

NASA CR-115064
TEXAS A & M N71-29228

TO: NASA Manned Spacecraft Center
Technical Information Dissemination Branch
Houston, Texas 77058
Attn. Retha Shirkey, Mail Code BM 6
Mark For: Contract NAS 9-10830

SUBJECT: Final Report on NAS 9-10830 Bacteriology of Select Aquatic
Hosts Utilized in Lunar Sample Exposure Studies

FROM: D. H. Lewis

Gentlemen:

As per contract schedule dated May 20, 1970 Attn: BB321, Mr. R. C. Bake, I wish to submit the final report on contract NAS 9-10830 for your consideration.

Sincerely,



D. H. Lewis, Ph.D.
Assistant Professor

DHL/mjm

cc with enclosures:
AAP
HOK
LCG

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Brief Description of Work:

A. Objectives: The contract was specifically designed to provide microbiological support to the invertebrate - lower vertebrate protion of the lunar receiving laboratory, Biological Protocol for Apollo 13 and succeeding missions. Technical consultation and assistance was sought by NASA for microbiologic support to the aquatic animals during Apollo 11 and 12 missions. This support was provided by the Department of Veterinary Microbiology, Texas A&M University.

B. Accomplishments: Microbiologic support embraces a multitude of categories. As applied to aquatic animals and specifically to the animals utilized in the NASA studies, the support consisted of screening the test animals for established pathogenic bacteria. Similar studies are rare, thus in addition to conducting routine bacteriologic analyses, some of the effort was of a developmental nature. Particular emphasis was placed upon developing methods for processing specimens in confining environments such as those of Class III cabinetry. Immunofluorescent techniques were developed which could: a) detect specific pathogens. and b) detect antibodies to those pathogens. These developments should be of value both to future similar testing procedures and to fisheries industries. Techniques applicable to processing large numbers of bacterial isolates in a limited space and with minimal amounts test media were also developed during the tenure of this contract. Two papers on the immunofluorescent technique: "An Immunofluorescent Technique for Detection of *Aeromonas liquefaciens* in Fish Utilized in Lunar Exposure Studies", D. H. Lewis and T. C. Allison and "Detecting Fish Antibodies to *Aeromonas liquefaciens* by an Indirect Fluorescent Antibody Technique", D. H. Lewis and N. L. Savage, have been prepared for submission to technical journals. The Lewis and Allison paper has been approved for publication in Transactions of the American Fisheries Soc. Approval for the Lewis and Savage paper is pending action by NASA, and the technical Journal. Two other manuscripts. "Device for Multiply Inoculating Bacterial Cultures", D. H. Lewis and "Bacteriologic Studies on Aquatic Hosts Used in Apollo 11-14 Lunar Soil Exposure Studies", D. H. Lewis are in final preparatory phase.

C. Results: Although the fish pathogen *A. liquefaciens* was observed in certain fish which were to be utilized in Apollo 13 and 14 missions, adverse effects were not observed in any of the test animals. The

Species	Log Concentration Aerobic Bacteria Per		
	Ml Aquarium Water	CM Body Length (Surface Load)	Gm Total Body Wt. Specimen
<u>Fundulus heteroclitus</u>	2.0+1	3.0+1	3.0+1
<u>Primephales promelus</u>	2.0+1	3.0+1	4.0+1
<u>Crassostrea virginica</u>	4.0+1	ND	ND
<u>Penaeus aztecus</u>	2.0+1	2.0+1	4.0+1

Table 1. Concentration of bacteria estimated by diluting sample 10-Fold in Trypticase Soy Broth (for Fundulus and Primephales specimens) and Trypticase Soy Broth + 1% NaCl (for Crassostrea and Panaeus specimens). Specimens were collected in sterile plastic bags and hosts were measured and weighed. The surface load was estimated by culturing washings of the various hosts. After washing, the specimens were immersed in 70% ethanol for 5 minutes, rewashed in sterile 1% saline and homogenized.

	Achromobacter	Cytophaga	Flavobacter	Pseudomonas	Vibrios	Enterobacteria	Bacillus and Gram pos. cocci
<u>Crassostrea virginica</u>							
Aquarium water	2		3	1		4	
Body Fluid	3		4	1	2		
<u>Panaeus aztecus</u>							
Aquarium water	2			1		3	
Surface	1	3	4	2			
Viscera	2		1	3	4		
<u>Fundulus heteroclitus</u>							
Aquarium water	2			1		3	
Surface	1	3	2	2			4
Viscera	2		3	1		4	
<u>Primephales promelus</u>							
Aquarium water	2			1		3	
Surface	1		3	2			4
Viscera	2		3	1	4		

Table 2. Distribution of the predominating types of bacteria on various aquatic hosts ranked in descending order of four most frequently identified genera.

microbial load of the animals and the bacterial concentrations of the aquarium were such as not to adversely affect the welfare of the test animals. Summary data on the host systems are presented in Tables 1 and 2.

Comments and Recommendations: Insofar as ascertaining "the presence of replicating" disease-producing agents, the basic experimental design of the testing protocol appears adequate. However, certain microbiologic aspects of the study could be improved.

1. The cabinetry is not adequately designed for performing microbiologic studies on the test hosts.

a. The distribution of samples within the enclosure could be facilitated by utilizing mechanized carrier systems. In the present system, samples are passed manually from one portion to other portions of the cabinetry "fire-brigade" style. This arrangement is awkward and could contribute to massive contamination of test animals.

b. Locks or other barrier provisions (eg laminar flow units) could be used to greater advantage behind the enclosures. Provisions are not made for streaking plates or processing specimens. Formalin fixed or autoclaved specimens are of no value for microbiologic examination.

2. Future studies on extraterrestrial materials should consider not only the potential pathogens but other replicating forms. Quite possibly these forms could only be revealed in living systems. Thus, greater emphasis could and should be placed upon utilizing hosts whose microflora are well defined.