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TITLE OF PROPOSED PROJECT Center for Microbial Oceanography: Research and Education \C-MORE\						
REQUESTED AMOUNT \$ 20,000,000		PROPOSED DURATION (1-60 months) 60 months		REQUESTED STARTING DATE 06/15/05		SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE 0337573
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☐ HUMAN SUBJECTS (GPG II.D.6) Exemption Subsection _____ or IRB App. Date _____ ☐ INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES INVOLVED (GPG II.C.2.j) _____ ☐ HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.E.1)						
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CERTIFICATION PAGE

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 04-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Drug Free Work Place Certification

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Appendix C of the Grant Proposal Guide.

Debarment and Suspension Certification

(If answer "yes", please provide explanation.)

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency?

Yes ☐

No ☒

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Debarment and Suspension Certification contained in Appendix D of the Grant Proposal Guide.

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding \$100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding \$150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure of Lobbying Activities," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than \$10,000 and not more than \$100,000 for each such failure.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE		SIGNATURE		DATE	
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10.b. PROJECT PERSONNEL

Last name	First name	Institution/organization
<i>Academic Institutions</i>		
Bidigare	Robert	University of Hawaii
Boyle	Edward	Massachusetts Institute of Technology
Chinn	Pauline	University of Hawaii
Chisholm	Sallie	Massachusetts Institute of Technology
Church	Matthew	University of California at Santa Cruz
DeLong	Edward	Massachusetts Institute of Technology
Dyhrman	Sonya	Woods Hole Oceanographic Institution
Helmreich	Stefan	Massachusetts Institute of Technology
Karl	David	University of Hawaii
Laws	Edward	University of Hawaii
Letelier	Ricardo	Oregon State University
Lunsford	Tami	Marine Advanced Technology Education (MATE) Center at Monterey Peninsula College
Rappé	Michael	University of Hawaii
Saito	Mak	Woods Hole Oceanographic Institution
Steward	Grieg	University of Hawaii
Vergun	Judith	University of Hawaii
Taylor	Craig	Woods Hole Oceanographic Institution
Waterbury	John	Woods Hole Oceanographic Institution
Webb	Eric	Woods Hole Oceanographic Institution
Zehr	Jonathan	University of California at Santa Cruz
<i>Non-Government Institution</i>		
Kolber	Zbigniew	Monterey Bay Aquarium Research Institute
Scholin	Chris	Monterey Bay Aquarium Research Institute

EXTERNAL ADVISORY COMMITTEE MEMBERS

Last name	First name	Affiliation
Abbott	Isabella	Bishop Museum / Stanford University
Hayes	John	Woods Hole Oceanographic Institution
Pace	Norman	University of Colorado, Boulder
Rubin	Eddy	Lawrence Berkeley Lab
Simon	Melvin	Agouron Institute / Cal Tech

2. PROJECT SUMMARY
CENTER FOR MICROBIAL OCEANOGRAPHY: RESEARCH AND EDUCATION
DAVID M. KARL, DIRECTOR
UNIVERSITY OF HAWAII

Intellectual merit: Life on Earth most likely originated as microbes in the sea. Over the past 3.5-4 billion years, microorganisms have shaped and defined Earth's biosphere, and created conditions that allowed the evolution of macroorganisms and complex biological communities. Today microbes inhabit and sustain all of Earth's biotopes, including those found in marine environments; they are truly the "unseen majority" (Whitman et al. 1998). They catalyze key biogeochemical transformations of nutrients and trace elements that maintain productivity of the oceans, form and consume greenhouse gases, and are a critical component of the food web linking dissolved organic matter to higher trophic levels. Until recently very little was known about the biology of the microorganisms *in situ*, their metabolic and genomic properties, or their temporal and spatial dynamics. Now there is an opportunity, using recent advances in molecular ecology, genomics, remote sensing and ecological modeling, to make great strides in the understanding of the biology, ecology and biogeochemical roles of the unseen majority in the world's largest biome: the global ocean. The goal of this project is to fuse these otherwise separate disciplines into a targeted inquiry into the microbial ecology of the oceans, to create synergy in the Center for Microbial Oceanography: Research and Education (C-MORE).

Although we know the identities of some of the key microbial "players" in marine ecosystems, the relatively recent explosion of biological, molecular and genomic approaches has provided evidence of how little we know about extant microorganisms and their metabolism, as well as indicating the potential knowledge to be gained using multiple approaches recently developed in a variety of disciplines. For example, the recent proliferation of cultivation-independent molecular identification techniques has resulted in the discovery of numerous clades of prokaryotic microbes that were not previously represented in culture collections. The present level of analytical sophistication and continued improvement of techniques are impressive and they hold great promise to yield new, fundamental information regarding the ecological role of microbial assemblages in the sea. According to a recent report of the American Academy of Microbiology (Stahl and Tiedje 2002), and by our own independent assessments, the fields of microbial ecology and genomics are currently poised at a "crossroads of opportunity."

It is critical to understand ocean processes and to put our new phylogenetic and genomic knowledge of marine microbes into the context of carbon (C), nitrogen (N), phosphorus (P) and related biogeochemical cycles and energy flow pathways if we ever hope to gain a full understanding of contemporary and probable future states of marine ecosystems. The scientific and societal benefits at stake in this research are profound. A full characterization of the global ocean microbial genome and the controls on its expression and regulation is a daunting task, by any measure. The human genome, approximately 30,000 separate genes, is now completely sequenced. By comparison, it has been estimated that there may be 10^{10} bacterial genes – many residing in the global ocean.

A comprehensive inventory of the ecologically important marine microbes and a complete understanding of their metabolic processes in a biogeochemical context are the formal scientific goals for the proposed Center. Research within the proposed Center will facilitate the achievement of a fundamental understanding of the genetic basis of marine biogeochemistry, from metabolic regulation and the environmental controls of gene expression to major element fluxes, including the net oceanic sequestration of atmospheric carbon dioxide. This new knowledge will serve as the basis for a comprehensive hypothesis-testing phase of research that will focus on at-sea, whole ecosystem manipulation experiments at a scale that would be difficult to achieve without a Center structure. We endeavor to combine the separate subdisciplines of marine genomics, systems biology, oceanography and ecology in an effort to provide novel insights into marine ecosystem processes. This scaling from

molecules-to-cells-to-ecosystems-to-biomes will form the basis of a unique collaboration among scientists who traditionally do not interact, and this should lead to new discoveries about the sea around us. A fundamental component of the success of this endeavor is the education of students in transdisciplinary efforts in the breadth and depth of the Center objectives. The establishment of a Center is necessary to facilitate this comprehensive research prospectus and its integrated education missions, including curriculum development and K-14 teacher training.

C-MORE will be established as a vehicle for progress in important areas of science and technology targeting representative coastal and open ocean biomes; the Center will facilitate a coordinated effort to explore, to experiment, and to educate. This collaboration will be led by the University of Hawaii (UH) with Massachusetts Institute of Technology (MIT), the Marine Advanced Technology Education (MATE) Center at Monterey Peninsula College (MPC), Oregon State University (OSU), University of California at Santa Cruz (UCSC), Woods Hole Oceanographic Institution (WHOI) and Monterey Bay Aquarium Research Institute (MBARI) as partner institutions. A central objective of C-MORE will be to increase our understanding regarding how biological diversity detected at the genomic level expresses itself at the ecosystem functional level and to transfer this knowledge to policymakers and the public at large.

Broader impacts: The environments of Earth, both terrestrial and marine, have changed radically during the past century. From historical records preserved in Antarctic ice cores we now know that Earth's climate has always been variable but for at least the last 400,000 years both the temperature and atmospheric burden of carbon dioxide are at unprecedented maxima, and rising. The contemporary, no analogue environmental conditions on Earth are certainly cause for societal concern. While we tend to think mostly about the plight of the human population, including the rapidly increasing rates of poverty, starvation and outbreaks of infectious disease, our new Center will have a deliberate focus on the microbial inhabitants of the sea. One reason for this is the fact that most microorganisms exhibit a complex set of behavioral responses to changes in their environment and, as such, may serve as model systems for understanding the impact of global environmental variability and change. Individual organisms, including microorganisms, perceive and respond to changes in their environment with a variety of sensory, control and feedback mechanisms, many of which are only beginning to be understood at an elemental, mechanistic level. The ensemble of these genetic mechanisms form a regulatory network with subtle and complex interconnections that are, for the most part, unknown or minimally understood.

Education, training and public outreach from "K-to-gray" including the establishment of active partnerships with programs for underrepresented and underprivileged students, will be fully integrated into the Center's mission. The proposed research of the Center will contribute to our understanding of the cycling of carbon in the marine environment, and will add to the rapidly growing environmental database emerging from complementary programs worldwide. By probing the activities of microorganisms in the sea we will be able to develop and refine meaningful predictive models of potential future states, linking climate variability to ecosystem structure and function. Through combined research and education, the Center will help to provide the intellectual resources needed to build a sustained ocean observing system for operational purposes. The broader societal benefits of our research are an increased understanding of the Earth's biologically driven elemental cycles, a greater knowledge of climate and ecosystem variability, including anthropogenic impacts and the education of younger generations and the public at large regarding the role of marine ecosystems in sustaining Earth's habitability.

4. PROJECT DESCRIPTION

4.a. RATIONALE FOR CENTER CONCEPT

Microorganisms are the foundation of life and are key to Earth's habitability and sustainability. In open ocean ecosystems, planktonic microbes dominate the living biomass, harvest light energy, produce organic matter and the oxygen we breathe, and facilitate the storage, transport, and turnover of key bio-elements. Their metabolic activities and specific growth rates are also responsible for the production and consumption of most of Earth's greenhouse gases. As microbiologist Louis Pasteur noted more than a century ago, "The very great is accomplished by the very small." To gain a full understanding of contemporary and probable future states of marine ecosystems, we need to understand the genetic basis of marine microbe biogeochemistry and ocean processes, and their response to climate change. Because of recent developments in molecular-based biotechnologies and their application to marine ecosystems, we are poised for rapid advances in our understanding of the integrated relationships among the previously isolated disciplines of genomics, biogeochemistry and ecology in the sea. The Earth's inhabitants need solutions to the global scale issues concerning the impact of the burgeoning human population, and this will require a comprehensive understanding of ocean habitat variability and biodynamics, including prognostic modeling of future ecosystem states. Without this information, we are at risk of making grievous mistakes in management and policy decisions that impact the current and future health of our ecosystems and all that they support. Furthermore, we need to communicate this information to others and include it in contemporary academic and community informal educational programs to increase public awareness about the importance of the research and to contribute to personal career choices, accountability and policymaking.

We propose to establish the Center for Microbial Oceanography: Research and Education (C-MORE) that will facilitate broad based research on marine microorganisms across geographical, disciplinary, and cultural boundaries. The proposed Center will be the entity to establish, monitor and manage the research, broker partnerships, conduct a comprehensive education and outreach program and facilitate the formative and summative evaluation necessary to obtain and apply the comprehensive new information about microbial life in the sea. The Center will bring together knowledgeable teams of scientists, educators and community members who otherwise do not have the opportunity to communicate, collaborate or design creative solutions to long-term ecosystem scale problems, and whose collective expertise will synergistically advance microbial oceanography beyond what an ensemble of individual researchers and teachers could accomplish. The Center will facilitate a range of activities, from genomics to instrument/sensor development for remote sensing, to mesocosm experimentation, to ecosystem modeling and, most importantly, integration of results from these diverse approaches. We believe the only way to achieve these interactions, and catalyze new transdisciplinary connections on large problems in a sustained effort, is under the auspices of a Science and Technology Center.

The establishment of C-MORE will provide novel and leveraged opportunities to enhance the coherent physical and biogeochemical datasets now being collected in the North Pacific Subtropical Gyre (NPSG) and in Monterey Bay. This will facilitate, largely for the first time, comprehensive cell-to-ecosystem-to-biome level analyses of key marine habitats and the populations that reside within them. This ecosystem scale of synthesis of the disciplines of oceanography, microbiology and systems ecology will lead to the design and implementation of whole ecosystem-scale perturbation/manipulation experiments that will serve to test meaningful hypotheses linking ecological structure to function. Ultimately, it will require unique opportunities and training like those proposed for C-MORE to lead the next generation of scientists, educators and policy makers on the path to describing new ways for understanding and managing the global marine ecosystem. Without a Center structure, it is doubtful that integrated research on this scale could ever be achieved.

4.b. MANAGEMENT PLAN FOR THE RESEARCH, EDUCATION AND KNOWLEDGE TRANSFER ACTIVITIES OF THE INTEGRATED CENTER

UH will assume the lead role in the coordination and management of the Center's activities. At UH the Center will become a new organized research unit within the School of Ocean and Earth Sciences and Technology (SOEST) and the Center Director will report to the SOEST Dean, presently Klaus Keil. The Center's education activities will be facilitated by SOEST's Department of Oceanography and by faculty from the UH College of Education. Key members of the C-MORE management team include:

- David M. Karl, UH-SOEST Professor of Oceanography and founding director in 1978 of the laboratory for microbial oceanography at UH will serve as Center Director. Karl received his Ph.D. degree from Scripps Institution of Oceanography in 1978 and joined the Department of Oceanography that same year. He has 30 years of field and laboratory experience including more than 3 years conducting research at sea. He has served as the principal investigator on more than 32 NSF grants, and since 1988 has served as one of the two co-directors of the interdisciplinary Hawaii Ocean Time-series (HOT) program. His research expertise is in the areas of marine microbial ecology and biogeochemistry. Karl is also a committed teacher, at all levels; earlier in his career he taught at an inner city vocational training high school in Buffalo, N.Y. and has maintained an active involvement in the classroom since moving to Hawaii. If C-MORE is selected for funding, Karl will devote his full-time effort to its management and to the conduct of Center-related education, research and knowledge transfer missions. His current research activities in the HOT program will be formally delegated to others so that he can give his undivided attention to the success of the Center. The Director will be responsible for: (1) administration of all management, scientific, financial and personnel aspects of the Center, (2) providing leadership to ensure the Center's effectiveness, improvement and success, (3) in conjunction with the Executive Committee (ExCom) and the External Advisory Board (EAB), judging the quality of and funding levels for the Center's incubator research projects (see below), (4) coordinating all Center activities including meetings, interim reports, formal evaluations and other Center metrics, (5) establishment and oversight of the Center's website and all interactions with senior personnel and other Center partners, and (6) the establishment and success of all education, public outreach and knowledge transfer activities.
- Judith Vergun holds a joint appointment with UH-SOEST and OSU-COAS. She will serve as the Center's Associate Director for Education, Public Outreach and Knowledge Transfer. Vergun received a dual Ph.D. in plant community ecology and science education from Oregon State University (OSU) in 1993. She has broad teaching and research experience including curriculum development. Vergun has previously served as a public information specialist, an assistant director in the film industry in Hollywood, and as a print and broadcast media specialist. She has extensive administrative experience serving previously as the project director of the Aldo Leopold Leadership Program administered by OSU on behalf of the Ecological Society of America. She currently serves as Director of the *Kumu Ola: Source of Knowledge Program*, a novel program that provides educational opportunity to underrepresented and underprivileged students in Hawaii, and as administrator/director of OSU's Native Americans in Marine and Space Sciences (NAMSS) program. If C-MORE is funded, Vergun will formally delegate her current responsibilities to others to be able to devote her full-time effort to the Center's education, public outreach and knowledge transfer missions.
- Edward Laws, UH-SOEST Professor and Chairman of the Department of Oceanography, will serve as the Center's Associate Director for Research and Technology. Laws received his Ph.D. degree in chemical physics from Harvard University before joining the UH faculty in 1974. He is currently serving in his third three-year term as Chairman of the Department of Oceanography; from 2001-2002 he served in the capacity of interim vice chancellor for research and graduate education at UH. In addition to his administrative experience, Laws has been an active and effective teacher, researcher and student mentor

for the past 30 years. In 1980 he received the prestigious Thomas Jefferson Award for his outstanding community service. His research expertise is in phytoplankton physiology and ecosystem modeling. Laws is also the lead P.I. on a recently funded NIEHS-NSF grant to establish the Pacific Research Center for Marine Biomedicine. This Center and C-MORE share several common research objectives and will use common facilities. Through Laws and other common investigators there will be a direct and vital collaboration between the two programs.

Together with Director Karl, Associate Directors Vergun and Laws will be responsible for the day-to-day activities of the Center. When the Director is out of town, either Vergun or Laws will serve in the capacity of Acting Director. An 8-person ExCom will consist of the two Associate Directors (Vergun and Laws), the Center's remaining co-P.I.s (J. Zehr, S. Chisholm and E. DeLong) and two elected members from the list of Center collaborators; the Director (Karl) will serve as an *ex officio* member. The two elected members will rotate on an annual basis. The ExCom will be responsible for: (1) ensuring the Center's effectiveness, improvement and success by providing guidance to the Director, (2) in conjunction with the EAB, evaluating the quality and determining the funding for the center's incubator projects, (3) assessing the performance of the Director and selecting a new Center Director if and when necessary.

The activities of the Center will be reviewed periodically by the EAB composed of at least six well respected scientists, educators, and science managers with expertise in the Center's overall mission. The EAB members will be from outside the affiliated organizations, and will be approved by NSF. The EAB will provide scientific and management guidance and independent oversight; they will review the Center's activities annually and provide a written report. We have already spoken to several world-class individuals who have expressed their willingness to serve on the Center's EAB if C-MORE is selected for funding. These individuals include: (1) Dr. Eddy Rubin, Director of the U.S. Department of Energy's Joint Genome Institute (JGI) at Lawrence Berkeley National Laboratory, (2) Dr. Melvin Simon, Director of the Agouron Institute and Professor of Biology at Cal Tech, (3) Dr. Norman Pace, Professor of Cellular and Developmental Biology at University of Colorado at Boulder, (4) Dr. John Hayes, Director of the National Ocean Sciences Accelerator Mass Spectrometer Facility located at Woods Hole, MA and (5) Dr. Isabella Abbott of the Bishop Museum. Collectively, their knowledge, experience and advice will be invaluable as we conduct and refine the research and education missions of C-MORE. For example, Dr. Abbott is the first woman of Hawaiian ancestry to receive a Ph.D. degree, and over her many years of teaching and research she has been a role model for this underrepresented group providing educational opportunities for at least two generations of young scientists. She is a professor emerita at Stanford University and recipient of the prestigious National Academy of Sciences Gilbert Morgan Smith Medal for her research on marine algae. Dr. Rubin currently heads the largest and most successful publicly funded microbial genomics program in the world. In addition to serving as JGI Director, Rubin (M.D./Ph.D.) also conducts research on gene regulatory sequences in mammals, and was involved in the Human Genome Project. Drs. Simon, Pace and Hayes are all elected members of the National Academy of Sciences; Drs. Simon and Pace have both received the National Academy's Selman A. Waksman award in microbiology, Simon for his discoveries in the field of bacterial chemotaxis and Pace for his pioneering research in bacterial phylogeny. We are very fortunate to have them as members of the C-MORE team. We are actively seeking one or more individuals to round out our EAB.

The Science Advisory Group (SAG) will consist of all senior personnel in the Center and will provide internal advice and guidance on all aspects of the Center's mission. Initially, monthly telecom meetings will be needed to get the stated objectives of the Center off the ground. After year one, we anticipate having bi-monthly telecom meetings in addition to a single annual meeting to discuss past research results and future plans. Finally, there will also be an Institutional Advisory Board (IAB) consisting of senior administrators from all partner institutions; at UH this would include the dean of SOEST, the dean of the UH College of Education and the vice chancellor for research and graduate education.

4.b.1. Effective Communication

Oceanography is inherently a geographically dispersed activity and yet is inherently collaborative. There is a strong tradition in oceanography for building successful and enduring research and education collaborations across great geographical distances. The ocean must be sampled and studied in place, and this demands collaborative expeditions aboard research vessels at sea. A significant component of the coordinated research activities of the Center will be conducted on the approximately semi-annual expeditions supported through the Center, as well as the more frequent cruises of opportunity that will be made available through collaborations established with the Hawaii Ocean Time-series and the Monterey Bay Time-series programs. In addition, the Center will host periodic visits by the mainland co-P.I.s, graduate students, teachers and staff technicians for the purpose of planning and conducting joint research and educational activities. Reciprocal exchanges will be arranged for members of the Hawaii team to help develop new methods, conduct laboratory-based experiments or to analyze collected samples.

The Center's directors (Karl, Vergun and Laws) will be responsible for creating an intellectual, administrative and scientific environment conducive to transdisciplinary investigation and reciprocally beneficial for all partner institutions. Synergy and integration are encouraged through strong leadership, pursuit of common goals, and the delineation of clear lines of authority, communication and responsibility. Regularly scheduled team meetings and educational events (e.g., symposia, research seminars, student research presentations) will facilitate interactions. Because the Center's investigators cover five time zones, we will schedule periodic (monthly) "all hands" meetings so that everyone can participate. Investigators and their students at mainland institutions will be encouraged to participate via teleconferencing facilities that will be established at each institution for this purpose. This will facilitate information exchange and planning. An annual meeting to review progress in the research and education missions of the Center will rotate between the lead institutions –UH, MIT, MBARI, UCSC, and OSU. The Center will also have a formal annual evaluation of their progress that will be conducted by The Institute for Learning, an Annapolis, MD-based non-profit educational research and development organization (see Budget Justification).

4.b.2. Resource Allocation and New Research Opportunities in the Center

It is impossible to predict the future research directions in the field of microbial oceanography; one needs only to look at the rapid progress over the past decade to realize that additional unexpected opportunities are likely to emerge. Therefore, in order for the Center to remain current and at the leading edge of this new sub-discipline, it will be necessary to have a fair and responsive resource management allocation plan in place when the Center is established. Although the main thrusts of the Center, namely biodiversity, metabolism and biogeochemistry, are not likely to change over the next decade, resource allocation within these broad areas may. Each Center investigator will be provided with a base budget to support his/her proposed research. To the extent possible, graduate students working on the Center's research and education missions will be provided funding to complete their degree requirements, even if their area of interest is de-emphasized in the overall research portfolio of the Center, except in the rare case where sufficient academic progress has not been made. Each year, at the Center's annual meeting, proposals for novel research and education opportunities will be presented and discussed by individuals or teams of Center investigators. These new "incubator" activities might also include collaboration with individuals outside the Center. The ExCom and EAB will meet to discuss these opportunities and will award funds – amounting to approximately 10% of the Center's funding base (up to \$400 K per annum) for these new projects. If successful, these incubator projects could lead to independent extramural funding from federal, state or private sources. The incubator awards will be given annually, usually for a period not to exceed two years.

4.c. RESEARCH OBJECTIVES OF THE INTEGRATED CENTER

The NSF-sponsored Joint Global Ocean Flux Study (JGOFS) program has just completed a 15-year field-oriented research program that was focused on marine biogeochemistry, especially the carbon cycle (Buesseler 2001; Fasham 2003). A living legacy of this program is the continuing ocean time-series sampling programs at Bermuda and Hawaii (Karl et al. 2001c). These ecosystem surveillance programs are predicated on the straightforward assumption that certain processes, such as species succession and climate variability, are long-term processes and must be studied as such. As the time-series data accumulate, new phenomena, previously “hidden in the present” (Magnuson 1990), become apparent and new understanding is derived. Already the Hawaii Ocean Time-series (HOT) research program has revealed subseasonal to interannual changes in the microbial community structure and functioning in the North Pacific Subtropical Gyre (NPSG) ecosystem (Karl 1999; Karl et al. 2001 a,b) that had previously been thought to be a time-invariant “climax” community. Because of the potential importance of the observed physical and biogeochemical variability to our understanding of the links between climate and ecosystem variability, the HOT program will continue to provide core physical and biogeochemical data sets through at least 2008. We propose to capitalize on the existence of HOT as well as similar programs in Monterey Bay and at several other sites around the world (Karl et al. 2003), by establishing a Center to collect meaningful and complementary data sets on microbial genomics and biogeochemistry and to support the conduct of large-scale open ocean perturbation experiments directed at testing relevant ecosystem-based hypotheses. The Center will also support the development of a new generation of technology to facilitate research in microbial oceanography, including the design and testing of novel genome- and microbial activity-based sensors, and a research component on ecosystem modeling (see Figure 1).

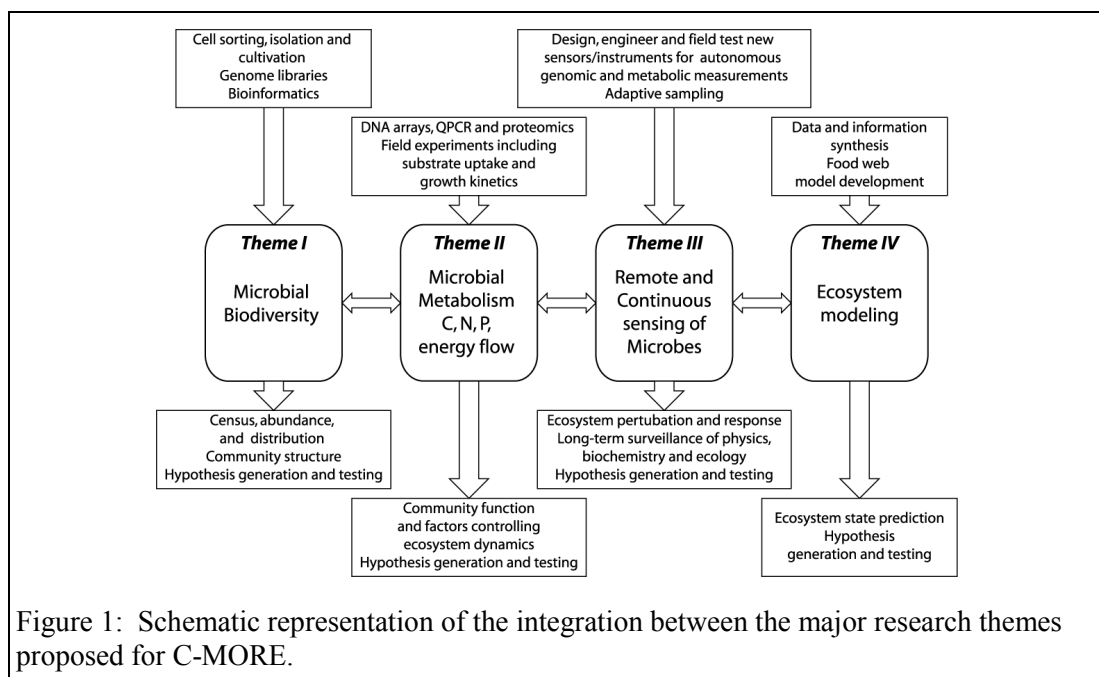


Figure 1: Schematic representation of the integration between the major research themes proposed for C-MORE.

C-MORE research will be organized around four interconnected themes: (I) Marine microbial biodiversity: From genomes and cultivation to ecology, (II) Microbial metabolism and the mechanisms of C, N, P and energy flow, (III) Remote and continuous sensing of microbial processes and links to climate variability, and (IV) Ecosystem modeling, computer simulation and prediction. The knowledge gained in our research will be incorporated into complementary field research and physical-

biogeochemical modeling efforts that are already funded by independent teams of investigators. The proposed science themes parallel contemporary research foci in marine sciences. They will steer our technology development requirements, and will serve as the basis for the development of new education and outreach programs. Much of this proposed research will build upon ongoing field and laboratory studies and will benefit from the logistical support infrastructures that are already in place in support of the HOT program and as part of the Monterey Bay Microbial Observatory and Monterey Ocean Observing System (MOOS) program in collaboration with F. Chavez and others.

In this proposal, a case will be made that the time is right for attacking major questions in microbial oceanography. First, recent advances in the application of molecular techniques to the study of microorganisms in the sea have provided an unprecedented view of community structure, diversity and potential ecological function. For example, from a single 200 liter water sample collected from the Sargasso Sea, C. Venter and his colleagues have recently reported the discovery of thousands of new bacterial species and more than one million new protein-coding genes, approximately one order of magnitude more genes than have been discovered to date (Venter 2003). By combining novel and rapidly evolving methods with more traditional techniques in microbiology, oceanography and ecology, it may be possible – for the first time – to address fundamental questions and to develop a meaningful predictive understanding of the ocean with respect to energy transduction, carbon sequestration, and bioelement cycling and their relationships to habitat variability and climate change. The strength of this forward-looking Center will reside in the synergy created by bringing together experts who traditionally have not worked together and this in turn will facilitate the creation and dissemination of new knowledge, the training of a new generation of ocean scientists, and the education of the public at large.

THEME I. MARINE MICROBIAL BIODIVERSITY: FROM GENOMES AND CULTIVATION TO ECOLOGY

I.a. Statement of the Problem: Cultivation-independent surveys of ribosomal RNA (rRNA) genes have greatly expanded our knowledge of microbial diversity on Earth (Pace 1997). Our perceptions of the distributions of major microbial groups in the sea, including *Archaea*, have been dramatically altered. Despite the impact of these discoveries, phylogenetic identification based on any single gene is insufficient to infer the major physiological, biochemical or ecological properties. Similarly, “functional gene” surveys (e.g., carboxylase, nitrogenase, etc.) can provide biogeochemically relevant diversity information, yet it can be difficult to place the diversity and distribution of “functional genes” into the larger ecosystem context. The current challenge is to integrate the now well-recognized microbial diversity into an ecological and ocean system perspective. Most of the numerically abundant microbes are presumably ecologically important, yet most have eluded isolation, and almost nothing is known about their role in bioelement cycling or ecosystem dynamics. The goal of this theme is to discover and culture novel microorganisms, and to establish several model biosystems to detect the existence of new metabolic pathways and other attributes that are relevant to global ocean ecology.

The exploration phase of Theme I will concentrate on the NPSG where an extensive set of environmental data already exists as part of the HOT program. It is hypothesized that microbial community structure there may be different from other more nutrient-replete regions, for example with significant changes in nutrient capture pathways and perhaps even basic pathways of light capture. While these expeditions will target microbial biodiversity estimation, establishment of BAC libraries (see below) and the cultivation of new strains, we will also collect a comprehensive suite of physical, chemical and biological data to place the microbial discovery data into an accurate ecological context. The expeditionary nature of these cruises will allow us to focus some of the broader educational activities around these trips including reports back to classrooms, near real time reports of discoveries, and dialogs with students. Teachers selected for the SEA program (see section 4.d) will participate in the field research and, thus, facilitate this information exchange. The research results from the NPSG will be

compared to data collected at our coastal study site in Monterey Bay to provide a comparative study of ecosystem dynamics and microbial controls of bioelement cycling and energy flow.

Model biosystems have been a mainstay of research in microbiology, including both basic and applied subdisciplines. *Escherichia coli* and *Salmonella typhimurium* are gram-negative bacteria of the family *Enterobacteriaceae*. Both species have been known since the emergence of microbiology as a modern scientific discipline, and both have been pre-eminent as model biosystems in research laboratories for nearly a century. The careful and systematic study of these bacteria led to major breakthroughs in physiology, metabolism and genetics, as well as viral morphogenesis. We now have a remarkably detailed, though still incomplete, picture of their biosynthetic pathways, metabolic regulons and growth processes (Neidhardt 1987 a,b). A primary goal in microbiology, development of a central dogma and complete cellular and subcellular paradigm, has been achieved largely through the study of these two model organisms. We endeavor to establish a diverse cohort of new model biosystems. Collectively, detailed laboratory study of these model microorganisms is likely to provide unique information about life and survival in the open ocean and this in turn will lead to new knowledge about our biosphere and the potential impacts of climate variability. The choice of this troupe of model biosystems is dictated by the facts that these are known to be key, numerically abundant and ecologically relevant organisms in the sea, and several of the Center's investigators, namely Waterbury, Chisholm, Rappé and Kolber, were responsible for the original discovery, isolation in pure culture, or successful laboratory propagation of one or more of these novel species. We recognize that the list of biogeochemically important (but currently unrecognized) marine microbial species will grow. Accordingly, we anticipate the target list of microbial groups will also grow along with the ecological context providing us the necessary information to help prioritize the overall research goals for the Center. The initial list includes:

(1) ***Prochlorococcus*** was discovered in the marine plankton less than 20 years ago, and is now recognized as a cosmopolitan clade of oxygenic phototrophs that dominates many open ocean marine ecosystems, especially the NPSG. Recent genome sequence analysis of two ecotypes, one adapted for growth in low light levels (MIT9313) and the other for growth in high light levels (MED4), indicates that the two genomes differ in size by 40% (Rocap et al. 2003); clearly these two strains have different gene inventories, physiologies and metabolic regulation and, hence, ecological niches. Whole genome arrays for MED4 and MIT9313 and the genomes of two viruses that infect them are available in Chisholm's laboratory.

(2) ***Synechococcus*** is represented by an extensive world-wide collection and can be cultured under defined laboratory conditions; there is also an extensive data set on their distribution, dynamics and growth in the global ocean (Waterbury et al. 1986). A genetic system for *Synechococcus* was developed by Brahamsha (1996) and the genome of strain WH8102 was recently sequenced and closed (Palenik et al. 2003). The whole genome array constructed by F. Morel at Princeton is available to Center investigators Saito and Waterbury for C-MORE-sponsored studies. As for *Prochlorococcus*, there are numerous clades (species?) with broad phenotypic and ecotypic diversity.

(3) ***Trichodesmium*** has been studied extensively since its discovery more than a century ago. A culture collection with representatives from all major ocean basins exists at WHOI (Waterbury). The distribution and ecology of this genus of filamentous N₂-fixing cyanobacteria has been studied extensively, including at Sta. ALOHA. The IMS-101 strain of *T. erythraeum* has recently been sequenced by JGI and a whole genome (7000 genes) array is under construction in Waterbury's laboratory.

(4) ***Crocospaera*** has been studied in the field (Zehr et al. 2001) and 12 axenic cultures are present at WHOI. This genus of unicellular cyanobacteria also fixes N₂; the genome of strain WH8501 is currently being sequenced by JGI.

(5) ***Proteorhodopsin-containing bacteria*** have recently been discovered based on sequence analysis of a Bacterial Artificial Chromosome (BAC) clone derived from a SAR86 bacterium. A new type of rhodopsin (proteorhodopsin), the first rhodopsin ever to be found in the domain *Bacteria* (Béjà et al.

2000a,b; Bèjà et al. 2001), has great potential significance. Biochemical and biophysical analyses of this novel bacterial rhodopsin now show that it functions as a light-driven proton pump (Bèjà et al. 2000b), and so has revealed a new type of phototrophy in the world's ocean. At present we have few data on the ecological significance of this discovery.

(6) *BChl a*-containing bacteria have recently been shown to account for a measurable amount of phototrophic electron transport in the sea, and may constitute a large fraction of marine bacterioplankton in marine surface waters (Kolber et al. 2000). Numerous BChl *a*-containing strains from surface waters of the coastal N. Atlantic Ocean, N. Pacific Ocean, equatorial Pacific, Southern Ocean and Mediterranean Sea have been isolated by Kolber et al. (2001). These microbes appear to be facultative anoxygenic phototrophs (Kolber et al. 2001), but their quantitative role in global ocean ecology is currently unknown.

(7) *SAR 11* is a representative of the α -proteobacterial lineage, and may be the most abundant group of heterotrophic microorganisms in the global ocean (Rappé et al. 2002; Morris et al. 2002). It has been estimated that members of the SAR 11 clade may constitute 30-50% of the total surface microbial assemblage, by numbers. Several strains of SAR 11 have recently been isolated in pure culture; they appear in the laboratory as tiny ($\sim 0.01 \mu\text{m}^3$) heterotrophs, but with significant phylogenetic and physiological differentiation within the clade. At 1.30 Mbp, the SAR11 isolates possess one of the smallest genomes currently known for a free-living microorganism (Rappé et al. 2002). The genome of SAR11 strain HTCC1062 has recently been sequenced to closure, and its annotation is in progress.

(8) *Planktonic crenarchaeota* comprise a large fraction of the total microbial plankton below the euphotic zone (depths > 200 m); they are nearly equivalent in cell numbers to bacteria at depths greater than 1000 m (DeLong et al. 1999; Karner et al. 2001). Despite the fact that planktonic crenarchaeota constitute 20% of all microbial cells in the global ocean, they remain a poorly understood component of Earth's carbon cycle. Throughout the mesopelagic and abyssopelagic zones (200-5000 m), crenarchaeota and heterotrophic bacteria co-exist and, presumably, compete or otherwise somehow partition the dissolved organic matter resource spectrum. Alternatively, it is conceivable that the crenarchaeota have a fundamentally different mode of metabolism, e.g., chemolithoautotrophy, or some yet to be described novel physiology.

In addition to the prokaryotes listed above, bacterial viruses (or phages) that infect each group will also be isolated and investigated. Phage infections can be a significant source of mortality for prokaryotic plankton (Wommack & Colwell 2000). Phage infections can also have important non-lethal interactions with their hosts such as mediating horizontal gene flow – a key process in microbial evolution – and altering host phenotype. In the latter case, a phage entering a host cell will integrate its DNA into the host chromosome. Subsequent expression of some phage genes can fundamentally alter the phenotype of the host in a process known as lysogenic conversion. As just one dramatic example, recent studies with *Vibrio cholerae* have documented a complex interaction involving the bacterium and two phages that results in the transformation of a benign bacterium into a serious human pathogen (Karaolis et al. 1999). Many other examples of lysogenic conversion have been described (Barksdale & Arden 1974), but to what extent this process is important for marine microbial ecology and biogeochemistry remains completely unknown. The dramatic examples of lysogenic conversion that have been studied, however, strongly suggest that a full understanding of the ecology, genetics and physiology of a given microbe may only be attained by considering the extended phenotype that is proscribed in part by these virus-host interactions. Despite the ecological importance of the model organisms being initially targeted as part of C-MORE, we know little about the impacts of phage on any of them. Waterbury and his colleagues (Waterbury and Valois 1993; Sullivan et al. 2003) have described cyanophages that infect *Prochlorococcus* and *Synechococcus* and this will become the first model system for investigation in C-MORE; others will be added as new viruses are isolated and identified.

Our approach to the documentation of novel marine microorganisms will employ both molecular-based and organism-based methods. The directed cultivation efforts will rely on the well known and successful approach of enrichment culture (Poindexter & Leadbetter 1986), but with several non-

traditional treatments to favor the proliferation of the novel, target microorganisms previously discussed. For example, we will employ high throughput single-cell sorting methods as well as long-term, *in situ* nutrient gradient techniques to enrich for specific microorganisms. For *Prochlorococcus*, in particular, cell sorting will be a useful tool as it has been applied successfully for this purpose in the past. Relatively pure samples of *Prochlorococcus* can thus be obtained, and used for the construction of BAC libraries (see below) for the analysis of the true genomic diversity within this group of organisms. This can also be used to isolate single cells for cultivation, thus expanding the culture collection in the Chisholm lab to include strains that would be unlikely to grow up in enrichment cultures. The strategy adopted for pure culture isolation will vary depending on the anticipated metabolic characteristics of each target group. We will also isolate lytic viruses for each of the model prokaryotes, and endeavor to describe any mitomycin C-inducible temperate viruses that these groups may harbor. Specific protocols will be tailored to the growth characteristics of each host species, but will essentially follow published protocols (Suttle 1993; Waterbury and Valois 1993; Sullivan et al. 2003).

We are aware of the limitations associated with the use of culture techniques in the isolation of marine microbes. For this reason we will also endeavor to describe the diversity of relevant eco-physiological genomes of yet uncultured species by constructing Bacterial Artificial Chromosome (BAC) libraries. The first steps in this direction used lambda (Schmidt et al. 1991) or cosmid-like vectors (Stein et al. 1996) to isolate large genome fragments from indigenous (but uncultivated) microbes. Now, large genome fragments in excess of 100 kilobase pairs in size can be isolated from uncultivated microbial groups (Béjà et al. 2000a). A few modest BAC libraries have already been prepared for surface waters at Sta. ALOHA (22°45'N, 158°W) by one of the prospective Center PIs (DeLong), but much more work is necessary to expand the genome content of these libraries and to analyze and interpret them properly. These open ocean BAC libraries will complement, and provide the basis for comparison, to four coastal ocean BAC libraries (1 m, 80 m, 100 m, 750 m) that are now under extensive analyses from Monterey Bay by DeLong and colleagues. The major focus will be on the organismal and gene content in the libraries, and fine scale genomic differences, within the target microbial groups mentioned above, and others yet to be identified. We will also specifically identify and analyze integrated phage sequences within the BAC libraries that may not be represented in the culture collections. While some phage-like sequences may turn up fortuitously, we will also screen BAC libraries using the phage integrase gene as a marker. Integrase is an enzyme mediating the insertion of phage DNA into the host genome. Integrase-like sequences will be identified among BAC library clones by multiplex PCR or by probing of high-density clone arrays. Integrase sequences are very divergent (Argos et al. 1986), so primer pairs will be designed for each integrase family known to have target phage representatives. These BAC libraries will serve as a source of genomic data, a source for generating and, in some cases, testing hypotheses. These libraries will also provide the basis for a new kind of science: genome-enabled comparative ecosystem analysis. This new endeavor should provide a much better appreciation of the variability of organisms and gene distributions, since most available data today provide us with only "snapshots" of microbial communities. The use of microarrays based on the BAC library sequences will define for the first time at high resolution the spatial and temporal variability of the major microbes in the ocean. In addition, universal and/or regional metabolic themes, novel biochemistries and physiologies, syntrophic interactions and metabolic complementarity, should also emerge from these comparative community genomics approaches.

THEME II. MICROBIAL METABOLISM AND THE MECHANISMS OF C, N, P AND ENERGY FLOW IN THE SEA

II.a. Statement of the Problem: C, N, P and energy flow are fundamental properties of biological systems. Most of our current understanding of marine ecosystem processes is based on "black box" biogeochemical transformation rate measurements, and descriptive biology of micro- and macroorganisms. As a recent American Academy of Microbiology report emphasizes, a complete

understanding of the role of microorganisms in nature will require much more than a biotic inventory – it demands a comprehensive understanding of gene expression patterns that underlie trophic interactions and biogeochemical cycling in the marine environment (Stahl and Tiedje 2002). Genome sequence analysis has revealed unexpected genetic plasticity, horizontal DNA exchange and metabolic/biochemical innovation, so we should anticipate ecosystem diversity and complexity. Major questions remain as to how biogeochemical cycles of N and P are linked to ecosystem carbon production, export and sequestration, the role of individual species and functional groups in ecosystem productivity and how biogeochemical cycles interact. Eventually, complete transcription profiles of selected, well-studied marine microbes in response to defined environmental perturbations will provide invaluable data on what we believe to be a complex and interactive set of genomic and ecophysiological phenomena. For example, the importance of P in microbial metabolism and, therefore, in ecosystem processes is best demonstrated by the impressive response of bacteria to *Pi*-stress or *Pi*-limitation. Under such conditions, substantial and coordinated changes occur that prepare the cells for competition and survival, including the synthesis of more efficient and specific *Pi*-capture systems, and the ability to utilize a broader range of potential P-containing organic substrates, including phosphonates. A comprehensive analysis of protein synthesis during *Pi* limitation and phosphonate metabolism was conducted for *E. coli* (VanBogelen *et al.*, 1996). The aggregate mass of proteins responding to *Pi* limitation or to growth on phosphonates was 30-40% of the total mass of the cell, once again emphasizing the key role of P in cellular metabolism. This impressive laboratory study can be used as a blueprint for bacterial growth in the marine environment, at least for the purposes of ecological prediction and hypothesis testing. In C-MORE, we will conduct similar studies using our model biosystems.

II.b. Objectives and Methods: A goal of Theme II is to investigate the pathways of nutrient and energy flux in the open (Sta. ALOHA) and coastal (Monterey Bay) ocean, using *in situ* field experiments and whole ecosystem perturbations as a means to test relevant ecological hypotheses. Both sites are well characterized and have ongoing ocean time-series programs in place that will facilitate the objectives of the Center. By targeting genes and proteins involved in specific biogeochemical functions, information on the identities, diversity and function of microorganisms involved in bioelemental and energy fluxes can be obtained and linked to rates of microbial growth and metabolism (Zehr and Hiorns 1998). Gene sequence diversity information can be used to design specific DNA arrays (DNA macroarrays and DNA “chips”) and quantitative PCR (QPCR) and RT-PCR approaches used for assaying temporal dynamics of populations and gene expression responses, and for screening genomic libraries. Using these tools it is possible to examine species distributions, and determine which microorganisms are actively transcribing genes, which makes it possible to determine how microbial community composition is linked to ecosystem dynamics and ultimately to identify the factors that constrain or regulate these processes.

Using combined molecular-genomic, biogeochemical and biophysical approaches new metabolic pathways (Béjà *et al.* 2000 a,b) and novel unicellular cyanobacteria involved in nitrogen fixation (Zehr *et al.* 2001), for example, have been identified. In the case of N₂ fixation, several of the prospective Center investigators have developed approaches for characterizing nitrogenase gene diversity using DNA macroarrays (Steward *et al.* 2004, Jenkins *et al.* 2004), assaying the distribution of specific phylotypes using QPCR (Short *et al.* in press) and examining gene regulation using quantitative RT-PCR (Zehr *et al.* 2001). We will continue to develop new procedures to determine how processes involved in other key steps of C, N and P assimilation are linked to microbial community composition. Our general approach is to proceed on development along three lines: (1) developing nucleic acid based tools for characterizing populations involved in biogeochemical fluxes, (2) examine short time scale population dynamics, building towards a long-term goal of using remote sampling technology and (3) perform microcosm and mesocosm experiments to determine the factors involved in shaping community composition through the responses of key genes involved in nutrient cycles.

These new methods will be used to complement traditional, population-based experimentation using specific fluorogenic-substrate additions, target enzyme assays and stable and radioactive tracers, and other common approaches to link processes involved in energy and nutrient metabolism to microbial biodiversity at a functional level (Karl 1986). Experimental manipulations will be an important component allowing us to test hypotheses on the factors regulating processes, and how microbial metabolism and biodiversity are related to biogeochemical fluxes. We will continue to examine recently discovered processes of aerobic anoxygenic photosynthesis, rhodopsin-mediated energy metabolism and new organisms involved in nitrogen fixation.

The availability of N and P are major controlling factors constraining ecosystem production, including the replication of viruses (Tuomi et al. 1995; Wilson et al. 1998). N₂-fixation, since it draws on the essentially inexhaustible reservoir of atmospheric nitrogen, can have wide-ranging effects on the nutrient status and even the structure of the food web. One major effect of the enhanced growth of N₂ fixing microorganisms, is the drawdown of P and the shift from N to P limitation (Karl 1999). There is some experimental evidence that limitation specifically by P, rather than N, controls viral replication as well (Bratbak et al. 1993; Wilson et al. 1996); a putative P-sensing mechanism has been reported for the marine Roseophage SI01 (Rohwer et al. 2000). We will examine the factors controlling N₂-fixation by different species (by examining the nutrient and light control of nitrogenase gene expression), determine how N₂-fixation is influenced by the relative supply of N, P and atmospherically derived iron (Fe), and investigate the possible role of biochemical interactions with N and P that may affect greenhouse gas formation, especially nitrous oxide and methane. Assays to address these research questions will include *in situ* measurements and experimental manipulations. Gene arrays, proteomics and rate measurements will be combined to determine how environmental factors control nitrogen fixation from the molecular to species and ecosystem levels. This research will be facilitated by the recent genome sequences of *Trichodesmium* and *Crocospaera*, two of our selected model biosystems. Finally, another major limitation to our understanding of microorganisms in the sea remains our extremely poor characterization of the large and vital pool of dissolved organic matter (DOM) in the sea (Hansell and Carlson 2002). The DOM pool links primary and secondary production, sustains the microbial loop and its attendant consequences to C-N-P cycles, controls the bioavailability of essential trace elements – among other roles – yet we still have a very poor understanding of DOM sources and sinks, molecular composition and size distribution of the bulk pool, and impact of DOM on the regulation of microbial processes in the sea. Studies of the metabolism of DOM using combined process-oriented and molecular-genetic-genomic tools is likely to yield exciting new information about this important, but largely unknown source of C, N and P.

Our integrated field experiments will first be performed in bottle (microcosm) assays, but in years 3 and 4 large-scale open-ocean mesocosm experiments will be constructed to test ecological predictions based on the results of theme I observations and theme II experiments. A set (at least 8) of large (4 m diameter x 30 m deep) marine enclosures (mesocosms) will be constructed and used to test these specific ecological hypotheses, e.g., the role of Fe on N₂ fixation, P-turnover and viral lysis rates, and hence ecosystem structure and function. Mesocosms have proven to be useful for the study of nutrient dynamics, organic matter production and recycling and the response of the microbial assemblages to short-term perturbations (Kuiper 1977; Menzel & Case 1977; Steele 1979; Davies 1984). The use of multiple enclosures will allow for replication and variable treatments for a given experiment. This “whole ecosystem” experimental approach is probably the most direct and most relevant for understanding ecosystem dynamics and for improving our prediction of the response of the ocean to natural and anthropogenic changes (Carpenter et al. 1995). As with the research expeditions in general, these large-scale perturbation experiments provide an educational opportunity for our K-12 and informal education programs as we use real-time reporting to allow students to follow the dynamics of the experiment to predict its outcome, based on information provided in the food web and nutrient dynamics lessons in their curriculum.

THEME III: REMOTE AND CONTINUOUS SENSING OF MICROBIAL PROCESSES AND LINKS TO CLIMATE VARIABILITY

III.a. Statement of the Problem: Oceanic ecosystems have changed radically during the past century, in part, due to anthropogenic impacts. Fossil fuel consumption, land use changes and numerous by-products from a manufacturing-based society have now begun to alter our seascapes and the microbial communities that inhabit them. Nevertheless, marine microorganisms are prepared for change; selection and evolution based on genetic variations of individuals are ongoing long-term (thousands to millions of years) processes that have continuously responded to perturbations in environmental forcing. What is less certain is the impact of these unprecedented climate variations on the shorter-term (decade to century time scales) stability and sustained and efficient functioning of natural ecosystems, and how they will be affected from individual microorganisms to large-scale biogeochemical cycles. In this theme we will focus on the development and field testing of novel sensors and instruments that will improve our observational capability, which in turn will advance our understanding of ecological processes in the sea and help improve the accuracy of our model-based predictions (also see Theme IV).

III.b. Objectives and Methods: Our goal in this theme is to develop a new class of sensors that can be used on a variety of emerging oceanographic platforms (e.g., moorings, drifters, autonomous vehicles) in support of a sustained mode of ocean observations with the ultimate goal being to understand microbial responses to habitat forcing on a range of time frequencies. Within this theme, we propose to: (1) design, construct and test a miniature Networked Oceanographic Sensor Array (NOSA) framework with well-defined communication protocols, and generic mechanical/electrical architecture that is adaptable to a variety of configurations and ocean sensing requirements, (2) design, construct and test a new generation Environmental Sample Processor (ESP) that automatically collects a water sample, concentrates the microorganisms therein and conducts an autonomous DNA (or probe)-based application to enable the remote time-series identification and quantification of a particular microbial group or process, and (3) refine the design and then field test a new generation Time-Series-Submersible Indicator Device (TS-SID) for autonomous high-frequency microbial rate estimation (Taylor and Howes 1994). These new technologies will be developed in conjunction with the science mission to provide novel data on microbial populations and dynamics. For example, the NOSA effort, directed by Center investigator Z. Kolber, will focus initially on sensors designed to measure: (1) key photosynthetic parameters including, the light-induced fluorescence transient response based on fast repetition rate fluorometry, (2) dissolved oxygen dynamics using novel sensors based on oxygen quenching of the phosphorescence of Pd-porphyrine encapsulated in a cellulose acetate butyrate matrix, (3) pCO₂ using a novel fluorescence resonance energy transfer (FRET) technique where a long-lifetime fluorophore and protonation-sensitive dye are both immobilized in a sol-gel matrix, and (4) nitrate based on ultraviolet absorption spectra. These and other sensors will further provide the means to explore the presence and evolution of chemical microgradients in marine environments and how they affect the magnitude and variability of *in situ* microbial processes. The ESP effort, directed by Center investigator C. Scholin, will focus initially on detection of the *nifH* gene as an indicator of N₂-fixation, but will eventually move in the direction of higher fidelity analyses such as the development of microarrays that target multiple expressed genes. The initial field testing of both the NOSA and the ESP arrays will be conducted on MBARI moorings and on the Monterey Bay cabled observatory. The TS-SID effort, directed by Center investigator C. Taylor, will initially focus on a mooring-compatible automated instrument that is capable of repeated *in situ* microbial rate experiments using selected stable or radioisotopic tracer additions. The TS-SID has already evolved to a point where it is capable of withstanding the rigors of long term deployment and we will continue to test and to refine its full potential to address Center goals. Once these new instruments and microbiogeochemical sensors are developed and field tested to establish precision, accuracy and stability we will deploy them on moorings, autonomous underwater vehicles, gliders and floats of opportunity and as part of our mesocosm arrays (see Theme II). We plan to use these data for synthesis and modeling efforts to

investigate the relationships between and among physical/anthropogenic forcing, primary and secondary bacterial production, and the exchange of carbon between the ocean and the atmosphere. These and other modeling efforts (see Theme IV), coupled to the data flows from moorings and other platforms can provide unique educational opportunities, particularly in museums and aquaria in Hawaii and coastal California.

THEME IV: ECOSYSTEM MODELING, COMPUTER SIMULATION AND PREDICTION

IV.a. Statement of the Problem: Beyond the challenge of understanding contemporary ecosystem dynamics is the anticipation and prediction of future changes in ocean ecology. There is now ample and growing evidence to suggest that major changes in the structure of the NPSG can occur over decadal time scales (Karl et al. 2001 a,b). This altered view of biogeochemical dynamics in the ocean may have a profound influence on how one thinks about marine planktonic ecosystems, including the potential impacts of natural or human-induced environmental change and its relationship to carbon sequestration. For example, N₂ fixation may be controlled by the atmospheric deposition of Fe and the degree of surface ocean stratification, both of which are variable, climate sensitive parameters. Total atmospheric dust transport is also affected by humankind, including population demographics, global economies and land use patterns. These complex natural and anthropogenic interactions, with multiple potential feedback loops, provide a mechanism for biogeochemical variability in otherwise “stable and homogeneous” biomes.

The response of marine food webs to environmental changes cannot be based on the predictions of static models whose parameters are chosen based on the goodness of fit of model output to currently observed phenomena. The reason is that communities of organisms are adaptive. In order to understand how biological communities adapt, it is first necessary to understand the principles that drive the organization of those communities. As noted by Patten (1993), “The admissibility of virtually any system configuration, within given physical and resource constraints, does not mean the processes of ecosystem organization are lawless. The challenge is to find the laws.”

Lotka (1922) argued more than 80 years ago that natural selection tends to maximize the energy flux through a system, so far as this goal is compatible with the constraints to which the system is subject. Odum (1983) extended Lotka’s ideas about energy flow. He argued that ecological succession is due to the maximum power principle applied to ecosystems, and that the process culminates in stabilized systems with maximum biomass and symbiotic function between organisms per unit of available energy flow. Based on theoretical work, Cropp and Gabric (2002) concluded that within the constraints of the external environment and the genetic potential of their constituent biota, ecosystems evolve to the state most resilient to perturbation. The idea that natural selection tends to favor biological communities that are stable within a given environmental framework is certainly not new (Steele 1974; May 1974), but direct field tests in open ocean ecosystems have never been conducted.

IV.b. Objectives and Methods: The challenge is especially difficult when predictive capability of biogeochemical systems- with their well known non-linearities, thresholds, and feedbacks- is sought. Laws et al. (2000) have developed an adaptive pelagic food web model that follows up on the logic presented above. The model gives remarkably good descriptions of the so-called export ratio and of the relative biomass of autotrophic and heterotrophic microbes in aquatic systems (Laws 2003). This model will be refined as part of the C-MORE effort, and data derived from the laboratory studies of the model biosystems and from the ongoing field observations will be used to improve the predictability of the model. Specifically, the refined model will: (1) include functional groups not considered by Laws et al. (2000), e.g., nitrogen fixers, (2) explore the possibility of simplifying portions of the model food web, e.g., consider the microbial loop as a single functional group, (3) explore the possibility of applying a similar approach to microbial food webs in other than pelagic environments, e.g., the mesopelagic. The

data sets from Themes I-III, above, will be used in combination with complementary ocean data sets obtained through collaborations with the Center partners. The simulations will lead to new hypotheses that will be tested using approaches described in the earlier sections of this preproposal.

Finally, the NOPP-funded PARTnerships for Advancing Interdisciplinary Global Modeling (PARADIGM) will serve as a key interface to C-MORE. PARADIGM is an ongoing broad-based, multi-investigator (16 co-P.I.s) ocean biogeochemistry modeling and prediction project led by L. Rothstein (U. Rhode Island). This research team is committed to building new advanced models of ecology and biogeochemistry for understanding and predicting the future states of the ocean. The PARADIGM group combines the expertise of observers and modelers, ecologists and physicists, biogeochemists and numerical specialists. A key scientific goal is to produce a rigorous, model/observation-based comparison of ecological and biogeochemical dynamics of the NPSG, the primary study area proposed for C-MORE. D. Karl, the proposed C-MORE director, is also a co-P.I. in PARADIGM. E. Laws, one of the two proposed associate directors of C-MORE who will lead the Theme IV efforts, along with Karl, will establish the main collaboration with PARADIGM. Over time it is expected that other Center investigators will also be actively involved in these ecosystem modeling efforts.

DATA AND INFORMATION MANAGEMENT AND DISTRIBUTION, AND BIOINFORMATICS

An important aspect of the overall vision of the Center includes an explicit requirement for data/information quality control and assurance, as well as for data archiving, management and distribution. All Center investigators will agree to provide common data sets and other products as quickly as possible for distribution to the other members of the Center's team, at most within 1 year of collection. The data would include the environmental, population and rate measurements from cruises, as well as all relevant laboratory results, including genomic sequence data and ecosystem model output. These data sets will be served from a workstation at the Center's administration facility at UH, and will be the vehicle for disseminating information and knowledge to K-12 students, teachers and the public at large. In all likelihood, C-MORE data and derived products will initially be built into the existing HOT program Data Organization and Graphics System (HOT-DOGS) which has been used for the past 15 years to provide unlimited access to HOT program data in a user-friendly format via the Internet. Over the longer term we will establish an OpenDAP system to serve our data. The data access protocol, OpenDAP software, is now freely available and since 1995 has served as the basis for data and information distribution for the National Virtual Ocean Data System.

We have not explicitly included bioinformatics as a central theme of C-MORE even though this will be an important component of our overall effort leading to new knowledge and the generation of novel hypotheses in the Center. The bioinformatics support will be derived from two independent but complementary programs, one at MIT (Chisholm and DeLong) and the other at UH (Rappé). C-MORE Co-P.I.s Chisholm and DeLong have a close working relationship with the faculty in the Bioengineering Division (DeLong will have a joint appointment with this division when he joins MIT later this year), which is one of the loci of bioinformatics at MIT. In particular, Chisholm and DeLong have been working with the CSBi group (Computational and Systems Biology), and D. Lauffenberger (Head of Bioengineering) to develop an interdisciplinary Center for Microbial Systems at MIT, which when established could be a great asset to C-MORE. Furthermore, at UH, M. Rappé is leading an effort to establish a collaboration with the Maui High Performance Computing Center (MHPCC). This cooperative agreement will provide a database and user interface for analyzing microbial genome data. Rappé will initially use this new facility to examine genomic heterogeneity within the SAR11 clade. The collaborative arrangement with MHPCC will provide a new server in Rappé's laboratory at HIMB, processor time at the MHPCC and a technician to build and administer the user interface for the database. This complementary proposal, if funded, would greatly enhance C-MORE.

4.d. EDUCATION AND HUMAN RESOURCE DEVELOPMENT OBJECTIVES OF THE INTEGRATED CENTER

4.d.1. Rationale of Education and Human Resource Development Plan

Students are inadequately prepared or completely unaware of marine sciences because teachers have not been trained to integrate marine science into their curricula. Also, there is a significant void in current marine science education because we have little understanding of the genetic basis of marine microorganisms and some ocean processes. Underrepresented and underserved students have few ethnic scientist or educator role models, and, local knowledge relevant to their lives is invisible in most curricula. The greater community has little opportunity to learn the value of integrated marine science research, education and culture due to a lack of information or effective communication and outreach.

Several recent changes position our proposed Center to successfully address these problems: UH-Manoa now offers undergraduate degrees in Marine Biology and Global Environmental Sciences (with a marine track) that encourage development of college preparatory marine science programs. Senior personnel of the proposed Center are members of the College of Education's Teacher Education Committee for Science that provides input on the content preparation of science teachers. New grants to the College of Education for K-12 science teacher education and curriculum development connect standards-based science to local culture and community; and new NSF-funded programs (GK-12, Tribal Colleges and Universities, Hawaii Networked Learning Communities, and the Louie Stokes Alliance for Minority Participation) support underrepresented students in STEM disciplines. To graduate from UH-Manoa, students are required to take 3 credits of a course addressing the intersection of Hawaiian, Pacific, and/or Asian cultures. C-MORE teams will develop a rigorous, culturally relevant 3-credit undergraduate oceanography course, integrating western science, traditional knowledge, technologies and practices, to be offered by the School of Ocean and Earth Sciences and Technology at UH-Manoa and to all partners.

The C-MORE Education goals and objectives template adapts proven principles of several key long-established (ten years or more) programs in the US that increase the number of underrepresented students earning BS degrees in science, technology, engineering and mathematics (STEM) and who are eligible for graduate schools; that enhance teacher STEM competencies; and, increase the greater society's awareness of the value of STEM (McManus et al. 2000, Cuker et al. 2001). One of these key programs is the Oregon State University Native Americans in Marine and Spaces Sciences (NAMSS). The current UH-Manoa (NSF-TCUP) Kumu Ola Program replicates the 14-year NAMSS program wherein 98% of all undergraduates have earned BS degrees in STEM within 4-5 years and more than half have continued in graduate schools. These statistics are better than in any community of learners, including Caucasian students, and strongly indicates that this model would be effective for all students, not just minorities. In this inquiry-based approach, students feel that they are doing something relevant with their lives and there is community understanding and participation at all levels. The basis for success is the multi-generational community of learners that form unbroken pathways of opportunity from Preschool to gray.

C-MORE's plan is a suite of programs similar to Oregon's that fall into five main categories:

1. Professional development opportunities for teachers
2. Curriculum adaptation, development, & distribution
3. Student recruitment and retention (K-12, undergraduate, and graduate)
4. Partnerships with existing programs to amplify educational outreach goals
5. Communication training to facilitate progress in the above areas

The integrated plan holds promise to increase the number of students who find school science engaging and relevant and are thus more likely to become members of a productive, diverse, and educated workforce. Communities stand to benefit from cultural values and practices that emphasize societal survival through sustainable practices and environmental ethics. Participants in the proposed Center's collaborative, cross cultural science programs will learn valuable technical, scientific, and

interpersonal skills enabling them to participate wisely and effectively in local and global multicultural worlds.

4.d.2. Goals and Objectives

Goal 1. Generate a pool of teachers who are highly qualified to teach science and relevant mathematics, and know how to integrate local values and knowledge into curricula.

Objectives to accomplish Goal 1:

a) Create teams of K-12 teachers, scientists, educators, undergraduate interns, graduate students, post-docs and community members to participate in the Center's research, fieldwork, labs, and curricula development and implementation. This includes teams at partner institutions.

a.1) Select K-12 teachers interested in becoming excellent marine science educators and becoming participatory members of the Center's teams.

a.2) Convene meetings to introduce the Center's mission and goals; discuss team focus options and opportunities. Collaboratively identify teams. Teams will have the flexibility to reorganize to meet needs of specific projects and programs as the Center evolves.

b) Provide credit for teachers and funds to facilitate and support their professional development training.

b.1) Create annual two-week C-MORE Scientist-Educator educator institutes for high school, college, and university faculty modeled after NSF's Teachers Experiencing Antarctica and the Arctic (TEA) program with a focus on microbial oceanography and the technologies used to explore and teach this subject. Educator travel costs and hotel accommodations will be covered by the Center. Educators will have the opportunity to participate fully in research including the research cruises. Necessary supplies and assistance will be provided to the teachers to facilitate implementation of their new knowledge into curricula. The institutes will be modeled after past workshops run by C-MORE partners, the MATE Center and Bio-link, who combined have worked with approximately 1800 educators.

b.2) Provide funds to support costs of traditional voyaging science training courses offered by C-MORE's partner the Polynesian Voyaging Society (PVS). Facilitated by C-MORE, teachers will study in PVS Crew Training classrooms and on one- or two-day voyages aboard the honored traditional voyaging canoe, the *Hokule'a*. Scientists, teachers, students, crew and traditional navigators will participate in C-MORE's research by collecting microbial samples during the *Hokule'a Statewide Education Sails* throughout the Hawaiian Islands, including the Northwest Hawaiian Islands. This will provide unique comparisons of ocean microbes in pristine areas with other inter-island microbial communities. Traditional voyaging techniques, scientific concepts and methods will be taught at sea while voyaging.

b.3) Provide funds to facilitate opportunities for teachers to further develop their ability to provide a cultural context to marine science by working with educators and voyagers from the Halau Ku Mana (HKM) public charter school. Teachers will hone their newly learned skills by apprenticing with Bonnie Kahape'a, Navigator and instructor and HKM's high school students aboard the school's floating classroom - the *Kanehunamoku*, a traditional voyaging canoe.

c) Work with the College of Education to modify teacher certification requirements so that a knowledge of marine science is strongly recommended for pre-service elementary teachers and required for secondary science teachers. Teachers' marine science knowledge will be upgraded by providing tuition and supplies for upper division/graduate courses focusing on Center research such as EDCS 433 Interdisciplinary Science Curriculum and EDCS 450 Materials and Methods in Science (Nationally accredited elementary education programs require only a semester each of biological and physical sciences. More is needed for our teachers to be marine science competent).

d) Provide funds to pay for substitute teachers during the absence of C-MORE team teachers from their regular classes. Lack of funds to support leave time is a persistent roadblock to teachers' professional development.

Goal 2. Infuse marine science research and education into curricula.

Objectives to accomplish Goal 2:

a) Develop curricula that integrate local values and knowledge into ocean science lessons.

a.1) Center teams will research available science curricula and determine what is missing with the intent to develop new curricula that integrates marine science with traditional knowledge and cultural values.

a.2) Convene curricula development workshops for C-MORE's teams. Polynesian Voyaging Society Master Navigator Nainoa Thompson, Mau Piailug, other Kupuna, Navigator Bonnie Kahape'a, scientists and educators from UH, MIT, Kamehameha and other K-12 schools will develop standards-based, culturally competent microbial oceanography curricula. Small- and large-group workshops will be offered at convenient locations for all partners, for example at UH-Manoa, the Maritime Museum, Hanauma Bay Education Center and at partner institutions.

a.3) Collaborate with NOAA National Education Coordinator Paula Keener-Chavis to enhance the Ocean Explorer curricula - *Learning Ocean Science through Ocean Exploration* and *The Northwest Hawaiian Islands Exploration - Educators Guide*. Center teams will develop *Microbial Oceanography: Science and Culture* - a series of lesson plans for grades 5-12 that meet National Science Education Standards.

a.4) Distribute the new curricula (including a resource directory of C-MORE personnel available to facilitate implementation of new curricula) through existing local and national venues - including as supplemental additions to the existing University of Hawaii Curriculum Research and Development Group's Fluid Earth and Living Ocean high school marine science exploratory texts.

Goal 3. Recruit and retain students interested in careers in science, technology, engineering and mathematics (STEM).

Objectives to accomplish Goal 3:

a) Provide academically enriching experiences beyond the classroom to K-14 students. Invite students, especially those from underrepresented communities, to visit and participate at appropriate levels in the Center's research and education activities.

a.1) Students will help collect and record data; work in laboratories; develop presentations to give in their own classrooms; develop science fair projects; attend professional meetings and participate in brainstorming discussions to help develop more interesting and engaging academic experiences.

a.2) Hands-on, field-oriented experiences will be provided to high school students by re-instating the Blue-water Marine Lab (BML) program that successfully ran in Hawaii from 1971-1984. Both daytime and extended overnight classes will be held on all the Hawaiian Islands with the sea as the classroom. Students who successfully complete the program will be trained and mentored by professional ocean scientists and will become "teachers" in the program.

b) Identify 6-12 students who are particularly interested in STEM; create near-peer mentoring teams; then network with and nurture those students along academically enriching pathways into the Center's recommended undergraduate courses that are rigorous and culturally relevant; and, into the Center's undergraduate research STEM internship program. Center teams will collaborate with K-12 teachers to tailor opportunities for individual students.

c) Establish the C-MORE undergraduate research internship program at UH-Manoa and partnering institutions. Carefully monitor student academic progress; provide free tutoring when needed; provide intensive mentoring and networking for the students.

c.1) Provide funding to the MATE Center to coordinate technical internships, placing students on C-MORE research cruises and in labs. Preference for at-sea technician positions will be given to MATE students, and Bio-link students will receive preference for biotechnology-related internships. Marine technology and biotechnology students will work side-by-side. This provides both student groups an experience of both career opportunities and links that they might not have otherwise considered. Bio-Link's North Central Center national internship initiative dovetails with MATE and the C-MORE plan.

c.2) Host summer interns at the Monterey Bay Aquarium Research Institute (MBARI). Individuals with a strong quantitative background in engineering or microbial oceanography will be recruited each year to participate in for a 10-week summer term to help develop sensors for monitoring microbial activity *in situ* and to participate in MBARI's research.

d) Engage students' families and other community members in C-MORE teams of K-12 teachers, researchers, undergraduate and graduate students. Invite them to participate in their students' activities,

especially to attend student presentations. Families and community members will be encouraged to participate in the Center's research, fieldwork, labs, and in Hawaii, help establish and run an education booth at SOEST's Annual two-day Open House for K-12 students and teachers.

d.1) Visit families in their homes, if invited, to deliver important information about academic opportunities. Center teams will be available by phone, email and personal visits to students' families for discussions and to answer academic questions that may arise.

d.2) Convene a community-based C-MORE Advisory Council knowledgeable in local values, cultural knowledge, work place needs and opportunities. Arrange periodic meetings of the council and the Center's teams to continually enhance pathways for information flow concerning the community and the Center's activities and resources. The council will have the option of participating in all Center programs.

e) Establish a student study and discussion room at C-MORE institutes. Graduate student tutors and faculty mentors will provide free tutoring during regularly posted hours. Peers will be encouraged to assist one another with homework and academic networking.

f) Help students prepare for graduate school by meeting with graduate students, and personnel in the Graduate Education Office; obtaining appropriate graduate school application materials; and, supporting travel to partnering institutions to interview faculty and learn about graduate opportunities.

Goal 4. Produce knowledgeable microbial oceanographers with the capability to become outstanding researchers and science educators.

Objectives to accomplish Goal 4:

a) Provide funds to support the education and training of graduate students and post-doctoral trainees at Center institutions. Involve graduate students and post-doctoral trainees in the education and training of secondary school students, undergraduates, and pre-service science teachers through: participation on collaborative teams to plan experiments (laboratory and fieldwork) and develop curricula, participation on resource teams to facilitate classroom activities and field trips and answer questions, participation on teams of teachers, researchers, undergraduates, and community members to plan educational exhibits, mentoring and counseling activities with undergraduates who are considering graduate school, participation on teams of teachers, researchers, undergraduates, and community members to plan educational exhibits, and serving as mentors to state science fair participants (grades 6 - 12).

b) Provide opportunities for graduate students from participating C-MORE institutions to work in laboratories of faculty members from other C-MORE institutions and to take courses at other C-MORE institutions.

b.1) Provide travel funds for graduate students to spend up to one semester at other C-MORE institutions taking courses and/or working with participating C-MORE scientists, and to participate in training internship at C-MORE affiliated biotechnology companies, e.g., Diversa Corporation.

b.2) Encourage the service of C-MORE scientists as outside members on the committees of graduate students from other C-MORE institutions by providing travel funds for faculty to be present at critical meetings including comprehensive examinations and defenses, and teleconferencing of student committee meetings.

c) Develop an intensive, high-impact, short course in microbial oceanography for graduate students from around the world. The Agouron Institute, a non-profit research organization, has expressed interest in sponsoring the creation of such a short course that would be field-oriented and patterned after their highly successful course in Geobiology.

d) Develop a web-based microbial oceanography syllabus, complete with Powerpoint presentations and lecture notes, that could serve as the basis for an advanced undergraduate or graduate-level course. This "virtual textbook" will be freely available and periodically updated by the Center Director as progress is made in this rapidly developing subdiscipline.

Goal 5. Create unbroken pathways between existing research and K-20 education programs and departments, and then enhance the network pathways with Center's activities and resources.

Objectives to accomplish Goal 5:

- a) Identify effective national and international scientific research and education programs to link with and enhance the Center's programs. C-MORE teams will develop and facilitate effective links that fill gaps in the existing network.
- b) Identify community-based science education organizations and school/teacher teams interested in long-term partnerships. Support the development of sites for dissemination of C-MORE's curricula, activities and resources.
- c) Facilitate student exchanges among partners and other institutions to participate in established programs, including the Shannon Point, WA *Minorities in Marine Science Undergraduate Program (MIMSUP)*; OSU's *Native Americans in Marine and Space Sciences Program* and the *Bridging Excellence in Science and Tradition Program*; Hampton University's *Multicultural students at Sea Together (MAST)*; and the *Quality Education for Minorities Network* in Washington, DC.

Goal 6. Provide communications training for Center's faculty and students.

Objectives to accomplish Goal 6:

- a) Provide communications training based on the Leopold Leadership Program model to improve the flow of accurate, credible scientific information to scientists, educators, students, policy makers, the media and the public.
- a.1) Train Center teams to be better communicators of complex scientific information about microbial oceanography, biogeochemistry and marine science in general.
- a.2) Offer intensive training in annual one-week sessions that feature "hands on," interactive modules led by communications trainers and the Center's specialists on local and national media. The focus of the training will be how to communicate effectively with non-scientists in terms they can understand and use.
- a.3) Provide monthly practice sessions at the Center to refine techniques. Presentations will be recorded on video and critiqued by media specialists and Center teams.
- a.4) Provide continuing access to trainers to mentor and guide Center's teams as they develop presentations, write stories for journals, and prepare for print and broadcast interviews.

M.S. and Ph.D. degrees completed during the past three years under the directorship of the proposed Center senior personnel (includes Karl, Laws, Chisholm, DeLong, Vergun and Zehr)

First name	Last name	Degree	Years	Gender	Ethnicity	Nationality
Jennifer	Brum	M.S.	2	F	Caucasian	U.S.
Christopher	Carrillo	M.S./Ph.D.	7	M	Caucasian	U.S.
Nicolas	Cassar	Ph.D.	5	M	Caucasian	Canadian
Elizabeth	Mann	Ph.D.	6	F	Caucasian	U.S.
Gabrielle	Rocap	Ph.D.	6	F	Caucasian	U.S.
Stephanie	Shaw	Ph.D.	5	F	Caucasian	U.S.
Jagat	Adihya	M.S.	2	M	Caucasian	India
Matthew	Sullivan	M.S.	2	M	Caucasian	U.S.
Anna	Rhodes	M.S.	2	F	Caucasian	U.S.
Mak	Saito	Ph.D.	5	M	Caucasian	U.S.
Victoria	Orphan	Ph.D.	6	F	Caucasian	U.S.
Bodie	Shaw	M.S.	2	M	Native American	U.S.
LoriAnne	Adams	M.S.	2	F	Native American	U.S.
Dawn	Anzinger	M.S.	2	F	Caucasian	U.S.
Enoma	Omoregie	M.S.	3	M	African	Nigeria
Angela	Gibson	M.S.	3	F	African American	U.S.
Natalie	Karouna	Ph.D.	6	F	Caucasian	U.S.

4.e. DIVERSITY OBJECTIVES OF THE INTEGRATED CENTER

In Hawaii, the past year's public school standardized test scores reveal that Asians (Japanese, Chinese, Koreans) scored highest for all grades tested in reading and mathematics followed by Caucasians. Last were Filipinos, Native Hawaiians, Pacific Islanders, Others. Test scores correlate with socioeconomic and second language status – students from poor and/or immigrant families are overrepresented in the trailing group. As test scores are frequently used to sort students into academic tracks, by the time students are in middle and high school Asians and Caucasians are overrepresented in college preparatory science courses while Native Hawaiians, Pacific Islanders, and Filipinos are overrepresented in low level science skills and special interest courses such as Marine Science, Aquascience, and Plants and Animals of Hawaii.

The underrepresentation in science of children from poor, immigrant, indigenous, and low status minority groups is found across the U.S. Inadequate K-12 science preparation leads to low ethnic diversity of post secondary students and faculty in science and science teacher preparation programs. Women are well represented among proposed Center personnel, including two of the four co-P.I.s, and their graduate students, but ethnic diversity of PhD students and proposed Center senior personnel is low.

Ethnographic research in science laboratories, schools, and communities suggests that scientists, science teachers, and students from groups underrepresented in science occupy different cultural worlds. Culture is defined in Webster's New World College Dictionary, 3rd Ed. as the "ideas, customs, skills, arts, etc. of a people or group that are transferred, communicated, or passed along...to succeeding generations." If science teaching may be thought of as cultural transmission of the ways that scientists think, communicate and operate in the science world, and underrepresented students do not take courses from teachers who teach the "ideas, customs, skills" of scientists, then these students are not likely to enter, much less succeed in postsecondary science programs.

Ironically, though the traditional knowledge of seafaring Native Hawaiians and Pacific Islanders is grounded in the same natural world studied by marine scientists, there is little inclusion of either traditional or current marine science knowledge in school curricula and teacher education programs. The transmission of Micronesian traditional navigation knowledge was essential to the non-instrumented voyage to the South Pacific of the first modern Hawaiian navigator Nainoa Thompson. But Hawaii's teachers do not know enough about Micronesian students to include their cultural resources in their learning. Instead, they attend workshops to learn how to work with this culturally and linguistically different population, the fastest growing segment of the K-12 population.

The cultural model of knowledge transmission that underlies constructivist, experiential, and interpersonal theories of learning provides the framework for designing the Center's educational and outreach programs to obtain its diversity goals of:

1. Increased participation of women and underrepresented minorities in Center postsecondary research and education programs.
2. Formation of a cadre of K-12 teachers knowledgeable in Center research and able to teach all students, especially underrepresented minorities, by connecting Center research to their lives, communities, and cultures.
3. Development and dissemination of curriculum presenting Center research in the context of Native Hawaiian and Pacific Islander cultural contexts through Center educational and community outreach programs.
4. Increased knowledge about and respect for different cultural perspectives and practices among Center higher education and research staff, teachers and underrepresented minority students and communities.

The Center proposes to achieve goal 1, increased participation of underrepresented minorities in Center postsecondary research and education programs, by providing schools and students with Center education programs and developing undergraduate oceanography courses that present content in the context of Native Hawaiian, Pacific Island and Asian cultures. The identification of schools with high proportions of underrepresented students is the first step to developing school and summer science enrichment. We will establish student research and mentoring programs, and help to identify role models from the underrepresented students' own communities. Helping students with college and scholarship applications, course selection, and program planning reduces barriers to entry (see relevant sections of educational objectives).

The Center proposes to achieve goal 2, increased numbers of K-12 teachers both knowledgeable in Center research and able to teach all students, by developing teacher education programs integrating culture and science and soliciting the participation of teachers who work with high proportions of underrepresented minorities. The formation of Center teams composed of members with cultural, educational, and research knowledge will allow Center research to be aligned with national and state science standards while being presented in relevant cultural contexts. Each member of the team serves as a "cultural translator." As team members co-teach, model and explain the values, language, and communication strategies of their group, they learn from each other as they prepare teachers to be more effective with diverse students.

The Center proposes to achieve goal 3, development and dissemination of curricula presenting Center research in the context of Native Hawaiian and Pacific Islander cultural contexts, by forming Center education and outreach teams with cultural, educational and science research knowledge and expertise. For Hawaii, teams will collaborate with teachers and knowledgeable members of local communities to develop curricula that may vary somewhat from one place to another, honoring and preserving the local knowledge necessary to understanding and sustaining marine resources.

The Center proposes to achieve goal 4, increased knowledge about and respect for different cultural perspectives and practices among Center higher education and research staff, teachers and underrepresented minority students and communities, by providing opportunities for sustained interpersonal interaction in informal as well as structured classroom or laboratory formats. These informal interactions may occur through field trips, voyages, service learning, and school or community-based immersions over a period of several days. Extended interpersonal contact is necessary for an outsider to begin to enter a new culture: for a teacher to become knowledgeable about Center research, for a student to learn about being a scientist, for a scientist to learn how traditional navigators set their course. Cultural knowledge and practices are gained in the context of participation and observation.

At MIT, Center investigators will participate in an ongoing program called "GEAR-UP," an after-school / summer program for underrepresented high school students with interests in science and engineering. Co-P.I.s Chisholm and DeLong, and their staff, will develop a microbial oceanography module for GEAR-UP based on recent Center activities and other advances in the discipline as a whole. This module, once developed and field tested, could be transported to other sites.

The benefits to society of the Center's diversity programs are as follows:

- Underrepresented minority students who find school science engaging and relevant are more likely to graduate, enter post secondary education, and become part of a productive, diverse, and educated workforce.

- K-20 science teachers who teach a curriculum that is rigorous in content yet relevant to their students' cultures, environment, and educational futures are likely to be more effective instructors and to find their work more personally and professionally meaningful.
- Minority communities rich in cultural knowledge but marginalized in educational and economic opportunities may grow stronger through Center programs that acknowledge culture as a resource and provide opportunities for educational and economic advancement.
- Center staff and programs stand to benefit from a more diverse scientific workforce bringing different questions, knowledge, and perspectives to research.
- Communities at large stand to benefit from Native Hawaiian and Pacific Islander cultural values that emphasize societal survival through sustainable practices and environmental ethics.
- Participants in the Center's collaborative, cross cultural science programs learn valuable technical, scientific, and interpersonal skills enabling them to participate wisely and effectively both in their immediate and global multicultural worlds.

A formal evaluation of how effectively the Center has attained its goals to achieve diversity will be conducted as part of the overall evaluation of C-MORE (see Budget Justification). Possible objective criteria include:

- Changes in the participation of women and underrepresented minorities in Center postsecondary research and education programs as measured from a baseline of gender and ethnicity data from the year before Center programs begin.
- Formation of a cadre of K-12 teachers knowledgeable in Center research and able to teach all students will be assessed through records of the number of teachers participating in Center programs and their ratings of how effectively the programs helped them to connect Center research to their minority as well as mainstream students' lives, communities, and cultures.
- Curriculum developed and disseminated through Center educational and community outreach programs will be assessed for the presence of diverse cultural perspectives, including those of Native Hawaiians and Pacific Islanders
- Increases in knowledge about and respect for different cultural perspectives and practices among Center higher education and research staff, teachers and underrepresented minority students and communities will be assessed through surveys, pre and post tests, interviews, and products produced by participants in Center activities.

4.f. KNOWLEDGE TRANSFER OBJECTIVES OF THE INTEGRATED CENTER

Several of the central research themes of the proposed Center, especially biodiversity and climate variability, are topics of great interest to the public; the sea has been vital to societies for the past millennium and has long been celebrated through literature, music and art. Ocean exploration and discovery provide vibrant images and novel information that capture the imagination of people from all walks of life and all age groups. We will capitalize on these opportunities in the design of our knowledge transfer and public outreach programs. We will endeavor to combine the excitement of exploration and discovery with the complex interdependency of global climate and marine ecosystem dynamics to educate a broad range of students (K-12), their teachers, as well as their parents/grandparents. This effort will include Hawaiian and Pacific Island students and minority students in coastal California who are underrepresented in the field of marine science despite their cultural connections to, and dependence on, the sea.

In addition to the traditional methods of dissemination of scientific results by peer-reviewed archival literature and presentations at scientific conferences, we will develop the following: (1) an accessible, interactive website for data/information exchange with the public that will also support the education programs mentioned above, (2) a public lecture series that is portable among the partner institutions, (3) public information materials that will be suitable for display at museums or other public venues, and (4) creation of a novel media approach for conveying the field of microbial oceanography to the public at large. We will work with partner museums and aquaria (Aquarium of the Pacific, Cabrillo Aquarium, Los Angeles County Natural History Museum, California Science Center), with MBARI and the Monterey Bay Aquarium and with the Waikiki Aquarium, Bishop Museum and the Hanauma Bay Education Center in Hawaii to create display materials that bring the excitement and content of our discovery to their visitors and students. At MIT, Center investigators will continue their ongoing collaboration with the Museum of Science in Boston to develop new programs in microbial oceanography for the Current Science and Technology Center. Some of these displays will be created directly with Center funds. However, we also plan to play a catalytic role where we create the relationships between our scientists and these informal education centers and stimulate them into seeking the large levels of funding required for professional level displays on these subjects.

4.f.1. Goals and Objectives

Goal 1. Produce traveling museum and aquaria exhibits and presentations, TV news segments, public service announcements, short and long documentaries and educational DVDs and videos about the Center's research and education activities.

Objectives to accomplish Goal 1:

- a)** Develop traveling exhibits for museums, aquaria, education centers and classrooms focusing on marine microbes and ocean processes. This will be new information about cutting edge research and education only now possible because of advances in technology and genetic research.
- b)** Spotlight the Center's researchers, educators and students in nightly TV news productions and public service announcements focusing on recent discoveries, innovative education methods, and the unique individuals involved. Make available to local TV Olole, NBC, ABC, FOX, affiliates in camera-ready form. These productions will be useful in classroom introductions to the Center's work, on our website, and in presentations to professional societies.
- c)** Participate in Haunama Bay's Thursday Night Film and Speaker Series, special education workshops, and the Education Center displays. C-MORE trained communications teams present and discuss C-

MORE Research and Education for the local community and visitors. Policymakers will be invited to join us to discuss C-MORE's contributions to possible solutions for current issues.

Goal 2. Develop a compelling, innovative media approach to engage Center teams, students and teachers at all academic institutions, local and national community in the value of microbial oceanography research and education.

Objectives to accomplish Goal 2:

a) Create a new community of super heroes to teach environmental lessons; inform the public; and, engage students, teachers and community in C-MORE activities. Super Marine Microbe Girls and Guys representing each of C-MORE's eight focal microbial groups (see Research Objectives of the Integrated Center) will have special powers unique to their own respective genome structure and functions.

a.1) Graphic artists, Design Engineer Richard Landon from Stan Winston Studios; Devon Kyle, Brainfreeze Studio; Shannon Vergun and Judith Vergun, Kumu Ola Media Labs will work with C-MORE scientists to create the superheroes.

a.2) C-MORE teams will develop a scientific and culturally-based strategy to infuse our superheroes into our center's education and outreach activities.

b) Launch a public relations campaign to develop science stories from a range of perspectives. For example, Super Marine Microbe Girl and Guy will have many adventures around the islands and beyond to tell microbial oceanography stories. C-MORE will sponsor competitions for best and most scientifically accurate adventure stories based on microbial oceanography. Prizes for best stories will include a tour of our partner studio – Stan Winston Studios in California; communications training at C-MORE communications lab; and sponsorship of the awardees to present their stories in academic institutions, museums and aquaria, and, professional society meetings. Students will be encouraged to compete at the annual Hawaii Science Fair, and at similar events at our partner institutions.

c) Partner with professional societies, especially the American Society for Microbiology and Michigan State University's Digital Learning Center to enhance existing educational materials such as the web program *Microbe Zoo*. Because our Center's activities will cover broad time and space scales from minutes to millennia and micrometers to full ocean basins there is an opportunity to create novel lessons based on "powers of ten," i.e., from satellites viewing the Earth to electron microscopes viewing subcellular structure.

d) Develop a series of media rich lessons that will be available on DVD at the Center's central office and on the website. C-MORE teams will work with Stan Winston Studios, Brainfreeze Studio and the Kumu Ola Media Labs to further develop the series. Include a resource list of related products and activities on DVDs, for example, the PBS production *Intimate Strangers: Unseen Life on Earth* with associated publications and lesson plans.

e) Offer internships to students in the new University of Hawaii Film School. Students interested in science education through digital media and filmmaking will have an opportunity to train with C-MORE teams and media productions.

4.f.2. New Partnerships and Co-ordination with Other National and International Research and Education Programs

The Center's investigators are collectively involved in numerous complementary research and education efforts that will greatly benefit the Center as a whole. Some are leading independent efforts in metagenomics and bioinformatics, as described previously, while others are engaged in the design and implementation of the next generation of carbon cycle research programs. At the NSF, a major ocean observatory initiative will benefit from the planned microbiological sensor development in the Center

and, likewise, our research prospectus will be facilitated by new, independent resources that will be invested in ocean observation platforms including moorings, drifters and cabled observatories. Several NSF-funded programs, including the MOSEAN mooring project (T. Dickey, UCSB) and the ALOHA cabled observatory (F. Duennebie, UH) near Hawaii and the MARS observatory (M. McNutt, MBARI) in Monterey Bay will be used extensively by C-MORE investigators. The ongoing Hawaii Ocean Time-series at UH will serve as a key logistical and time-series data support partner for C-MORE objectives. Finally, a new 5-year Oceans and Human Health project has recently been funded at UH (E. Laws, P.I.; Pacific Research Center for Marine Biomedicine). This research will focus on harmful algal blooms, water- and vector-borne diseases and marine-derived pharmaceuticals. Analytical and microorganism culturing facilities, as well as common molecular biology-based techniques and instrumentation will enhance the proposed objectives of C-MORE.

The central theme of C-MORE, microbial oceanography, is a relatively new and very important subdiscipline of marine sciences. We know of only one academic institution in the world, The International Max Planck Research School of Marine Microbiology in Bremen, Germany, that awards advanced (M.Sc./Ph.D.) degrees in this field of study. As one important part of C-MORE's combined research, education and knowledge transfer mission, we will partner with scientists in the School of Marine Microbiology (R. Amann and N. Dubilier, program coordinators) for the mutual benefit of our faculty, staff and students and theirs. The partnership could involve a graduate student exchange program, jointly sponsored research expeditions and coordinated workshops and symposia.

4.f.3. Evaluation of Knowledge Transfer

As one part of the formal annual evaluation of C-MORE we will assess the effectiveness of our Knowledge Transfer programs by the following means:

- Creation and dissemination of knowledge via peer-reviewed presentations and publications related to Center programs.
- Development and utilization of an interactive website supporting Center educational and outreach programs through the lifetime of the project, and establishment of new Center-related research and educational partnerships and coordination with other national and international programs.
- Delivery of public lectures presenting Center research activities at participating institutions, and displays of Center research activities at a range of venues including museums and aquaria.
- Production and dissemination of media intended to increase student and public knowledge and interest in microbial oceanography, Center research, and activities.
- Production and dissemination of pre- and post-secondary curricular materials intended to increase student interest in microbial oceanography, Center research and activities.
- Records of the number of science fair projects and other student projects related to microbial oceanography in communities hosting Center institutions and outreach activities.

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