,000), high cysteine (23-33%) and metal content as well as known homology at the structural or functional level to the well characterized mammalian MT (Hamer 1986). Most research regarding MTs has centered upon the study of eukaryotic systems and much is known regarding the structure and metal binding capacity of these compounds. In addition, various aspects of the genetics and molecular biology of the proteins have been detailed (Hamer 1986). While a number of proteins with metal binding capacity have been found in bacteria, a distinct prokaryotic MT has been reported only recently. This unique protein has been isolated and characterized from the freshwater unicellular cyanobacterium *Synechococcus* spp. PCC 6301 (Olafson et al. 1988). In addition, the operon encoding both the MT structural gene as well as a regulatory protein has been cloned (Robinson et al. 1990, Gupta et al. 1993). There is a profound increase in transcription of the structural gene upon exposure to sublethal quantities of several metals (Gupta et al. 1993). *Synechococcus* spp. PCC 6301 is closely related to the marine coccoid cyanobacteria found in the lower Chesapeake Bay as well as other unicellular cyanobacteria found throughout the estuary. These organisms have been shown to be numerically as well as metabolically important to the trophic dynamics of the Bay (Affronti and Marshall 1993, Ray et al. 1989). The advances made at the molecular biological level for the freshwater species provide a powerful set of tools to examine how metals impact these marine and estuarine cyanobacteria. Discerning variations in the transcription of the MT gene in natural populations of Chesapeake Bay *Synechococcus* spp. will yield a unique and sensitive measure of the stress imposed by metals on the autotrophic community.

MT in *Synechococcus* spp. Detailed characterization of the *Synechococcus* sp. MT revealed that while the protein had some structural likenesses and distinct functional similarities with known MT's, it was unique and previously uncharacterized (Olafson et al., 1988). There was very little homology between the amino acid sequence of this MT and others, although short clusters (i.e., Cys-Xaa-Cys and Cys-Cys) common to all MT's were

found. A set of degenerate oligonucleotide primers were designed based upon the amino acid sequences of the terminal portions of the protein. They were used in conjunction with the polymerase chain reaction (PCR) to amplify the gene encoding MT in *Synechococcus* sp. PCC 6301 (previously *Anacystis nidulans*; Robinson et al. 1990). DNA sequence analysis of the cloned PCR product confirmed its identity and the gene was designated *smtA*. *SmtA* was expressed in *Escherichia coli* and a variety of metals including Cd, Cu, Hg, and Zn were found to bind the protein with very high affinities (Shi et al. 1992). Probes generated from the cloned gene were used to isolate the same gene from a second strain, *Synechococcus* sp. PCC 7942 (formerly *A. nidulans* R2) which was more amenable to genetic studies (Robinson et al. 1990). Analysis of the pattern of transcription of *smtA* revealed that Cd, Cr, Cu, Hg, Ni, Pb, and Zn acted as inducers. Transcription increased from 1.5 to 40-fold over basal levels when metals were added at the maximum concentrations permissive for growth (i.e., 0.025μM for Hg to 25 μM for Cr). However, distinct increases in transcript abundance were noted for most metals with additions of 1.0 to 10.0μM.

Additional sequence analysis of the coding region located just upstream of *smtA* revealed a second gene, *smtB* which was homologous to other genes known to have regulatory functions in two bacterial metal efflux systems (Gupta et al. 1993). When *smtB* was inactivated in Zn-induced *Synechococcus* PCC 7942, transcription of *smtA* increased 4-fold over the maximal level observed in wild type cells, and 20-fold over uninduced cultures. The data indicates that *smtB* encodes a repressor.

Synechococcus spp. cells exposed to metals are known to develop an enhanced tolerance to metal stress. Examination of the *smt* operon in *Synechococcus* sp. PCC 6301 suggests that two interrelated events act to confer this tolerance. Initial studies demonstrated that adaptation to sublethal levels of Cd (1.7μM) caused the number of *smtA* genes in the chromosome to increase about 4-fold (Gupta et al., 1992). The amplification was not associated with selection

of particular variants within the culture. In addition to the increase in gene dosage, Cd-tolerant cells had a distinct change in the *smt* operon itself (Gupta et al., 1992). These cells were found to have a truncated coding region (Gupta et al., 1993). Sequence analysis revealed that tolerant *Synechococcus* sp. PCC 6301 had lost approximately 350 base pairs of the region upstream of *smtA*; hence inactivating *smtB*, the repressor. Thus, tolerant cells have increased both MT gene dosage as well as the capacity for its transcription.

The implication of these findings are quite important. They suggest that cells in the environment will have a distinct genetic and physiological signature after chronic exposure to metal stress.

Marine Coccoid Cyanobacteria Chroococcoid cyanobacteria are widely distributed in the marine environment (Glover 1985, Waterbury et al., 1987) and are prominent members of the autotrophic community in Chesapeake Bay (Ray et al. 1989, Affronti and Marshall 1993). Picoplankton accounted for as much as 17% of total carbon fixation in the Bay during the summer and *Synechococcus* spp. comprised as much as 51% of the total picoplankton biomass (Ray et al. 1989). Two *Synechococcus* spp. characterized by their major pigmentation are found in Chesapeake Bay. During the summer a phycocyanin (PC) dominant strain is most abundant while a phycoerythrin (PE) dominant is most prevalent during the winter (Affronti and Marshall 1993). These strains are easily identified using standard epifluorescent microscopy and can be isolated from other components of the plankton by simple differential filtration techniques. Such methods have been utilized to isolate RNA from *Synechococcus* spp. found in coastal waters off N. Carolina and direct probing has revealed a distinct diel periodicity in rRNA content linked to phasing of cell division and growth over the course of the day (Kramer and Singleton submitted). Representatives of both these strains are readily cultured in the laboratory and are amenable to a wide range of molecular biological studies.

Genomic libraries have been constructed from both, and several metabolically important genes isolated. (Kramer 1988, Kramer et al., in prep)

The ecological importance of *Synechococcus* spp., and the fact that these organisms are accessible in their natural environment as well as the laboratory, combine to make them nearly ideal candidates for studies of how chronic exposure to toxic metals affects the autotrophic community of Chesapeake Bay.

Relevance to the Problem Completion of this research program will yield two highly relevant results. First, this study will result in a better understanding of the impact of metals upon members of the autotrophic community in Chesapeake Bay. This will help in the assessment of the extent and severity of the stress imposed by metals. Second, this study will develop and apply a molecular biological approach. This will enable very sensitive measurements to be made of cellular characteristics indicative of ongoing metal stress as well as previous exposure to this class of toxic materials. The use of these techniques has great relevance and promise for studies of metal stress in the physically and chemically complex estuarine environment of Chesapeake Bay. In a broader context, this research program is highly relevant to studies throughout the U.S. It is an approach that does not rely upon high, lethal dosages of toxic compounds to reveal a cellular response. Rather it is an effort in "molecular toxicology" that promises to assay metal toxicity in natural populations with true, environmentally significant exposures. Such techniques are at the forefront of environmental toxicology and will ultimately be of great use to scientists and managers alike (Marshall 1993).

OBJECTIVES AND HYPOTHESIS

Hypothesis The underlying hypothesis of this research program is that marine *Synechococcus* spp. will respond to the stress imposed by chronic exposure to sublethal concentrations of toxic metals by altering the rate of transcription of the MT structural gene (*smtA*). Comparisons of these rates in different locations in Chesapeake Bay will give a sensitive measure of the impact of metals upon the autotrophic picoplankton and will provide a benchmark for understanding how low levels of these toxic impact the microbial food web.

Overall Objectives The overall objectives of this program will be to isolate and clone *smtA* from two marine *Synechococcus* spp. strains found in Chesapeake Bay. These cloned genes will be used as probes to examine variations in MT transcription after exposure to selected metals in laboratory studies. The same probes will then be applied to the ultimate objective of examining variations in transcription patterns of natural populations of *Synechococcus* spp. in the lower Chesapeake Bay as well as the Elizabeth and James Rivers.

1994 Objectives The specific objectives for the first year of the program are;

1. To isolate and clone *smtA* from *Synechococcus* spp. WH 5701 and WH 7803,
2. To examine how *smtA* transcription varies after exposure to, Cd, Cr, Cu, Hg, Pb and Zn and,
3. To determine the minimum concentration of each metal that induces *smtA* transcription.

1995 Objectives The specific objectives for the second year of the program are;

1. To conduct two surveys to collect *Synechococcus* spp. from the lower Chesapeake Bay, the Elizabeth River and the James River. RNA purified from

these cells will be probed for *smtA* transcript levels. Surveys will be conducted once in the spring and once in the late summer.

2. To determine through laboratory experiments if marine *Synechococcus* spp develop tolerances to metals through molecular genetic pathways similar to those discerned in the freshwater models.

METHODOLOGY

To accomplish the specific objectives of this program I will build a conceptual and methodological base through laboratory studies. This base will then be applied to examinations of natural populations of cyanobacteria in Chesapeake Bay.

Cloning *smtA* From Representative Cyanobacteria The cyanobacteria chosen for the laboratory portion of this program are both found in Chesapeake Bay. *Synechococcus* sp. WH 5701 is a PC-dominant strain that is closely related to the freshwater *Synechococcus* spp. utilized in the original isolation of *smtA*. *Synechococcus* sp. WH 7803, a PE-dominant, is considered to be a true marine strain (Waterbury and Rippka 1989). I have previously constructed genomic libraries for both strains in the phage vector, LambdaGem11. These libraries will be screened with a probe for *smtA* obtained from the group who originally cloned the gene. Once localized, the gene will be subcloned into an appropriate plasmid vector (pBluescript or pUC19) and the identity of the ca. 160 bp coding region confirmed by DNA sequencing using the Sequenase protocol. I will also pursue an alternate approach based upon amplification of the *smtA* coding region using PCR and the degenerate primers noted by Robinson et. al. (1990). Products from the amplification will be blunt-end ligated into pBluescript and sequenced. These two approaches are complementary and maximize the opportunity to expeditiously isolate the gene from the marine strains. These techniques are routinely done in my laboratory and have been utilized successfully to isolate genes such as

glnA (encoding the glutamine synthetase structural protein) and the a rRNA operon from the target cyanobacterial strains. Comparisons will be made of the sequences of the cloned *smtA* genes using the computerized Wisconsin Package of sequence analysis software available at C.O.M.B., and if possible specific probes for each species designed. Since it is most probable that these species-specific probes will be oligonucleotides, the hybridization characteristics of each will be evaluated, as will the degree of heterologous binding to the other cyanobacteria as well as representative heterotrophic marine bacteria.

Transcriptional Analyses To assess the degree that the *smtA* gene responds to metal exposure, I will conduct a series of experiments designed to determine the minimal level of metal that causes an increase in transcription over basal levels. I will also determine the level that causes the highest rate of transcription. The metals to be assayed include: Cd, Cu, Cr, Hg, Pb and Zn. These studies will be conducted on both *Synechococcus* sp. WH 5701 and WH 7803. The organisms will be cultured in a fully defined medium and exposed to each metal for periods ranging from 2-24 h. Variations in transcription rate will be assayed by probing RNA samples with the *smtA* genes from each strain. Northern hybridization of ^{32}P-labelled probes to dot blots will be used and quantified by direct scintillation counting of individual dots. Because physiological state may change with metal exposure, replicate dot blots will be probed with a 16S rRNA probe (Kramer and Singleton 1992, submitted). Variations in *smtA* transcription will be normalized to the changes in rRNA content to account for global variations in transcription rates and cell growth rates. Changes in cell division rate will be made by direct cell counts using standard epifluorescent microscopy.

Studies of Natural Populations in Chesapeake Bay Variations in the level of *smtA* transcription in natural populations of cyanobacteria will be made twice during the second year of the program. Collections will be made in the lower Bay and at various locations in the Elizabeth and James Rivers. In order to assess variability during periods of high and low

freshwater input to the system, studies will be conducted in the early spring and in the late summer to early autumn. Sampling will be conducted from the University of Maryland's *RV Aquarius* over the course of 2 days during each season. It is anticipated that there will be considerable interaction between this program and other CBEEC researchers during this phase of the study.

Picoplanktonic cyanobacteria will be collected on 0.6 m Nucleopore polycarbonate membrane filters after removing larger organisms by prefiltration through 2.0 μm membranes. Samples in the range of 10-20 l are anticipated. Preliminary studies using multiple 47 mm filters accomplished similar filtrations in approximately 30 min. We currently have large format filtration systems (142 and 243 mm diameter) that will be used to facilitate rapid sampling. Filters containing the so-called "*Synechococcus*-fraction" will be placed in RNA extraction buffer and stored frozen on dry ice for subsequent laboratory based RNA purifications. Studies conducted in the coastal waters off North Carolina (Kramer and Singleton, submitted) demonstrated that intact cyanobacterial RNA of sufficient quality for Northern hybridizations can be readily isolated with this technique.

Dot blots prepared from the RNA purified from *Synechococcus* spp. from various locations in Chesapeake Bay and the tributaries will be probed with *smtA* from both laboratory strains as well as a probe generated from the freshwater *Synechococcus* spp. Because of the possibility of "contamination" of the 0.6 to 2.0 μm fraction with heterotrophic bacteria, separate, parallel blots will be probed with a cyanobacterial as well as a "universal eubacterial" 16S rRNA probe to establish a baseline value for the amount of *Synechococcus* spp. RNA on the filters. These measures will also provide a relevant physiological base for variations in global transcription rates (Kramer and Singleton 1992, submitted). Given that the *smtA* gene appears to be quite unique at this time it is unlikely that there will be heterologous hybridization to the heterotrophic background. However, a separate collection of heterotrophic bacteria contained

in a 0.22 to 0.6 μm fraction will be made at representative stations. RNA purified from these samples will be probed as described previously and the degree of heterologous hybridization to natural communities of heterotrophic bacteria evaluated. At all locations, samples will be taken for cell counts by epifluorescent microscopy. Cyanobacteria will be evaluated by counting autofluorescing cells; PC and PE dominants will be discriminated with appropriate excitation and emission filter sets. Total bacterial cell numbers will be evaluated by acridine orange direct counts.

Development of Metal Tolerance in Marine Synechococcus spp. In the final stage of the program I will use laboratory culture studies to establish how metal tolerance is achieved in the marine cyanobacteria. Culture of each strain will be exposed to increasing dosages of two of the metals to be designated after completion of the primary transcriptional analyses. Increased exposures will be made in a stepwise fashion. Once tolerant cultures are established, DNA will be purified and the gene dosage of *smtA* assayed by quantitative Southern hybridization (dot blot protocols). In addition, I will examine how the strains respond after transfer back to a metal-free medium. In particular, it will be important to compare how transcription of the *smtA* gene varies in tolerant and non-tolerant strains as well as to determine what the net effect of tolerance is on the growth of cells after the metal stress is removed.

Basic Methodologies All of the procedures utilized for cyanobacterial studies are routinely done in my laboratory. Protocols for the purification of nucleic acids involve slight modifications of standard methodologies and are very efficient for the strains chosen. These techniques have been adapted with success to studies of natural communities of *Synechococcus* spp. (Kramer 1988, Kramer 1990, Kramer and Morris 1990, Kramer and Singleton 1992 and submitted). Techniques for gene isolation, cloning and sequence analysis as well as all hybridization protocols are taken from the standard manuals (Ausebel et al. 1990, Sambrook

et al. 1989). During the course of all experiments there will be sufficient replication of samples as well as assays to allow appropriate statistical analyses to be made.

EXPECTED RESULTS

It is anticipated that this research effort will yield a better understanding of the impact of metals upon marine *Synechococcus* spp and the autotrophic community of the lower Chesapeake Bay. Hence the results will yield both fundamental molecular biological/toxicological information as well as applied, environmentally relevant data for a particular ecosystem under stress. The approach taken is novel and exploits molecular biological methodologies to examine a specific metal responsive gene in a target organism of considerable significance to the Bay. Successful completion of this program will result in two developments, both of which are important to the CBEEC Toxics Research Program. First, will be a detailed picture of how stress occurs in the target community. In addition, the particular molecular biological signature of these impacted organisms will potentially serve as a biomarker that may be traced as the cells are transported into the estuary. The second important result of this study will be the development of new, high resolution techniques to assess low level or chronic stress imposed by metals. The application of these techniques to the study of natural communities impacted *in situ* will be a significant accomplishment with great potential for elucidating the degree and relevance of metal stress in other marine and aquatic systems.

DISSEMINATION OF RESULTS

The primary vehicle for the dissemination of the results of this study will be through publication in peer-reviewed journals. It is anticipated that findings will also be presented at

least once per year at a national conference. Because the techniques to be utilized in this program are novel, efforts will be made to develop a readily adaptable series of protocols that can be used by other researchers studying metal stress to cyanobacteria in other aquatic systems. As part of this effort, the genes isolated and cloned as a result of these efforts will be made available to others for use as probes.

RELATIONSHIP TO OTHER WORK

Relationship to other CBEEC Programs The proposed program is designed to complement and add to the ongoing efforts of the CBEEC Toxics Research Program. The approach taken will provide a new means to identify when autotrophic populations have been impacted and will lead to the development of the capability to identify metal exposed cells by their molecular biological signature. Taken together, this information will be very useful in determinations of how the net effect of a stress is transferred in the biota from an impacted area to other portions of the estuary. The target organism hence becomes an ecological indicator of the occurrence of metal stress, its physiological/molecular biological ramifications and ultimately, its dissemination into other portions of the ecosystem. Therefore, the study fits well with ongoing efforts in the water-column bioprocessing group. Because it is focussed upon a target organism, the approach is quite specific on the molecular biological level. In that respect it is related to studies of more easily sampled benthic organisms. Indeed the emphasis on studying the MT of *Synechococcus* spp. complements similar efforts utilizing molecular biological tools to examine MT and stress responses in oyster larvae and spat. Our efforts will be a primary attempt to bring the specificity and resolution of a molecular approach to studies of water column organisms.

Relationship to Ongoing Research Over the past several years, the main focus of my research has been centered upon the physiology and molecular biology of marine

Synechococcus spp. The central theme of this research has been to describe how the regulation of key genes is related to cell growth and division. My approach has been to model various aspects of this process in laboratory studies and subsequently examine natural populations of *Synechococcus* spp. in the field. To facilitate this work I have isolated and cloned several physiologically important genes from these marine cyanobacteria. One of the primary goals of these efforts is to assemble a bank of probes for genes involved in central metabolic pathways in *Synechococcus* spp. Recent efforts have been directed to genes involved in nitrogen assimilation. Hence I have cloned *glnA*, the structural gene for the enzyme glutamine synthetase and am presently in the process of isolating *narG*, the structural gene for nitrate reductase. Genes relating to the photosynthetic process are available from collaborating laboratories, as is a cloned rRNA operon which I isolated several years ago. Preliminary efforts to apply these tools to studies of natural populations have been completed and have shown great promise (Kramer and Singleton, submitted). High quality RNA from *Synechococcus* spp. populations was isolated and hybridization to a 16S rRNA probe. The results revealed a distinct diel variability in the ribosome content of the cells. This periodicity was related to phasing of cell growth and division over the course of the daily cycle of irradiance.

My intent is to use these techniques to examine how irradiance and nitrogen supply regulate the growth of natural populations of *Synechococcus* spp. To that end, proposals are pending with the National Science Foundation (Biological Oceanography Program) and planned for impending deadlines with the U.S. Department of Energy (Environmental Sciences Division). Because these techniques are designed to give an accurate indication of physiological state and changes therein, they lend themselves to the assessment of the toxic impacts on these cyanobacteria. Therefore, a proposals has been submitted to the Environmental Protection Agency (Office of Exploratory Research) to investigate how these measure may be used to assay sublethal effects of toxic compounds in marine *Synechococcus* spp.

In total these efforts have given me a strong methodological and theoretical foundation to undertake the research proposed in the present effort. They will serve to complement and strengthen the investigation on many levels.

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PROJECT NUMBER: R/CBT-23

PROJECTED TIME SCHEDULE

PROJECT ACTIVITIES	YEARS 1994	1995			
1. smtA isolation and cloning	xxxxx				
2. Sequence analyses	xxx				
3. Studies of transcription patterns in metal exposed laboratory cultures		xxxxxxxxxxxx			
4. Spring collection of cyanobacteria from Chesapeake Bay and tributaries		x			
5. Analysis of spring samples		xx			
6. Summer/Autumn collections		x			
7. Analysis of summer/autumn samples		xx			
8. Tolerance studies in laboratory		xxx			
PROJECTED BUDGET: FEDERAL	$ 37,668	$ 50,332	$	$	
MATCH	$	$	$	$	

NOAA FORM 90-2 (ADAPTED) U.S. DEPARTMENT OF COMMERCE

*** SEA GRANT PROJECT SUMMARY 1994***

Title: A risk assessment for Dimilin use in the northern Chesapeake Bay. A model study for non point-source runoff.

Project Number: R/CBT-24
Grant Number:
Sub Program:

Revision Date:
Initiation Date: 01/01/94
Ccompletion Date: 12/31/95

Principal Investigator: David A. Wright
Affiliation: Chesapeake Biological Laboratory
Man-Months: 1

Principal Investigator: Rodger Dawson
Affiliation: Chesapeake Biological Laboratory
Man-Months: 1

Proposed Fed Funds: $90,217
Current Fed Funds: $0
Fed Fund to Date: $0
Current pass through:

Proposed Match Funds: $0
Current Match Funds: $0
Match to Date:

Related Proj:
Parent Proj:
Sea Grant Classification #: 45

Keywords:

Objectives: Specific objectives will be to map the usage patterns of Dimilin in the northeast Chesapeake Bay catchment area, and monitor Dimilin run-off at two field sites. Bioassay results from form two crustacean species at these field sites will be compared with bioassay data from a (Dimilin-free) reference site. End points from these and laboratory bioassays will characterize the role of food in Dimilin bioavailability and will form the basis of a hazard assessment for this compound's specific mode of action. Chemical application, run-off and toxicity data will be combined as a model risk assessment for non-point source pesticide run-off .

Methodology: Chemical analysis for Dimilin residues in water and sediments using a combination of HPLC and C-HS techniques will be coupled with sensitive laboratory and field bioassay procedures employing as end points mortality, fecundity and cuticular abnormalities of two crustaceans. Field bioassays will be coordinated with Dimilin applications adjacent to watersheds of rivers in the Northern Chesapeake Bay. Data from the field application of Dimilin, run-off levels, environmental half-life, ecology of test animals and bioassays will be used to construct a risk assessment for this pesticide in these locations.

Rationale: In order to construct a risk assessment, two components are necessary: exposure assessment and hazard assessment. In constructing a model for a non-point-source risk assessment it is important to choose a compound and test organism(s) for which there is a high probability of achieving good quantitative estimates of both of these parameters. There are several reasons why Dimilin and the test species proposed here form an excellent basis for such a model.

Exposure Assessment relies for success on accurate data for both environmental chemical loading and distribution of test organisms. In the case of Dimilin, usage in Maryland is carefully monitored and controlled by the agency responsible for its application (MD. Department of Agriculture). Detailed spatial and temporal records are kept and recorded centrally.

With respect to species distribution, unusually detailed records are available from several sources for both *E. affinis* and *L. plumulosus*.

NOAA FORM 90-4 U.S. DEPARTMENT OF COMMERCE

GRANTEE: Period: 1994 GRANT/PROJ. NO: RCBT-24
University of Maryland, Center for Environmental and Estuarine Studies
PRINCIPAL INVESTIGATORS: David A. Wright
Rodger Dawson
DURATION (MOS.): 12 Months

BUDGET CATEGORY	MAN-MONTHS SEA GRANT	MAN-MONTHS GRANTEE	SEA GRANT FUNDS	GRANTEE SHARE
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Invest.	1.2		6,050	
b. Associates	1.2		6,490	
Sub Total			12,540	0
2. OTHER PERSONNEL				
a. Professionals				
b. Research Associates	12.0		12,000	
c. Res. Asst. Grad. Std.				
d. Prof. School Students				
e. Pre-Bac Students				
f. Secretarial-Clerical	1.2		2,000	
g. Technical-Shop				
Total Salaries/ Wages			26,540	0
B. FRINGE BENEFITS			8,964	
Total Salaries,Wages and Fringe Benefits			35,504	0
C. PERMANENT EQUIPMENT			6,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT			6,000	
E. TRAVEL				
1. Domestic	1,200			
2. International				
Total Travel			1,200	
F. PUBLICATIONS AND DOCUMENTATION COSTS			600	
G. OTHER COSTS				
1. Computer costs				
2. Copying,Library and Communication			800	
3. Analytical and Shop Services			12,000	
4. Fuel,Boat Time and Vehicle Usage			1,500	
5. Equipment Use and Maintenence				
6. Subcontracts				
7. Service Contracts			984	
8. Waste Disposal				
9. Other			2,000	
Total Other Costs			17,284	0
TOTAL DIRECT COSTS			66,588	0
INDIRECT COSTS				
(On Campus) : 39 % of 60,588			23,629	
(Off Campus) : % of				
Total Indirect Costs			23,629	0
TOTAL COSTS			90,217	0

A RISK ASSESSMENT FOR DIMILIN USE IN THE NORTHERN CHESAPEAKE BAY. A MODEL STUDY FOR NON POINT-SOURCE RUNOFF.

David A. Wright
Rodger Dawson

INTRODUCTION

The C.B.E.E.C. Toxics Research Program has identified the need for information on the fate and effects of toxic chemical runoff in the Chesapeake Bay catchment area at environmentally realistic levels, and the effect which trophic transfer is likely to have on bioavailability (and toxicity). The problem essentially has the elements of a risk assessment in that chemical application is related to exposure concentration which is, in turn, compared with toxicity data. Pesticides are excellent candidates for risk assessment particularly where exposure and toxicity data overlap.

Rationale

In order to construct a risk assessment, two components are necessary: exposure assessment and hazard assessment. In constructing a model for a non-point-source risk assessment it is important to choose a compound and test organism(s) for which there is a high probability of achieving good quantitative estimates of both of these parameters. There are several reasons why Dimilin and the test species proposed here form an excellent basis for such a model.

Exposure Assessment relies for success on accurate data for both environmental chemical loading and distribution of test organisms. In the case of Dimilin, usage in Maryland is carefully monitored and controlled by the Agency responsible for its application (Md. Department of Agriculture). Detailed spatial and temporal records are kept and recorded centrally.

With respect to species distribution, unusually detailed records are available from several sources for both E. affinis and L. plumulosus (see pages 5 and 6).

Hazard Assessment: Data which have been gathered in this laboratory are virtually unique in the Chesapeake system in demonstrating toxicity of Dimilin to a widespread indigenous species (E. affinis) at very low levels which have been documented in the ambient aquatic environment (Smucker 1988; Wright et al 1992; Savitz et al. 1993; Table 1). Toxicity data were reported from 10‰ (Savitz et al 1993). Although we have yet to investigate Dimilin toxicity in the lower salinities/freshwater found at the study sites proposed here, we anticipate the toxicity would be even greater. The characteristic cuticular abnormality seen in Dimilin-exposed E. affinis (Savitz et al 1993) is likely to prove particularly useful in identifying specific Dimilin toxicity and provide a means of differentiating it from other potentially toxic agents in the water. Spatial and temporal considerations of test-site selection should also minimize complicating effects of other toxic agents. Information from laboratory experiments will further refine the hazard assessment.

We therefore anticipate a high probability of obtaining precise data for both components of the Risk Assessment. The high, and characteristic, toxicity of Dimilin in this regard, give the study a good probability of achieving characteristics of a "worst case" model and one which may aid in similarly modelling other non-point source toxics.

Dimilin Usage and Toxicity

Diflubenzuron (DFB), marketed under the trade name Dimilin has been approved by the U.S. Environmental Protection Agency for control of several insect pests including the gypsy moth (1976) and foliar feeders on soybeans (1982). It is presently in wide use in Maryland for gypsy moth (Lymantria dispar (Smucker and Speith, 1987). In aquatic habitats DFB is present on particulates, in sediments, and in the water itself as a result of mobilization from forests that have been aerially sprayed (Smucker and Speith 1987, Smucker 1988). In 1988 Maryland Department of Agriculture listed 28,000 lbs. (active ingredient) Dimilin usage. More current figures are currently in preparation but are not yet available.

DFB (TH-6040, or 1-(4-chlorophenyl)-3-(2,6-difluorobenzoylurea) is an insect growth inhibitor, specifically targeting the molting process. Following the use of DFB as a pest control measure, residues may enter the aquatic environment in runoff, or by direct over aquatic systems. Because of its specific mode of action, there is little concern over direct effects on non-target organisms, such as birds and mammals, which do not have chitin-containing exoskeletons (Mauchamp and Perrineau 1987, Granett 1987). Similarly, effects on fish occur only at extremely high concentrations (Julin and Sanders 1978, Lee and Scott 1989). Chitin production in diatoms and

fungi surprisingly is not affected by DFB treatment (Booth et al 1987; Mauchamp and Perrineau 1987). In crustaceans, however, the mode of action of DFB is similar to that in insects (Christiansen et al 1984; Antia et al 1985). In the past it was thought that DFB directly inhibited the enzyme chitin synthetase which is required for arthropod molting (Gijswijt et al 1979). However, recent studies have demonstrated that its action is less direct. For example, it may act as a serine protease, inhibiting the activation of the chitin synthetase (Clarke and Jewess 1990; Lee et al 1990), or it may affect hormonal processes in the molting cycle such as B-ecdysone metabolism (Gulka et al. 1980; Lee et al. 1990). Alternatively, it may affect substrate or cofactor availability (Marks and Ward 1987). Finally, a post-synthetic process such as chitin polymerization, deposition or integration may be the target (Antia et al. 1985; Cohen 1987; Grosscurt and Jongsma 1987). Impairment of chitin incorporation may, in turn, be the result of effects on transport or synthesis of chitin precursors or cuticular proteins (Lee et al 1990). Histological studies have shown that deposition of the endocuticle is disturbed by DFB in both crustaceans as well as insects (Mulder and Gijswijt 1973; Christiansen and Costlow 1982; Gulka et al 1982; Retnakaran and Wright 1987).

Because of its mode of action Dimilin is likely to elicit some highly characteristic symptoms in exposed test organisms. This facilitates a highly specific hazard assessment, even in the presence of other toxic chemicals. Savitz et al (1993) and Savitz and Wright (unpublished) have demonstrated that Dimilin is toxic to the copepod Eurytemora affinis at very low concentrations. Survival and reproductive performance were both affected at Dimilin concentrations $< 1 \mu g l^{-1}$ and some of the data have been summarized in Table 1. Additionally, cuticular separation in E.

TABLE 1

Effects of Diflubenzuron on Maturating and Reproductive Success of *Eurytemora affinis* (from Savitz and Wright 1992)

	Continuous Exposure				Early Exposure				Late Exposure			
	Control	0.5 µg/L	1.0 µg/L	1.25 µg/L	Control	0.5 µg/L	1.0 µg/L	1.25 µg/L	Control	0.5 µg/L	1.0µg/L	1.25 µg/l
Percent Survival±S.D	96.25±4.79	98.33±2.89	67.5*±8.66	22.5*±21.02	90.00±7.07	87.50±6.46	41.25*±7.50	8.75*±4.79	86.25±8.54	92.50±11.90	83.75±11.09	82.50±11.
Percent Adults ± S.D.	93.40±3.00	98.33±2.89	71.78*±11.00	6.35*±7.45	95.59±5.63	97.21±3.22	86.11±13.98	62.50±47.87	100%	96.25±3.80	80.80±7.90	81.90±6.9
Percent Females w/Broods ±SD	70.80±10.69	52.30±20.18	0.	0.	81.55±17.86	58.33±21.51	45.83±41.67	37.50±47.87	50.60±41.48	81.61±12.76	0*	6.25*±0.
Nauplii/Female					9.51±4.16	13.00±9.48	2.56±5.13	0*	10.27±13.85	7.30±5.94	0	0
Percent Exuviae attached	0.00	0.00	13.00	8.25	0.00	0.00	0.00	0.00	0.00	0.00	17.00	30.80
Total / Survivors n=80	77	59(n=60)	60	18	74	70	33	7	69	74	67	66

*Significantly different from control at $p<0.05$

affinis was shown to be characteristically associated with Dimilin exposure (Savitz et al., 1993). Field concentrations of Dimilin, following spraying, have been shown to be within the range of toxic concentrations reported in Table 1 (Smucker 1988).

Test Organisms

Brownlee and Jacobs (1987) found that E. affinis made up 20% of the mesozooplankton in the Chesapeake Bay when abundance was averaged over a full year, ranking second in number to the calanoid copepod Acartia tonsa. Together, these two species comprised 65% of the total mesozooplankton collected. While E. affinis dominates in the oligohaline, mesohaline and polyhaline regions mainly in the spring months, it is the dominant copepod in freshwater portions of the Bay throughout the year (Brownlee and Jacobs 1987). Information on distribution and abundance of the species in the Chesapeake Bay is available from Allan et al 1976; Setzler-Hamilton et al. 1981b; Storms 1981; Brownlee and Jacobs 1987; Olsen 1987. In addition to this large body of information, there are several other reasons why E. affinis is an appropriate organism for this study. This species has proven highly sensitive to Dimilin in controlled assays involving mortality, fecundity and cuticular abnormalities (Savitz et al 1993; Savitz and Wright, unpublished). Additionally, Hartwell et al (1993) have demonstrated the utility of a E. affinis fecundity assay as a means of assessing toxic stress in the ambient environment. E. affinis has a short generation time of approximately two weeks at 20°c (Heinle and Flemer 1975) and it has been successfully cultured in the laboratory. Moreover, its preference for salinities <15 ppt (Jeffries 1962, from Katona 1970), along with its presence in early spring (Heinle 1972; Bradley 1977; Brownlee and Jacobs 1987; Olsen 1987) make it vulnerable to pest control practices during

spring months in the relatively freshwater regions of the Bay and its tributaries.

Leptocheirus plumulosus Shoemaker is an ecologically important infaunal inhabitant of both oligohaline and mesohaline portions of the Chesapeake Bay where nearly 20 years of (almost monthly) population data are now available (Holland et al 1988; Kerhin et al 1988). Its use as a bioassay organism for sediment toxicity testing in the Chesapeake Bay was recently recommended by Schlekat et al. (1992) who demonstrated its wide salinity tolerance.

OBJECTIVES AND HYPOTHESIS

Hypothesis

We hypothesize that receiving waters adjacent to areas subjected to Dimilin spraying will have concentrations of the pesticide higher than water at a reference site of similar characteristics but distant from Dimilin application.

We hypothesize that test crustaceans entrained in ambient water containing close to 1μg L^{-1} will show significant toxic effects (mortality, reproductive impairment, cuticular abnormalities) relative to animals from reference sites.

Laboratory bioassays simulating field conditions will be used to refine toxic end-points and investigate the part played by particulate adsorption in Dimilin bioavailability. We anticipate that both environmental half-life and bioavailability of Dimilin will be significantly affected by particulate adsorption. The effect of Dimilin adsorption to particulates on its bioavailability (and

toxicity) will be investigated in the laboratory.

We anticipate that a combination of Dimilin runoff data, test species ecology and results from in situ and laboratory bioassays will enable risk assessment for this pesticide which will act as a model for other non point-source compounds.

Overall Objectives

Specific objectives of this study will be to:

1) Map the usage patterns of Dimilin in the northern Chesapeake Bay catchment area, document the loading and runoff patterns in the northwest segment of the Bay between the Elk and the Choptank rivers and determine which areas represent the most likely recipients of non-point source runoff from this activity.

2) Evaluate the effects of Dimilin runoff on Eurytemora affinis, and Leptocheirus plumulosus as determined by mortality, reproductive performance and molting, using a combination of in situ and laboratory bioassays. (Hyalella azteca may be used as an additional bioassay organism in freshwater environments).

3) Examine the role which particulates (food) play in the uptake of Dimilin by planktonic and benthic crustaceans.

4) Evaluate the most sensitive crustacean screening assay among those tested and use those indicators to characterize the extent of Dimilin effects down a concentration gradient established as a result of preliminary chemical analyses.

Year One Objectives

The primary focus of the study during the first year will be to document Dimilin application in the catchment area of the northeast Chesapeake Bay region in a quadrant between the Elk and the Chester Rivers and to assess the proportion of the applied compound which reaches the receiving waters. Effects of Dimilin will be assessed using in situ bioassays employing both species.

Work will begin on laboratory bioassays using amphipods (Leptocheirus plumulosus) designed to test effects of sediment characteristics (particulate profile, TOC, etc.) on Dimilin bioavailability.

Year Two Objectives

Copepod bioassays will test the effect of particulate adsorption on Dimilin toxicity. Both inorganic (Kaolin) and organic (algae) particulates will be used, effectively characterizing the role of food in Dimilin bioavailability.

The most sensitive end-points from Year One and Year Two laboratory bioassays will be incorporated into a series of field bioassays positioned along a Dimilin concentration gradient (transect) established through prior chemical monitoring.

METHODOLOGY

Year One

Documentation of Pesticide Application and Loading

Dimilin usage in Maryland is documented by the Maryland Department of Agriculture. This agency keeps an inventory of the amount of Dimilin used and coordinates flight paths and patterns. A detailed application record is, therefore, available which can be coordinated with runoff to the Elk, Bohemia, Sassafras, Chester, Wye and Choptank Rivers. Flow data available from the U.S. Geological Survey will be used in conjunction with field chemical analyses of Dimilin in constructing a budget for Dimilin runoff.

Field Analyses of Dimilin

Analyses of water and sediment will be made at two sites in the study area, selected on the basis of their proximity to spraying activity. One site will be in the Elk river and the other selected from the Wye, the Chester or the Choptank Rivers. One hour integrated water samples will be collected daily over a two week period following a spraying event. Daily sediment samples will be collected from the same site over this 14 day period and weekly thereafter over the next 56 days. Aquatic Dimilin concentrations will be used to estimate runoff. Daily analysis of a static water sample from the field will be used (together with sediment data) to estimate the environmental half-life of the compound. Known breakdown products such as 4-chlorophenylurea, 2,6-difluorobenzoic acid and 4-chloroaniline will also be monitored, although these may also originate from other benzoylurea pesticides (e.g. linuron) in an area impacted by Dimilin spraying they would largely be attributed to this parent compound.

Dimilin is concentrated from natural waters using c-18 solid phase extraction (SPE) after adjustment of pH and the addition of acetonitrile to the samples. After clean-up of interfering compounds using selective elution, Dimilin is detected by Reversed Phase HPLC with detection by UV spectrophotometry and Photo Diode Array. Detection limits are below 100ppt when using stable, sensitive detectors. Sediments are extracted by exhaustive sonication with acetonitrile-water mixtures, subjected to a multi-stage SPE clean-up and further analyzed by HPLC as above. Recoveries from sediments are quite matrix dependant and have been shown to range from 60-90% (Smucker, 1988) which requires that spiked samples should be analyzed at each sediment sampling site to constrain the range of expected dimilin recovery.

With the acquisition of an electrospray LC-MS interface (scheduled for mid 1994) Dimilin will also be measured by a technique currently being developed in collaboration with the National Research Council of Canada (Drs Boyd, Dawson and Smucker) which promises to provide a more sensitive and unambiguous detection system less prone to interferences with other compounds in environmental matrices. This technique will also enhance our ability to follow the production of products of Dimilin, specifically the formation of parachloraniline which has been detected by HPLC in environmental samples (Smucker,1988).

Field Bioassays

Copepods and gammarids will be entrained in containers at the field sites which will allow free water circulation and access to particulate food but will exclude predators. Similar containers will be entrained at a reference site of similar water quality, but away from Dimilin spraying activity. Copepod (E. affinis) containers will be similar to those described by Savitz et al (1993) and Hartwell et al (1993). Basically they consist of PVC tubing sealed at the lower end by Nitex mesh. For field deployment the tubes will be mounted in a floating wooden frame designed to ensure immersion at constant depth.

At the start of the assay, test containers will be inoculated with nauplii larvae filtered from a recently cultured population. After 14 days six replicate containers will be taken from each test site (and reference site) and the copepods examined for mortality, presence of egg-bearing females (number of eggs) and cuticular abnormalities.

Gammarids (L. plumulosus) will be entrained in cages set in shallow water on the sediment surface. Cages will have a fine mesh bottom to prevent escape (and denying access to predators) and will be buried to a depth of at least 1" for a period of one week prior to adding animals. Juvenile L. plumulosus taken from laboratory cultures will be used to start each assay. Animals from test and reference sites will be examined (quadruplicate containers) after 2 weeks and 4 weeks from the start of the assay. Toxic end points will be mortality, growth, numbers of females with eggs, number of eggs/female, cuticular abnormalities. Water and sediment samples at all bioassay sites will be monitored for a broad range of trace metals and organic chemicals

using atomic absorption spectroscopy and GC-MS. Organic carbon content and grainsize characteristics of sediments will also be analyzed.

Laboratory Bioassays

Amphipods

Sediment toxicity bioassays will be conducted in the laboratory under controlled conditions and using a range of Dimilin concentrations encompassing those found under field conditions. Physico-chemical characteristics of sediments will be matched to field conditions. Methodology will essentially follow that used by Schlekat et al (1992), although experiments will last 2 weeks. A range of 5 Dimilin concentrations plus controls will be used and assays will be conducted in triplicate. Dimilin will be analyzed in both sediment and biota using HPLC and LC-MS. As with field assays, survivorship and fecundity will be measured, and particular attention given to molting problems and cuticular abnormalities. A dose response will be developed for all these parameters.

Year Two

Laboratory Assays

Laboratory assays will be conducted using Eurytemora affinis to determine the effect of Dimilin adsorption to particulates on its bioavailability/toxicity. Both inorganic (kaolin) and organic (algae) particles will be studied. Savitz et al. (1993) used a 1:1 mixture of Thallasiosira fluviatilis and Isochrysis galbana for E. affinis culture. Kaolin will be used as the inorganic

particulate in view of the fact that Dimilin is normally applied as a kaolin-adsorbed powder or suspension. Two assays will be performed:

1. The toxicity of 1μg L^{-1} Dimilin will be tested as (a) pure compound, (b) pure compound plus kaolin suspension (same density ad field application), (c) pure compound plus algal density of 10^5 cells/ml, (d) pure compound plus algal density of 10^4 cells/ml, (e) kaolin only control, (f) algal control (10^5 cells/ml). Mortality of nauplii will be followed in (6) replicated containers over a 6-day period (Savitz et al 1993).
2. Toxicity of 1μg L^{-1} Dimilin will be tested as (a) pure compound, (b) pure compound plus kaolin suspension, (c) pure compound plus kaolin suspension plus algae (10^5 cells/ml), (d) kaolin only control.

Field Assay (Transect)

Initial chemical analyses will be performed in the field in order to characterize a concentration gradient for Dimilin, following a spraying event. A 3-station transect will be established down this gradient and in situ bioassays will be conducted at these sites using end-points characterized in Year One and refined in the light of laboratory experiments on both test species. Mortality, growth, fecundity and cuticular abnormalities will all be considered as end points.

Statistical Analyses

Results obtained from all field and laboratory bioassays will be analyzed using appropriate parametric or non-parametric statistics, dependent on the requirements of each data set.

Whenever feasible, analysis of variance will be used to compare sites or between treatments to detect significant differences ($p<0.05$). The 96h LC_{50} and EC_{50} values and 95% confidence limits will be determined for toxicity tests using parametric (probit) and non-parametric (Trimmed Spearman Karber) procedures.

EXPECTED RESULTS

In striving towards complex risk assessments for the Chesapeake Bay involving chemical mixtures, some toxic, some not, it seems rational to start with a toxic pesticide for which there are precisely quantified application data, and for which there is recorded evidence of biological effects at documented environmentally realistic levels. Results from this work should fulfill both of these criteria and afford us an excellent opportunity to construct a risk assessment which will (a) serve as a model for other pesticides and (b) form a basis for the construction of more complex models involving chemical mixtures.

DISSEMINATION OF RESULTS

Results will be reported to the funding agency in the form of annual and final reports as well as peer-reviewed literature. Reports will also be submitted to appropriate State Agencies in the tri-state area. At least two oral presentations are anticipated in conferences related to pest management.

RELATIONSHIP TO OTHER WORK

Results will be compatible with the models currently being developed by Kemp and co-workers describing the fate and effects of organic contaminants in the Chesapeake Bay.

This study has strong connections with the EPA-funded MEERC project currently in the developmental phase. It extends current work in this laboratory on methods development for the positive identification and quantification of modern pesticides in aquatic systems, and ongoing toxicological studies using non-target organisms. It complements the chemical monitoring effort for Dimilin currently conducted by the MD Department of Agriculture by providing an additional assessment of potential biological impacts.

PERMANENT EQUIPMENT

Funds are requested ($4,500) for the purchase of a Soutar box corer in Year 1. This sampling device facilitates the sampling of small undisturbed box cores and allows for core sectioning in horizons of a few mm (Shaw, 1989). $1,500 is also requested to construct a submersible, non-contaminating sampling pump with all Teflon supply hoses suitable for deployment from small boats with DC power supplies.

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PROJECT NUMBER: R/CBT-24

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1994	1995
Manufacture of experimental containers for laboratory and field assays	Jan-April	
Culture of experimental animals and algal food	Mar-April	
Examination of Md. Dept. of Agriculture records of Dimilin application, projected spraying patterns, selection of field/reference sites	Mar-April	
Field bioassays. Dimilin and other chemical analyses.	Apr-June	
Laboratory bioassays (Amphipods). Dimilin analyses.	June-Oct	
Collation of results, species distribution data, and preparation of annual report	Oct-Dec	
Laboratory bioassays (Copepods/ Particulates)		Mar-May
Field sampling. Dimilin analyses. Selection of Dimilin gradient/ transect. Field (transect) bioassays.		Apr-July
Preparation of final report		Aug-Dec
PROJECTED BUDGET: FEDERAL	$90,217	$89,216
PROJECTED BUDGET: MATCH		

SEA GRANT PROJECT RECORD FORM SG-SID-No.:_____
Specialist:_____
SG Class: _____

I. PROJECT SUMMARY INFORMATION

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Organic contaminant metabolite production, elimination,and bioavailability in benthic macrofauna of lower Chesapeake Bay

PROJECT NUMBER: R/CBT-25
PROJECT STATUS: 1
SUB PROGRAM: Toxics/CBEEC

REVISION DATE: 06/15/93
INITIATION DATE: 01/01/94
COMPLETION DATE: 12/31/95

PRINCIPAL INVESTIGATOR: Rebecca M. Dickhut
AFFILIATION: Virginia Institute of Marine Science
EFFORT: 0.6
AFFILIATION CODE: 5101
CO-PRINCIPAL INVESTIGATOR: Linda Schaffner
AFFILIATION: Virginia Institute of Marine Science
EFFORT: 0.6
AFFILIATION CODE: 5101

S.G. FUNDS: 0
LAST YEAR'S SG FUNDS: 0
PASS-THROUGH FUNDS: $ 104,751
STATE MATCHING FUNDS: 0
LAST YEAR'S MATCH FUNDS: 0
LAST YEAR'S PASS-THROUGH: 0
RELATED PROJECTS: All R/CBT projects
PARENT PROJECTS: R/CBT-15
SEA GRANT CLASSIFICATION: Pollution-other-toxics (45)
KEYWORDS: organic contaminants, PAH, PCB, macrofauna, metabolism

OBJECTIVES: Using Chesapeake Bay macrofauna as representative estuarine species we will (1) quantitatively evaluate the rates of uptake, transformation, metabolite binding and elimination of a series of representative organic contaminants; (2) examine the rates of uptake and metabolism of representative organic contaminants as a function of food source material; and (3) determine the fate of organic contaminant metabolites produced for a series of representative organic contaminant parent compounds.

METHODOLOGY: A microcosm approach will be used to evaluate uptake, transformation, binding and elimination processes in selected estuarine benthic macrofauna as a function of food source and contaminant properties. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls will be selected for study based on their hydrophobicity, anticipated difference in uptake, transformation, and elimination rates. Microcosms prepared with various combinations of sediment or sterile, solvent extracted fine sand substrate, presence of absence of benthic macrofauna and contaminated food source material will be used to resolve microbial versus macrofauna release of metabolites from the sediment into the water column. Elimination rate experiments will be used to assess rates of contaminant elimination from body tissues.

RATIONALE: The proposed research emphasizes quantification of the rates of organic contaminant uptake, and subsequent metabolite production, binding and elimination by representative benthic organisms. Our ongoing research (R/CBT-15) indicates that metabolite production and subsequent cycling is a potentially important process governing contaminant transfer and fate at the sediment-water interface. The biogeochemical cycling of metabolites is virtually unknown but has major implications for a variety of ecosystem processes including trophic transfer. This effort will allow determination of the disposition of organic contaminant parent compound and metabolites within the benthic region and delineation of associated risks after initial exposure of the benthos to organic pollutants. The proposed research is highly relevant to understanding the bioavailability of organic contaminants from the benthos to demersal predators.

**** SEA GRANT BUDGET ****

GRANTEE:
Virginia Graduate Marine Science Consortium

PROJECT NO: R/CBT-25

PROJECT TITLE:
Organic contaminant metabolite production, elimination, and bioavailability in benthic macrofauna of lower Chesapeake Bay.

PROJECT STATUS: 1

PRINCIPAL INVESTIGATORS:
R. M. Dickhut and L. C. Schaffner

DURATION: 1/1/94-12/31/94

A. SALARIES AND WAGES	No. of Person.	Months	Sea Grant Funds	Grantee Funds
1. Senior Personnel				
a. Prin. Investigator	2	1.20	8000	0
b. Associates:			0	0
Sub Total:			8000	0
2. Other Personnel				
a. Professionals			0	0
b. Research Assoc.			0	0
c. RA Grad. Stud.	2	12.0	27400	0
d. Prof. School Stud.			0	0
e. Pre-Bac. Stud.			0	0
f. Secret./Clerical			0	0
g. Technical/Shop	1	4.0	7666	0
h. Hourly Labor	1	3.0	2880	0
Total Salaries and Wages			45946	0
B. FRINGE BENEFITS			4450	0
Total Sal. Wages & Fringe Benefits (A+B)			50396	0
C. PERMANENT EQUIPMENT			21500	0
D. EXPENDABLE SUPPLIES			11500	0
E. TRAVEL				
1. Domestic - US & Possessions	1. 2800		700	0
2. International	2. 0		0	0
Total Travel			700	0
F. PUBLICATION AND DOCUMENTATION COSTS			0	0
G. OTHER COSTS				
1. Vessel rental			500	
2. Tuition			1400	
3. Gas cylinder rental			144	
4. Chemical/solvent waste disposal			450	
5. Photocopying			300	
6. Graphic Arts			250	
7.				
8.				
9.				
10.				
Total Other Costs			3044	0
TOTAL DIRECT COSTS (A through G)			87140	0
INDIRECT COSTS: On Campus:(5% of A2c plus 45% of A1a-b,A2a-b,d-h,B,D,E,F,G3-5)			17611	0
TOTAL INDIRECT COSTS			17611	0
TOTAL COSTS			104751	0

ORGANIC CONTAMINANT METABOLITE PRODUCTION, ELIMINATION, AND BIOAVAILABILITY IN BENTHIC MACROFAUNA OF LOWER CHESAPEAKE BAY

Rebecca M. Dickhut, Assistant Professor of Marine Science, Dept. of Physical Sciences, School of Marine Science, The College of William and Mary, Virginia Institute of Marine Science

Linda C. Schaffner, Assistant Professor of Marine Science, Dept. of Biological Sciences, School of Marine Science, The College of William and Mary, Virginia Institute of Marine Science

INTRODUCTION

Benthic macrofauna are known to influence the fate and transport of sediment-associated contaminants via a variety of mechanisms. Karickhoff and Morris (1985) noted that tubificid oligochaetes cause increased transport of sorbed contaminants to the sediment surface through bioturbation. More recently, McElroy et al. (1990) noted an increased flux of a polycylic aromatic hydrocarbon (benz[a]anthracene) from sediment and increased rates of microbial mineralization of benz[a]anthracene to CO_2 in the presence of a tubiculous polychaete. In ongoing research in our laboratory (Schaffner and Dickhut, 1991) we have observed both increased loss of sediment-associated organic contaminants from surface sediment and increased subduction of contaminants deep into the sediment column via the activities of intact benthic communities from lower Cheasapeake Bay.

In addition to demonstrating the role of benthic biota in the physical transport of sediment sorbed contaminants, our work (Schaffner and Dickhut, 1991) and that of others (e.g. McElroy 1985 and 1990) illustrate the strong capability of a variety of benthic macrofauna to biotransform organic contaminants, in particular, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Specifically, our studies indicate that a variety of benthic fauna from lower Chesapeake Bay rapidly (i.e. within 3 days of initial exposure) exhibit detectable levels (i.e. > 3 times background) of aqueous soluble metabolites of selected PAHs (pyrene and benzo[a]pyrene) and PCBs (2-chlorobiphenyl and 2,2',5,5'-tetrachlorobiphenyl) (Table 1). The contribution of metabolites to the total contaminant body burden measured varies between organisms and with the nature of the organic pollutant. Nonetheless, PAH metabolite body burdens in *Paraprionospio pinnata*, an important benthic food resource for demersal fish predators in Chesapeake Bay, ranged from 30-40% of the total measured PAH body burden by day 3 of organic contaminant exposure increasing to between 60-90% after 21 days of exposure (Figure 1). PAH metabolite body burdens in *Loimia medusa*, a functionally important benthic species in Chesapeake Bay, were similarly large as were PCB metabolite body burdens (Figure 2). However, a slower increase in 2,2',5,5'-tetrachlorobiphenyl metabolites and rapid loss of 2-chlorobiphenyl metabolites were observed in this organism (Figure 2).

McElroy (1985, 1990) has shown that the polychaete *Nereis virens* rapidly metabolizes benz[a]anthracene with corresponding accumulation of conjugated (water soluble) and nonextractable (bound) metabolites. In McElroy's studies, the bound benz[a]anthracene metabolite fraction generally contributed as much as the water soluble metabolite fraction to the total *Nereis* body burden of contaminant. This information indicates that metabolite body burdens in Chesapeake Bay benthic macrofauna upon exposure to organic contaminants will likely be even greater than measured in our study. Moreover, these studies show that large fractions of organic contaminants in benthic macrofauna exist as metabolic products and little is

Table 1. Presence/Absence of Organic Contaminant Parent Compound and Aqueous Soluble Metabolites in Chesapeake Bay Benthic Macrofauna after 3 Days of Exposure to Contaminanted Sediments*

Species	2-chlorobiphenyl		Pyrene		2,2',5,5'-TCB		Benzo[a]pyrene	
	Parent	Metab.	Parent	Metab.	Parent	Metab.	Parent	Metab.
Acteocina canaliculata	-	-	+	-				
Balanoglossus balanoglossus	+	-	+	+	+	-	+	+
Bhawania heteroseta	-	-	+	-	-	-	+	-
Edwardsia elegans	-	-	+	-				
Loimia medusa	+	+	+	+				
Microphiopholus atra	-	-	+	-				
Macroclymene zonalis	+	-	+	+	+	-	+	-
Macoma tenta					+	-	+	+
Nemertinea	-	-	+	+	+	-	+	-
Nephyts spp.	+	-	+	+	+	-	+	+
Nereis sp.					+	-	-	-
Pectinaria gouldii	-	-	-	-				
Phoronida	-	-	+	-	-	-	-	-
Paraprionospio pinnata	+	+	+	+	+	-	+	+
Pseudeurythoe paucibranchiata	+	-	+	+	+	-	+	+
Sigambra tentaculata	-	-	+	-	+	-	+	+
Turbonilla interrupta	-	-	+	-				
Yoldia limatula	+	-	+	+				

* + indicates presence, - indicates absence, blanks indicate no organisms available for sampling

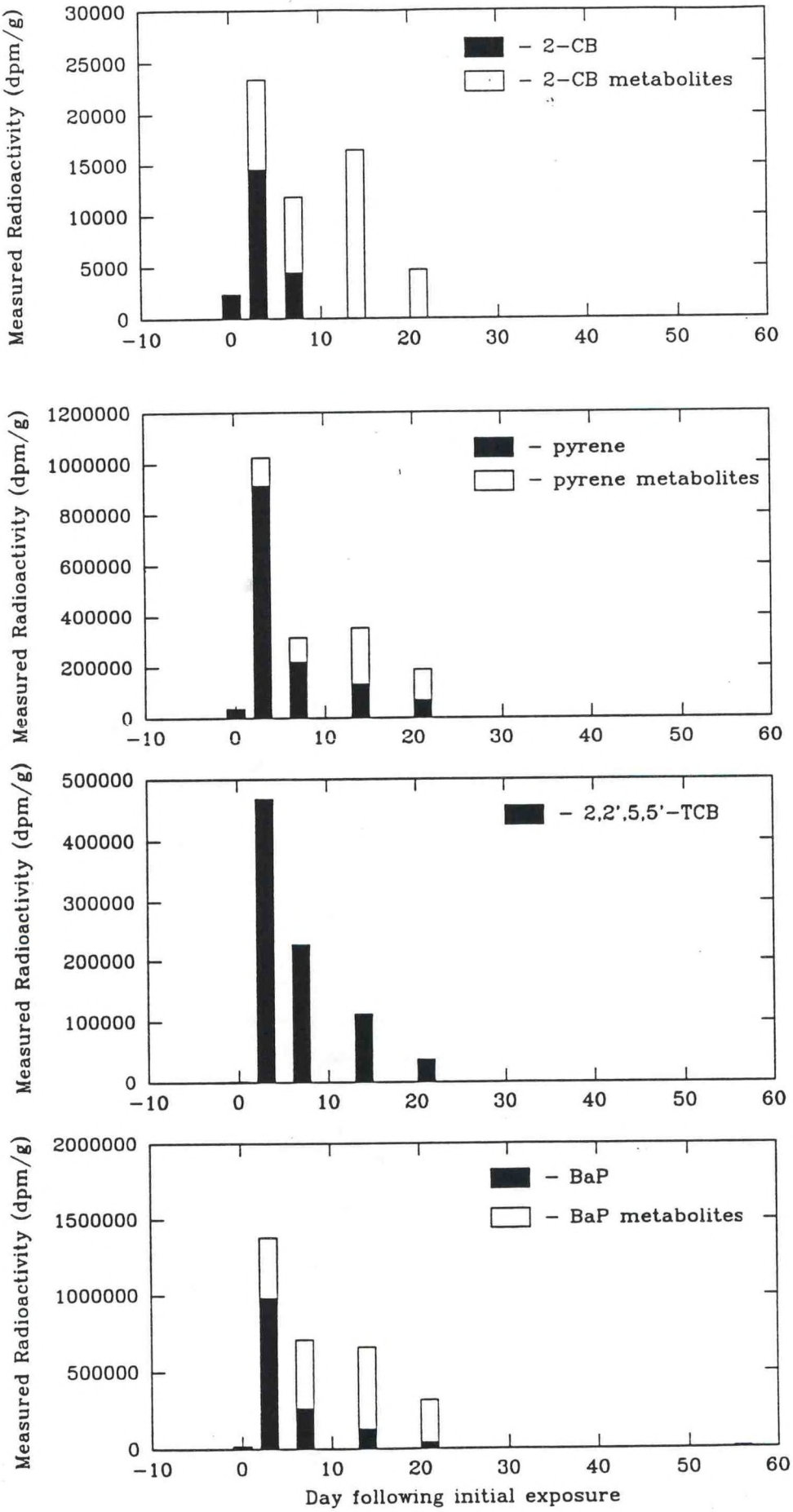

Figure 1. Measured organic extractable parent compound (plus metabolites) and water soluble metabolites in *Paraprionospio pinnata* after exposure to organic contaminant spiked sediments.

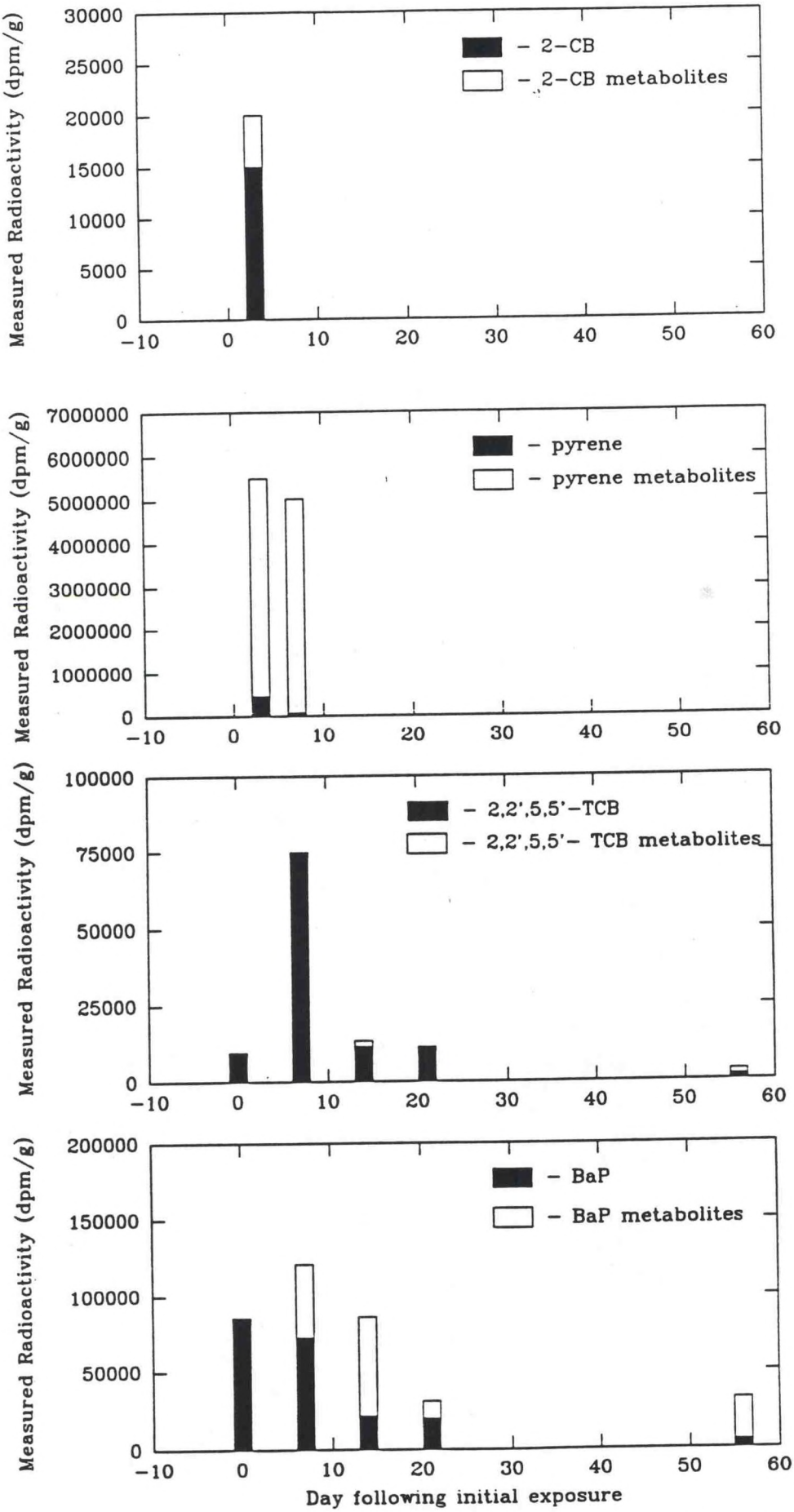

Figure 2. Measured organic extractable parent compound (plus metabolites) and water soluble metabolites in *Loimia medusa* after exposure to organic contaminant spiked sediments.

known about the bioavailability and effects of these substances.

Metabolites resulting from benthic macrofauna biotransformation of organic contaminants in sediments can potentially adversely effect aquatic organisms through a variety of processes:

a. metabolites may have direct effects on benthic macrofauna,

b. metabolites produced by benthic macrofauna may be released from the organisms followed by transport and uptake by aquatic organisms not involved in the initial transformation with subsequent effects produced in the secondary animal,

c. metabolites generated by benthic macrofauna may potentially be transfered through trophic interactions with subsequent effects on the consumer.

Thus, to fully understand the effects of organic contaminants on benthic organisms and the influence of benthic biota in the transfer of toxic substances to other aquatic species, it will be necessary to quantitatively evaluate the production, binding and elimination of organic contaminant metabolites in benthic macrofauna.

Evidence suggests that the major potential for deletorious direct effects of organic contaminants on benthic organisms may result as a consequence of a rise in mutagenic potential after metabolic activation to reactive intermediates (Sims and Grover, 1974; Jerina and Daley, 1974). Reactive metabolites can bind to cellular macromolecules and interfer with DNA, RNA or enzyme function. Consequently, if metabolites are not excreted, direct toxic effects on the metabolizing organism are possible.

A toxicological risk may also exist due to metabolites excreted from benthic macrofauna. Our data indicates that water soluble metabolites of a relatively hydrophilic organic pollutant (2-chlorobiphenyl) may be rapidly excreted from an abundant benthic organism (*Loimia medusa*) in lower Chesapeake Bay

(Figure 2). Further, McElroy et al. (1990) suggest that excretion of water soluble metabolites by worms could augment flux of PAH from sediment. However, to date, no extensive research on this potential fate and transport mechanism for organic contaminant metabolites produced by benthic fauna can be found in the literature. Quantification of elimination rates of water soluble metabolites from benthic organisms is required to discern resultant exposure levels of biologically transformed chemical species at the sediment/water interface.

Trophic transfer of organic contaminant metabolites between benthic marine organisms has been examined recently by McElroy and coworkers (1989 and 1991). Their results demonstrate that PAH metabolites associated with the benthic polychaete *Nereis virens* fed to bottom-feeding fish (winter flounder - *Pseudopleuronectes americanus*) can be accumulated through the diet and further metabolized by the consumer organism to form DNA adducts. Also, the bioavailability of the PAH metabolites can differ from that of the parent compound. Therefore, PAH metabolite burdens in benthic organisms that serve as prey for higher trophic level species should be considered when assessing the ecological risk of contaminants. The resultant impact of diet on the higher trophic level organism will be dependent on the form of the contaminant which was ingested.

It is evident from our data (e.g. Figures 1 & 2) that large fractions of organic contaminant metabolites are rapidly produced in benthic macrofauna from lower Chesapeake Bay. Additionally, potential effects of organic contaminant metabolite production in benthic organisims are apparent as outlined above. Therefore, the rates of organic contaminant uptake, and production, binding and elimination of organic contaminant metabolites by benthic macrofauna should be determined in order to resolve the relative amounts of parent toxicant and metabolites available to cause biological effects.

Relevance to the Problem

The proposed research emphasizes quantification of the rates of organic contaminant uptake, and subsequent metabolite production, binding and elimination by selected benthic macrofauna from lower Chesapeake Bay. Our ongoing research indicates that metabolite production and subsequent cycling is a potentially important process governing contaminant transfer and fate at the sediment-water interface. The biogeochemical cycling of metabolites is virtually unknown (McElroy et al., 1990) but has major implications for a variety of ecosystem processes including trophic transfer. This effort will allow determination of the disposition of organic contaminant parent compound and metabolites within the benthic region and deliniation of associated risks after initial exposure of the benthos to organic pollutants. The proposed research is highly relevant to understanding the bioavailability, including trophic transfer capabilities of organic contaminants from the benthos to demersal predators.

OBJECTIVES AND HYPOTHESIS

Hypothesis

The primary hypothesis of the proposed study is that sediment-associated organic contaminants are rapidly metabolized in ecologically important benthic macrofauna of Chesapeake Bay with production, binding and elimination rates of the resultant metabolites dependent upon the characteristics of the chemical contaminants, benthic macrofauna, and contaminant source material.

Overall Objectives

Using Chesapeake Bay macrofauna as representative estuarine species we will:

[1] quantitatively evaluate the rates of uptake, transformation, binding and elimination of a series of representative organic contaminants;

[2] examine the rates of uptake and metabolism of representative organic contaminants as a function of food source material;

[3] determine the fate of organic contaminant metabolites produced for a series of representative organic contaminant parent compounds.

1994 Objectives

[1] Develop analytical protocol to quantify organic contaminant parent compounds, polar and conjugated metabolite fractions, and nonextractable, bound organic contaminant fractions in benthic organisms.

[2] Initiate microcosm studies to evaluate organic contaminant uptake, metabolite production, binding and egestion in selected benthic macrofauna from lower Chesapeake Bay.

[3] Initiate organic contaminant tissue metabolite elimination rate studies.

1995 Objectives

[1] Complete microcosm and elimination rate studies.

[2] Conclude data analysis and prepare manuscripts for publication.

METHODOLOGY

Toxic Organic Chemicals

Polycyclic aromatic hydrocarbons (PAHs - e.g. pyrene, benzo[a]pyrene) and polychlorinated biphenyls (PCBs - e.g. 2-chlorobiphenyl and 2,2',5,5'-tetrachlorobiphenyl) will be selected for study based on their hydrophobicity (octanol/water partition coefficient - K_{ow}), anticipated difference in uptake, transformation and elimination rates, and availability as ^{3}H and/or ^{14}C radiolabeled compounds. PAHs and PCBs represent trace organic pollutants with a variety of sources and physical-chemical properties. PAHs and PCBs tend to be hydrophobic, and hence, associate with sediments in aquatic systems and have the ability to bioaccumulate. Many individual PAHs and PCBs are known to be toxic. Several CBEEC sponsored studies of individual components from these different classes of toxic organic contaminants are ongoing; research on PAHs and PCBs will provide information on pollutant behavior which encompasses numerous related chemical species.

Microcosm Exposure Experiments

Each experiment will consist of benthic microcosms prepared using defaunated York River sediment or sterile, organic solvent-extracted sand substrate with acclimated individual species of benthic macrofauna from lower Chesapeake Bay. Benthic macrofauna will be selected for study based on ecological importance, abundance, and demonstrated ability to metabolize organic contaminants. An appropriate number of microcosms to insure a sufficient number of replicates per chemical treatment, controls (i.e. no macrafauna) and a blank (i.e. no chemicals) per sampling time will be used. Randomly selected microcosms will be treated with food source material (see below) containing radiolabeled organic

contaminants. Four to six sampling times between 0 and 60 days will be utilized to evaluate rates of organic contaminant uptake, transformation, and metabolite binding in the selected benthic macrofauna.

Microcosms prepared with various combinations of sediment or sand substrate, presence or absence of surface-deposit feeding benthic macrofauna, and contaminated food source material will be used to resolve microbial versus macrofauna release of metabolites from the sediment into the water column. Using sand substrate, benthic macrofauna and contaminated food, rates of macrofauna excretion of organic contaminants and metabolites will be assessed. Similarly, sediment controls will be used to evalute microbial release of organic chemicals and degradation products, while sediment-worm-contaminant systems will be used to assess total liberation of contaminants including any enhanced or interactive release of degradation products in the presence of both worms and microbes (see McElroy et al., 1990). Experiments will be conducted under controlled laboratory conditions using short-term static systems followed by use of flow-through conditions as required for long-term experiments.

At each sampling time an appropriate number of replicate samples will be collected to assess sampling and analytical variance. Samples will be analyzed immediately or stored in a freezer (-20°C) until processing. Samples to be collected from each microcosm at each sampling time will include: (1) sediment and pore water from two composite cores from each microcosm, (2) benthic macrofauna species, (3) benthic macrofauna tube material, (4) fecal pellets/mounds from benthic macrofauna species. Overlying water will be sampled at selected time intervals.

Elimination Rate Experiments

Elimination rates of organic contaminants and metaboilites from selected species will be determined.

Animals removed from microcosms at selected sampling times will be placed into clean sediment and elimination will be followed for ca. 30 days following the method outlined in Landrum (1988). At time 0, sediments remaining in organism guts will be allowed to evacuate for 6 hours and then remaining material will be removed by dissection.

Organic Contaminant Source Materials

Contaminated food source materials for benthic macrofauna will be prepared using both relatively refractory (i.e. surficial sediments) and highly labile (i.e. phytoplankton) organic carbon reservoirs. These substances represent typical food source materials available to benthic macrofauna. It is proposed that the efficiency of uptake, and consequently, production, binding and elimination of contaminant metabolites in benthic macrofauna will be dependent upon the refractory nature of the source material. Consequently, the influence of contaminant source material will be evaluated through comparison of two widely diverse, but ecologically relevant, carbon sources.

Surficial sediment will be collected from the York River, organisms removed via sieving, and homogenized. Phytoplankton suspensions will be otained from in house cultures. Radiolabeled organic contaminants will be added to the sediment/phytoplankton by mixing aqueous slurries with organic contaminant spikes (in methanol) in glass jars. The aqueous slurries will be held at constant polyhaline salinity (i.e. 18-25 ppt) approximately equal to the salinity for the study site and microcosm experiments. The organic contaminant spikes will be added to the system such that initial contaminant concentrations do not exceed one half the aqueous solubility for the chemical. The sediment/phytoplankton concentration and amount of radiolabeled compound utilized will be adjusted in order to obtain appropriate trace level food source material spikes (see Landrum, 1989 and Weston, 1990). All preparative and analytical

procedures will be performed under gold fluorescent lights to avoid PAH photodegradation.

Sampling Procedures

On each sampling date, replicate sample, control, and blank microcosms as defined above will be sacrificed. The water overlying each microcosm will be siphoned off and surface sediments subsampled for grain size, water content and organic carbon, and radiolabeled organic chemical analyses. Samples for water/organic carbon content and radiolabeled organic chemical analyses will be centrifuged to separate sediment and pore water. Particulate organic carbon will be determined using a Carlo-Erba CHN analyzer following the procedure outlined in Hedges and Stern (1984). Extraction and analysis of radiolabeled organic chemicals will be done as described below.

After sediment/pore water sampling, one side each microcosm will be removed and organism or structure samples will be collected by dissection or sieving. Organisms will allowed to evacuate their guts prior to sacrifice. Samples will be analyzed for radiolabeled organic parent compound and metabolite activities or lipid content (Gardner et al., 1985). Samples of tubes and fecal pellets or mounds will be collected and treated as for sediment samples.

Radiolabeled Organic Contaminant Parent Compound and Metabolite Analyses

Radiolabeled organic chemical analyses will be performed using the methods described by McElroy (1991). Four major classes of compounds are considered: [1] parent compound, [2] primary metabolites which are more polar than the parent compound but still extractable with organic solvents, [3] secondary, conjugated metabolites which are water soluble, and [4] bound metabolites which are resistent to

extraction. Briefly, organic contaminants and degradation products are extracted using a combination of water, methanol and chloroform, followed by removal of sample residue via filtration or centrifugation and reextraction of the sample with chloroform. Residual tissue is then dryed and combusted at 1000°C with evolution of bound radioactivity (e.g. as $^{14}CO_2$) which is trapped in phenethylamine and subsequently quantified. The extracts are combined and fractionated into organic and aqueous soluble components by the addition of water, with subsequent radioactivities determined via a combination of high performance liquid chromatography (HPLC) and liquid scintillation counting (LSC). Parent compounds and polar metabolites associated with the organic fraction are resolved using HPLC. Polar metabolites elute prior to parent compounds on a reversed phase column which allows for separation and fraction collection for LSC analysis. Evaluation of radioactivity in the aqueous fraction results in quantifacation of the secondary, conjugated metabolites. Radiolabeled organic chemical activity will be measured for the extract fractions using a Beckman Model LS 5000TD liquid scintillation system. Additionally, a Beckman Model LS-150 liquid scintillation system is also available for analyses.

Background Chemical Analysis

Background PAH and PCB concentrations in ambient sediments and organisms will be measured utilizing a combination of gas chromatography/mass spectrometry and gas chromatography/electron capture analysis. Briefly, samples are freeze dried and subsequently solvent extracted for 48 hr with dichloromethane using a soxhlet apparatus. Clean-up of sediment samples is achieved by gel permeation chromatography for removal of organic polymers and liquid chromatography for separation of aliphatics and polar compounds (e.g. Bieri et al., 1986). The extract is then concentrated using rotoevaporation, followed by evaporation under purified nitrogen and solvent exchange into hexane.

PAHs will be identified and quantified using a combination of gas chromatography (GC) and mass spectrometry (MS). Subsequent to PAH analysis, the remaining extract, is fractionated by solid-liquid chromatography using a Florisil column (1.25% water deactivated) eluted with hexane followed by 10% (v/v) diethylether in hexane to separate PCBs and interfering compounds. PCBs are analyzed by high resolution GC with electron capture detection.

Data Analyses

The data from this study, will be used to compare organic contaminant uptake, metabolite formation, elimination and binding rates for a series of organic contaminants ranging in hydrophobicity (e.g. log K_{ow}'s between 4 and 7) in selected benthic organisms from Chesapeake Bay. Each process will be examined for relationships to species and chemical contaminant characteristics. Quantitative information on organic contaminant and metaboite pools in benthic organisms, as well as rates of generation of these fractions, will be determined.

EXPECTED RESULTS

Data from the proposed research will provide insight regarding the importance of benthic macrofauna on influencing organic toxicant bioavailability and effects. The ability of ecologically important macrobenthic organisms to transform organic pollutants and eliminate or bind metabolites will be determined. Rates at which benthic biota produce, sequester or excrete organic contaminant metabolites will be quantified. This will allow for evaluation/inclusion of organic contaminant metabolite influence in risk assessment calculations, in particular, with respect to designating contaminant exposure levels in the benthos and estuarine food webs.

Our ongoing work strongly suggests that metabolite formation in benthic organisms is potentially a major process influencing organic contaminant transport and fate, particularly with respect to bioavailability and exposure levels of parent compounds and metabolites. Adequate modelling of organic contaminant fate and effects in the Chesapeake Bay ecosystem will depend on an understanding of rates of metabolite formation and exchange between benthic organisms and other ecosystem components.

DISSEMINATION OF RESULTS

The results of this work will be of interest to those involved in making management decisions for the Chesapeake Bay and to the scientific community in general. Results will be distributed to appropriate agencies and individuals as advised by the local Sea Grant office. Results will also be presented at national scientific meetings (e.g. Society of Environmental Toxicology and Chemistry and Estuarine Research Foundation). Manuscripts will be prepared for publication in the appropriate peer-reviewed scientific journals.

RELATIONSHIPS TO OTHER WORK

Our project will complement ongoing CBEEC funded studies, in particular, those of Capone et al., Newell et al,, Chu and Hale, and should augment the modeling work of Kemp et al. This proposal is an extension of our previous work examining the role of benthic communities in sediment-associated toxic organic chemical fate and transport in lower Chesapeake Bay. The proposed research will further our understanding of biologically-mediated processes and aid in developing our understanding of organic contaminant effects on benthic biota and the influence of benthic macrofauna on organic contaminant bioavailability and effects on other aquatic organisms. Information obtained from this study will have

general applicability in other systems where biological mediation of benthic processes is important.

PERMANENT EQUIPMENT

Permanant equipment requested for this project include: an automated biological oxidizer ($12,000) for tissue combustion and analysis bound organic contaminant metabolites, an Isco model 2360 HPLC gradient programer ($3725) for solvent gradient programming required for separation of polar metabolites from parent compounds, an incubator ($2400) to maintain phytoplankton cultures exposed to radioactive organic contaminants, a drying oven ($1100) to dry samples for water content and prior to combustion analysis, and a YSI oxygen meter ($1700) for evaluation of O_2 in microcosms.

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PROJECT NUMBER: R/CBT-25

PROJECTED TIME SCHEDULE

PROJECT ACTIVITY	YEARS 1994	1995
Develop analytical protocol for analysis of radiolabeled organic parent compound and metabolites.	———	
Perform microcosm studies examining organic contaminant uptake and metabolite formation, binding and elimination in benthic macrofauna from lower Chesapeake Bay.		———
Evaluate data and prepare manuscripts for publication.		———
PROJECTED BUDGET: FEDERAL	$104,751	$90,425
MATCH		

SEA GRANT PROJECT RECORD FORM
I. PROJECT SUMMARY INFORMATION INPUT

SG-SID-No.:_____
Specialist:_____
SG Class: _____

INSTITUTION: Virginia Graduate Marine Science Consortium
ICODE: 5100
TITLE: Chesapeake Bay Toxics Research Program:
New Project Initiatives

PROJECT NUMBER: M/PD-CBT-1 **REVISION DATE:** 07/01/93
PROJECT STATUS: 2 **INITIATION DATE:** 09/01/88
SUB PROGRAM: Program Admin. **COMPLETION DATE:** 12/31/94
PRINCIPAL INVESTIGATOR: William L. Rickards **EFFORT:** as needed
AFFILIATION: Virginia Graduate Marine Science Consortium
AFFILIATION CODE: 5100
CO-PRINCIPAL INVESTIGATOR: Christopher D'Elia **EFFORT:** as needed
AFFILIATION: Maryland Sea Grant College Program
AFFILIATION CODE:
S.G. FUNDS: $197,462 **MATCHING FUNDS:** 000
LAST YEAR'S SG FUNDS: $64,812 **LAST YEAR'S MATCH FUNDS:** 000
PASS-THROUGH FUNDS: 000 **LAST YEAR'S PASS-THROUGH:**000
RELATED PROJECTS: all
PARENT PROJECTS:
SEA GRANT CLASSIFICATION: Program Development (81)
KEYWORDS: DEVELOPMENT

OBJECTIVES: To provide the capability to develop new studies related to the impacts of ecological processes and water quality conditions upon the living resources of the Chesapeake Bay. Previous CBEEC research has focused on the study of Bay hypoxia, however current CBEEC research is being focused on the role of toxics and their effects on ecological processes in Chesapeake Bay.

METHODOLOGY: Proposals for studies related to the impacts of environmental conditions upon organisms and/or populations will be received and evaluated for submission to the National Sea Grant College Program. The Directors of both the Virginia and Maryland Sea Grant College Programs will participate in the evaluation process.

RATIONALE: Studies of the transport, fate and effects of toxic contaminants on the biota of the Bay, and on the relevant ecosystem-level processes and pathways, are necessary for determining how these impact the populations of organisms of commercial and ecological importance in the Chesapeake Bay.

ACCOMPLISHMENTS: To be updated.

BENEFITS: To be updated.

NOAA FORM 90-4 (ADAPTED) NOAA, U.S. DEPT OF COMMERCE

SEA GRANT BUDGET

GRANTEE:
Virginia Graduate Marine Science Consortium

GRANT/PRO. NO:
M/PD-CBT-1

PRINCIPAL INVESTIGATORS:
W.L. Rickards and C.F. D'Elia

DURATION:
1/1-12/31/94

A. Salaries and Wages 1. Senior Personnel	No.	Man-Mo.	Sea Grant Funds	Grantee Funds
a. Prin. Investigator	3		20000	
b. Associates:			0	
Sub Total:			20000	0
2. Other Personnel				
a. Professionals				
b. Research Assoc.				
c. RA Grad. Stud.	2		25000	
d. Prof. School Stud.				
e. Pre-Bac. Stud.				
f. Secret./Clerical				
g. Technical/Shop				
h. Hourly Labor				
Total Salaries and Wages			45000	0
B. Fringe Benefits			5400	0
Total Sal. Wages & Fringe Benefits			50400	0
C. Permanent Equipment			0	0
D. Expendable Supplies			3988	0
E. Travel				
1. Domestic - US & Possessions		1.	5948	
2. International		2.		
Total Travel			5948	0
F. Pub. and Documentation Costs			4000	0
G. Other Costs				
1. Workshops, conferences			7126	
2. Telephone, postage			1000	
3. CBEEC staff assistance			25000	
4. two projects under revision			100000	
5.				
6.				
7.				
8.				
9.				
10.				
Total Other Costs			133126	0
TOTAL DIRECT COSTS (A through G)			197462	0
Indirect Costs: On Campus: *see below			0	
Off Campus:				
Total Indirect Costs			0	0
TOTAL COSTS			197462	0

* indirect costs included here as direct costs

CHESAPEAKE BAY TOXICS RESEARCH PROGRAM: NEW PROJECT INITIATIVES

William L. Rickards, Director
Virginia Sea Grant College Program

Chris D'Elia, Director
Maryland Sea Grant College Program

BACKGROUND

Each year since initiation of the Toxics Research Program, funds have been set aside under a "New Project Initiatives" project to support the initiation and/or development of research projects, workshops or other efforts related to ongoing studies which are relevant to the Program's emphasis on the effects of toxics upon the Chesapeake Bay ecosystem.

As the Toxics Research Program matures, the Environmental Effects Committee continues the process of developing research which will provide the management agencies and Bay Program Executive Committee with information needed to make decisions regarding restoration efforts in the Bay. Having the capacity for relatively quick response to emerging resource problems or the need for educational efforts related to the environmental effects program continues to be a worthwhile and increasingly integral part of the overall Toxics Research Program.

PROGRESS TO DATE

As of this date, program development funds have been used to: 1) initiate an ecosystem modeling project which includes the effects of toxics upon functions within the system; 2) conduct the annual workshops involving all Toxics Research Program investigators, state and federal management agency personnel, and interested academic researchers; and 3) provide budget supplements for two projects (R/CBT-2 and 3) which needed additional funds in order to complete the proposed work plans.

FUTURE DIRECTIONS

Since the initial Congressional appropriation, the Chesapeake Bay Environmental Effects Studies program has followed a progression from investigating the processes and mechanisms responsible for the creation and maintenance of the low dissolved oxygen phenomenon, to investigating the effects of the low dissolved oxygen conditions on the biota in the Bay. For the past three years, emphasis has been placed on the transport, fate and effects of toxics upon the Bay ecosystem.

Throughout this program's duration, efforts have been made to disseminate and distribute the information gained to various audiences for their use. Such activity will continue to be supported during the coming year. For example, a document which summarizes the second year progress of the research projects is nearing completion. Project development funds will be used to help support the final production of this publication as well as the document expected from the third workshop which will be held during 1994.

As the present research package was reviewed and assembled, two proposals were judged to be worthy of funding if the work plans were amended in response to comments from the reviewers. We anticipate using approximately $100,000 of the funds set aside for New Project Initiatives to be used for these two projects once they are revised to the satisfaction of the Environmental Effects Committee. Full copies of the revised proposals will be processed through the National Office of Sea Grant for final approval before funds are awarded to the investigators.

It has become increasingly evident over the past two years, that the Environmental Effects Committee needs assistance and input from a person who is scientifically credible, well-versed in matters related to toxics research, and familiar with the Bay Program's management structure. This assistance is especially critical during the preparation of the Request for Proposals as well as during the year when technical interaction occurs with such groups as the Toxics Subcommittee and/or the research community. We intend to allocate approximately $25,000 during 1994 in order to cover the costs associated with acquiring such assistance for CBEEC. A proposal for this activity will require final approval from the National Office of Sea Grant before a final agreement is reached with the person to be involved.

The balance of the New Project Initiatives funding will be used to develop additional new projects related to the overall goals of the Toxics Research Program. As with other initiatives, approval of the National Office of Sea Grant will be sought, as required, prior to allocation of the funds.

APPROACH

Access to the new project initiation funds will be possible for all researchers in Virginia and Maryland. In fact, the CBEEC encourages the development of inter-institutional, interstate or regional investigations.

We anticipate that specific proposals will be developed and submitted to the local Sea Grant College Programs for initial evaluation. Following this evaluation, the proposals would be submitted to the National Sea Grant College Program for final review and evaluation. Proposals submitted could either be fully developed research activities or "seed" efforts which would be more fully developed through subsequent funding. Successful

projects would be incorporated into the Chesapeake Bay Toxics Research Program, and they would be monitored accordingly for research and fiscal accountability.

Approval of this funding request will permit the development of research which the NOAA Chesapeake Bay Environmental Effects Committee has encouraged for studies in the Chesapeake Bay and will be applicable to issues identified as important by the Chesapeake Bay Stock Assessment Committee, the Chesapeake Bay Program's Research Priorities Work Group, and the Bay Program's Toxics Subcommittee.

Schedule of Indirect Costs

Maryland

University of Maryland College Park on Campus research	46%
Benedict Estuarine Research Laboratory On Campus Research	60.21%
Maryland Biotechnology Institute COMB on Campus Research	46%
University of Maryland CEES on Campus Research	39%

Virginia

Virginia Institute of Marine Science, College of William and Mary On-campus Research	45.0%

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Curriculum Vitae

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EDUCATION

B.S., Drexel University, Philadelphia, PA, 1961
M.S., Hahnemann Medical University, Philadelphia, PA, 1968
Ph.D., University of Delaware, Newark, DE, 1971

EXPERIENCE

1970-1973, Postdoctoral Fellow, Department of Pathology, University of Minnesota

1973-1982, Laboratory Head, Sloan-Kettering Institute of Cancer Research, Rye, NY.

1975-1982, Assistant Professor, Cornell University Graduate School of Medical Sciences, New York, NY.

1982-1986, Research Biologist/Immunologist, Biotechnology Division, U.S. Army Aberdeen Proving Ground, MD.

1986-present, Professor, Chesapeake Biological Laboratory, University of Maryland System, CEES, Solomons, MD.

RESEARCH INTERESTS

Comparative immunology and pathology. Effects of environmental pollutants on immunocompetency of aquatic invertebrates and fish. Comparative hematology, with emphasis on functional studies of phagocytic cells and their role in controlling infectious diseases. Neoplastic disease of aquatic invertebrates and the biotransformation of environmental carcinogens.

PROFESSIONAL ACTIVITIES

Member of American Association of Immunologists, American Society of Zoologists (Program Officer, 1981-3; Public Relations Committee, 1991-3), International Society of Developmental and Comparative Immunology, Society for Invertebrate Pathology (Chair, Membership Committee, 1986-88; Secretary, 1988-90, Trustee 1992-95), Society of Sigma Xi, Society of Toxicology, and National Shellfisheries Association.

Member of NIH Tropical Medicine and Parasitology Study Section (AHR), 1985-present

Editorial Board Membership: Journal of Invertebrate pathology (1976-83; 1987-90), Journal of Developmental and Comparative Immunology (1977-83), Reviews in Aquatic Sciences (1986-93), and Reviews in Fisheries Science (1992-present).

PUBLICATIONS (Relevant to this proposal; >70 total publications to date):

1. Anderson, R.S. 1987. Immunocompetence in invertebrates. pp. 93-110 In: C.S. Giam and L.E. Ray (eds.) Pollutant Studies in Marine Animals. CRC Press, Inc., Boca Raton, FL.

2. Anderson, R.S. 1988. Effects of anthropogenic agents on bivalve cellular and humoral defense mechanisms. In: W.S. Fisher (ed.) Disease Processes in Marine Bivalve Molluscs, American Fisheries Society. Special Publication 18:238-242.

3. Anderson, R.S., L.M. Oliver, and D. Jacobs. 1992. Immunotoxicity of cadmium for the Eastern oyster (Crassostrea virginica [Gmelin, 1791]): effects on hemocyte chemiluminescence. *J. Shellfish Res.* 11:31-35.

4. Anderson, R.S., K.T. Paynter and E.M. Burreson. 1992. Increased reactive oxygen intermediate production by hemocytes withdrawn from Crassostrea virginica infected with Perkinsus marinus. *Biol. Bull.*, in press.

5. Anderson, R.S., L.M. Oliver, and L.L. Brubacher. 1992. Superoxide anion generation by Crassostrea virginica hemocytes as measured by nitroblue tetrazolium reduction. *J. Invertebr. Pathol.* 59:303-307.

6. Anderson, R.S. and L.L. Brubacher. 1992. In vitro inhibition of medaka phagocyte chemiluminescence by pentachlorophenol. *Fish Shellfish Immunol.* 2:299-310.

7. Anderson, R.S. 1993. Modulation of nonspecific immunity by environmental stressors. pp. 483-510 In: J.A. Couch and J.W. Fournie (eds.). *Patholbiology of Marine and Estuarine Organisms*. CRC Press, Inc., Boca Raton, FL.

Curriculum Vitae

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Professor
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EDUCATION

B.S.—Eastern Oregon State College, LaGrande, OR, 1966
M.S.—Oregon State University, Corvallis, OR, 1973
Ph. D.—Oregon State University, Corvallis, OR. 1975.

EXPERIENCE

1992–present, Professor, School of Marine Science, VIMS, College of William and Mary, Gloucester Point, VA.

1986-1992, Associate Professor, School of Marine Science, VIMS, College of William and Mary, Gloucester Pt., VA 23062

1981-1985, Assistant Professor, School of Marine Science, VIMS, College of William and Mary, Gloucester Pt., VA 23062.

1977-1980, Senior Marine Scientist and Program Manager, BLM Outer Continental Shelf Environmental Studies, Virginia Institute of Marine Science, Gloucester Pt., VA

1975-1977, Program Manager, Seabrook Ecological Studies, Normandeau Associates, Inc., Bedford, NH.

RESEARCH INTERESTS

Biology of marine fish and shellfish parasites, with emphasis on factors that affect the pathogenicity and abundance of protozoan pathogens of oysters. Enhancement of natural disease resistance in oysters through selective breeding.

PROFESSIONAL ACTIVITIES

Member of National Shellfisheries Association, Society of Protozoologists, Helminthological Society of Washington, Estuarine Research Federation and International Association of Leech Scientists (Newsletter Editor, 1992+).

SELECTED PUBLICATIONS (Relevant to this proposal):

Burreson, E. M. 1988. Use of immunoassays in haplosporidan life cycle studies. Pgs. 298-303 In: Fisher, W. S. (Ed.) Disease Processes in Marine Bivalve Molluscs. Amer. Fish. Soc. Spec. Publ. 18. 315p.

Burreson, E. M., M. E. Robinson and A. Villalba. 1988. A comparison of paraffin histology and hemolymph analysis for the diagnosis of *Haplosporidium nelsoni* (MSX) in *Crassostrea virginica* (Gmelin). J. Shellf. Res. 7: 19-23.

Burreson, E. M. and J. D. Andrews. 1988. Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Oceans 88 Proc. Vol. 3: 799-802.

Burreson, E. M. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: I. Susceptibility of native and MSX-resistant stocks. J. Shellfish Res. 10(2): 417–424.

Paynter, K. T., Jr. and E. M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. J. Shellf. Res. 10(2): 425–432.

Meyers, J. A., E. M. Burreson, B. J. Barber and R. Mann. 1991. Susceptibility of diploid and triploid Pacific oysters, *Crassostrea gigas*, and eastern oysters, *Crassostrea virginica*, to *Perkinsus marinus*. J. Shellfish Res. 10(2): 433–438.

Anderson, R. S., K. T. Paynter and E. M. Burreson. 1992. Increased reactive oxygen intermediate production by hemocytes withdrawn from *Crassostrea virginica* infected with *Perkinsus marinus*. Biol. Bull. (In Press).

Ragone, L. M. and E. M. Burreson. 1993. Effect of salinity on infection progression and pathogenicity of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica* (Gmelin). J. Shellfish Res. (In Press).

La Peyre, J. F., M. Faisal and E. M. Burreson. 1993. In Vitro propagation of the protozoan *Perkinsus marinus*, a pathogen of the eastern oyster, *Crassostrea virginica*. J. Eukaryotic Microbiol. (In Press).

Biographical Sketch

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EDUCATION:

A.B. Middlebury College, Vermont (Biology), 1968
Ph.D. University of Georgia, 1974 (Zoology)
Dissertation: Aspects of the phosphorus flux of scleractinian corals.

EXPERIENCE:

1989-	Director, Maryland Sea Grant College, H.J. Patterson Hall, University of Maryland College Park, College Park, Maryland, 20742.
1988-	Professor, Chesapeake Biological Laboratory, University of Maryland Center for Environmental and Estuarine Studies, Solomons, Maryland, 20688-0038.
1987-1989	Program Director, Biological Oceanography Program, Division of Ocean Sciences National Science Foundation, Washington, D.C. 20550. (Rotator, on leave of absence from the University of Maryland, June, 1987-February, 1989.)
1983-1988	Associate Professor, Chesapeake Biological Laboratory, University of Maryland Center for Environmental and Estuarine Studies, Solomons, Maryland, 20688-0038.
1982-1983	Associate Curator and Distinguished Ruth Patrick Scholar in Aquatic Studies, Division of Environmental Research, Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania. (On leave of absence from the University of Maryland, December, 1982-January, 1984.)
1984-Present	Research Associate, Division of Environmental Research, Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.
1983	Adjunct Associate Professor, Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania.

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EDUCATION

1971 B.Sc. Chemical Oceanography Honors First Class, University of Liverpool

1974 Ph.D. University of Liverpool, Chemical Oceanography Thesis Title: I. Adsorption of radionuclides by sediments and phytoplankton II. Chlorinated hydrocarbons in the environment

PROFESSIONAL EXPERIENCE

1985-present	Associate Professor. Center for Environmental & Estuarine Studies, Chesapeake Biological Laboratory, University of Maryland, Solomons, MD.
1983-85	Assistant Secretary. Intergovernmental Oceanographic Commission (IOC) UNESCO, Paris
1982-83	Consultant. Marine Pollution Research and Monitoring, IOC/UNESCO
1974-83	Section Leader. Marine Organic Chemistry, Sonderforschungsbereich 95, University of Kiel
1969-71	Demonstrator. Department of Oceanography, University of Liverpool
1971-72	Research Assistant. IAEA Laboratory, Musee Oceanographique, Monaco
1967-68	Laboratory Assistant. Imperial Chemicals Industries (ICI)

RESEARCH INTERESTS

Marine organic chemistry of natural products, biogeochemical cycling, Marine pollution research and monitoring, and analytical chemistry.

SELECTED PUBLICATIONS

1980 Mopper, K., R. Dawson, G. Liebezeit and V. Ittekott. The mono-saccharide spectra of natural waters. Mar. Chem. 10: 55-66.

1982 Boelter, M. and R. Dawson. Heterotrophic utilization of biochemical compounds in Antarctic waters. Neth. J. Sea. Res. 16: 316-332.

1983 Dawson, R. and G. Liebezeit. Determination of amino acids and carbohydrates. Pages 319-340 In: K. Grasshoff, M. Ehrhardt and K. Kremling (eds.), Methods of Seawater Analysis, Second Edition. Verlag Chemie, Weinheim.

1985 Dawson, R., J. Kalbfleisch, G. Liebezeit, C.A. Llewellyn, R.F.C. Mantoura, F. Moreau and S.A. Poulet. Analyses of dissolved free amino acids, pigments and vitamins in plankton and particles using HPLC methods. Actualites Biochimie Marine: Vol. 7. Oceanis ll, Fasc. 5: 521-531.

1986 Mopper, K. and R. Dawson. Determination of amino acids in seawater - Recent chromatographic developments and future directions. Science of the Total Environ. 49: 115-131.

1986 Smucker, R.A. and R. Dawson. Products of photosynthesis by marine phytoplankton: Chitin in TCA 'protein' precipitates. J. Exp. Mar. Biol. Ecol. 104: 143-152.

1988 Bianchi, T.S., R. Dawson and P. Sawangwong. The effects of macrobenthic deposit-feeding on the degradation of chloropigments in sandy sediments. J. Exp. Mar. Biol. Ecol. 122: 243-255.

1990 Marsh, A.G., A. Gremare, R. Dawson and K.R. Tenore. Translocation of algal pigments to oocytes in Capitella sp. I (Annelida: Polychaeta). Mar. Ecol. Prog. Ser. 67: 301-304.

1990 Dawson, R., T.S. Bianchi, P. Sawangwong and C.E.F. Orano-Dawson. Production flux and fate of photosynthetic pigments in estuaries. Pages 824-844 in: Y. Guohui, J-M. Martin and Z. Jiayi (eds.), Biogeochemical Studies of the Changjiang Estuary. China Ocean Press, Beijing.

1990 Ehrhardt, M., G. Wattayakorn, and R. Dawson. GC/MS based analyses of individual constituents of Chao Phrya River water and estimated discharge rates into the Upper Gulf of Thailand. Estuarine Coastal Shelf Sci. 30:439-451. CEES Contribution No. 2170.

1992 Carpenter, E.J., B. Bergman, R. Dawson, P.J.A. Siddiqui, E. Soederbac and D.G. Capone. 1992. Glutamine synthetase and nitrogen cycling in the marine diazotrophic cyanobacteria Trichodesmium spp. Appl. and Environ. Microbiol, 3122-3129.

1992 Mopper, K., C.A. Schultz, L. Chevolot, C. Germain, R. Revuelta and R. Dawson. (1992). Determination of sugars in unconcentrated seawater and other natural waters by liquid chromatography and pulsed amperometric detection. Environ. Sci. Tech.26:133-138.

STUDENT ADVISEES

Gerd Liebezeit, Ph.D 1980
Marion Schumann, Ph.D 1985
Pichan Sawangwong, Ph.D 1991
Andrew Rogers, Ph.D. in progress

NAME: Rebecca Marie Dickhut **TITLE:** Assistant Professor

BORN: July 31, 1960 **SOCIAL SECURITY NUMBER:** 388-76-2801

EDUCATION AND EXPERIENCE:

The College of William and Mary - Virginia Institute of Marine Science - Assistant Professor - 1989 to present

University of Wisconsin - Madison - Ph.D. - 1989; M.S. - 1985
Major: Water Chemistry; Minor: Chemical Engineering
Wisconsin Sea Grant Research Assistant 1983-1988
Teaching Assistant - Water Analysis 1983
Water Analyst - University of Wisconsin Environmental
Health and Safety Department 1982-1983

St. Norbert College - De Pere, Wisconsin - B.S. - 1982
Major: Natural Sciences and Mathematics
Teaching Assistant - Mathematics 1981-1982

PUBLICATIONS

Dickhut, R.M., A.W. Andren, D.E. Armstrong. 1986. "Aqueous Solubilities of Six Polychlorinated Biphenyl Congeners at Four Temperatures." Environ. Sci. Technol. 20:807-810.

Andren, A.W., W.J. Doucette, R.M. Dickhut. 1987. "Methods for Estimating Solubilities of Hydrophobic Organic Compounds: Environmental Modeling Efforts." In Sources and Fates of Aquatic Pollutants, R.A. Hites and S.J. Eisenreich (eds.). Advances in Chemistry Series 216, American Chemical Society: Washington, D.C., Chapter 1.

Andren, A.W., R.M. Dickhut, W.J. Doucette, L.P. Burkhard. 1987. "Chemical Property Estimation Techniques for Environmental Modeling." In Oceans '87 Proceedings: The Ocean - An International Workplace. The Institute of Electrical and Electronics Engineers; IEEE Catalogue No. 87-CH2498-4. pp. 1761-1764.

Dickhut, R.M., A.W. Andren, D.E. Armstrong. 1989. "Naphthalene Solubility in Selected Organic Solvent/Water Mixtures." J. Chem. Eng. Data. 34:438-443.

Dickhut, R.M., D.E. Armstrong, A.W. Andren. 1991. "The Solubility of Hydrophobic Aromatic Chemicals in Organic Solvent/Water Mixtures: Evaluation of Four Mixed Solvent Solubility Estimation Methods." Environ. Toxicol. & Chem. 10:881-889.

Gustafson, K.E. and R.M. Dickhut. 1993. "Molecular Diffusion of Selected Organic Chemicals in Aqueous Solution." In preparation (submitted for internal review) for J. chem. Eng. Data.

Gustafson, K.E. and R.M. Dickhut. 1993. "Molecular Diffusion of Selected Organic Chemicals in Air." In preparation (submitted for internal review) for J. chem. Eng. Data.

Dickhut, R.M., J.E. Baker and D.L. Leister. 1993. "Design and Field Validation of an Automated Precipitation Sampler for Hydrophobic Organic Contaminants." In preparation (submitted for internal review) for Atmos. Environ.

Liu, K. and R.M. Dickhut. 1993. "Vapor Pressures and Thermodynamic Properties of Selected Chlorinated Benzenes." In preparation for J. chem. Eng. Data.

CURRICULUM VITAE

NAME: GILMOUR, CYNTHIA C. **TITLE: Assistant Curator**

BORN: 11/15/58 **SOC. SECURITY NO: 187-52-7101**

EDUCATION:

1985 Ph.D., University of Maryland, Marine, Estuarine and Environmental Sciences
1980 B.A., Cornell University, Biochemistry

PROFESSIONAL EXPERIENCE:

1990-Present Assistant Curator, The Academy of Natural Sciences, BERL
1988-1990 Patrick Scholar, The Academy of Natural Sciences, BERL
1988-Present Associate of the Division of Applied Sciences, Harvard University
1986-1990 Adjunct Assistant Professor, Marine Sciences Research Center, State University of New York
1985-1987 Postdoctoral Research Fellow, Harvard University

FIVE SELECTED RELEVANT PUBLICATIONS:

Gilmour, C.C., E.A. Henry and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26:2281-2287.

Gilmour, C.C. Effect of acid rain on microbial processes in soil and water. 1992. In: "Environmental Microbiology," R. Mitchell, (ed.), Wiley-Liss, Inc., NY. pp. 33-57.

Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Poll. 71:131-169.

Gilmour, C.C., M.L. Leavitt and M.P. Shiaris. 1990. Evidence against incorporation of exogenous thymidine by sulfate-reducing bacteria. Limnol. and Oceanogr. 35:1401-1409.

Gilmour, C.C. and J.H. Tuttle. 1987. Anaerobic microbial methylation of inorganic tin in estuarine sediment. Microbial. Ecol. 14:233-242.

JONATHAN G. KRAMER

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Education

1988	Ph.D.	Marine Estuarine Environmental Sciences Minor in Microbial Physiology The University of Maryland, College Park, Maryland Dissertation: Macromolecular Bases of Growth Regulation in Marine *Synechococcus* spp.
1982	M.S.	Marine Environmental Sciences The State University of New York, Stony Brook, New York Thesis: Seasonal Aspects of Carbon Metabolism in *Fucus vesiculosus*
1979	B.S.	Environmental Sciences The University of Massachusetts, Amherst, Massachusetts Honors Thesis: Immediate Physiological Effects of Cadmium on *Chlorella vulgaris*

Professional Experience

1992-Present	Research Assistant Professor; Center of Marine Biotechnology
1992	Instructor; Applications of Mol. Biol. in Marine Sci., MEES Program, Univ. Md.
1988-1992	Faculty Research Associate/Postdoctoral Fellow; Center of Marine Biotechnology
1989	Instructor; Introduction to Molecular Genetics, Community College of Baltimore
1987-1988	Visiting Scientist; Dept. Biol. Sci., Univ. of Warwick, Coventry, U.K. (With Prof. N.G. Carr)
1983-1988	Graduate Research Assistant; Horn Point Environ. Laboratory, U. MD. (With Prof. I. Morris)
1983	Research Associate; Marine Sciences Research Center, SUNY Stony Brook
1979-1982	Graduate Research Assistant; Marine Sciences Research Center, SUNY Stony Brook
1979	Research Assistant; Environmental Sciences Dept., Univ. Massachusetts

Research Interests

Physiological and biochemical adaptations of prokaryotes to life in the oceanic environment. Mechanisms controlling growth and macromolecular synthesis. Molecular biology and physiology of marine cyanobacteria (*Synechococcus* spp.). Application of molecular biological theories and techniques to the study of prokaryotic growth regulation and physiology in marine systems

Professional Activities

American Society of Microbiology
American Society of Limnology and Oceanography
American Society for the Advancement of Science

Publications

Furhman, J.A., H.W. Ducklow, D.L. Kirchman, J. Hudak, G.B. McManus, J. Kramer. 1986. Does Adenine Incorporation into Nucleic Acids Measure Total Microbial Production ? Limnol. and Oceanogr. 31:627-636.

Kramer, J.G. 1990. The Effects of Irradiance and Specific Inhibitors on Protein and Nucleic Acid Synthesis in the Marine Cyanobacterium *Synechococcus* sp. WH 7803. Arch. Microbiol 154:280-285.

Kramer, J.G. and I. Morris. 1990. Macromolecular Bases of Growth Regulation in Irradiance Limited Marine *Synechococcus* spp. WH 7803. Arch. Microbiol. 154:286-293.

Kramer, J.G. and F.L. Singleton. 1992. Variations in rRNA Content of Marine *Vibrio* spp. during Starvation-Survival and Recovery. Appl. Environ. Microbiol. 58:201-207.

Kramer, J. G. and F.L. Singleton. (In press) Measurement of rRNA variations in natural communities of microorganisms on the southeastern U.S. continental shelf. Appl. Environ. Microbiol.

Kramer, J. G. and F.L. Singleton. (In prep.) Cloning and Sequence Analysis of the Structural Gene Encoding Glutamine Synthetase (*glnA*) in Marine *Synechococcus* spp.

Collaborators

Dr. Harold J. Schreier, Center of Marine Biotechnology, MBI, Univ. of Maryland System
Dr. Michael Wyman, Plymouth Marine Laboratory, Plymouth, England
Dr. Thomas Malone, Horn Point Environmental Laboratory, CEES, Univ. of Maryland System
Dr. Stephen Giovannoni, Oregon State University
Dr. Hugh Ducklow, Horn Point Environmental Laboratory, CEES, Univ. of Maryland System

Advisors

Dr. Ian Morris; Advisor for Ph.D.

Dr. Fred L. Singleton; Postdoctoral Advisor

WILLIAM L. RICKARDS
Social Security Number: 221-28-3691

October 27, 1941
Wilmington, Delaware

TITLE: Director, Virginia Graduate Marine Science Consortium

EDUCATION AND EXPERIENCE:

A.B.	University of Delaware, 1963 (Biology)
M.S.	University of Georgia, 1966 (Zoology)
Ph.D.	University of Miami, 1971 (Marine Sciences - Fisheries)

1960 (Summer)	- Research Assistant, University of Delaware Marine Laboratories.
1963 (Summer)	- Research Assistant, University of Delaware Marine Laboratories.
1963-1965	- Research and Teaching Assistant, University of Georgia
1965-1971	- Research Assistant, University of Miami, School of Marine and Atmospheric Sciences.
1971-1973	- Research Associate, Dept. of Environmental Sciences and Engineering, University of North Carolina; Assistant Coordinator, UNC Sea Grant Program.
1973-1981	- Visiting Assistant Professor, Zoology, N.C. State University, Associate Director, UNC Sea Grant Program.
1981	- Director, Virginia Graduate Marine Science Consortium Director, Virginia Sea Grant Program Research Professor, Dept. of Environmental Sciences, University of Virginia

PUBLICATIONS:

Gopeland, B. J., W. L. Rickards, and D. R.Berg. 1975 N. C. Coastal Resources and Short Term Needs: a view of priorities. University of North Carolina Sea Grant Pub. UNC-SG-75-14, 45pp

Rickards, W. L., J. E. Foster and W. R. Jones. 1978 A feeding tray for use in eel farming. University of North Carolina, Sea Grant Pub. UNC-SG-78-04,11 pp.

Rickards, W. L., W. R. Jones and J. E. Foster, 1978 Techniques for culturing the American eel. Proc. World Mariculture Society, Vol. 8, pp. 641-646

Rickards, W. L., ed. 1978. A diagnostic manual of eel diseases occuring under culture conditions in Japan. University of North Carolina Sea Grant Pub. UNC-SG-78-06. 88 pp.

Rickards, W. L., 1981. A discussion of aquaculture candidate species for eastern North Carolina. UNC Sea Grant Working Paper, UNC-SG-WP-81-2. 10 pp.

NAME: Gerhardt F. Riedel **TITLE: Senior Scientist**

BORN: 7/8/51 **SOCIAL SEC. NO: 546-80-8349**

EDUCATION:

1983	Ph.D., Biological Oceanography, Oregon State University
1978	M.S., Biological Oceanography, Oregon State University
1974	B.A., Biology, Humboldt State University
1974	B.S., Oceanography, Humboldt State University

PROFESSIONAL EXPERIENCE:

1987-1993	Senior Scientist, The Academy of Natural Sciences, BERL
1985-1987	Postdoctoral Investigator, The Academy of Natural Sciences, BERL
1983-1985	Postdoctoral Fellow, Harbor Branch Foundation

FIVE SELECTED RELEVANT PUBLICATIONS:

Sanders, J.G., G.F. Riedel, and D.P. Ferrier. 1991. Changes in community structure of Chesapeake Bay phytoplankton when exposed to low levels of trace metals: Management implications. in J.A. Mihursky and A. Chaney (eds.) New Perspectives in the Chesapeake System: A Research and Management Partnership. Chesapeake Research Consortium Publication No. 137.

Sanders, J.G., G.R. Abbe, and G.F. Riedel. 1990. Silver uptake and subsequent effects on growth and species composition in an estuarine community. Sci. Tot. Environ. 97:761-769.

Sanders, J.G., R.W. Osman and G.F. Riedel. 1989. Pathways of arsenic uptake and incorporation in estuarine phytoplankton and the filter-feeding invertebrates, Eurytemora affinis, Balanus improvisus, and Crassostrea virginica. Mar. Biol. 103:319-325.

Riedel, G.F., J.G. Sanders, and R.W. Osman. 1989. The role of three species of benthic invertebrates in the transport of arsenic from contaminated estuarine sediments. J. Exp. Mar. Biol. Ecol. 134:143-155.

Riedel, G.F., J.G. Sanders, and R.W. Osman. 1987. The effect of biological and physical disturbances on the transport of arsenic from contaminated sediments. Est. Coastal Shelf Sci. 25:693-706.

CIRRICULUM VITAE

NAME: James G. Sanders **TITLE: Curator**

BORN: 6/10/51 **SOCIAL SEC. NO: 239-88-1054**

EDUCATION:

1978	Ph.D.	Marine Sciences, University of North Carolina
1975	M.S.	Marine Sciences, University of North Carolina
1973	B.S.	Zoology, Duke University

PROFESSIONAL EXPERIENCE:

1990-Present	Curator, The Academy of Natural Sciences, BERL
1983-92	Laboratory Director, The Academy of Natural Sciences, BERL
1985-90	Associate Curator, The Academy of Natural Sciences, BERL
1981-85	Assistant Curator, The Academy of Natural Sciences, BERL
1981-89	Adjunct Professor, University of Maryland, CEES, CBL
1980-81	Visiting Assistant Professor, University of Maryland, CBL
1978-80	Postdoctoral Investigator, Woods Hole Oceanographic Institution

FIVE SELECTED RELEVENT PUBLICATIONS

Sanders, J.G., G.F. Riedel, and R.W. Osman. 1993. Arsenic cycling and impact in estuarine and coastal marine ecosystems. In: J.O. Nriagu, ed., Arsenic in the Environment. Advances in Environmental Sciences and Technology, J. Wiley & Sons, in press.

Sanders, J.G. and G.F. Riedel. 1992. Sources, cycling and fate of contaminants in Chesapeake Bay. Water Sci. Technol. 26:2645-2652.

Sanders, J.G., G.F. Riedel, and D.P. Ferrier. 1991. Changes in community structure of Chesapeake Bay phytoplankton when exposed to low levels of trace metals: Management implications. Pages 451-460 in J.A. Mihursky and A. Chaney (eds.) New Perspectives in the Chesapeake System: A Research and Management Partnership. Proceedings of a Conference 4-6 December 1990, Baltimore MD. Chesapeake Research Consortium Publication No. 137.

Connell, D.B., J.G. Sanders, G.F. Riedel and G.R. Abbe. 1991. Pathways of silver uptake and trophic transfer in estuarine organisms. Environ. Sci. Technol. 25:921-924.

Sanders, J. G., R. W. Osman and G. F. Riedel. 1989. Pathways of arsenic uptake and incorporation in estuarine phytoplankton and filter-feeding invertebrates, Eurytemora affinis, Balanus improvisus, and Crassostrea virginica. Mar. Biol. 103:319-325.

BIOGRAPHICAL SKETCH

Name: **LINDA C. SCHAFFNER**

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EDUCATION: 1987 Ph.D., The College of William and Mary
1981 M.A., The College of William and Mary
1976 B.A., Drew University

RELEVANT PUBLICATIONS

Schaffner, L. C., R. J. Diaz, C. R. Olsen, and I. L. Larsen. 1987. Faunal characteristics and sediment accumulation processes in the James River Estuary, Virginia. Estuarine, Coastal and Shelf Science 25:211-226.

Schaffner, L.C. 1990. Small-scale organism distributions and patterns of species diversity: evidence for positive interactions in an estuarine benthic community. Marine Ecology Progress Series 61:107-117.

Pihl, L., S. P. Baden, R. J. Diaz and **L. C. Schaffner. 1992.** Hypoxia-induced change in the diet of bottom feeding fish and Crustacea. Marine Biology 112: 349-361.

Schaffner, L. C., P. Jonsson, R. J. Diaz, R. Rosenberg and P. Gapcynski. 1992. Benthic communities and bioturbation history of estuarine and coastal systems: effects of hypoxia and anoxia. Science of the Total Environment Suppl. 1001-1016.

Olsen, C. R., I. L. Larsen, P. J. Mulholland, K. L. Von Damm, J. M. Grebmeier, **L. C. Schaffner**, R. J. Diaz and M. N. Nichols. Equilibrium surface applied to particle sources and contaminant distributions in estuarine sediments. (in press, Estuaries)

Mayer, M. M., **L. C. Schaffner** and W. M. Kemp. Effects of macrobenthic community composition on nitrogen cycling and transformation in estuarine sediments: a site comparison (in review)

Mayer, M. M. **L. C. Schaffner** and W. M. Kemp. Nitrification activity of macrofaunal tubes and burrows: effects of environmental conditions and animal behavior. (in review).

Boesch, D. F., **L. C. Schaffner**, M. A. Bowen, J. van Montfrans, and R. C.Swartz. Benthos community dynamics following a plankton bloom and hypoxia in the New York Bight (in revision, Estuarine, Coastal and Shelf Science)

WALCH, MARIANNE

Research Assistant Professor
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Education:

B.S., University of Maryland, College Park, 1980
S.M., Harvard University, 1981
Ph.D., Harvard University, 1986

Experience:

Materials Research Engineer/Microbiologist, Biotechnology Program Office, Naval Surface Warfare Center, Silver Spring, MD, 1990-Present.

Research Assistant Professor, Center of Marine Biotechnology, University of Maryland, 1988-Present.

Research Associate, Center of Marine Biotechnology, University of Maryland, 1986-1988.

Research Assistant, Harvard University, Division of Applied Sciences, 1983-1986.

National Science Foundation Predoctoral Fellow, Harvard University, 1980-1983.

Teaching Fellow, Harvard University, 1981-1983.

Biological Aid, U.S. Fish and Wildlife Service, Migratory Bird and Habitat Research Laboratory, 1979-1980.

Research Interests:

The ecology of surface-associated microorganisms and applied aspects of their growth and activity. Current projects include:

- microbiologically influenced corrosion.
- biomagnification of pollutants by microbial films.
- role and regulation of emulsifier production by hydrocarbon-degrading microorganisms.
- interactions between invertebrate larvae and biofilms of specific marine bacteria.
- genetic engineering of bacterial photopigments for use in molecular computing and holographic applications.

Professional Activities:

American Association for the Advancement of Science; American Society for Microbiology; National Shellfisheries Association; National Association of

Corrosion Engineers; Marine Technology Society; Maritime Archaeological and Historical Society; Participant, ONR-DELMANC Biosurfaces Study Group; Executive planning committee, National Collaborative Study of the Relationships of Indicators, Human Enteric Pathogens and Potential Health Risks in Shellfish Growing Waters; Delegate, UNESCO Workshop on Teaching and Training in Marine Science for the Year 2000, Paris, June 6-10, 1988; Invited participant, Dahlem Workshop, "Structure and Function of Biofilms," Berlin, November 27 - December 2, 1988; Guest editor, Marine Technology Society Journal, September 1990 issue on marine biological corrosion; *Ad hoc* reviewer for Microbial Ecology, Biofouling, Sea Grant, American Chemical Society; Small Business Innovative Research (SBIR) Program Reviewer and Contract Officer's Technical Representive, Naval Surface Warfare Center; Co-Director, Molecular Computing Program, Naval Surface Warfare Center, February 1991-Present; Consultant to Maryland Historic Trust Archaeological Conservation Laboratory; Scientific advisor to the Maritime Archaeological and Historical Society; Chair, Biological and Medical Sciences Session, Navy Research and Development Exchange Conference, Silver Spring, Maryland, April 8-10, 1992; Graduate student major advisor: 1 Ph.D., Graduate student committees: 2 Ph.D., 1 M.S.;

Selected Publications:

R.M. Weiner, M. Walch, M.P. Labare, D.B. Bonar, and R.R. Colwell. 1989. Effect of biofilms of the marine bacterium Alteromonas colwelliana (LST) on the settlement and metamorphosis of oysters (Crassostrea gigas and C. virginica). J. Shellfish Res. 8(1): 117-123.

T.E. Ford, M. Walch, R. Mitchell, M.J. Kaufman, R. Vestal, and M.A. Lock. 1989. Microbial film formation on metals in an enriched artic river. Biofouling 1:301-311.

M. Walch. 1989. Spatial distribution of biotic and abiotic components in the biofilm. In: W.G. Characklis and P.A. Wilderer, (eds.), Biofilm Structure and Function, pp. 165-191. Wiley, New York.

D.B. Bonar, S.L. Coon, M. Walch, R.M. Weiner, and W.K. Fitt. 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bull. Mar. Sci., 46(2): 484-498.

W.K. Fitt, S.L. Coon, M. Walch, R.M. Weiner, R.R. Colwell, and D.B. Bonar. 1990. Settlement behavior and metamorphosis of larvae of Crassostrea gigas in response to bacterial supernatants. Marine Biology 106: 389-394.

S.L. Coon, M. Walch, R.M. Weiner, R.R. Colwell, and D.B. Bonar. 1990. Ammonia (NH_3) induction of oyster larval settlement behavior. Biol. Bull. 179: 297-303.

M. Walch. 1991. Microbiological influences on marine corrosion. Sea Technology 32(8): 31-34.

BIOGRAPHICAL SKETCH

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EDUCATION

B.S.	1964	Brooklyn College, Brooklyn, New York
M.S.	1967	Long Island University, Brooklyn, New York
Ph.D.	1970	Iowa State University, Ames, Iowa, W. Lockhart, Advisor

POSITIONS

Sept 1969 - Sept 1970	Instructor, Department of Bacteriology, Iowa State University, Ames, Iowa
Sept 1970 - Sept 1975	Assistant Professor, Department of Microbiology, University of Maryland, College Park, Maryland
Sept 1975 - Sept 1986	Associate Professor, Department of Microbiology, University of Maryland, College Park, Maryland
Oct 1980 - Oct. 1981	Acting Chairman, Department of Microbiology, University of Maryland, College Park, Maryland
Sept 1986 - present	Professor, Department of Microbiology, University of Maryland, College Park, Maryland
Jan 1987 - present	Joint appointment with Center of Marine Biotechnology
Jan 1990 - present	Professor in Cell and Molecular Biology Program of the University of Maryland

SELECTED PROFESSIONAL ACTIVITIES:

American Society for Microbiology; Fellow, American Academy for Microbiology, Danforth Colloquium; Visiting Scientist, Natl. Inst. of Health; Fulbright Scholar Award, 1990; Five patents pending or issued.

UNIVERSITY AND TEACHING ACTIVITIES:

Graduate student major professor: Ph.D., 16; M.S., 13; Postdoctoral fellows, 7; Graduate Teaching: Seminar (MICB 688); Biotechnological approaches to the study of Marine Microbiology (MICB 788D) Undergraduate Teaching: Microbial Ecology (MICB 480); General Microbiology (MICB 200); Premedical internship (MICB 388P); Texts and manuals written: Microbiology, McGraw Hill; Laboratory Manual in Microbiology; Molecular Approaches to Marine Ecology, ed; Chair or member of over 60 University committees.

PUBLICATIONS: Over 60 peer reviewed journal articles, 14 book chapters, over 80 seminars with abstracts at national or international meetings, 14 invited presentations at Symposia.

PROJECT RELATED/SIGNIFICANT PUBLICATIONS:

Moore, R. and R. Weiner. 1989. The Hyphomonas. pp 1906-1910. In: Bergey's Manual of Determinative Bacteriology. 9th ed., Vol 3, Williams and Wilkins.

Shen, N., L. Dagasan, D. Sledjeski, and R. M. Weiner. 1989. Major outer membrane proteins unique to reproductive cells of Hyphomonas jannaschiana. J. Bacteriol. 171:2226-2228.

Weiner, R., M. Walch, M. Labare, D. Bonar and R. Colwell. 1989. Effect of biofilms of the marine bacterium Alteromonas colwelliana on set of the oysters Crassostrea gigas (Thunberg) and C. virginica (Gmelin). J. Shellfish Res. 8:117-123.

Devine, R. and R. Weiner. 1990. Hyphomonas species metabolize amino acids using Krebs cycle enzymes. Microbios. 62:137-153.

Abu, G., R. Weiner, J. Rice, R. Colwell. 1991 Properties of an extracellular adhesive polymer from the marine bacterium, Shewanella colwelliana. Biofouling. 3, 69-89.

Fuqua, W., V. Coyne, D. Stein, C. Lin, and R. Weiner. 1991. Characterization of mel A; a gene encoding melanin biosynthesis from the marine bacterium Shewanella colwelliana. Gene. 109:131-136.

Labare, M., and R. Weiner. 1991. Interactions between Shewanella colwelliana biofilms oyster larvae and hydrophobic organic phosphate pesticides. Appl. Environ. Microbiol. 56, 3817-3821.

Sledjeski, and R. M. Weiner. 1991. Homogeneity of R-Type lipopolysaccharides of marine bacteria. appl. Environ. Microbiol. 57, 2094-2096.

Tritar, S., D. Prieur and R. Weiner. 1992. Effects of bacterial films on the settlement of the oysters Crassostrea gigas and Ostrea edulis and the scallop, Pecten maximus. J. Shellfish Res. 11:325-330.

Weiner, R., E. Quintero and D. Sledjeski. 1992. Regulation of Synthesis Novel Complex Marine Polysaccharides by two periphytic marine bacteria. pp 459-470 In: Developments in Marine Biotechnology. C. Nash, Ed. W.C. Brown.

Sledjeski, D.P. and R.M. Weiner. 1993. Production and characterization of monoclonal antibodies specific for Shewanella colwelliana exopolysaccharides. Appl. Environ. Microbiol. 59: (In press).

Fuqua C. and R. Weiner. 1993. The melA gene is essential for melanin biosynthesis in Shewanella colwelliana. J. Gen. Microbiol. 139: (In press).

DAVID A. WRIGHT

BIOGRAPHIC SKETCH

Associate Professor
University of Maryland System, Center for Environmental and Estuarine Studies
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EDUCATION:

1969 - Honors B. Sc., University of Newcastle-upon-Tyne
1973 - Ph.D., University of Newcastle-upon-Tyne

EXPERIENCE:

1988- Assistant Professor, 1979-1988; Associate Professor, Chesapeake Biological Laboratory, University of Maryland System, Solomons, MD 20688
1989-1990 - Visiting Professor, Institute for Environmental Studies, University of Toronto, CA.
1974-1978 - Senior Demonstrator, University of Newcastle-upon-Tyne, U.K.
1972-73 - Lecturer, Northern Counties College of Education, Northumberland, U.K.
1972-74 - Research Associate [Alcan (U.K.)], University of Newcastle-upon-Tyne, U.K.

RESEARCH INTERESTS:

Comparative physiology; effects of inorganic pollutants on marine and estuarine organisms; biotoxicity assays.

PROFESSIONAL ACTIVITIES:

- Editorial Board, Marine Pollution Bulletin, 1985-1991.
- Member: Society for Experimental Biologists, Cambridge, England; American Association for the Advancement of Science; American Society of Zoologists; Atlantic Estuarine Research Society; Estuarine Research Federation.

REFERRED JOURNAL PUBLICATIONS: (LAST 4 YEARS)

Meteyer, M.J., D.A. Wright and F.D. Martin. 1988. Effect of cadmium on early development stages of the sheepshead minnow (Cyprinodon variegatus). Environ. Toxicol. Chem. 7:321-328. Contrib. No. 1854.

Wright, D.A. 1988. Dose-related toxicity of copper and cadmium in striped bass larvae from the Chesapeake Bay: Field considerations. Water Sci. Tech. 20:39-48. Contrib. No. 1819.

Wright, D.A. and D.J.H. Phillips. 1988. Chesapeake and San Francisco Bays: A study in contrasts and parallels. Mar. Pollut. Bull. 19:425-431. Contrib. No. 1909.

Sinex, S.A. and D.A. Wright. 1988. The distribution of trace metals in the sediments and biota of Chesapeake Bay. Mar. Pollut. Bull. 19:425-431. Contrib. No. 1897.

Hall, L.W., Jr., S.J. Bushong, M.C. Ziegenfuss, W.E. Johnson, R.L. Herman and D.A. Wright. 1988. Chronic toxicity of tributyltin to Chesapeake Bay biota. Water, Air and Soil Pollut. 39:365-376.

Pinkney, A.E., L.L. Matteson and D.A. Wright. 1990. Effects of tributyltin on survival growth, morphometry and RNA:DNA ratio of larval striped bass, Morone saxatilis. Arch. Environ. Contam. Toxicol. 19:235-241. Contrib. No. 2006.

Wright, D.A. and C.D. Zamuda. 1991. Copper contamination in the Patuxent River, Maryland. Hydrobiologia 215:31-41. Contrib. No. 2178.

Wright, D.A. and C.D. Zamuda. 1991. Use of oysters as indicators of copper contamination in the Patuxent River, Maryland. Hydrobiologia 222:39-48. Contrib. No. 2213.

Wright, D.A., P.M. Welbourn and A.V.M. Martin. 1991. Inorganic and organic mercury uptake and loss by the crayfish (Oreonectes propinquus). Water Air Soil Pollut. 56:697-707. contrib. No. 2281.

Wright, D.A. and P.M. Welbourn. 1991. Effects of mercury on unidirectional sodium and calcium influx in Asellus aquaticus. Arch. Environ. Contam. Toxicol. 21:567-570. Contrib. No. 2230.

GRADUATE STUDENTS SUPERVISED:

Lobel, P.B., Ph.D., 1981
Alliegro, M.C., M.S., 1981
Hetzel, E.W., M.S., 1983
Krygsman, A., M.S., 1983
Mountford, N.K., M.S., 1984
Zamuda, C.D., Ph.D., 1984
Becerra, R., M.S., 1984
Pinkney, A., Ph.D., 1988
Jepson, M., M.S., 1988
Greer, L.E., Ph.D., 1989
Savitz, J.D., M.S., 1991

COLLABORATORS: - last 5 years

Dr. Chares Hocutt, University of Maryland, CEES, Horn Point Environmental Laboratory
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Dr. Victor S. Kennedy, University of Maryland, CEES, Horn Point Environmental Labortory
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Professor Stuart Tanner, University of Sheffield, Medical School, Dept. of Pathology
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