From: Office of Naval Research Resident Representative, Seattle
To: ONR Scientific Officer, Dr. Randall S. Alberte, Code 1123B, Oceanic Biology, Office of the Chief of Naval Research, Ballston Tower

Subj: REQUEST FOR FINAL TECHNICAL APPROVAL, GRANT N00014-89-J-1048, R&T PROJECT CODE: 423a001---02; THE UNIVERSITY OF WASHINGTON; PRINCIPAL INVESTIGATOR IS DR. JODY W. DEMING, SCHOOL OF OCEANOGRAPHY

1. This office is in the process of closing subject contract. We have been advised that the final technical report has been submitted.

2. So that closeout may continue, please provide this office with information as to whether technical requirements have been performed satisfactorily.

ELEANOR A. DIXON
Procurement Assistant

CC:
DTIC (w/copy of final technical report)

FIRST ENDORSEMENT on ONR ltr., 27 February, 1992

From: ONR Scientific Officer, Dr. Randall S. Alberte, Code 1123B
To: Office of Naval Research, Seattle

1. Returned for necessary action.

2. I certify that all technical requirements under subject contract are:

   ___ Satisfactory
   ___ Unsatisfactory
   ___ Comments:

Date 92-05937

Scientific Officer
The goals of this project centered around two areas of research: 1) the study of hyperthermophilic archaebacteria from submarine hydrothermal vents on the East Pacific Rise; and 2) the study of physiologically diverse microbial populations associated with a whale carcass partially buried in sediments of the Santa Catalina Basin. Specific objectives of the first area of research included the following:

1) Completion of analyses of all geochemical, biochemical and microbiological measurements made on smoker fluid samples obtained during ALVIN dives to the Endeavour Segment of the Juan de Fuca Ridge (under earlier funding) in order to assess the potential existence of a subcrustal biosphere;
2) Further characterization of DNA from some of those smoker samples and selected isolates to assess phylogenetic origin;
3) Determination of the upper temperature and pressure limits for growth and survival of hyperthermophilic archaebacteria obtained during earlier cruises; and
4) Tests for the production of thermostable extracellular enzymes during growth and survival studies at extreme conditions;

The specific objective of the second area of research included tests of two hypotheses:

1) Physiological types of bacterial-invertebrate symbioses in the deep sea directly reflect available energy sources; and
2) Deep-sea conditions of low temperature and elevated pressure do not limit bacterial responses to intense organic loading.

ACCOMPLISHMENTS:

With regards to research on hyperthermophilic vent organisms, we successfully completed analyses of the DNA and corresponding geochemical data obtained from smoker samples during the 1988 ALVIN dive program on the Endeavor Segment. These analyses resulted in: 1) the first statistically significant evidence for a vast subsurface biosphere at deep-sea spreading zones; 2) an important section in an invited book chapter (Baross and Deming, 1992; see below) that reviews all available information on thermophilic bacteria from hydrothermal environments; 3) an invited presentation at the 1991 GSA meetings in San Diego that is now the basis for a manuscript in preparation; 4) an invitation to join the French microbiology expedition MICROSMOKE, planned for Mid-Atlantic vents in 1994, to again deploy our in situ Ti incubator at active smokers on the seafloor in search of microbial activity at superheated temperatures.

The temperature and pressure growth characteristics of two strains of hyperthermophilic archaebacteria were determined and published, along with the prediction (based on those characteristics) that microbial habitats exist deep below seafloor spreading zones. Another manuscript, identifying the organisms as members of the genus Thermococcus on the basis of 16S rRNA sequences, is in draft form.

The study the thermostability of proteins produced by hyperthermophilic bacteria in culture evolved into more fundamental studies of protein formation and stability under extreme conditions. In collaboration with geochemist M. Engel at the University of Oklahoma, organic chemist S. Macko at the University of Virginia and geophysicist E. Schock at Washington University in St. Louis, we conducted a series of high temperature/pressure experiments in my laboratory on the stability and racemization of amino acids and peptide bonds under simulated hydrothermal conditions, including the presence of solid minerals. This work has resulted in: 1) evidence that peptides are more stable under extreme conditions than isolated amino acids, in support of J. Baross' (University of Washington) theory on the origin of life and in keeping with H. Helgeson's (Berkeley) theory on metastable conditions in the subsurface regime; 2) a joint presentation at the 1991 GSA meetings in San Diego; 3) a joint manuscript in preparation; and 4) plans to submit a joint proposal to explore further both the experimental and theoretical possibilities.

In pursuit of molecular studies of vent (and other) bacteria, a masters-level molecular biologist (S. Carpenter) was
hired and trained in the art of culturing "extremophiles" from the deep sea. She, in turn, established connections to the molecular biology facility in the Department of Botany, where the equipment we lack is available, and completed the extraction and purification of DNA from all of our hyperthermophilic cultures, as well as from previously collected smoker fluids, in preparation for more sophisticated work to identify their phylogenetic and some physiological characteristics. The pay-off from this ambitious goal lies well beyond the timing and funding constraints of this ONR grant; however, we have made a notable beginning.

With regards to research on the impact of heavy organic loading at the seafloor, my laboratory group joined the C. Smith (University of Hawaii) NSF ALVIN project at the dead whale site in the Santa Catalina Basin in Feb 1991. Our efforts during and after this cruise resulted in: 1) the documented persistence of a chemoautotrophic basis for invertebrate communities on the whale bones; 2) experiments to confirm the simultaneous presence (and novelty) of endosymbiotic nitrifiers and sulfur-oxidizers in animal tissues (analyses ongoing); 3) manuscripts in preparation on the effects of organic enrichment on microbial parameters in these deep-sea sediments; and 4) a unique educational opportunity for my graduate students to adapt intertidal microbiological techniques to research on deep-sea sediments (methods manuscript in preparation).

PUBLICATIONS RESULTING FROM THE GRANT:


MANUSCRIPTS IN PREPARATION:


ABSTRACTS:


