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DEMONSTRATION OF A VIRUS HOST SYSTEM FOR VIRUS REMOVAL STUDIES.(U)
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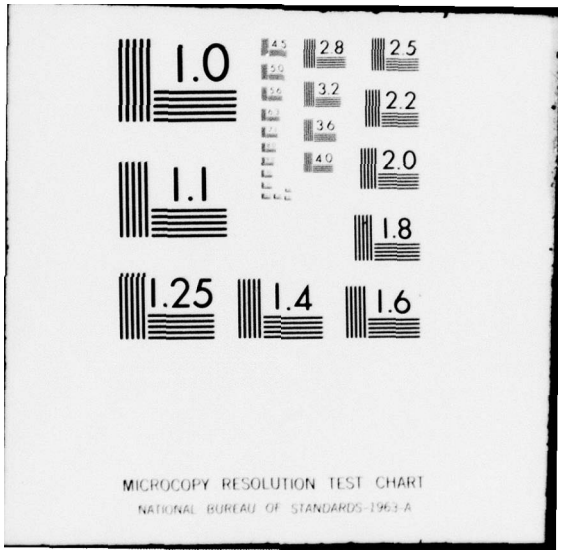
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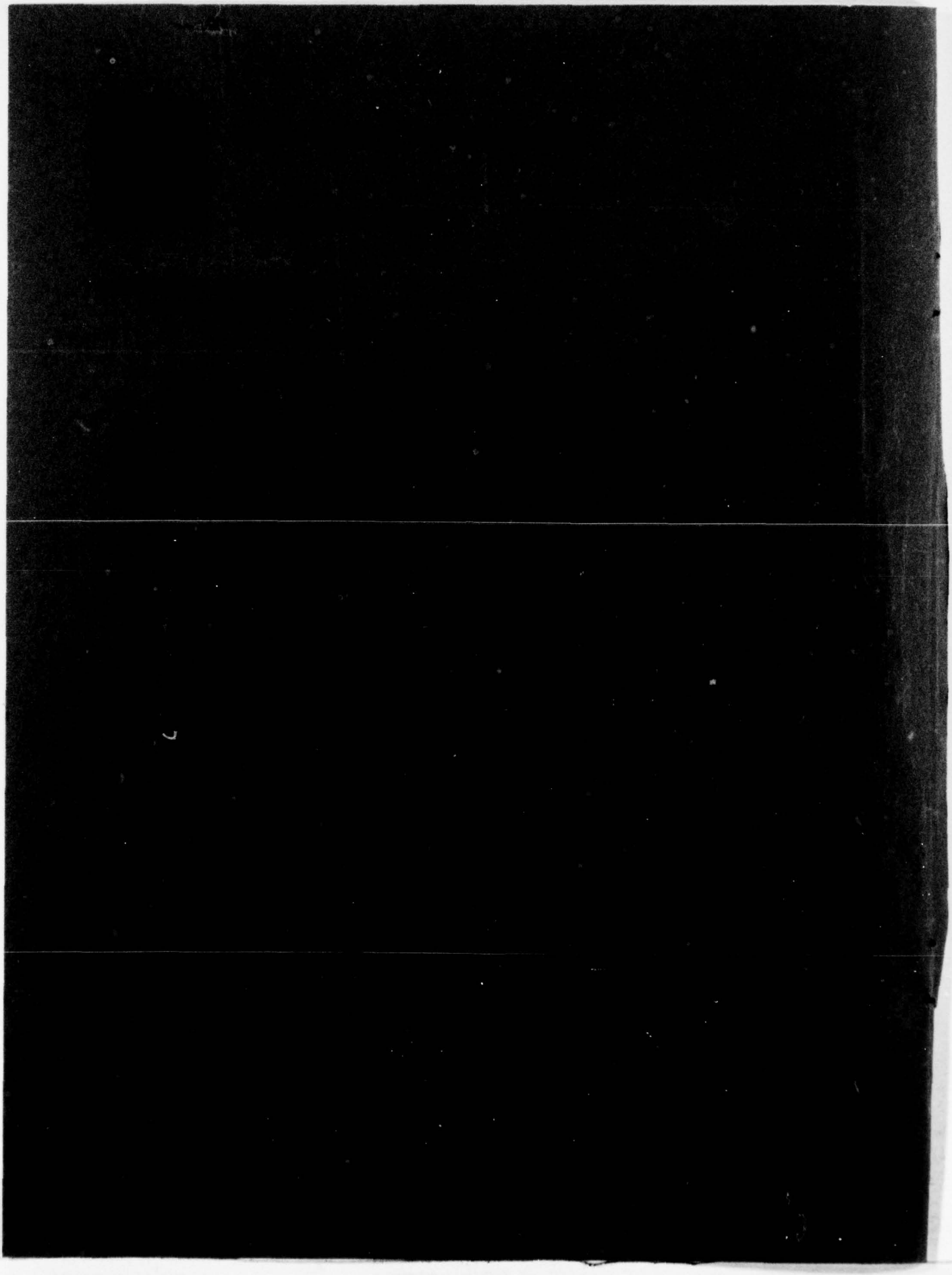

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The feasibility of a virus-host system (LPP-1/Plectonema boryanum) as an indicator of virus removal by wastewater treatment processes is reported. Full-scale virus studies, along with bacterial characterization of test effluents, were carried out at the Naval Coastal Systems Center treatment plant. The system is a useful tool for modeling studies because (1) the techniques are simple and inexpensive; (2) problems, such as bacterial contamination and plaque enumeration, do not occur; and (3) the test virus is a "safe" agent from both the environmental and human health point of view.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

BACKGROUND

Recently the Navy and other governmental agencies, in addition to public health and water supply professionals, have become interested in the elimination of the threat of contaminating the coastal environment by the disposal of domestic wastes. Small concentrations of waterborne pathogens, especially viruses, may survive effluent discharge or leakage to cause severe epidemics. Thirty outbreaks of hepatitis in the U.S. between 1961 and 1970 have been attributed to water systems[1]. The fact that naval shore facilities and particularly naval ships operating in coastal waters may be involved in the disposal of such pathogenic agents opens the service to censure by ecological groups at home and abroad.

Although the need is widely recognized[2], the requirement for a simple, safe method of evaluating the virus threat has not been fulfilled. Until recently the difficulties of working with viruses, their pathogenicity, and tediousness of culture techniques, have hindered the development of a good quantitative assay for water systems. The discovery of algal viruses and the subsequent development of techniques for their culture and assay, has provided an important new indicator for pollution studies.

DISCOVERY OF LPP-1

The first virus known to attack blue-green algae was isolated by Safferman and Morris in 1963[3]. Obtained from an Indiana waste stabilization pond, and named LPP-1 because it displayed lytic activity for the blue-green algae genera Lyngbya, Plectonema, and Phormidium.

DISTRIBUTION

Researchers widely separated geographically[4][5][6] soon showed the range of this virus-host system to be worldwide. Francis Drouet[7] suggested the ubiquitous nature of the cyanophage by describing its

host, which he reclassified as a single species, Schizothrix calcicola, as "perhaps the most widely distributed and most frequently encountered blue-green algae on earth."

Prior to 1970, it was generally accepted that cyanophages were limited to fresh water habitats. Braswell[8] tested the validity of this assumption in a general survey of the Delaware Bay estuary. In this survey, naturally occurring cyanophages of Plectonema boryanum were isolated from widely distributed stations throughout the Delaware Bay estuary. Laboratory and field studies, undertaken to elucidate the nature and ecology of this estuarine cyanophage, demonstrated a virtually ubiquitous presence of the phage throughout the bay in concentrations of sufficient magnitude to preclude the assumption of a solely riverine origin. Additionally, discrepancies in seasonality observed between the occurrence of riverine and estuarine phages contradicts the hypothesis of simple transport into the estuary. Results of antigenic analysis of the bay cyanophage distinctly demonstrated the LPP-1 nature of the phage. Laboratory experiments involving virus survival and replication, coupled with high concentrations of LPP-1 in the Delaware Bay, suggest that cyanophages may play an important role in saline as well as fresh water ecology.

CHARACTERIZATION

Electronmicroscopic investigations of the morphology of the virus, and the infection and replication processes, have been reported by Smith, et al[9] and, Smith, Brown, and Walne[10]; and reviewed by Brown[11], and Padan and Shilo[12]. It is reported to be in the size range of 56 to 66 mμ. The virus particle consists of a polyhedral head piece and a relatively long tail. It is a DNA virus and shows a resemblance to bacterial viruses, particularly the T7 coliphage[13][14]. LPP-1 virus has been found to be exceptionally stable over a pH range from 7 to 11, which is significantly greater than that reported for bacteriophages (pH 5-8)[12] and considerably greater than the maximum growth range for its host, P. boryanum (pH 7011)[14][15][16]. Thermal inactivation of the virus has been observed to occur at 60°C or above[14][16]. LPP-1 can be concentrated by acetone precipitation without significant loss in activity[14][17].

USE OF LPP-1 AS AN INDICATOR VIRUS

In 1967, Shane, Wilson, and Fries[18] investigated the use of the LPP-1 virus-host system as a laboratory tool to evaluate the effect of different unit processes in the removal of this virus. From their studies, it was clear that a great deal of information concerning virus removal in water and sewage threatment processes could be obtained

through the use of the LPP-1 virus-host system. The technique is simple and inexpensive. Enumeration of plaque-forming units (pfu) is not complicated by high bacterial counts because bacterial contamination of the synthetic growth medium of the host alga is not a problem.

For large scale testing, animal virus systems which present greater health hazards and require expensive tissue culture techniques, are not competitive with this algal virus indicator. The use of LPP-1 as an indicator system in the analysis of virus elimination in sewage and water treatment processes involves the implementation of inexpensive and uncomplicated culture, recovery, and assay techniques. Since LPP-1 has been demonstrated to occur in widely distributed fresh water and saline habitats, the threat of introducing foreign organisms into the environment is minimized (Appendix A).

Furthermore, the use of LPP-1 relies upon a system demonstrated to possess a high degree of host specificity. LPP-1 is known to infect only a single species of blue-green algae and its use is unlikely to present a hazard to human health. The ease with which this cyanophage is inactivated by heat or chlorine provides an additional margin of safety in its use as an indicator virus.

The present effort to demonstrate the feasibility of using LPP-1 as a virus-host system for large-scale testing was begun in 1973 at the Naval Coastal Systems Center. Funded by the Navy's Bureau of Medicine and Surgery, this work constituted a portion of an exploratory development effort in virus elimination technology and constituted the virus model used in the Naval Sea Systems Command effort.

METHODS AND MATERIALS

CULTURE, MAINTENANCE, AND STORAGE TECHNIQUES

Techniques were devised for full-scale testing of wastewater treatment plants with the virus-host system. For large volume production of suspensions with high titres of LPP-1, 5-gallon (18.9 litre) Pyrex carboys containing 15 litres of Chu #10 medium 19 were used. Following sterilization, the growth medium was inoculated with 200 millilitres of a 2-week old culture of Plectonema boryanum (ATCC* #18200). The algae were agitated by vigorous aeration while it grew at room temperature for 2 weeks under cool, white fluorescent lights (at intensities of 100-125 foot candles). Figure 1 is a drawing of the culture apparatus.

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*ATCC - American Type Culture collection Reference number.

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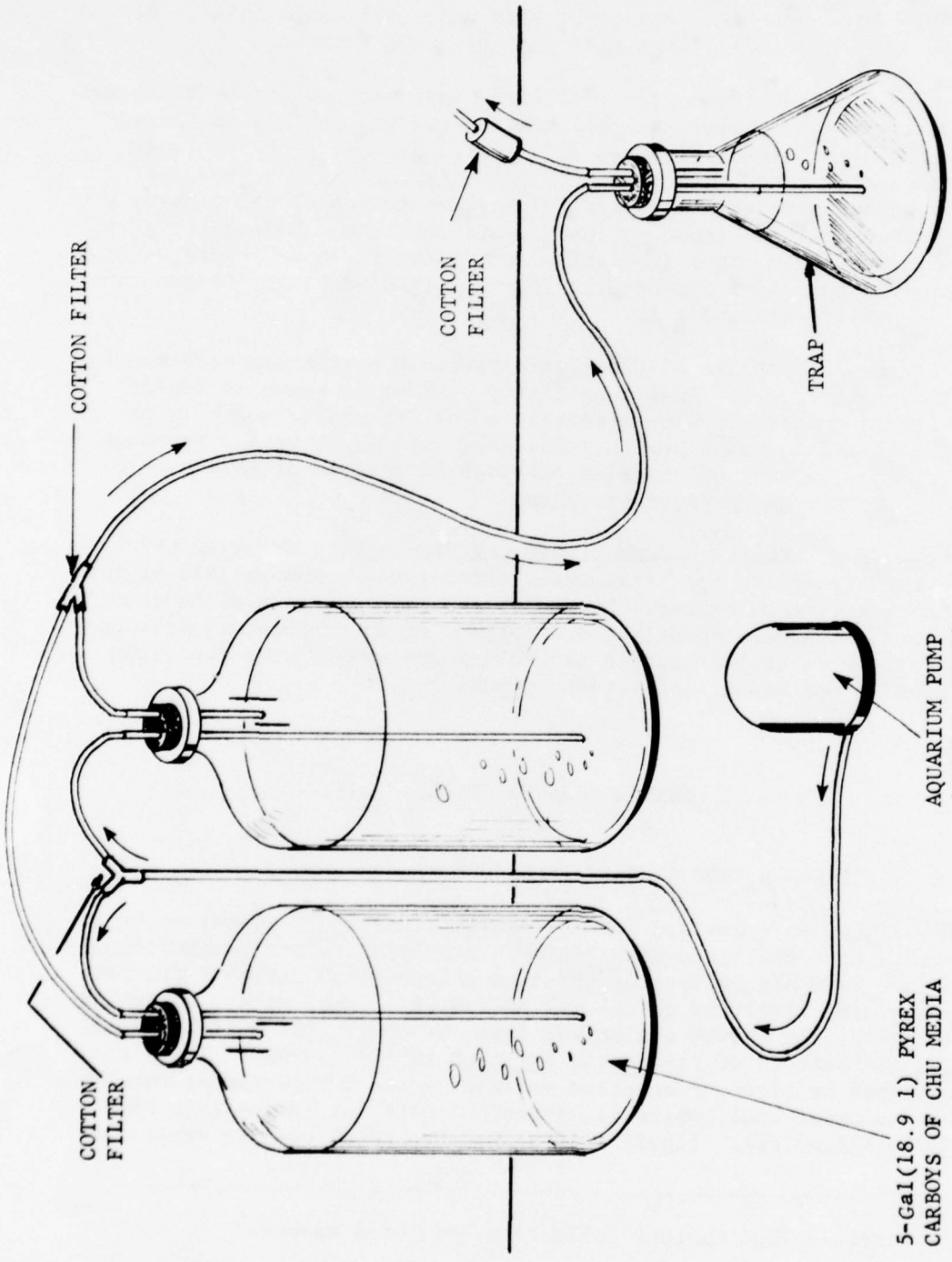

FIGURE 1. BATCH PRODUCTION OF LPP-1

After 14 days of incubation, 100 millilitres of a suspension which contained the virus (ATCC #18200 - B1) was introduced into the algal cultures. To develop this innoculum, 250 millilitre Erylenneyer flasks containing the 100 millilitres of P. boryanum culture (approximately 10^6 cells per millilitre) was infected with the virus (2 millilitres of suspension at approximately 10^7 PFU per millilitre). Lysis was complete after 48 to 72 hours. At 72 hours after introduction of the virus, contents of the Erylenmeyer flasks were poured into the 5-gallon (18.9 litre) carboys of media.

Aeration of the carboy contents was continued while these containers were kept at room temperature for 96 hours after the virus was added. Carboys were then refrigerated at 10°C until use.

ASSAYS

Plaque-forming units (pfu) of the virus were determined through assays as described by Safferman and Morris[3]. All samples were assayed in duplicate. Viral titres usually ranged between 10^3 and 10^8 pfu per millilitre. Figure 2 presents a typical dilation series where cyanophage concentration approaches 0.001 pfu/ml.

In all phases of the study, aseptic techniques were practiced; and experiments were repeated to ensure reproducibility. Glassware was cleaned as prescribed by Standard Methods[19] with the addition of a final rinse in Haemosol,* a coating compound to reduce virus absorption.

A typical experiment involved addition of LPP-1 of known titre and volume into the treatment or holding facility under test. Following a suitable exposure time, samples were taken for quantitative assay of the virus concentration. Facilities representative of the major types of naval sewage disposal systems were tested.

RESULTS

FULL SCALE TESTING OF NCSC SEWAGE TREATMENT PLANT

The NCSC Sewage Treatment Facility, showing sampling stations is diagrammed in Figure 3. The facility consists of a comminutor, bar

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*Haemo-Sol - Scientific Products, Division of American Hospital Supply Corporation. 1/2 oz to 1 gallon of water.

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pfu/1.0 ml pfu/0.1 ml pfu/0.01 ml pfu/0.001

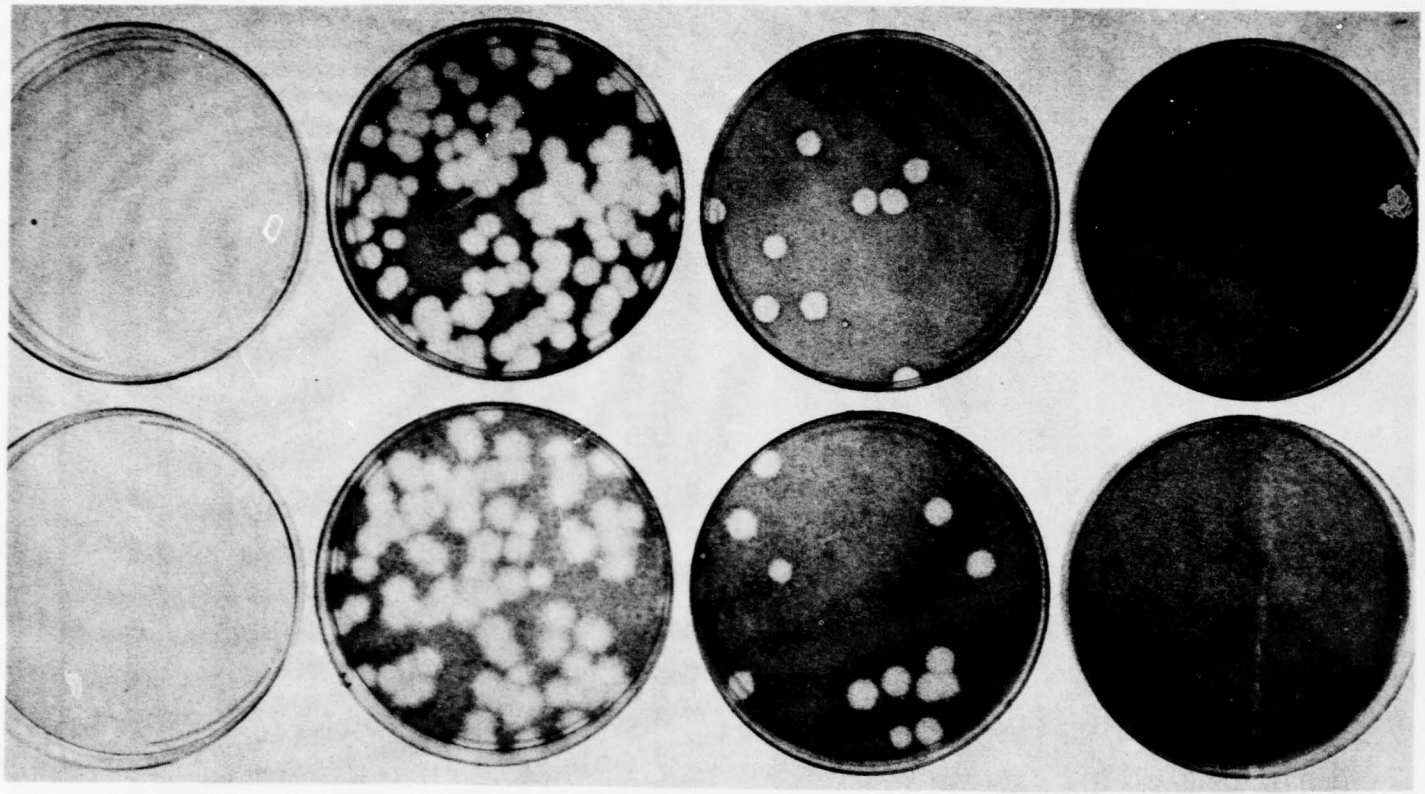

FIGURE 2. TYPICAL DILUTION SERIES WHERE CYANOPHAGE CONCENTRATE APPROACHES 0.001/PFU/ML

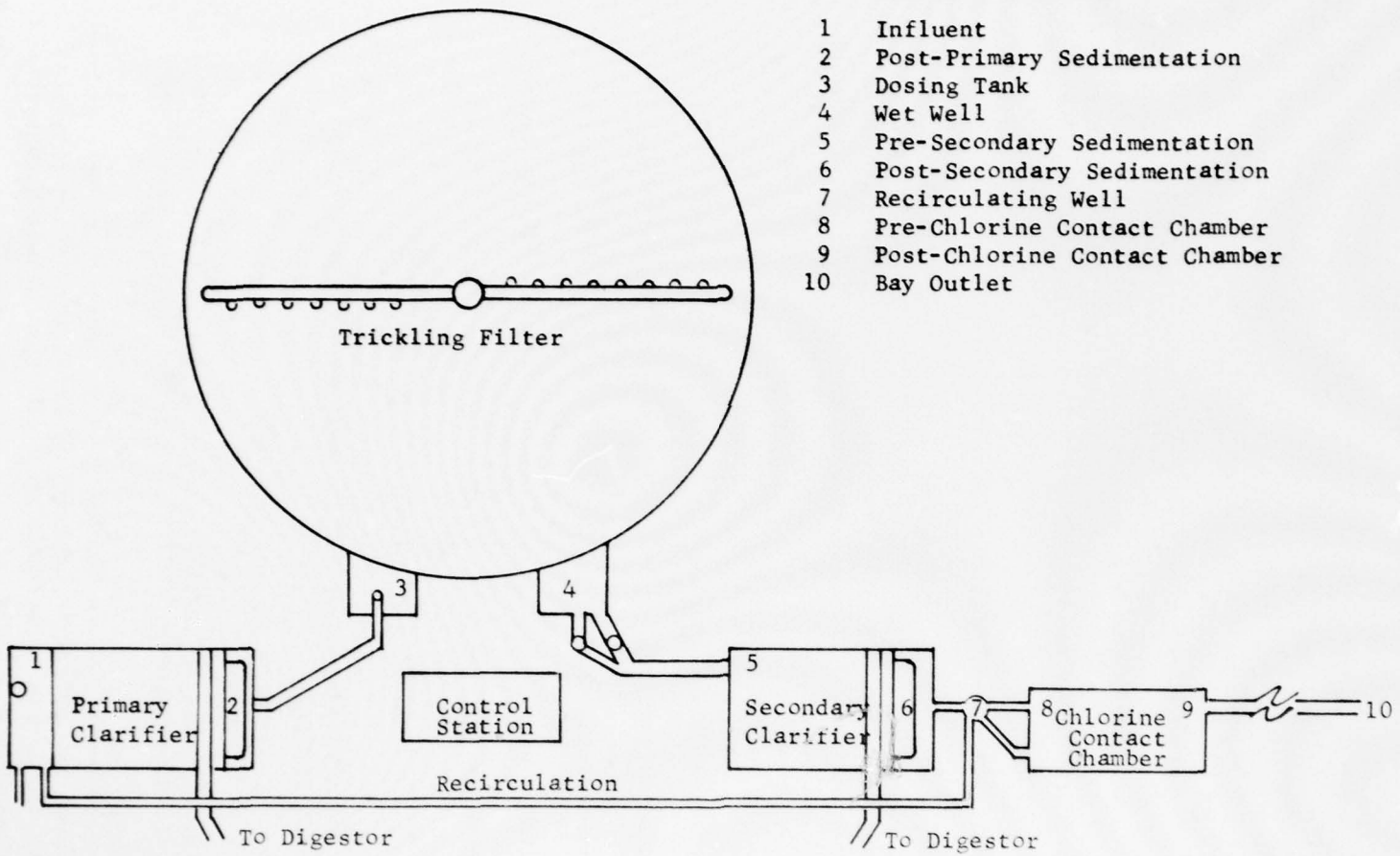

FIGURE 3. NCSC SEWAGE TREATMENT FACILITY

screen (used only when the comminutor is inoperable), a primary settling tank, a trickling filter, a secondary clarifier, and a chlorine contact chamber. Recirculation of effluent (for wetting of the trickling filter during slack periods) into the primary clarifier is approximately 43,000 gal/day (162,755 1/day). Average sewage flow is 70 percent of the maximum capacity of 150,000 gallons per day (567,750 1/day). Chlorine is introduced for terminal disinfection, and ahead of the primary settling tank (by recirculated effluent) for odor control.* the chlorine contact chamber provides a 20 minute holding time with total chlorine residuals averaging 1.0 mg/1. The chlorinated effluent passes through approximately 50 metres of pipe before it is discharged into the St. Andrew Bay.

SEWAGE CHARACTERISTICS - CHEMICAL/PHYSICAL

The sewage at NCSC consists of (1) waste water from shops, laboratories, and maintenance areas, (2) domestic sewage from housing areas, and (3) sewage from the U.S. Coast Guard Station on Alligator Bayou. Sewage flow averages 105,000 gallons per day (416,350 1/day) with the following characteristics:

Settleable Solids (raw)	2-35 ppm
Settleable Solids (final)	trace
Suspended Solids (raw)	5-150 ppm
Suspended solids (final)	5-15 ppm
BOD_5 (raw)	100-200 ppm
BOD_5 (final)	3-15 ppm
pH	6.7-7.2

BIOLOGICAL CHARACTERIZATION OF SEWAGE INFLUENT AND EFFLUENT AT NCSC TREATMENT FACILITY

Bacteria

Experiments 1 and 2 used Plate Count Agar (total bacteria)[19][21] M-ENDO LES (coliforms-total)[19][21] and M-Enterococcus Agar (fecal

*Although fresh raw sewage is usually odorless, the influent to this plant contained inputs from shipboard holding tanks which contributed to occasional odor problems.

streptococci)[21][22]. A composite sample was established by combining 100 ml aliquots at hourly intervals over an 8-hour period. The composite sample also contained 0.2 µl of 10 percent sodium thiosulfate to eliminate residual chlorine activity. Several dilutions were made, and appropriate samples were millipore filtered using phosphate-buffered water for wash procedures. Cultures were incubated at 37°C for 48 hours.

Results in Table 1 indicate initial bacterial concentrations of approximately 10^6 organisms per ml. Total coliform counts averaged 10^4 colony forming units per millilitre (cfu/ml), and fecal streptococci approximately 10^3 cfu/ml. Primary sedimentation, trickling filtration, and secondary sedimentation generally reduced the coliform count by an order of magnitude each. Effluent assays indicated 100 cfu/ml, 10 fecal streptococci per millilitre, and no coliforms. Chlorine activity resulted in one order of magnitude reduction in total bacteria.

TABLE 1

INITIAL BIOLOGICAL CHARACTERIZATION OF
NCSC TREATMENT FACILITY

Station	Total Bacteria (cfu/ml)	Total Coliforms (cfu/ml)	Total Streptococci (cfu/ml)
1	1.6×10^6	1.4×10^5	4.8×10^3
2		5.5×10^4	
3		4.0×10^4	
4		4.0×10^3	
5		6.0×10^3	
6		8.9×10^3	
7		5.0×10^3	
8		1.0×10^2	
9	1.0×10^2	0	<1.0

Experiment 3 attempted to further characterize the bacterial input and output of the sewage treatment facility. Media used for isolation included Salmonella-Shigella Agar (SS Agar)[19][22], M-Enterococcus Agar[21][22], M-Pseudomonas aeruginosa Agar (mPA)[23], Pseudosel

Agar[24][25], Mannitol Salt Agar[20][21][26], and Staphyloccus 110 Agar[21][26]. All media were inoculated and incubated at 37°C for 24-48 hours. Results of a composite versus a grab sample are shown in Table 2. Although no attempt to differentiate between staphylococcus

TABLE 2

BACTERIAL CHARACTERIZATION OF COMPOSITE VERSUS GRAB SAMPLES

Medium	Composite Sample*		Grab Sample**	
	Influent (cfu/ml)	Effluent (cfu/ml)	Influent (cfu/ml)	Effluent (cfu/ml)
M-Enterococcus	335	<1.0	900	<1.0
Pseudosel	90	0	250	0
mPA	60	0	<10.0	0
Staph 110	4200	<1.0	1270	<1.0
Mannitol Salt Agar	1330	<1.0	2230	<1.0
SS Agar (non-lactose)	overgrowth	0	0	<1.0

*8-hour Composite Sample - 100 ml taken at hourly intervals
**Grab Sample - Single 100 ml sample

species was made, levels of total staphylococci averaged 10^3 organisms per millilitre. Pseudomonas data were variable depending on the medium used for primary isolation. mPA results showed Pseudomona saeruginosa numbers of approximately 50 organisms per millilitre. Total Pseudomonas levels approached 100 organisms per millilitre. Results with SS Agar for non-lactose fermenters (Salmonella-Shigella, etc.) appear inconclusive and were repeated later. Effluent characteristically contained less than 10 cfu/100 ml each of Pseudomonas, fecal streptococci, and staphylococci. Results further indicate significant differences between samples. A representative sample was difficult to secure during any test period and meaningful conclusions regarding the sample characteristics for a particular test period were difficult to formulate.

Since initial tests with SS Agar were inconclusive, an expanded series of tests were performed (Table 3). In addition to the above media, Pfizer Selective Enterococcus Agar (PSE)[19][27][28]; KF Streptococcal Agar[19][28], Modified Pseudosel (containing Nalidixic Acid) Agar[30][31], Violet Red Bile Agar-1[30], and Violet Red Bile Agar-2[31]

TABLE 3

BACTERIAL CHARACTERIZATION OF INFLUENT VERSUS EFFLUENT

Medium	Influent* (cfu/ml)	Effluent* (cfu/ml)
M Enterococcus	5450	0
PSE	4400	0
KF Streptococcal	7450	<1.0
mPA	300	0
Pseudosel + Nalidixic Acid	365	0
SS Agar (non-lactose fermenters)	29000	0
VRB-1	24500	0
VRB-2	25000	0
Staph 110	<100	<10.0

*Grab Sample
**PSE-Pfizer Selective Enterococcus; mPA-m Pseudomonas aeruginosa Agar; SS Agar - Salmonella-Shigella Agar; VRB-1 - Violet Red Bile-1; VRB-2 - Violet Red Bile-2.

were used. Sewage influent contained 10^4 cfu/ml streptococci, 10^2 cfu/ml Pseudomonas aeruginosa, 10^4 cfu/ml coliforms, 10^2 cfu/ml staphylococci, and 10^4 non-lactose fermenters (non proved to be Salmonella or Shigella). Effluent contained both the streptococci (< 1.0 cfu/ml) and staphylococci (< 10 cfu/ml).

Cyanophage

Plaquing for the presence of LPP-1 phycovirus, in order to determine background counts, yielded variable results as indicated in Table 4. Background levels of phycorivus could be detected throughout the plant but always in concentrations of less than 10 pfu/ml. LPP-1 was never detected in the chlorinated effluent.

Repeated assays for background levels of the LPP-1 virus showed results comparable to those reported in Sample 2, Table 4. Again, frequency of the virus varied throughout the facility at counts of approximately 10 pfu/ml. Consistent with earlier findings, LPP-1 was not recovered from the post-contact chamber effluent.

TABLE 4

BACKGROUND COUNTS FOR LPP-1 (NCSC SEWAGE TREATMENT FACILITY)

Station	Sample 1 (pfu/ml)	Sample 2 (pfu/ml)
1	0	1
	0	2
2	0	4
	1	5
3	8	3
	3	9
4	8	11
	9	12
5	2	9
	6	10
6	3	9
	3	10
7	0	0
	0	1
8	0	0
	0	1
9	0	0
	3	3
10	0	0
	0	0

FULL SCALE CYANOPHAGE TESTS

Inoculation of the treatment facility influent with 16 litres of suspension containing 10^8 pfu/ml of LPP-1 was undertaken to follow reduction in viral numbers by various components of the treatment facility. Over a period of three days, sampling was conducted at each of the 10 test points throughout the plant. The results are shown in Table 5 along with corresponding data for background bacterial presence. First day reductions in the frequency of LPP-1 of approximately 1 to 2 orders of magnitude were observed at both the primary and secondary sedimentation stages. At no point were counts greater than 10 pfu/ml detected beyond the secondary sedimentation stage. No LPP-1 presence was detected in the final effluent at any time (Table 6).*

*Samples from the effluent receiving waters in St. Andrew Bay were checked for presence of the cyanophage before, during, and after the study. Station locations for this 2-year sampling effort are shown in Appendix A.

(Text Continued on Page 14)

TABLE 5

RECOVERY OF LPP-1 FROM SEWAGE TREATMENT PLANT*

	Day 1 Sample				Day 2 Sample LPP-1 (pfu/ml)	Day 3 Sample LPP-1 (pfu/ml)
	Total Coliforms (cfu/ml)	Fecal Streptococci (cfu/ml)	Total Bacteria (pfu/ml)	LPP-1 (pfu/ml)		
Influent	1.5×10^5	4.4×10^3	3.7×10^6	1.0×10^5	1.2×10^4	$<1.0 \times 10^2$
Post-primary	1.9×10^5	1.2×10^4	1.2×10^7	1.0×10^4	1.2×10^3	$<1.0 \times 10^2$
Dosing Tank	1.7×10^5	3.0×10^3	2.2×10^7	1.0×10^4	4.3×10^3	ND
Wet Well	ND	ND	ND	ND	7.0×10^2	$<1.0 \times 10^2$
Pre-secondary	2.0×10^4	6.5×10^2	1.1×10^5	1.2×10^4	1.3×10^4	$<1.0 \times 10^2$
Post-secondary	2.0×10^4	3.0×10^2	ND	1.3×10^2	2.2×10^3	$<1.0 \times 10^2$
Recirculating Well	1.8×10^4	1.6×10^2	ND	ND	$<1.0 \times 10^1$	ND
Pre-contact	1.0×10^2	1.0×10^0	1.0×10^4	ND	ND	ND
Post-contact	2.0×10^0	0	1.3×10^2	ND***	ND	ND
Bay outlet	NDONE**	NDONE	NDONE	ND	NDONE	NDONE

*Three-day test—Frequency of Recovery. Added 16 litres of 10^8 pfu/ml LPP-1.
**Not Done (NDONE).
***Not Detected (ND).

TABLE 6

INFLUENCE OF CHLORINE CONTACT CHAMBER ON LPP-1*

Post-Inoculation (min.)	Dilution			
	10^1	10^2	10^3	10^4
0	0	0	0	0
	0	0	0	0
10	0	0	0	0
	0	0	0	0
20	0	0	0	0
	0	0	0	0
30	0	0	0	0
	0	0	0	0
45	0	0	0	0
	0	0	0	0
60	0	0	0	0
	0	0	0	0
90	0	0	0	0
	0	0	0	0
120	0	0	0	0
	0	0	0	0
180	0	0	0	0
	0	0	0	0

*Chlorine Contact Chamber
Initial LPP-1 Concentration 10^4 pfu/ml
Holding Time: 20 minutes

Although the purpose of this study was to demonstrate the feasibility of using this virus-host system rather than elucidate the performance of the NCSC treatment plant, the test was repeated. As would be expected, two subsequent tests yielded significant variations in viral counts for various components of the plant. However, no problems were encountered in the technique.

DISCUSSION AND CONCLUSIONS

In their recommendation of LPP-1/Plectonema boryanumas as an indicator for virus studies, Shane, Wilson, and Fries stated that much information concerning virus removal in water and sewage treatment processes could be gained by using this virus-host system. The work presented here demonstrates the use of LPP-1 for such a study, and documents the feasibility of employing this simple system for modeling studies with viruses.

Smedberg and Cannon have proposed the use of this cyanophage as a pollution indicator. However, serious objections have been raised to using LPP-1 as an indicator of fecal pollution . While the system may not meet reasonable criteria for an indicator of sewage presence, it clearly might serve as a model in studies of the effectiveness of various treatments for eliminating viruses. For military purposes, studies which take advantage of the easy culture and assay of this cyanophage may be useful for determining the vulnerability of facilities to attack with biological agents.

The technique is simple and inexpensive. Problems involving bacterial contamination and plaque enumeration which complicate the use of bacteriophages and animal viruses do not occur with this system. Furthermore, its wide distribution and characterization as a relatively "safe" agent from both the environmental and human health point of view strongly recommend this virus-host system as a tool.

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APPENDIX A

STUDY AREA AND ENVIRONMENT

AREA DESCRIPTION

The St. Andrew Bay estuarine system is located on the northwest coast of Florida, between latitudes $30^{o}00'$ and $30^{o}20'$N and longitude $085^{o}20'$ and $085^{o}53'$W. This system consists of four coastal plain bays - North, East, West, and St. Andrew Bay. This study was conducted along the northwestern shore of St. Andrew Bay, adjacent to the Naval Coastal Systems Center (Figure A1). St. Andrew Bay is the center of this estuarine system, and of the four bays listed above, it is the only one with openings to the Gulf of Mexico. The average depth of St. Andrew Bay is 5 metres ($16\frac{1}{2}$ ft)[A1].

The turbidity of this estuarine system is low due to the low fresh water inflow and the porosity of the soils. The clarity of this water is not too different from that of open sea water[A2].

The St. Andrew Bay system contains areas of submerged vegetation. Species consist of Thalassia testudinum, turtle grass, Syringodium filiforme, manatee grass, and Diplantera wrightii, shoal grass[A3].

ENVIRONMENTAL DATA

Sampling was conducted periodically between November 1973 and February 1975 to provide data before, during, and after the study dates. Sampling times were 0800 and 1200 hours each sampling day. Samples for dissolved oxygen, Biochemical Oxygen Demand (BOD), turbidity, and pH were taken just below the water's surface with an 8 litre water sampler. Salinity and temperature were determined in situ with a Beckman R55-3 portable salinometer (accuracy $\pm 0.5^{o}$C and ±0.3 percent). The pH was determined with an Orion Model 404 portable pH meter (accuracy ±0.05 pH units) and turbidity was determined with a Hack Model 2100A turbidometer (accuracy ±0.02 JTU). The modified Winkler method was used for the dissolved oxygen and BOD determinations (accuracy ±0.05 ppm)[A4]. Total bacterial numbers were obtained by cultural methods. Pour plates consisting of plate count agar with 75 percent aged sea water were used for bacterial enumeration. Plates were incubated 37^{o}C for 24 hours[A5]. Total coliforms were determined according to Standard Methods[A6]. These data are presented in Table A1.

(Text Continued on Page A-4)

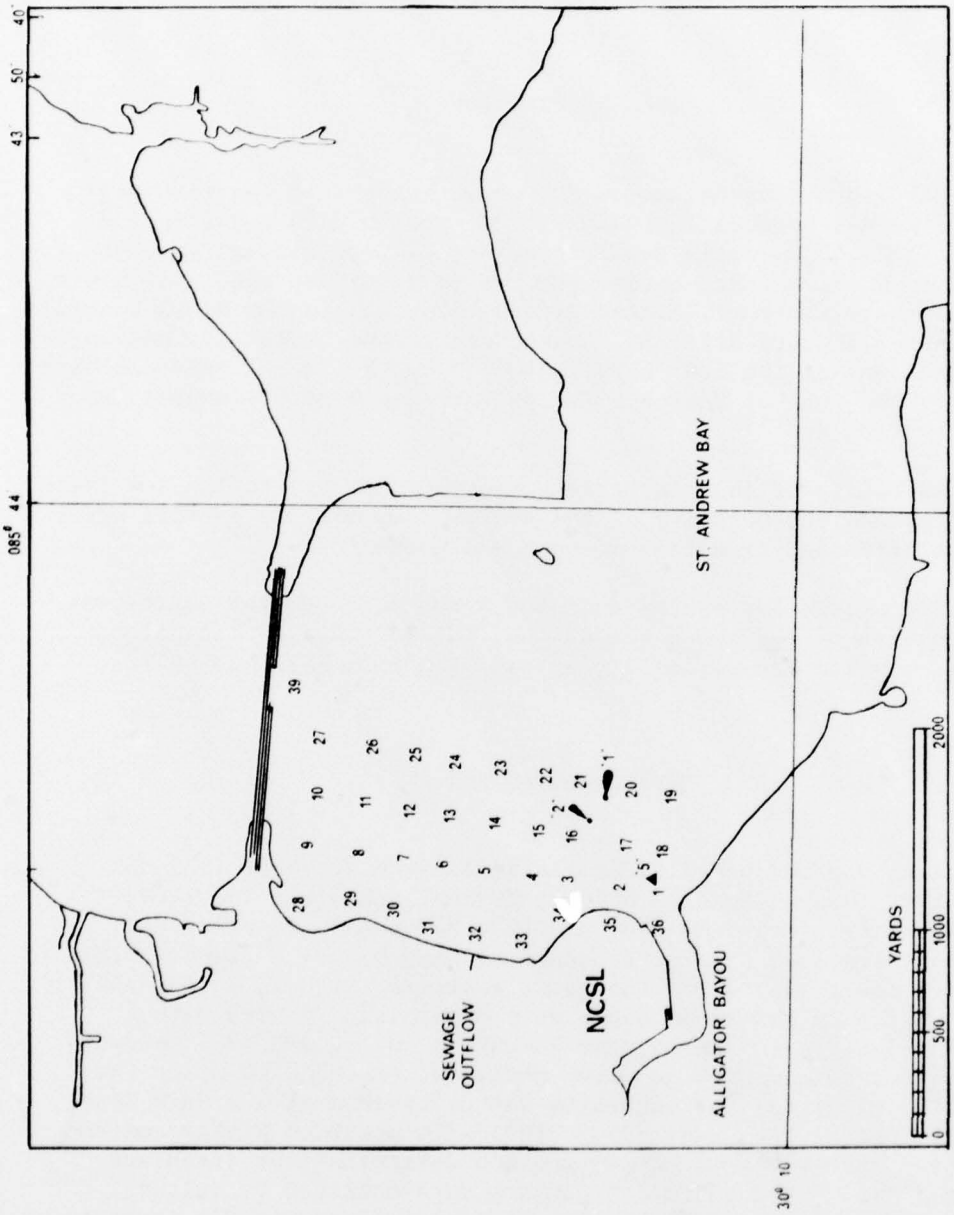

FIGURE A1. LOCATION OF SAMPLING STATIONS OFF NCSC

TABLE A1

SURFACE WATER QUALITY - ST. ANDREW BAY

Parameter	-	1973 Nov	1974 Feb	1974 May	1974 Sept	1975 Feb
Acidity (pH)	$\bar{x}$	8.2	8.2	8.2	8.1	8.2
	s	0.09	0.04	0.10	0.16	0.08
	n	32	35	36	36	36
Salinity (ppt)	$\bar{x}$	28.4	22.8	28.5	21.1	23.0
	s	2.6	1.9	0.8	0.6	2.5
	n	36	36	36	35	36
Water Temperature (^{o}C)	$\bar{x}$	20.3	13.3	25.1	28.6	16.1
	s	0.4	0.8	0.5	0.1	0.2
	n	29	36	36	36	28
Air Temperature (^{o}C)	$\bar{x}$	24.4	18.2	25.4	27.4	12.8
	s	2.3	4.2	1.0	1.7	1.5
	n	36	36	36	36	28
Turbidity (JTU*)	$\bar{x}$	0.82	0.61	0.46	0.92	1.5
	s	0.42	0.34	0.16	0.96	1.0
	n	35	36	36	35	35
Dissolved Oxygen (ppm)	$\bar{x}$	8.3	9.4	6.8	6.4	8.7
	s	0.8	0.3	0.5	0.5	0.3
	n	10	10	10	10	10
Biological Oxygen Demand (ppm)	$\bar{x}$	1.4	1.3	1.6	2.3	2.4
	s	0.6	0.3	0.4	1.0	0.6
	n	10	10	10	10	10
Total Bacteria (cfu/ml)	$\bar{x}$	43.4	50.4	161.8	216.4	141.9
	s	116.8	77.3	230.1	467.8	394.8
	n	11	17	19	19	19
Total Coliform Bact. (cfu/ml)	$\bar{x}$	1.6	1.6	0	7.8	0
	s	0.8	1.9	0	16.8	0
	n	7	17	19	19	19

*JTU - Jackson Turbidity Units
$\bar{x}$ - Mean
s - Standard deviation
n - Number of stations sampled

Both direct and indirect methods were used to assay for LPP-1[A7]. Although the virus is widespread in nature, it was never isolated from St. Andrew Bay. However, experiments with the virus do not pose a problem to this estuarine environment because chlorination rapidly inactivates laboratory strains of the virus[A7] and neither virus nor host are pathogenic to man.

APPENDIX A - REFERENCES

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