HALL

OCCURRENCE OF SALMONELLA AND VIBRIO PARAHAEMOLYTICUS IN THE NEW BEDFORD AREA OF BUZZARD'S BAY

A Thesis

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of Southeastern Massachusetts University

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by

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BIOGRAPHICAL SKETCH

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	8
of microorganisms from prepared samples	12
RESULTS	26
Delineation of the study area	26
Physicochemical parameters of the seawater	26
Microbiological parameters	36
DISCUSSION	52
Distribution and occurrence of potentially pathogenic bacteria	52
Distribution and occurrence of indicator bacteria	
The influence of season and source(s) of contamination	55
The influence of precipitation and wind on the distribution of indicator bacteria	56
Conclusion	60
LITERATURE CITED	61
APPENDICES	74
A. Predicted contours of dilution after 49.5 hours	74
B. Vibrio parahaemolyticus-Review	82
C. Survival of enteric bacteria in seawater	88

Page

D. Laboratory studies - Vibrio parahaemolyticus . 119

1. Growth of Vibrio parahaemolyticus in filter-sterilized seawater 119

2. Comparison of Thiosulphate Citrate Bile Salts agar and Starch-Galactose agar . . 119

3. Growth of Vibrio parahaemolyticus in Tryptic Soy Salts broth with 0.85, 3.0, and 7.0% sodium chloride at 37 C and 41 C 123

LIST OF TABLES

Table		Page
1.	Comparison of the composition of the Twedt-Novelli, Levin-Watkins m-VP and the proposed Starch-Galactose mediums	23
2.	Characteristics of the Vibrio parahaemolyticus type strain (EB 101) ATCC 17802	24
3.	Physicochemical parameters of the seawater . . .	31
4.	Microbiological parameters of the seawater . . .	39
5.	Total coliform counts of marine invertebrates compared with the overlying seawater . . .	44
6.	Biochemical and serological characteristics of Salmonella isolated	49
7.	Growth of V. parahaemolyticus at 20 C in filter-sterilized New Bedford seawater and a comparison of Thiosulfate Citrate Bile Salts and Starch-Galactose agars	51

LIST OF ILLUSTRATIONS

Figure — Page

1. Predicted contours of dilution after 49.5 hours (tidal current only; end of flood). (After Marini, 1974) 75
2. Predicted contours of dilution after 49.5 hours (tidal and northwest current; end of flood). (After Marini, 1974) 77
3. Predicted contours of dilution after 49.5 hours (tidal and northwest current; end of flood). (After Marini, 1974) 79
4. Predicted contours of dilution after 49.5 hours (tidal and southwest current; end of flood). (After Marini, 1974) 81
5. Methods for isolation of Salmonella spp. and Vibrio parahaemolyticus; and methods for determination of total and fecal coliforms from seawater 14
6. Methods for isolation of Salmonella spp. and Vibrio parahaemolyticus; and for determination of total and fecal coliforms from marine invertebrates 16
7. Flow diagram of the confirmation of presumptive Salmonella cultures isolated 19
8. General study area showing location of sampling sites 28

Figure Page

9. Primary New Bedford study area showing location of sampling sites 30

10. Seasonal water temperature 38

11. Coliform counts at increasing distance from outfall 58

12. Typical survival curves for enteric bacteria in seawater (After Orlob, 1956) 93

13 A. July *in situ* survival of *E. coli* ATCC 11775 and *S. enteriditis* T59/21a in seawater . . 102

13 B. Tidal cycle calculated from National Ocean Survey Tide Tables 102

13 C. Air and water temperature recorded in degrees Centigrade 102

13 D. Salinity recorded in parts per thousand . . . 104

13 E. Concurrently recorded pH 104

13 F. Total coliforms and fecal coliforms per 100 ml seawater 106

13 G. Presumptive vibrios and presumptive *Vibrio parahaemolyticus* 108

13 H. Dissolved oxygen recorded as mg O_2/liter . . . 108

13 I. Biochemical oxygen demand shown as mg O_2/liter 108

13 J. Dissolved organic nitrogen recorded as microgram-atoms/liter 108

13 K. Nitrites as microgram-atoms NO_2-N/liter . . . 110

13 L. Nitrates as microgram-atoms NO_3-N/liter . . . 110

Figure Page

13 M. Orthophosphates as microgram-atoms PO_4-P/liter 110

14 A. September *in situ* survival of *E. coli* ATCC 11775 and *S. enteriditis* T59/21a in seawater 112

14 B. Tidal cycle calculated from the National Ocean Survey Tide Tables 112

14 C. Water temperature recorded as degrees Centigrade 112

14 D. Air temperature recorded as degrees Centigrade 114

14 E. Salinity recorded as parts per thousand . . . 114

14 F. Concurrently recorded pH 114

14 G. Nitrites reported as microgram-atoms NO_2-N/liter 114

14 H. Nitrates reported as microgram-atoms NO_3-N/liter 116

14 I. Orthophosphates recorded as microgram-atoms PO_4-P/liter 116

14 J. Total and fecal coliforms per 100 ml seawater. 116

14 K. Presumptive vibrios and presumptive *Vibrio parahaemolyticus* plotted as log number organisms per 100 ml seawater 118

15. Growth of *Vibrio parahaemolyticus* in filter-sterilized seawater collected from Clarks Cove 122

Figure Page

16. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in Tryptic Soy Salts Broth with 0.85% sodium chloride 125

17. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in Tryptic Soy Salts Broth with 3.0% sodium chloride 127

18. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in Tryptic Soy Salts Broth with 7.0% sodium chloride 129

APPENDICES

APPENDIX Page

A. Predicted contours of dilution after 49.5 hours 74

B. *Vibrio parahaemolyticus* - Review 82

C. Survival of enteric bacteria in seawater . . 88

D. Laboratory Studies - *Vibrio parahaemolyticus*. 119

1. Growth of *Vibrio parahaemolyticus* in filter-sterilized seawater. 119

2. Comparison on Thiosulphate Citrate Bile Salts agar and Starch-Galactose agar. . . 119

3. Growth of *Vibrio parahaemolyticus* in Tryptic Soy Broth with 0.85, 3.0, and 7.0% sodium chloride at 37 C and 41 C . . 123

OCCURRENCE OF *SALMONELLA* AND *VIBRIO PARAHAEMOLYTICUS* IN THE NEW BEDFORD AREA OF BUZZARD'S BAY

Abstract

The incidence and occurrence of indicator organisms and potential pathogens were studied at 17 different stations in Buzzard's Bay. Samples were collected primarily from Clarks Cove New Bedford area from July 1974 through September 1975. The highest frequency of occurrence of total and fecal coliforms was at the marine outfall of the New Bedford municipal sewage plant. The second highest frequency of occurrence of these organisms was in the northernmost part of Clarks Cove. The possibility of an overflow condition in this area during periods of significant rainfall and the implications are discussed.

Salmonellae were isolated from New Bedford raw sewage by the Moore swab technique, but not by the catch or high-volume filtration methods. Salmonellae were not recovered from seawater by any of these techniques. One *Mercenaria mercenaria* (quahog) sample yielded a *Salmonella enteriditis*, serotype C_2 isolate. No salmonellae isolates were recovered from other environmental sources.

Vibrio parahaemolyticus was not isolated from any environmental source during the study. Laboratory studies indicated that the organism was capable of survival and growth in the New Bedford marine waters. However, it was concluded that the normally high salinity (32 parts per thousand) and

pH (pH 8.1) prevent the establishment of the annual cycle of this pathogenic vibrio in Clarks Cove.

To assess the effects of New Bedford marine waters on bacterial indicators and pathogens, in situ survival of Escherichia coli ATCC 11775 (laboratory culture) and Salmonella enteriditis T59/21a, serotype Group C_2 (freshly isolated from sewage) was examined in relationship to several physicochemical and microbiological parameters. Viable cell counts were determined from aliquots of inocula contained within dialysis sacs suspended in seawater. Physicochemical parameters of the seawater recorded simultaneously were: water temperature, salinity, pH, dissolved oxygen (DO), and biochemical oxygen demand (BOD_5). Microbiological parameters included: total colony-forming bacteria per 100 ml on Thiosulphate Citrate Bile Salts agar (TCBS), counts per 100 ml of sucrose negative colony-forming bacteria on TCBS, and total and fecal coliforms counts per 100 ml seawater. Survival of both E. coli ATCC 11775 and S. enteriditis T59/21a was higher in September than in July. Survival of S. enteriditis was significantly higher than E. coli during both the July and September studies. Results: water temperature, salinity, pH, DO, and BOD_5 suggested an inverse relationship to percent survivals. Nitrates, nitrites, dissolved organic nitrogen, and phosphates generally showed an inverse relationship to percent survivals. Total and fecal coliforms counts exhibited a delayed inverse relationship to survival curves. Both total and sucrose negative

colony counts from TCBS showed a directly proportional relationship to percent survivals.

The results of this study indicate some specific problems encountered in coastal cities, such as New Bedford, which depend on marine disposal of sewage. The recovery of a pathogen from shellfish in this area and the persistence of this pathogen in in situ survival studies is particularly relevant, in view of the frequency of illegal shellfishing in this area, either for personal consumption or public sale.

INTRODUCTION

The impact of man's disposal of domestic and industrial sewage in the marine environment has been increasing at an alarming rate. The economic and social impacts have been well documented. By 1948, over 60 percent of the clam-producing areas in Massachusetts had been closed. It was predicted that more clam beds would be closed as the marine disposal of wastes continued. The closing of shellfish beds in New Bedford in April 1967 and in nearby Fairhaven in 1971 by the Massachusetts Department of Public Health appears to confirm that prediction. The closing of these beds represented a substantial economic loss to the area. According to Mr. Romeo Mosakowski, New Bedford Shellfish Constable, shellfish with a potential market value of about $350,000 remain in the area (personal communication). Due to economic pressures and local tastes, illegal shellfishing has not been uncommon, according to Mr. Mosakowski and local residents (personal communications).

Man has long assumed that the sea has an almost infinite capacity to distribute and assimilate waste materials without environmental impact. It has become increasingly clear that this assumption is incorrect. Perfect distribution does not occur in either coastal areas or the open sea. Waste materials may be accumulated or concentrated by physical or biological processes.

In 1971 a water quality survey was conducted in the New Bedford, Massachusetts, area (82). It was found that coliform

counts decreased with increasing distance from the sewage outfall.

In 1974 Camp, Dresser and McKee (48) a Boston engineering firm, studied the distribution and dilution of New Bedford sewage following marine disposal. The results shown in Figures 1, 2, 3, and 4 (Appendix A) agree with the bacteriological data of the earlier water quality survey.

It is clear that "perfect" distribution does not occur in the New Bedford area. The distribution of sewage in the marine environment depends on factors such as tidal and meteorological conditions and currents. Poor dilution and distribution of sewage along coastal areas such as New Bedford can endanger the public health.

The majority of sewage effluents discharged into marine waters are untreated or minimally treated. The New Bedford sewage treatment plant provides only primary treatment and chlorination. The plant affords primary treatment to an average dry-weather flow of 30 million gallons of sewage per day (mgd) (48). The plant was designed to treat all flow transported by the interceptor, but combined dry-weather and stormwater flows exceed the interceptor capacity of 120 mgd and result in overflows. Although the plant is capable of removing the larger suspended material from the wastewater, very little of the finer solids and none of the dissolved material are removed. New Bedford's sewage is comprised of both domestic and industrial wastes.

This study was initiated to determine the occurrence of

Salmonella spp., a bacterial pathogen commonly associated with sewage, and of Vibrio parahaemolyticus, a bacterial pathogen of marine origin often found associated with sewage-contaminated marine waters (47). Although this objective was of the major importance, several minor studies were also conducted in support of this objective. These studies will be included in the appendices and referred to in the appropriate sections. The results of this study address an academic problem. However, implications relate to public health problems facing many coastal cities.

In addition to Salmonella, the most common water-borne pathogens found in sewage-polluted waters are: Shigella, Leptospira, enteropathogenic Escherichia coli, Pasteurella, Vibrio, Mycobacterium, human enteric viruses, cysts of Endamoeba histolytica, and hookworm larvae (31). The source of these pathogens may be pets, livestock, poultry, the wild animal community, or human infections. Salmonella strains have been isolated from sewage, streams, irrigation waters, wells and tidal waters. The sources of these strains may depend on their host adaptation (8). Detection of the other pathogenic organisms from the water environment has been less frequent because of the difficulties in their isolation and identification. Salmonella typhi has been brought under control in the United States and, in 1970, there were only 533 isolations of Salmonella typhi, of which 147 were asymptomatic carriers. The carrier state of salmonellosis is temporary for most patients. Permanent carriers primarily

occur with the typhi and paratyphi groups. Saphra and Winter (8) found a difference in carrier prevalence among different serotypes and estimated a 0.2% carrier rate in the general population. For a city the size of New Bedford with a population of 100,000, the carrier rate, based on this estimate, would be approximately 200 persons shedding salmonellae at any one time. This source, combined with the animal sources indicated previously, would contribute to the number of salmonellae found in the sewage and to the potential hazard of the marine disposal of sewage effluent.

An important route of reinfection of man, by salmonellae entering the marine environment, is the consumption of raw or inadequately cooked shellfish which have been exposed to sewage-contaminated water. The relation between ingestion of raw shellfish and enteric diseases such as typhoid fever has been recognized since the early seventeenth century according to Ratzan et al. (64). Sporadic outbreaks of typhoid fever following the consumption of raw shellfish occurred often in the United States in the early 1900's (58). In 1924 there was an outbreak of 1,500 cases of typhoid fever, with 150 deaths directly associated with the ingestion of raw oysters obtained from Raritan Bay, New Jersey. This incident was instrumental in the establishment of the National Shellfish Sanitation Program of the Public Health Service (80). In 1924 the city of New Bedford reported 18 cases of typhoid fever, with six deaths related to consumption of raw quahogs taken from the Clarks Cove-Buzzard's

Bay area (21). Several other specific incidents were cited in 1965, where consumption of shellfish harvested from sewage-polluted areas resulted in a number of persons contracting typhoid fever (80).

To determine more specifically the potential public health hazards of polluting shellfish-growing waters by domestic sewage, Metcalf et al. (51) conducted an extensive 6-year study of a New Hampshire estuary. At times, they isolated salmonellae from one of every three samples examined. The occurrence of salmonellae in shellfish was not found to parallel occurrences in overlying waters. Furthermore, the number of total or fecal coliforms found in shellfish-growing waters often did not reflect the presence or the absence of salmonellae in shellfish. Mussels, oysters and cockles have been reported to filter out and accumulate material, including pathogens. Mussels have been considered particularly dangerous because they are relatively insensitive to low salinities and mild pollution (61).

Sewage pollution of marine recreational waters is yet another area of public health concern. British authorities generally consider direct contamination of bathers by sewage-derived pathogens to carry little risk unless one is exposed to relatively heavily polluted waters (61). Cabelli et al. (11) discussed the available information on the subject and the difficulties of evaluating observations. A progress report on their ongoing study in 1974 (81) reported that ... "a statistically significant increase in the incidence of

gastrointestinal symptoms among swimmers relative to non-swimming beach goers was observed at ... a barely acceptable beach." The severity index (stayed home, stayed in bed, visited a physician) was found to be higher among swimmers than among non-swimmers at the barely acceptable beach.

The public health hazards of consuming shellfish harvested from sewage-contaminated waters and the recreational use of these waters have been discussed in view of pathogens originating from sewage. The organic component of sewage may, under certain conditions, contribute to another potential health hazard associated with the marine environment. Certain indigenous marine bacteria can utilize the nutrients introduced by disposal of wastes into the marine environment. The genus Vibrio has the biochemical versatility to utilize these nutrients.

A marine halophilic pathogen, Vibrio parahaemolyticus, which has received much attention in recent years, has been suspected of a close association with areas of high organic content or chitinous material, such as found in marine waters polluted with domestic or industrial wastes. Mackowiak (23) estimated that at least 2% of all foodborne outbreaks of gastroenteritis in the United States are caused by this marine pathogen. In areas in which seafood is consumed in large quantities, the incidence is probably considerably higher. It has been suggested that the overall biochemical versatility of the genus Vibrio correlates well with limited data indicating that V. parahaemolyticus and related vibrios

are found in higher numbers in waters which receive animal wastes than in waters of low organic content. Vasconcelos et al. (87) were unable to isolate vibrios from stations remote from canning or processing operations, but recovered the organisms from waste discharges, seawater and sediments near plants. The increase in organic content attended by the disposal of seafood wastes through outfalls a few meters off shore was suggested as more favorable to the existence of the vibrios (87). It has been observed in the Puget Sound region that V. parahaemolyticus seemed to be more numerous in inshore areas and particularly in harbor areas. It was noted that V. parahaemolyticus appeared to be limited to estuarine waters which had higher concentrations of organic matter than open ocean (36).

The distribution of reports of Vibrio parahaemolyticus, both geographically and ecologically, suggests that the vibrio might be found in the New Bedford area (Appendix B).

MATERIALS AND METHODS

Seawater. Catch samples were collected and transported according to the guidelines set by the American Public Health Association (APHA) (2,3). Gauze swabs were suspended in seawater for periods of 5 to 10 days as a seawater sampling technique (2,3,19,46). On collection, each swab was placed into a sterile Whirl-pak (NASCO, Fort Atkinson, Wis.) and transported on ice in an insulated container to the laboratory. Swabs were either placed directly into appropriate enrichment broths or the fluid expressed and used in enrichment or enumeration techniques.

Volumes of up to 20 liters were examined qualitatively by using a high-volume filtration (HVF) method described by Levin et al. (45) for the enumeration of micoorganisms present in very low numbers in seawater. After filtering the seawater through a Balston Type AA cartridge filter (2.5 X 6.4 cm) held by a Type 90 filter holder (Balston Filters, Inc., Lexington, Ma.), the filter was aseptically placed into appropriate enrichment media.

Salinity was determined by a salinity hydrometer (Oceanography Unlimited, Incorporated, Paterson, N.J.) or a Salinometer, Model RS5-3 (Beckman Instruments, Inc., Cedar Grove, N.J.).

A Corning Research pH Meter, Model 12 (Corning Scientific Instruments, Corning, N.J.) was used to determine pH. Biological oxygen demand (BOD_5) was determined using the standard 5-day incubation period (2,3). Dissolved

oxygen (DO) was determined using the Azide Modified Winkler Method (2,3). Dissolved organic nitrogen (DON), nitrites (NO_2), nitrates (NO_3), and orthophosphate (PO_4) were determined according to the procedures outlined in Practical Handbook of Seawater Analysis (79).

Tidal cycles were calculated from the Tide Tables prepared by the National Ocean Survey for 1974 and 1975 (79).

Shellfish. Samples of shellfish stock were collected using shellfish tongs from a boat or collected by hand in the intertidal zone or with SCUBA depending on the species and accessibility of the shellfish. Samples were placed into new polyethylene bags and immediately into an insulated container on ice for transportation to the laboratory. All shellfish samples were processed within 6 hours of collection and most were processed within 3 hours.

Bivalves sampled included Mercenaria mercenaria, Mytilus edulis, and Modiolus demissus (71). The shellfish were prepared for opening according to the procedures recommended by the APHA (2). The tissues and fluid were collected in a sterile beaker and weighed. The samples were then transferred to a sterile Waring Blender, Model DS-7 (Waring Products Corporation, Winsted, Conn.), with an equal amount, by weight, of the appropriate sterile phosphate buffered dilution water (pH 7.0) (2,3). The samples were ground for 60 to 90 seconds at high speed, ca. 14,000 RPM. The resulting shellfish homogenate (SFH) was used in subsequent culturing techniques.

The gastropods Littorina littorea and Crepidula

fornicata (71) were scrubbed as effectively as possible under tap water with a sterile brush and then allowed to drain briefly. In preparing L. littorea samples, each individual was crushed with a pair of sterile pliers and collected in a sterile beaker. After weighing the samples, with shells, the procedure was continued as for bivalves in preparation of SFH. C. fornicata "stacks" were separated and the animals removed from the shells with a sterile knife. The shellfish were then weighed without shells and the SFH prepared as for bivalves.

Crustacean shellfish consisting of Neopenope texana and Carcinus maenas (71) were rinsed under running tap water and allowed to drain. After weighing the crabs, the appropriate salinity phosphate buffered dilution water (PBDW) was added and the sample blended intact to prepare the SFH.

The barnacle Balanus amphitrite niveus (71) was processed by first weighing out a sufficient number of individuals and then blending the animals intact with an equal amount by weight of the appropriate salinity PBDW. The resultant SFH was used in the various culturing techniques.

Sewage. Untreated sewage was sampled by catch and the gauze swab methods (3,19,46). Catch samples were examined for salmonellae using the HVF method (45) with subsequent culturing of the filters in enrichment broths. Gauze swabs were autoclaved in paper bags and placed in the sewage flow near Station 10 at the Screen House. All city sewage flows through this point to the municipal sewage plant located on

Clarks Point. After 5 days the swabs were collected and placed immediately into appropriate enrichment media and transported to the laboratory within 1 hour of collection. Catch samples were transported at ambient temperature and processed within 1 hour.

FIG. 6. Methods for isolation of Salmonella spp. and Vibrio parahaemolyticus; and for determination of total and fecal coliforms from marine invertebrates.

Legend

HE - Hektoen Enteric agar

MPN - Most Probable Number technique

SEF - Selenite F broth

SG - Starch-Galactose agar

SS - Salmonella-Shigella agar

TCBS - Thiosulphate Citrate Bile Salts agar

TSSB - Tryptic Soy Salts Broth

TTB - Tetrathionate Broth

Methods and Media for Enumeration and Isolation of Microorganisms from Prepared Samples

Determination of Total Coliforms. Seawater was examined for total coliform counts using the membrane filter technique as outlined by the APHA (2,3) (Figures 5 and 6). Membrane filters, HAWP 47mm diameter, 0.45 micron porosity (Millipore Corp., Bedford, Ma.) were used routinely throughout the study. Filters were placed onto pads containing ca. 2.5 ml m-Endo Broth MF (Difco Laboratories, Detroit, Mich.) and incubated at 37 C for 18-24 hours.

Total coliform counts were also determined by the multi-tube Most Probable Number technique. Lauryl Tryptose Broth (Difco) was inoculated with selected dilutions and incubated at 37 C.

Determinations of Fecal Coliforms. Fecal coliform counts were determined for seawater by the membrane filter technique using m-FC agar (Difco). The filters were incubated at 44.5 C in a water bath for 24 hours as directed by the APHA (2,3).

Fecal coliform counts of shellfish homogenate were determined by the use of the MPN procedure in conjunction with EC medium as formulated by the APHA (2,3). Incubation was at 44.5 C in a water bath for 24 hours.

Isolation of Salmonella spp. Two enrichment media were used during the study, Tetrathionate Broth (BBL, Cockeysville, Md.) and Selenite F Broth (BBL). One to three ml of SFH or gauze swab fluid were added per 10 ml of enrichment broth.

FIG. 5. Methods for isolation of Salmonella spp. and Vibrio parahaemolyticus; and methods for determination of total and fecal coliforms from seawater.

Legend

HE - Hektoen Enteric agar

HVF - High Volume Filtration

M-C - Membrane filter technique for total coliforms

M-F - Membrane filter technique for total coliforms

MPN - Most Probable Number technique

SEF - Selenite-F enrichment broth

SG - Starch-Galactose agar

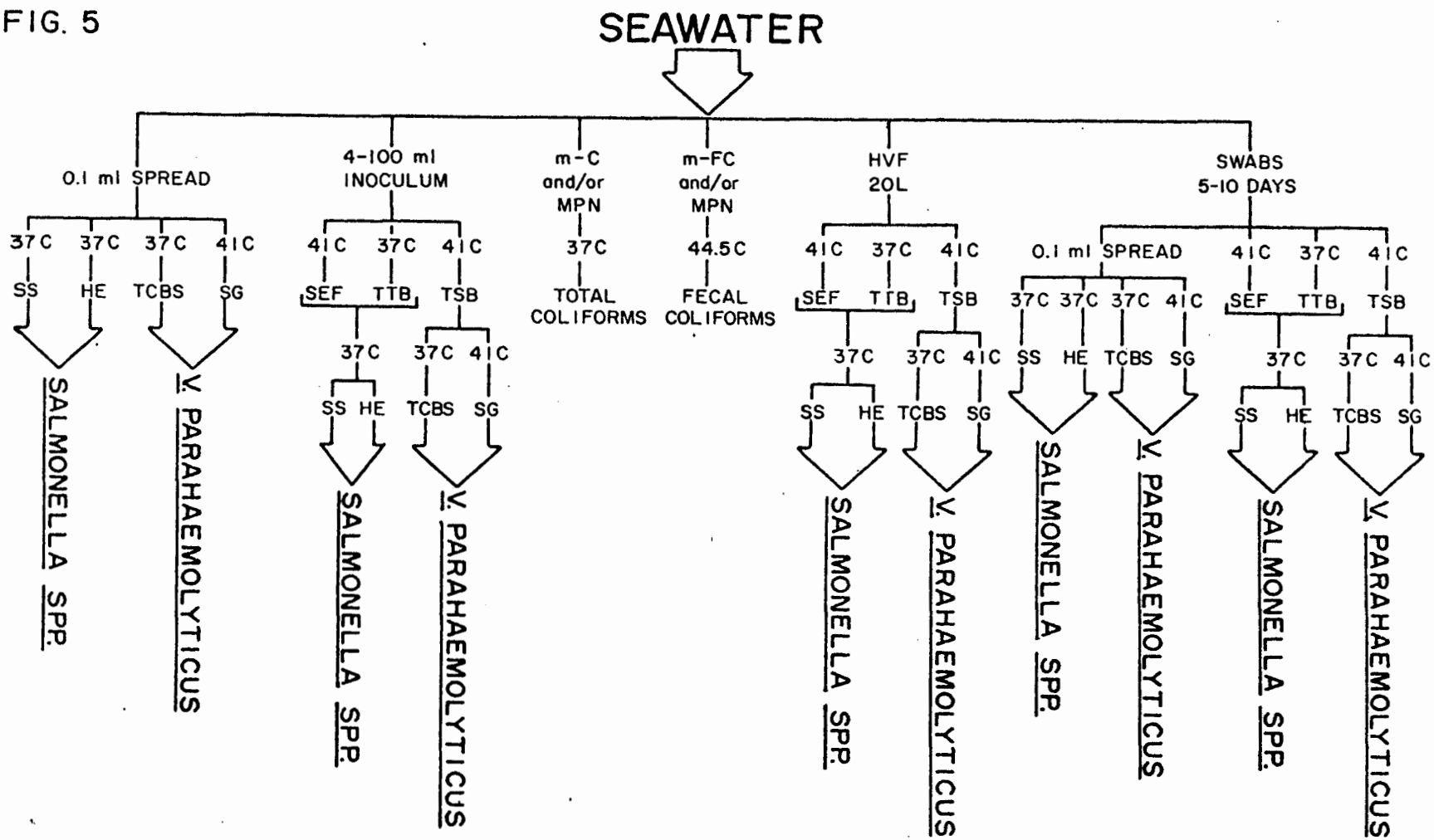
SS - Salmonella-Shigella agar

TCBS - Thiosulphate Citrate Bile Salts agar

TSB - Tryptic Soy Salts Broth

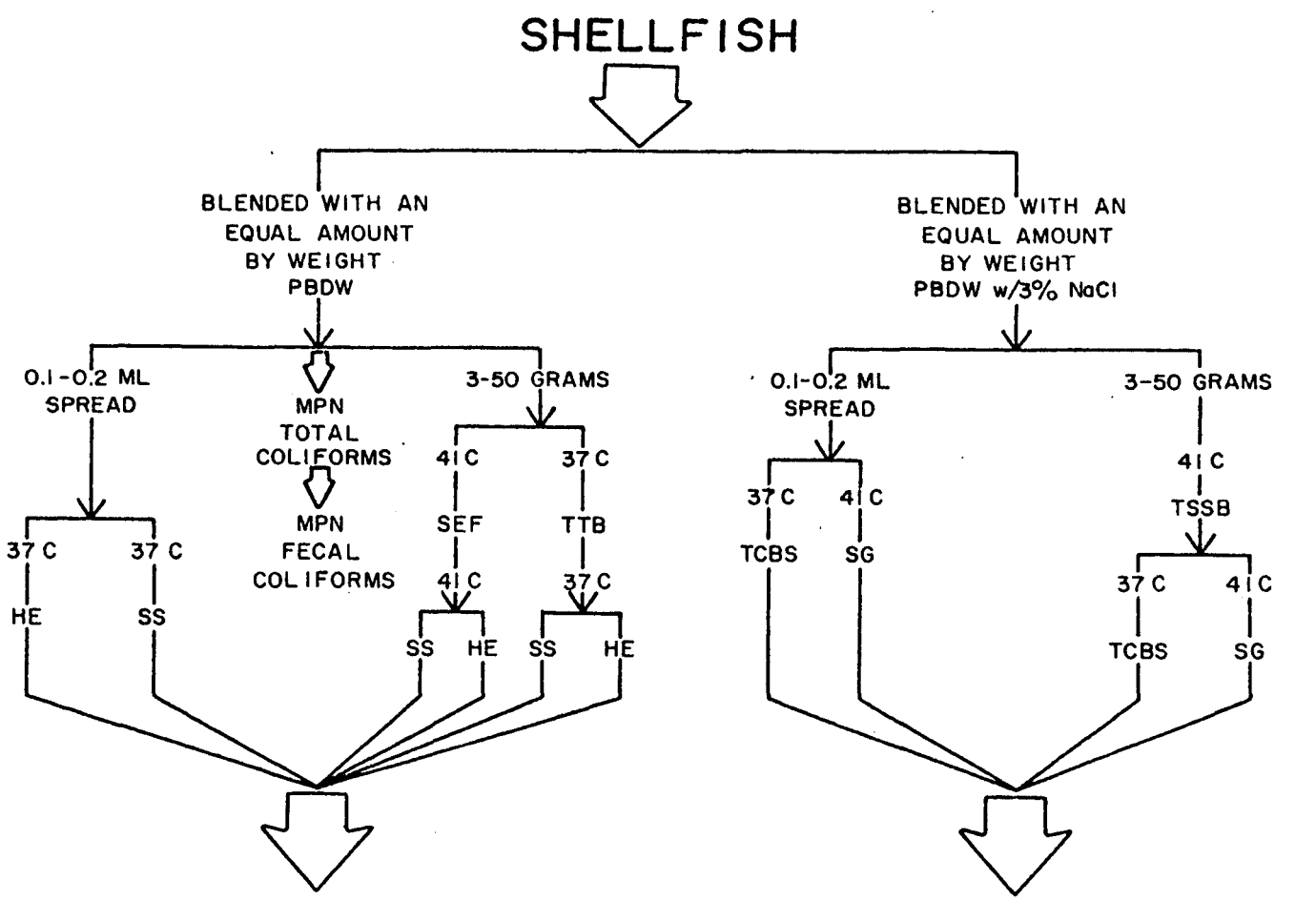
TTB - Tetrathionate Broth

FIG. 5



METHODS FOR ISOLATION OF SALMONELLA SPP. AND VIBRIO PARAHAEMOLYTICUS; AND METHODS FOR DETERMINATION OF TOTAL AND FECAL COLIFORMS FROM SEAWATER.

FIG. 6



METHODS FOR ISOLATION OF SALMONELLA SPP. AND VIBRIO PARAHAEMOLYTICUS; AND METHODS FOR DETERMINATION OF TOTAL AND FECAL COLIFORMS FROM MARINE INVERTERBRATES.

After inoculation, the Tetrathionate Broth was incubated at 37 C for 18-24 hours and the Selenite F Broth was incubated at 41 C for 24 hours as directed by the APHA (2,3). Gauze swabs and Balston Filters were placed directly into the enrichment broths and agitated to insure thorough mixing (45).

Two selective plating media were used, Salmonella-Shigella agar (SS,BBL) and Hektoen Enteric agar (HE, Difco). Enrichments were spread and/or streaked onto the agar surfaces. Salmonella-Shigella agar was incubated at 37 C for 24 hours. Opaque, transparent, or translucent uncolored colonies with or without black centers were picked from SS agar as suspect salmonellae and restreaked to MacConkey agar (MA, Difco). Blue-green to blue colonies with or without black centers were chosen from HE as suspect salmonellae and restreaked to MA.

Biochemical tests were then conducted to confirm the identification of Salmonella spp. (25), (Fig. 7). Well-isolated colonies were transferred to Triple Sugar Iron agar (TSI, Difco) and incubated at 37 C for 18-24 hours. Those cultures exhibiting an alkaline slant and an acid butt, with or without hydrogen sulfide or gas production, were considered presumptive of Salmonella Spp. and further examined. Growth from TSI slants were used to inoculate the following prepared media: Urease Medium (BBL), Phenylalanine-Malonate Broth (Difco), Lysine Decarboxylase Broth (Difco) or Pathotec Lysine Decarboxylase test papers (Warner-Chicott, Morris

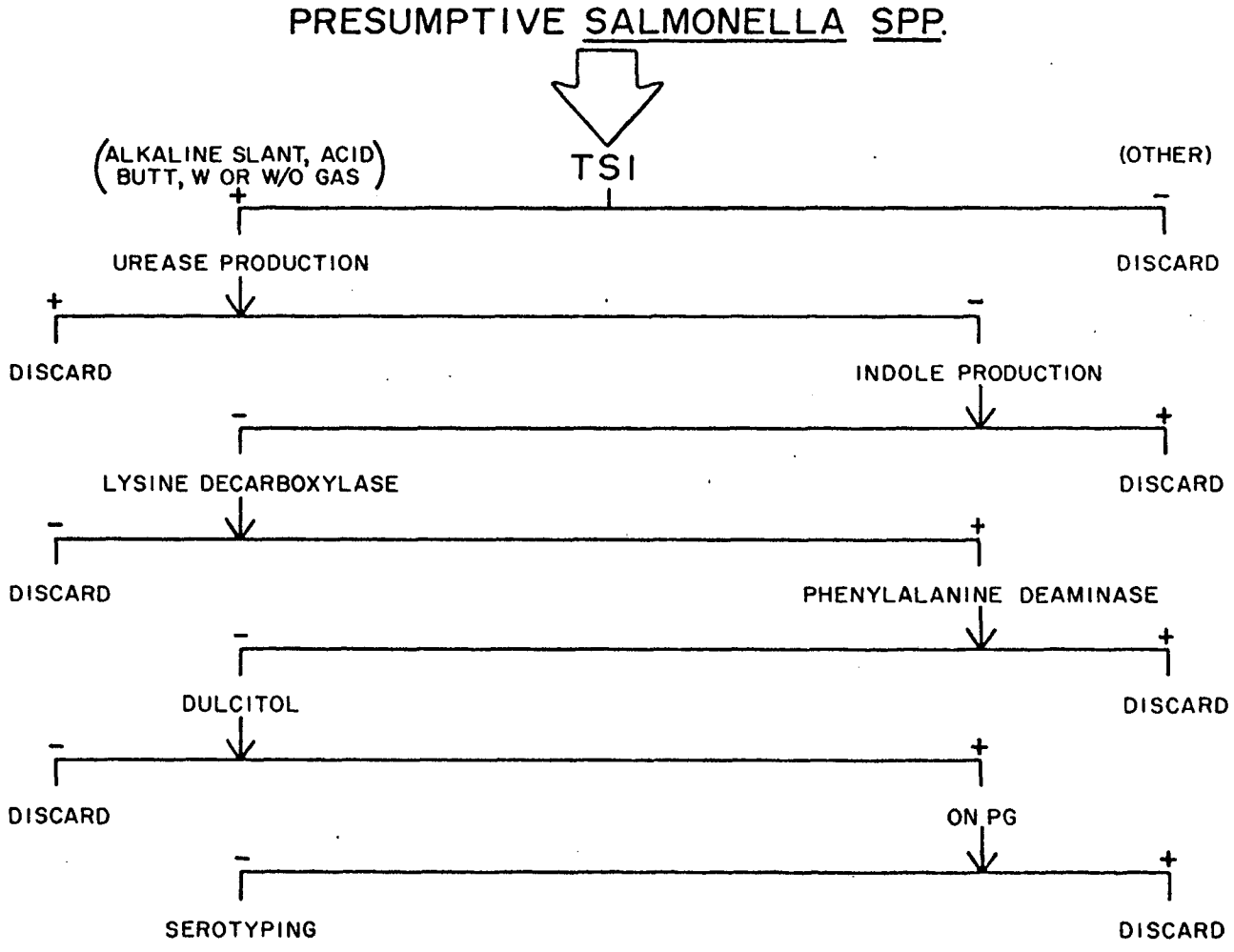
FIG. 7. Flow diagram of the confirmation of presumptive *Salmonella* cultures isolated.

Legend

TSI - Triple Sugar Iron agar

ONPG - Ortho Nitrophenol Galactodidase

FIG. 7


PRESUMPTIVE SALMONELLA SPP.
(ALKALINE SLANT, ACID BUTT, W OR W/O GAS)
TSI
(OTHER)
+
-
UREASE PRODUCTION
DISCARD
+
-
DISCARD
INDOLE PRODUCTION
-
+
LYSINE DECARBOXYLASE
DISCARD
-
+
DISCARD
PHENYLALANINE DEAMINASE
-
+
DULCITOL
DISCARD
-
+
DISCARD
ON PG
-
+
SEROTYPING
DISCARD

Plains, N.J.), 1% Trytone Broth (Difco), Purple Carbohydrate Broth (Difco) with 1% dulcitol and Ortho Nitrophenol Galactodidase (ONPG, Difco).

Serotyping by somatic group was accomplished using growth from a Nutrient agar (NA, Difco) slant which was incubated for 18-24 hours. Growth from slants was suspended in 0.5 ml 0.85% normal saline. A drop of each of the Salmonella spp. antisera (Difco) was used to determine somatic groups.

Isolation of Vibrio parahaemolyticus. Tryptic Soy Broth supplemented with 7% NaCl (TSSB 7%, Difco) was used in the enrichment for V. parahaemolyticus as suggested by Vanderzant & Nickolson (84). Aliquots of SFH and fluid expressed from swabs were added to this enrichment media and incubated at 41 C for 18-24 hours. Filters and swabs were placed directly into the broth and incubated as above.

Two different selective plating media were used for the recovery of V. parahaemolyticus. Thiosulphate Citrate Bile Salts agar plates (TCBS, Difco) were spread and/or streaked with SFH, fluid expressed from swabs, and enrichment broths. Thiosulphate Citrate Bile Salts agar plates were incubated for 18 to 24 hours at 37 C. Green to blue-green colonies typical for V. parahaemolyticus were picked as presumptive and restreaked to TCBS one or more times to insure a pure culture. All organisms forming colonies on TCBS were designated presumptive vibrio (PV) and organisms forming colonies typical of V. parahaemolyticus were designated presumptive

V. parahaemolyticus (PVP) after Kaneko and Colwell (38). Isolated cultures were maintained on Tryptic Soy agar supplemented with 3% NaCl (TSSA 3%, Difco).

A selective plating media was developed to recover V. parahaemolyticus but effectively inhibit organisms other than V. parahaemolyticus from environmental samples. This medium was designated Starch-Galactose agar and is essentially a combination of the media described by Twedt and Novelli (104) and Thomas et al. (100). Shown in Table 1 is a comparison of the three media. Starch-Galactose agar plates were enclosed in a plastic bag to prevent dehydration and incubated at 41 C for 18-24 hours. Typical V. parahaemolyticus colonies on S-G were bright yellow (galactose positive) with a 1-3 mm zone of clearing when viewed by transmitted light (amylase positive). Suspect colonies were restreaked to S-G to insure a pure culture and then transferred to a Tryptic Soy Salts agar slant.

Biochemical and morphological characteristics of isolated cultures examined were: Gram stain, plemorphism, motility, growth on TSI, salt tryptone broth-3%, 7%, peptone broth, production of indole, methyl red test, Voges-Proskauer test, possession of decarboxylase-dihydrolase for (L-Arginine·HCl, L-Histidine·HCl, and L-Tyrosine), fermentation of carbohydrates (sucrose, glucose, galactose, l-arabinose, Ribitol, D-mannitol, L-(-)rhamnose, inositol, D-fructose, D(+)trehalose, maltose, d(+)mannose, raffinose, and D-xylose) and halophilism (29). See figure 5 for outline of methods for seawater and

figure 6 for outline of methods for shellfish. Characteristics were compared with the type strain GB101 (Table 2).

TABLE 1. Comparison of the composition of the Twedt and Novelli (90), Levin-Watkins m-VP medium (77) and the proposed Starch-Galactose medium.

INGREDIENTS	TWEDT-NOVELLI MEDIUM	LEVIN-WATKINS m-VP MEDIUM	STARCH-GALACTOSE MEDIUM
Polypeptone		10.0 grams[a]	
Peptone	20.0 grams		20.0 grams
Yeast extract	2.0 grams	0.7 grams	
Galactose		10.0 grams	10.0 grams
Cornstarch	5.0 grams		5.0 grams
Sodium Chloride	30.0 grams	30.0 grams	30.0 grams
Bromthymol Blue		0.025 grams	0.025 grams
Copper Sulfate		1.5 grams	
Sodium Cholate		1.5 grams	1.5 grams
Penicillin	2000-5000 units[b]		
Agar	15.0 grams	15.0 grams	15.0 grams
pH	8.0	8.5	8.5

[a]Grams per liter.

[b]Units per liter.

TABLE 2. Characteristics of the type strain of *Vibrio parahaemolyticus*. (After Fujino et al., 1974).

Characteristics	Type strain EB 101
Gram-negative, asporogenous	+
Motility	+
Polar monotrichous	+
Growth in 1% Trypticase broth with:	
0% NaCl	-
8% NaCl	-
10% NaCl	-
Growth at 42 C	-
Growth on TCBS agar	+
Indophenol oxidase	+
Catalase	+
Nitrate to nitrite	+
Nitrate to N_2 gas	-
Indole	+
Acetylmethylcarbinol	-
Hydrogen sulfide (Kligler)	-
Phenylalanine deaminase	-
Lysine decarboxylase	+
Arginine dihydrolase	-
Ornithine decarboxylase	+
Urease (Christensen)	-
Gelatinase	+
Chitinase	(+)
Citrate (Simmons)	+
Malonate	-
Alginate	-
Starch hydrolysis	+
Casein hydrolysis	+
Glucose, gas	-
Acid under petrolatum seal from:	
Glucose	+
Arabinose	+
Cellobiose	(+)
Galactose	+
Lactose	-
Fructose	+
Maltose	+
Mannose	+
Melezitose	-
Melibiose	-
Raffinose	-
Rhamnose	-
Ribose	+
Sorbose	-

TABLE 2. (Con't).

Characteristics	Type strain EB 101
Sucrose	-
Trehalose	+
Xylose	-
Adonitol	-
Dulcitol	-
Erythritol	-
Inositol	-
Mannitol	+
Sorbitol	-
Salicin	-
Alpha-methyl-D-glucoside	-
Beta-methyl-D-glucoside	+
Esculin hydrolysis	+
Kanagawa reaction	-
H antigens specific for V. parahaemolyticus	+

[a] +, Positive reaction within 48 hours of incubation; (+), positive reaction after 48 hours or more of incubation; -, no reaction.

RESULTS

Delineation of the Study Area

Shown in figure 8 are the locations of sampling sites within the general study area. The accompanying figure 9 depicts the primary study area and sampling sites.

Physicochemical Parameters

Given in Table 3 are the results of the physicochemical parameters of the seawater samples examined during the study.

Salinity. As can be seen the salinity of the seawater ranged from 26.1 to 34.0 parts per thousand with an average of ca. 32 parts per thousand. The lowest salinity was observed on February 28, 1975, at Station 11. Since there is no influx of fresh water from an established stream or river in this area, the lower salinity can best be explained by the 2.34 inches of precipitation which fell 23-25 February. The high salinity of 34.0 parts per thousand was recorded only once during the course of the study. It can be explained by the fact that the sample was collected at Station 8 (Figure 8,9) located in the northwesternmost area of Clarks Cove on August 15, 1974. There was very little precipitation recorded during the preceding 30 days. The prevailing winds from the southwest, coupled with the historically poor circulation in the area, would tend to increase the salinity of the surface waters. Salinity appears to be relatively stable within the area studied, remaining near the average of 32.0 parts per thousand throughout the year.

FIG. 8. General study area showing sampling sites.

FIG. 8

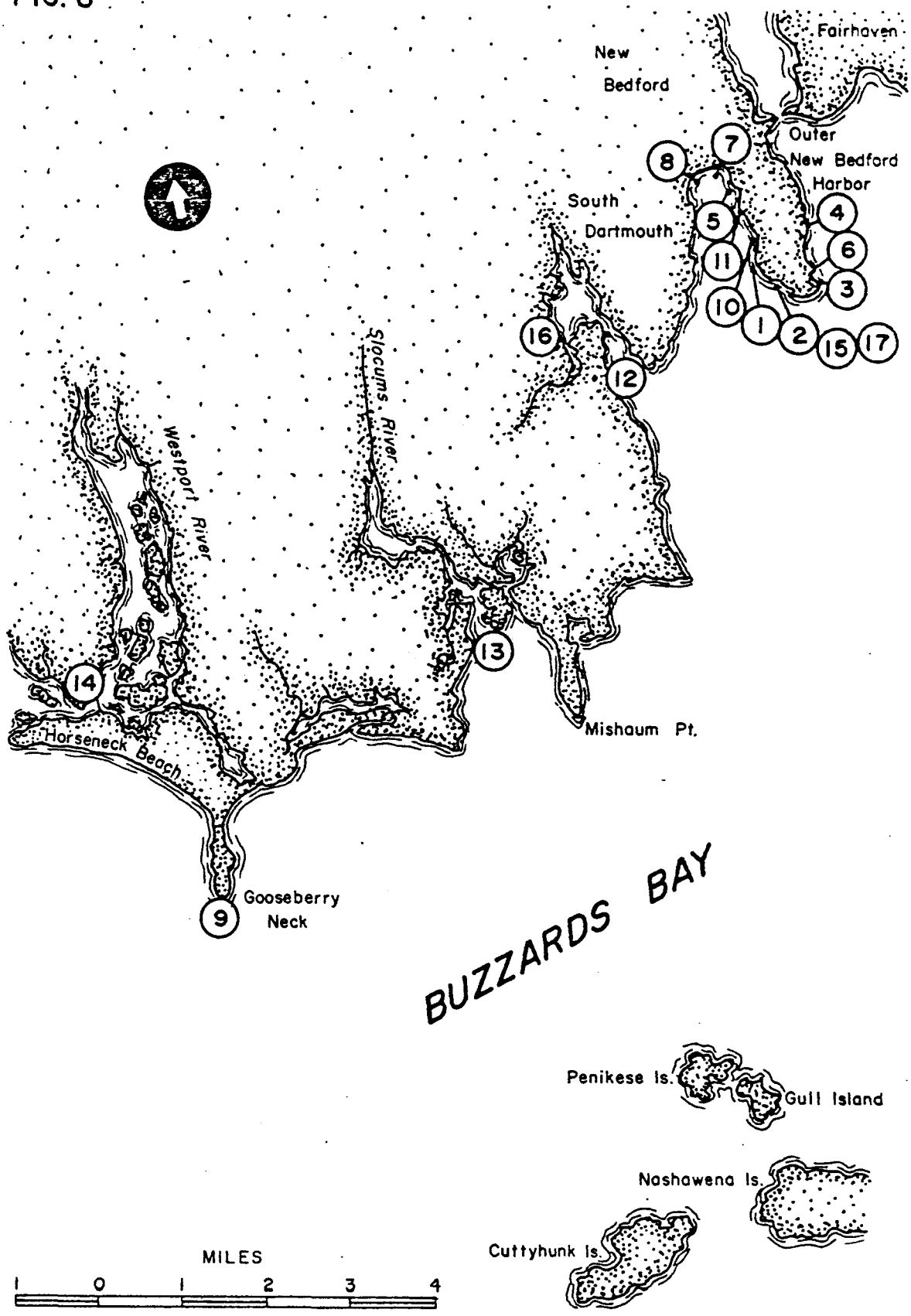

Fairhaven
New Bedford
Outer New Bedford Harbor
South Dartmouth
Slocums River
Westport River
Horseneck Beach
Gooseberry Neck
Mishaum Pt.
BUZZARDS BAY
Penikese Is.
Gull Island
Nashawena Is.
Cuttyhunk Is.
MILES
1 0 1 2 3 4

FIG. 9. Primary New Bedford study area showing location of sampling sites (1-15) and sewage overflow structures (a-h).

FIG. 9

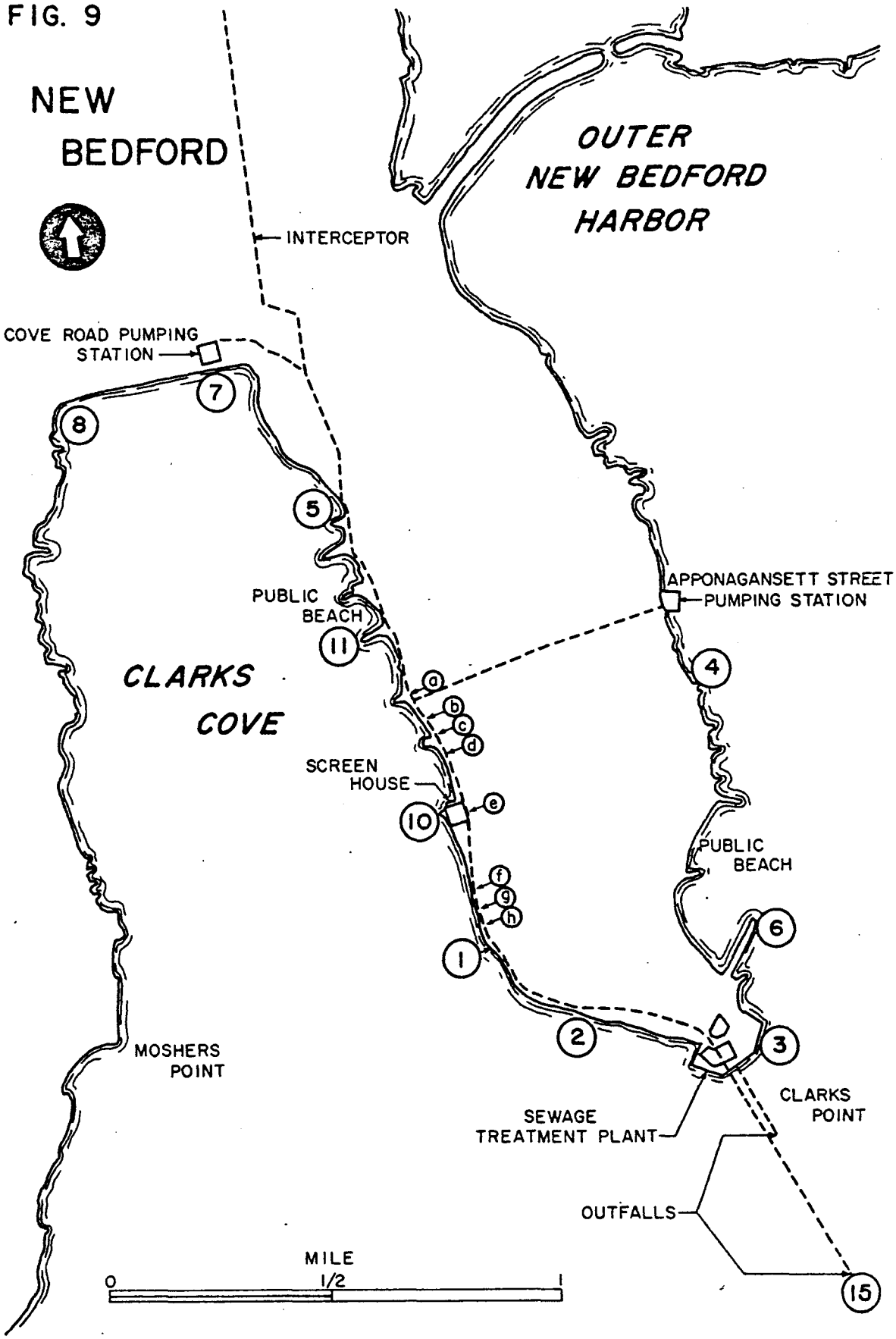

NEW
BEDFORD
OUTER
NEW BEDFORD
HARBOR
INTERCEPTOR
COVE ROAD PUMPING
STATION
APPONAGANSETT STREET
PUMPING STATION
PUBLIC
BEACH
CLARKS
COVE
SCREEN
HOUSE
PUBLIC
BEACH
MOSHERS
POINT
SEWAGE
TREATMENT PLANT
CLARKS
POINT
OUTFALLS
MILE
0
1/2
1
7
8
5
11
4
a
b
c
d
10
e
f
g
h
1
6
2
3
15

TABLE 3. Physicochemical parameters of the seawater.

Station	Date	Salinity ($^0/_{00}$)	pH	Temperature (C) Air	Temperature (C) Water	Tidal Cycle
1	7/20/74	32.0	--a	26.0	22.0	Low
	7/25/74	33.0	--	25.0	23.0	Low
	1/20/75	32.5	8.0	-3.0	2.0	Low
	5/22/75	32.0	8.0	21.0	16.5	Low
	6/12/75	31.0	8.1	17.0	17.0	Low
	6/22/75	32.0	8.2	25.0	23.0	½
2	7/20/74	32.0	--	25.0	23.0	Low
	7/25/74	32.0	--	25.0	24.0	Low
3	7/20/74	32.0	--	24.0	22.0	Low
	7/25/74	32.0	--	23.0	22.5	Low
	8/2/74	32.0	--	31.0	24.0	Low
	8/8/74	31.0	--	26.0	24.0	¼

Station	Date	Salinity ($^0/_{00}$)	pH	Temperature (C) Air	Temperature (C) Water	Tidal Cycle
3 (Cont.)	8/15/74	32.0	--	27.0	25.0	Low
	9/19/74	32.0	--	23.0	21.0	¼
	7/11/75	32.0	8.1	20.0	21.0	High
	7/11/75	32.0	8.1	25.0	22.0	¼
	7/11/75	32.0	8.2	25.0	22.0	¼
	7/11/75	32.0	8.12	21.0	21.0	High
	7/11/75	32.0	8.0	20.5	21.0	Low
	7/12/75	32.0	8.0	20.5	21.0	½
	7/12/75	32.5	8.0	20.0	21.0	High
	7/13/75	32.5	7.9	20.0	21.25	High
	7/14/75	32.5	8.0	21.5	22.0	3/4
	9/9/75	32.3	8.1	23.0	20.9	3/4
	9/9/75	32.25	8.2	21.0	21.0	Low
	9/9/75	32.6	8.19	14.5	20.0	High
	9/10/75	31.4	8.06	21.0	22.0	High
	9/11/75	32.2	8.12	24.5	20.7	High
	9/12/75	32.5	7.90	21.5	19.6	3/4

Station	Date	Salinity (0/00)	pH	Temperature (C) Air	Temperature (C) Water	Tidal Cycle
4	7/25/74	31.0	--	23.0	23.0	Low
	8/8/74	32.5	--	24.5	24.0	¼
	8/15/74	31.0	--	26.0	25.0	Low
5	7/25/74	32.0	--	23.0	23.0	Low
	7/31/74	32.5	8.1	--	25.4	3/4
6	8/2/74	32.0	--	30.5	24.0	Low
7	8/8/74	31.0	--	24.0	24.0	½
8	8/15/74	34.0	--	27.0	26.0	Low
	9/19/74	32.0	--	19.0	19.0	High
	7/31/75	32.0	8.1	30.0	26.4	½
9	8/22/74	33.0	8.1	26.5	24.0	Low
	6/22/75	32.0	8.1	23.0	19.0	3/4

Station	Date	Salinity (0/00)	pH	Temperature (C) Air	Water	Tidal Cycle
10	9/25/74	32.0	--	19.0	19.0	High
	5/22/75	32.0	8.0	21.0	17.0	Low
	7/31/75	32.0	8.0	--	26.4	3/4
	7/31/75	30.0	8.2	--	26.4	½
11	10/23/74	--	--	13.0	9.0	Low
	11/4/74	--	--	15.0	13.0	High
	2/22/75	31.0	8.0	6.5	3.5	Low
	2/28/75	26.1	--	4.0	4.0	Low
	3/17/75	28.8	--	0.0	3.0	Low
	4/6/75	31.0	8.0	6.0	5.5	Low
	4/16/75	32.0	8.0	8.0	6.0	Low
	5/22/75	32.0	8.0	21.0	17.0	¼
	7/4/75	32.0	8.2	21.0	22.5	Low
12	10/3/74	32.0	--	11.0	15.0	Low
	2/22/75	27.0	--	10.0	9.0	¼

Station	Date	Salinity(0/00)	pH	Temperature (C) Air	Water	Tidal Cycle
13	10/3/74	32.0	--	12.0	14.0	Low
14	10/16/74	30.0	--	--	--	½
15	5/12/75	31.2	--	--	12.7	Low
16	10/3/75	32.0	--	11.0	14.0	Low
	3/24/75	--	8.0	10.0	5.5	Low
17	7/31/75	32.0	8.1	--	26.4	½

Surface water temperature. Temperature ranged from a low of 2.0 C in January to a high of 26.4 in July, showing the expected seasonal change (Figure 10).

pH. The pH of the seawater ranged from 7.9 to 8.2 with an average of ca. pH 8.1.

Winds. The prevailing winds observed during the study were from the south and southwest, although other wind directions were noted. It is expected that changes in wind direction would cause significant changes in surface currents with corresponding changes in the distribution of microbial and chemical indicators of water quality. Winds from the south and southwest have a tendency to concentrate debris and other material in Clarks Cove, particularly in the northern part.

Microbiological Parameters

Presented in Table 4 are the Microbiological parameters of the seawater samples examined, given by station and date. Total coliform counts of the marine invertebrates compared with total coliform counts of the overlying seawater are given in Table 5.

Total Coliforms (TC). Total coliform counts of the seawater were found to be from less than 1/100 ml to a high of 15,000/100 ml, with the highest count being found at the outfall (Station 15, Figure 8,9) of 280,000/100 ml (Table 4). The highest count, other than at the outfall, was found at Station 8 in September 1974 (Figure 8,9). As noted earlier, there is often poor circulation in this area with a tendency

Figure 10. Seasonal water temperature.

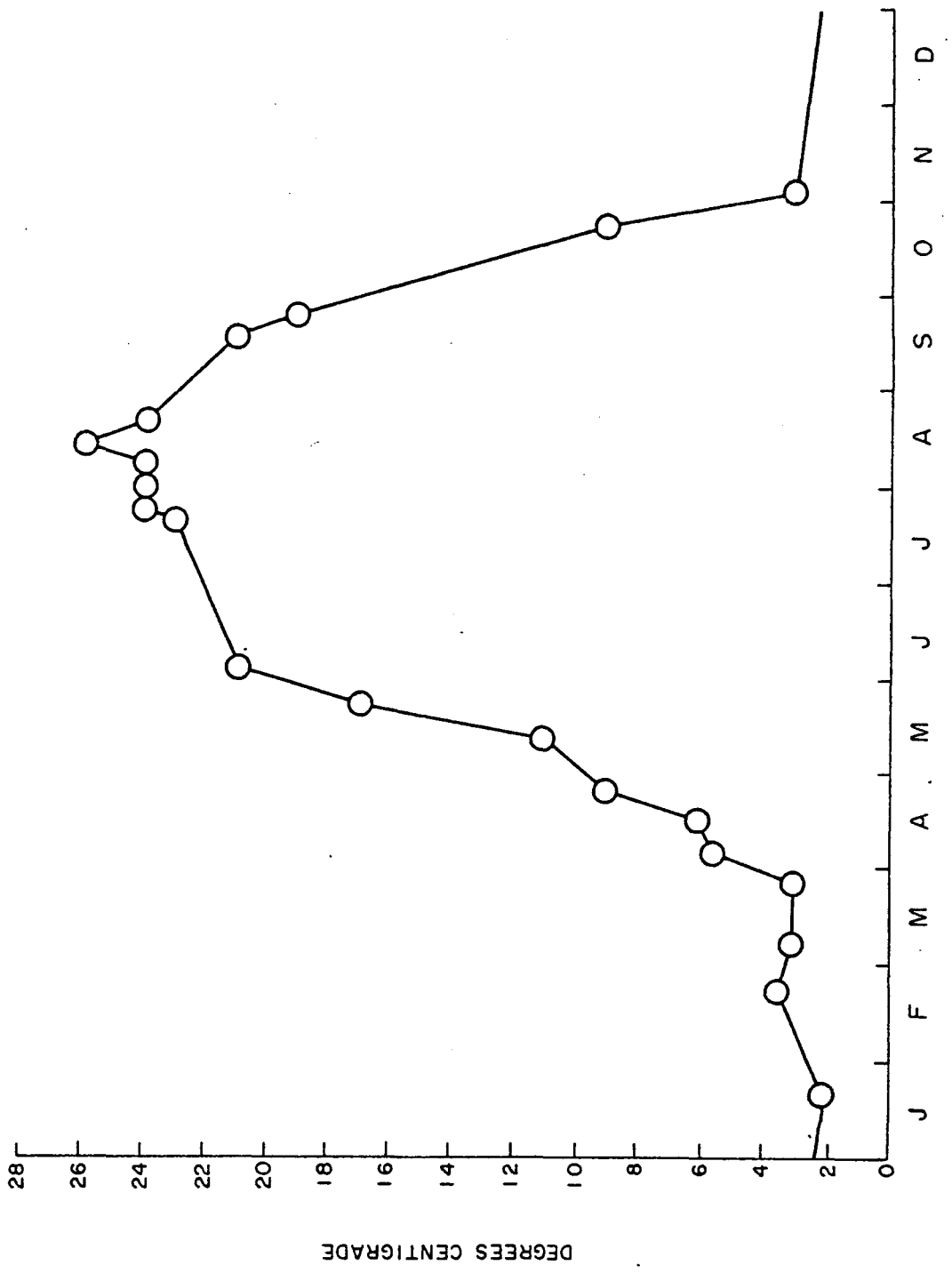

DEGREES CENTIGRADE
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
J
F
M
A
M
J
J
A
S
O
N
D

FIG. 10

TABLE 4. Microbiological parameters of the seawater.

Station[a]	Date	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	Tidal Cycle
1	7/20/74	190	---[b]	Low
	7/25/74	10	---	Low
	1/20/75	1	<1	Low
	5/22/75	100	---	Low
	6/12/75	50	8	Low
2	7/20/74	300	---	Low
	7/25/74	1	<1	Low
3	7/20/74	24	---	Low
	7/25/74	70	---	Low
	8/02/74	40	10	Low
	8/08/74	60	5	¼
	8/15/74	120	---	Low
	9/19/74	10	6	¼

Station	Date	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	Tidal Cycle
3 (Cont.)	7/11/75	44	40	High
	7/11/75	8	4	¼
	7/11/75	14	14	¼
	7/11/75	500	270	High
	7/11/75	20	2	Low
	7/12/75	10	10	½
	7/12/75	190	<1	High
	7/13/75	130	<1	High
	7/14/75	3,000	<1	3/4
	9/09/75	20	10	3/4
	9/09/75	60	20	Low
	9/09/75	10	10	High
	9/10/75	20	10	High
	9/11/75	20	20	High
	9/12/75	200	38	3/4

Station	Date	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	Tidal Cycle
4	7/25/74	20	<1	Low
	8/08/74	130	<1	¼
	8/15/74	TNTC	2	Low
5	7/25/74	24	---	Low
	7/31/74	10	<1	3/4
6	8/02/74	10	<1	Low
7	8/08/74	10	---	½
8	8/15/74	10	<1	Low
	9/19/74	15,000	1700	High
	7/31/75	10	10	½
9	8/22/74	10	6	Low
	6/22/75	---	---	3/4

Station	Date	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	Tidal Cycle
10	9/25/74	<1	<1	High
	5/22/75	45	---	Low
	7/31/75	130	<1	3/4
	7/31/75	400	2	½
11	2/22/75	---	---	Low
	3/17/75	780	---	Low
	4/06/75	45	<1	Low
	4/16/75	70	70	Low
	4/26/75	130	---	¼
	5/22/75	40	---	Low
12	10/03/74	170	---	Low
	2/22/75	---	---	¼
13	10/03/74	60	---	Low

Station	Date	Total Coliforms per 100 ml	Fecal Coliforms per 100 ml	Tidal Cycle
14	10/03/74	200	10	½
15	10/16/74	280,000	130,000	Low
	5/12/75	3,500	---	Low
16	10/03/75	---	---	Low
17	5/12/75	80	---	½

[a]See Fig. 8 for location of stations.
[b]No data.

TABLE 5 Total coliform counts (TC) of marine invertebrates compared with TC of the overlying seawater.

Date	Marine Invertebrates	No.	Weight (grams)	Total Coliforms per 100 grams	Seawater	
					TC[a]	FC[b]
7/20/74	Mytilus edulis	14	93.6	---[c]	24	--
7/25/74	" "	14	95.7	---	70	--
8/02/74	" "	14	92.1	---	40	10
8/02/74	" "	14	150.0	---	10	<1
8/08/74	" "	14	138.0	9,100	60	5
8/08/74	" "	14	144.4	460,000	130	<1
8/15/74	" "	14	119.3	240,000	10	<1
8/15/74	" "	14	184.4	9,100	10	<1
8/15/74	" "	14	180.2	9,100	Confluent	2
8/22/74	" "	14	137.1	---	10	6
8/22/74	" "	14	142.6	23,000	10	6
5/22/75	" "	16	100.0	2,400	40	--
10/23/74	Mercenaria mercenaria	14	1,100.0	4,500	--	--
10/23/74	" "	14	1,154.0	1,800	--	--
11/04/74	" "	9	329.0	3,300	--	--
11/04/74	" "	10	594.0	140	--	--
7/31/75	" "	10	884.0	---	130	<1
7/31/75	" "	10	624.0	---	10	<1
7/31/75	" "	10	820.0	---	10	10
7/31/75	" "	<1	100.0	---	400	2

TABLE 5 (Con't)

Date	Marine Invertebrates	No.	Weight (grams)	Total Coliforms per 100 grams	Seawater	
					TC	FC
8/08/74	Modiolus demissus	14	135.7	93,000	TNTC	7500
2/22/75	Littorina littorea	--[d]	127.0	18,000	--	--
3/24/75	" "	--	107.0	---	--	--
3/27/75	" "	--	131.0	---	780	--
3/27/75	Crepidula fornicata	--	44.6	---	780	--
5/22/75	Crustacean shellfish[e]	14	82.5		--	--
2/22/75	Barnacle	--	150.0	22,000	--	--

[a]Total coliform counts per 100 ml seawater.
[b]Fecal coliform counts per 100 ml seawater.
[c]No data.
[d]Not counted.
[e]Carcinus maenas and Neopenope texana.

toward accumulation of material, so it is not surprising that the highest coliform count would be found in this location. Point or non-point sources of pollution in this area could be responsible for the high coliform count at Station 8.

The highest coliform count (460,000/100 grams) in marine invertebrates was observed in Mytilus edulis (blue mussel) (Table 5). The high for Mercenaria mercenaria (quahog) was 4,500/100 grams. Barnacles had 22,000/100 grams. Crepidula fornicata yielded a total coliform count of 1715/100 grams. The periwinkle was found to have 18,000/100 grams and crabs 3,300/100 grams, which is interesting because these animals are not filter-feeders and, therefore, would not be expected to accumulate coliforms. This may be due to the fact that they are bottom feeders and may accumulate coliforms which have settled out of the seawater. The higher coliform counts of the filter-feeders are functions of filtering-rate which is primarily dependent upon temperature and the concentration of coliforms in the seawater during a given period of time. During the study, total coliform counts were used as a rough measure of fecal pollution because of limited time and materials. Fecal coliform counts are generally considered a more reliable indication of recent fecal pollution and of possible presence of pathogens. It is now recognized that the relationship between fecal coliforms and pathogens is not absolute; the presence or absence of fecal coliforms does not preclude the presence or absence of pathogens, but the presence of fecal coliforms indicates the possibility of

pathogens being present (51).

Fecal coliforms (FC). Fecal coliform counts of the seawater ranged from less than 1/100 ml to 7500/100 ml with the highest count of 130,000/100 ml at the outfall. The low was observed, at least once, at Stations 1, 2, 3, 4, 6, 8, 10, and 11 (Figures 8,9; Table 4). The highest count, other than at the outfall, occurred at Station 7 which is located in the northeasternmost part of Clarks Cove. This is not surprising since this station is under the same influences as Station 8 mentioned previously.

Distribution of potentially pathogenic bacteria. Salmonellae were recovered from raw sewage by the swab technique. Four cultures belonging to Salmonella enteriditis Groups C_1 and C_2 were isolated. These cultures were designated T59/16, T59/22a, T59/22 and T59/36. Biochemical and serological characteristics are shown in Table 6. Catch samples of raw sewage processed directly or by using high-column filtration did not yield salmonellae. No salmonellae were isolated from seawater using the techniques employed. One Salmonella spp. was isolated, however, from an environmental source. A quahog sample designated "54" was collected on October 23, 1974, at Station 11 located near the New Bedford Municipal Beach (Figures 8,9). The culture designated "T54/1b" was found to be a Salmonella enteriditis belonging to Group C_2. It is interesting to note that another quahog sample collected nearby in the same area, "53", was found to have a total coliform count of 4,500/100

grams, but no salmonellae were recovered. Sample "54" had a total coliform count of less than 1800/100 grams. In view of the limited recovery of Salmonella, an in situ survival study was conducted to determine survival potential. Results are included in Appendix C.

Vibrio parahaemolyticus was not isolated from any samples during the course of this study. Closely related organisms were isolated, but eliminated from consideration as V. parahaemolyticus on the basis of biochemical tests.

Growth of V. parahaemolyticus in New Bedford seawater was examined to test the hypothesis that New Bedford seawater might have an inhibitory effect on the growth and survival of V. parahaemolyticus. The results of that study are included in Appendix D. No inhibitory effect appeared to be exerted by New Bedford seawater. Included in Appendix D are results of the evaluation of the TSSB enrichment media and the starch-galactose plating media. The enrichment media appeared to support good growth of the competing Presumptive Vibrio parahaemolyticus (PVP) group, which indicated that the medium is not selective for V. parahaemolyticus. It does support good growth of V. parahaemolyticus at 37 C and 41 C so perhaps the addition of an inhibitor, such as sodium cholate, could improve the selectivity of the medium.

The starch-galactose plating medium proved to be very effective, both in the recovery and selectivity of V. parahaemolyticus, compared to TCBS.

TABLE 6. Biochemical and serological characteristics of *Salmonella* isolated.

Test or substrate	T54/1b	T59/16	T59/21a	T59/22	T59/36
Triple Sugar Iron Agar	AG/NC[a]	AG/NC	AG/NC	AG/NC	AG/NC
Hydrogen sulfide (TSI)	+[b]	+	+	+	+
Indol	-[c]	-	-	-	-
Urease	-	-	-	-	-
Methyl Red	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-
Simmon's Citrate	+	+	+	+	+
Motility	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+
Arginine dihydrolase	NT[d]	-or(+)[e]	+	-or(+)	-or(+)
Ornithine decarboxylase	+	+	+	+	+
Phenylalanine deaminase	-	-	-	-	-
Glucose	+	+	+	+	+
Lactose	-	-	-	-	-
Sucrose	-	-	-	-	-
Mannitol	+	+	+	+	+
Dulcitol	+	+	+	+	+
Salicin	NT	-	-	-	-
Adonitol	NT	-	-	-	-
Inositol	NT	+	+	+	+
Sorbitol	+	+	+	+	+
Arabinose	NT	+	+	+	+
Raffinose	NT	-	-	-	-
Rhamnose	NT	+	+	+	+
Malonate	-	-	-	-	-
Maltose	+	+	+	+	+
Xylose	+	+	+	+	+
Trehalose	NT	+	+	+	+
Cellobiose	NT	-or(+)	-	-	-
Glycerol	NT	-	-	-	-
Esculin	NT	-	-	-	-
ONPG	-	-	-	-	-
Serology-*S. enteriditis* Group	C_2	C_1	C_2	C_1	C_1

[a]Acid butt/alkaline slant, gas.
[b]Positive for given reaction.
[c]Negative.
[d]Not tested.
[e]Negative or delayed positive.

In laboratory studies (Appendix D), the percent recovery of V. parahaemolyticus on medium compared with TCSB ranged from 17.6% to 110.0%, with an average of 66.6% (Table 7). It was found that incubation at 41 C had a tendency to dehydrate the surface of the S-G agar, thus reducing the plate count. Enclosure within a plastic bag helped retain moisture and would be expected to improve viable counts. Percent recovery would then be expected to also be higher than the 66.6% average obtained in this study. To fully evaluate the proposed medium, it was compared against TCBS for examination of marine environmental samples. The S-G agar reduced the "background" count by an average of 85%. In addition, it reduced the total number of false presumptives of V. parahaemolyticus by 92%.

Table 7. Growth of V. parahaemolyticus at 20 C in filter-sterilized New Bedford seawater and a comparison of TCBS and S-G agars.

TIME-DAYS	Counts per ml[a]		Ratio
	TCBS	S-G	S-G/TCBS/100[b]
0	440	240	54.5%
3	$2.1X10^5$	$3.7X10^4$	17.6%
6	$2.1X10^5$	$2.3X10^5$	110%
7	$2.2X10^5$	$1.5X10^5$	68.2%
10	$1.7X10^5$	$1.4X10^4$	82.4%
17	$4.5X10^4$	$1.5X10^4$	33.3%

[a]Average of three plates of the appropriate dilutions.
[b]Average percent recovery of S-G agar compared to TCBS was 66.6%.

DISCUSSION

Distribution and Occurrence of Potentially Pathogenic Bacteria

Salmonella. The only *Salmonella* isolated from the New Bedford marine environment was from a quahog sample collected at Station 11 on October 23, 1974. This isolation may have been related to the precipitation followed by overflow conditions. On October 15-17, 1974, there were 1.94 inches of precipitation; 1.86 inches fell during October 16 alone. If the percent survivals of *S. enteriditis* T59/21a during both the July and September survival studies are averaged and projected from 3 days to 6 days, an estimated 20% of the cells would still be viable. Therefore, if an overflow did occur on October 16, 1974, it is theoretically possible to have recovered this organism from quahogs 6 days later. The quahog sample which yielded the *Salmonella* culture was collected within less than 500 meters north of the Screen House, a suspected source of overflow. It should be noted that one of the *S. enteriditis* cultures isolated from raw sewage in December, 1974, was of the same serotype group (C_2) as the culture isolated earlier from the quahog sample.

Further evidence implicating the Screen House as a source of sewage pollution was provided by informal SCUBA surveys. These surveys, conducted in the area from Station 1 to Station 10, revealed an apparent reduction in marine life near Station 10 as compared with the surrounding areas.

This would be expected for an area sporadically receiving a combined sanitary and industrial effluent (65).

Vibrio parahaemolyticus was not found to occur in the study area during the study. It was initially thought that this organism would be found in this area because of favor- (35,36,38,87). From these studies it has been generally accepted that this marine vibrio inhabits estuarine and coastal waters, particularly at the mouths of rivers. Geographically, V. parahaemolyticus has been isolated as near as Cape Cod on the east (24) and Narragansett Bay to the west (74).

The ecology of the vibrio has been reported to be linked to an annual cycle dictated by temperature and associated with plankton (38). Over-wintering in the sediments, the organism reenters the water column during the early summer when the water temperature rises from 14 C to 19 C. The return to the water column appears to be dependent on attachment to plankton.

Seasonal temperatures in Clarks Cove were well within the range tolerated by V. parahaemolyticus. The pH and salinity were within the conditions allowing the survival and growth of the organism (49).

Growth studies indicated that New Bedford seawater is a suitable environment for the vibrio. V. parahaemolyticus

was able to survive and grow in this seawater at a temperature of 20 C (Appendix D).

Related organisms of the PVP group were consistently present in samples during the warmer months, declining as the temperature decreased in late fall. A strong incidental relationship between PVP and V. parahaemolyticus has been suggested (35) and direct enumeration of PVP has been considered a useful indicator for the presence of V. parahaemolyticus (19). This apparently is not true in New Bedford waters.

Some factors limiting the distribution of V. parahaemolyticus were suggested in a report by Kaneko and Colwell (36). They were unable to detect V. parahaemolyticus in the Atlantic Ocean 4 to 10 miles from shore where salinities were from 28.0 to 34.0 parts per thousand. During a baywide survey, however, they were able to isolate the organism from sediment and plankton in Chesapeake Bay during May, 1971. During this survey salinities ranged from 4.0 to less than 12.0 parts per thousand.

In Rhode Island, V. parahaemolyticus was recovered during July through September, 1976, at salinities of from 14-23 parts per thousand (Watkins, personal communication).

Recent information indicated that the ability to adsorb onto plankton plays an important role in the annual cycle of V. parahaemolyticus (49). The effect of salinity and pH on this adsorption phenomenon was studied by Kaneko and Colwell (37). They found that adsorption of V. parahaemolyticus to

at 15 parts per thousand, adsorption dropped [illegible] pH 8.0 to 9.0 was found to reduce adsorption; whereas, a pH of less than 8.0 enhanced adsorption. This information is significant in view of the fact that salinities within the New Bedford study area ranged from 26.1 to 34.0 parts per thousand and averaged 32.0 parts per thousand--well above that necessary for successful adsorption onto plankton by the vibrio. The average pH (8.1) for the area is also less than optimum for adsorption.

The results of the present study of the New Bedford area support the premise of Kaneko and Colwell (36) that, "... the distribution of V. parahaemolyticus is restricted to estuaries, because of the effects of salinity, pH, temperature, and as yet unidentified factors on the attachment, survival and growth of this organism." This study provides a further indication of the limited ecological niche and estuarine nature of this pathogenic vibrio.

Distribution of Indicator Bacteria

The influence of season on the distribution of indicator bacteria. There was no definite seasonal variation observed in the number of indicator bacteria within the study area, but only a "low background level of coliforms with sporadic increases occurring throughout the year." This result is similar to the data reported by Carney et al. (24).

Several factors could have been responsible for these sporadic occurrences: influence of the marine outfall (Station 15), other point or nonpoint sources of contamination, and hydrological and/or meteorological conditions.

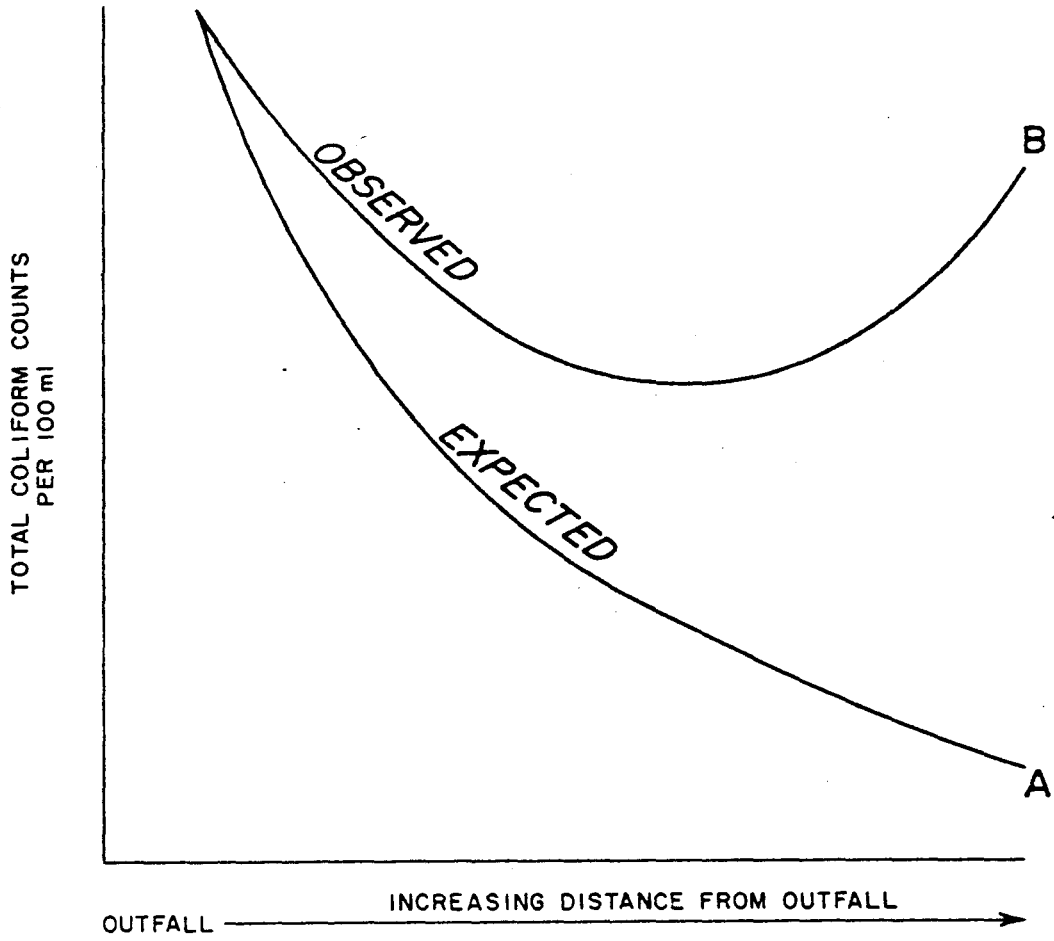
Careful examination of the influence of the marine outfall did not reveal an acceptable explanation for the observed distribution. If an average of coliform counts at each station is plotted against increasing distance from the outfall, the relationships would be as shown in curve A; however, curve B was the observed relationship (Figure 11). This evidence suggests the presence of a source of pollution in Clarks Cove other than that found at the sewage plant outfall.

The second source(s) of sewage contamination is probably overflow outlet(s). Defective tide gates and overflow weirs may contribute to the problem. Although the precise locations of these appurtenances were not given, examination of blueprints provided by the New Bedford Engineering Department showed the location of some overflow outlets. Overflow outlets were shown to be located at Capitol, Calumet, Aquidneck, Bellevue, Portland, Hudson, Seymour and Bayview Streets and at the Screen House on the east shore of Clarks Cove (Fig. 9).

The influence of precipitation and wind on the distribution of indicator bacteria. It has already been mentioned that precipitation as stormwater flow contributes to overflow conditions. A specific example of this relationship occurred during the summer of 1975. On August 7, 1975,

FIG. 11. Coliform counts at increasing distance from outfall.

FIG. 11


TOTAL COLIFORM COUNTS
PER 100 ml
OBSERVED
EXPECTED
B
A
OUTFALL
INCREASING DISTANCE FROM OUTFALL

counts of indicator bacteria for East Beach were 2,800 and 3,000 (personal communication). The beach was closed on August 8, 1975. During the period of August 5-9, 1.37 inches of rain fell; 1.10 inches on August 7, 1975. The contribution of the stormwater flow apparently resulted in an overflow condition. The normal sewage flow combined with stormwater flows exceed the interceptor capacity of 120 mgd (48). David K. Whittaker, assistant director of public health, said that West Beach had also been closed intermittently for short periods over the years during periods of minor pollution (personal communication). During the course of this study higher coliforms counts, that may have been linked to precipitation, occurred on October 16, 1974; March 17, 1975; and July 11, 12, and 14, 1975 (Table 4).

Wind can cause resuspension of sediments and associated bacteria, particularly in the shallower Clarks Cove. Prevailing winds during the summer observed during this study were from the south to southwest. This is in agreement with the United States Coast Pilot 2 (78). Wind from the south to southwest would have a tendency to concentrate materials suspended in the surface waters in the northernmost part of Clarks Cove.

CONCLUSION

The results of this study indicate a problem which needs to be addressed. The recovery of a bacterial pathogen from shellfish in the study area and the apparent survival capability of this pathogen in these waters emphasizes the danger of consuming shellfish taken from these waters. New Bedford shellfish beds were closed in 1967; however, it should be noted that enforcement is difficult. A public awareness program of the dangers of consuming shellfish from New Bedford marine waters may be more effective than current enforcement measures.

Although the hazards of bathing in sewage-contaminated waters is only recently becoming documented (11), it is becoming more apparent that this activity can be a public health hazard. With the close relationship between rainfall and overflow conditions in New Bedford, a program could be planned to close the beaches when the amount of rainfall would result in an overflow. Continued closure of the beaches could be contingent on results of a follow-up water test. This would reduce the possibility of the public's bathing in sewage-contaminated waters and better protect the public health.

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APPENDIX A

Predicted contours of dilution after 49.5 hours.

FIG. 1. Predicted contours of dilution after 49.5 hours (tidal current only; end of flood). (After Marini, 1974).

FIG. 1

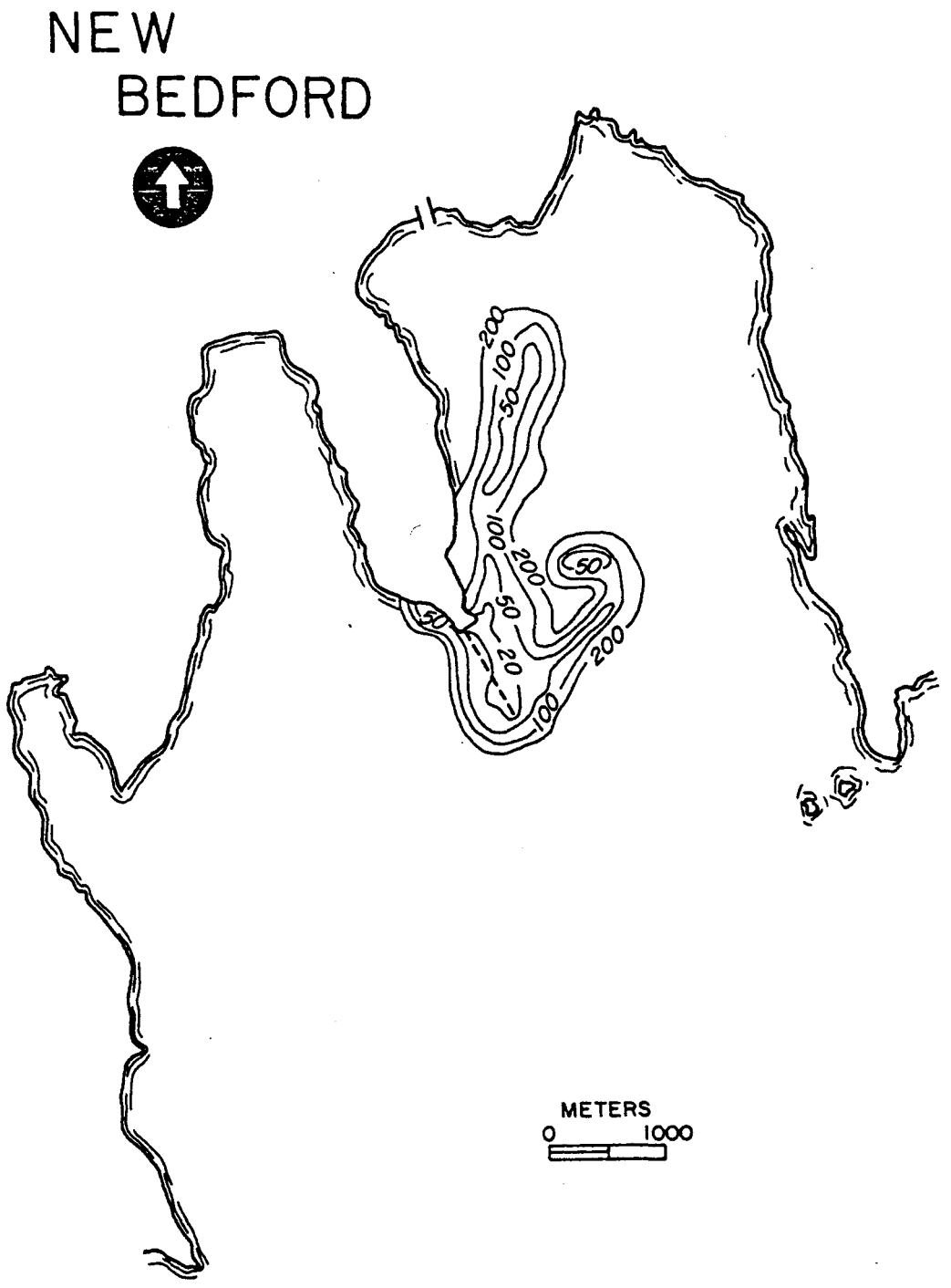


FIG. 2. Predicted contours of dilution after 49.5 hours (tidal and northwest current; end of flood). (After Marini, 1974).

FIG. 2

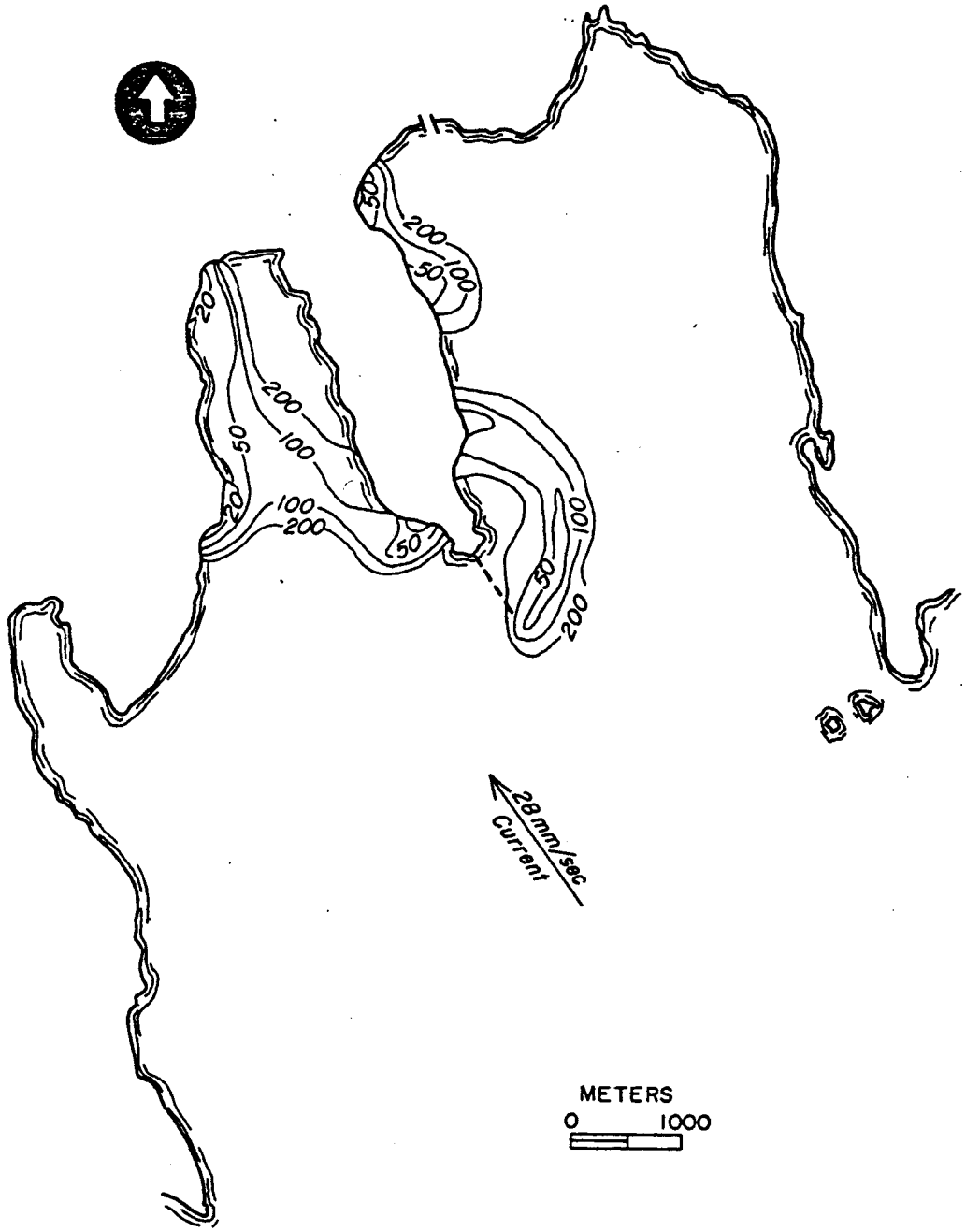

50
200
100
50
20
200
50
100
20
100
200
150
50
100
200
28 mm/sec
Current
METERS
0
1000

FIG. 3. Predicted contours of dilution after 49.5 hours (tidal and northeast current; end of flood). (After Marini, 1974).

FIG. 3

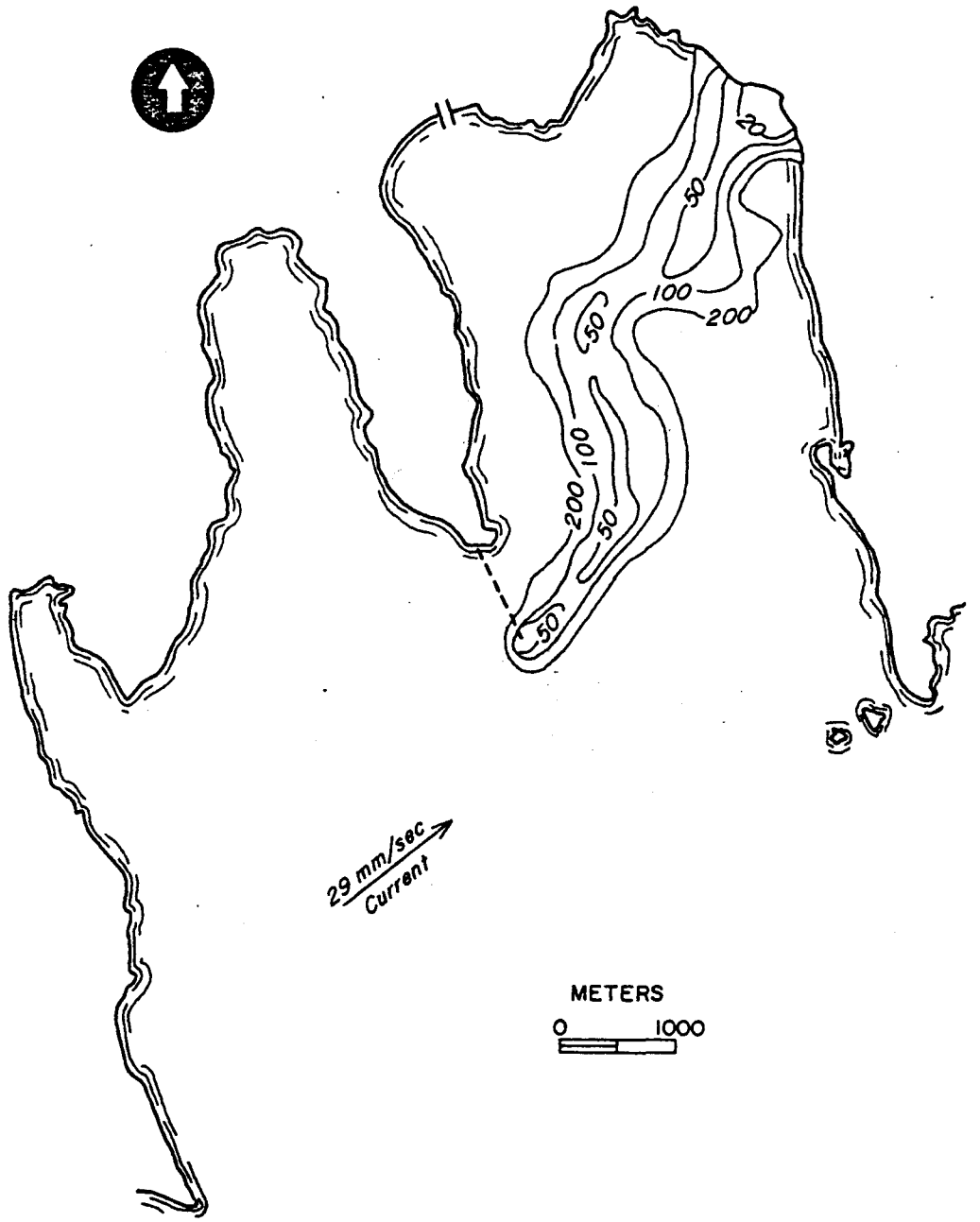
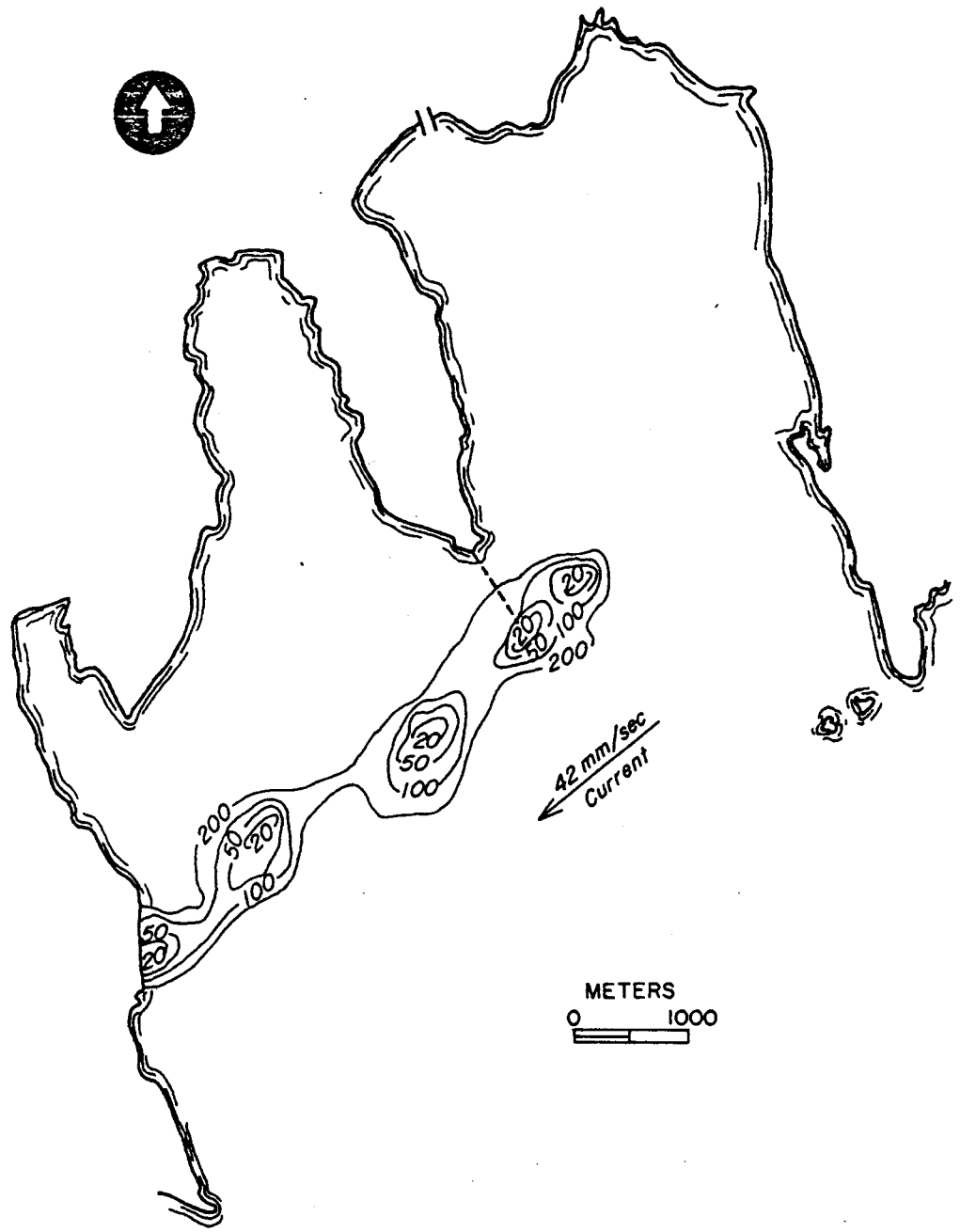


FIG. 4. Predicted contours of dilution after 49.5 hours (tidal and southwest current; end of flood). (After Marini, 1974).

FIG. 4


20
20
40
100
200
20
50
100
200
50
20
100
50
20
42 mm/sec
Current
METERS
0
1000

APPENDIX B

VIBRIO PARAHAEMOLYTICUS -- A Review

On October 21, 1950, two hundred and seventy-two persons in the southern part of Osaka, Japan, became ill with acute gastroenteritis. Cases were characterized by acute abdominal pain, vomiting, and watery or bloody diarrhea described as "explosive" on onset. There were twenty deaths recorded in this outbreak of food poisoning. It was found that "Shirasu", a small sardine, Engraulis japonica Houttyyn, eaten in a partially dried state after boiling in salt-water was the food implicated in this outbreak (22). T. Fujino, et al. (22, 47) investigated this outbreak and were able to isolate a hemolytic rod-shaped bacterium. Further study revealed that it had bipolar staining characteristics, lacked curvature, did not react to Vibrio cholerae antisera and yet was very pathogenic to mice (22). On the basis of these characteristics, Fujino et al. (22) designated the new species, Pasteurella parahemolytica.

In 1955 one of the largest outbreaks occurred, involving about 20,000 residents of the Niigata Prefecture (1, 22, 47). During the fall of 1955, 155 patients of the National Yokohama Hospital became victims of food poisoning. Using a 4% salt medium that is normally used to isolate Staphlococcus, Takikawa (22) was able to isolate a Gram-negative rod possessing a single polar flagellum as the causative organism. The food implicated in this outbreak was "Asazuke", a brine

cucumber or pickle, which was contaminated with mackerel thought to be the source of the "pathogenic halophilic bacterium". Takikawa noted the similarities between his isolate and the culture isolated by Fujino, but classified the causative organism as Pseudomonas enteritis. According to Colwell (22), Dr. Takikawa "can be credited with establishment of the pathogenicity and medical importance of this organism."

Miyamoto et al. (22, 58) found the organism serologically different from Pseudomonas and suggested that it should be placed in the genus Aeromonas on the basis of its fermentation of glucose. Vibrio had been discarded as a possible genus for the organism, because pathogens, such as Vibrio cholerae, could grow on media with or without 3% NaCl. They proposed the name Oceanomonas parahaemolytic because a survey had established the marine origin of the bacterium.

The public health problem having been recognized, the Japanese Ministry of Health and Welfare organized a committee to study the "pathogenic halophile" in 1960 (22).

Sakazaki et al. conducted a detailed study of 1,072 Gram-negative short rods. They found these cultures to be motile, positive for indol production, nitrate reduction, gelatin liquefaction, cytochrome oxidase, and glucose fermentation. This study revealed three subgroups or biotypes based on differences of growth in peptone water with 7% and 10% NaCl, Voges-Proskauer reaction, and fermentation of sucrose, arabinose, and cellobiose. Biotype 3 did not grow in 7%

NaCl and was finally considered a Vibrio anguillarum-like organism and removed from the pathogenic halophile group. Biotypes 1 and 2 were designated Vibrio parahaemolyticus. Biotype 2 grew in both 7% and 10% NaCl, whereas, biotype 1 grew only in the 7% NaCl. Biotype 1 cultures were almost exclusively from feces of human patients suffering from gastroenteritis. Those of Biotype 2 were mostly isolated from marine sources (22, 58). Sakazaki et al. (22) concluded that the genus should be Vibrio and proposed the name Vibrio parahaemolyticus. So Vibrio parahaemolyticus in 1963 was divided into biotypes 1 and 2 with biotype 1 officially called "Choen Vibrio" in Japanese, meaning gastroenteritis causing Vibrio (22).

In 1965, Zen-Yoji et al. (22) applied numberical taxonomy to the study of V. parahaemolyticus and came to the conclusion that biotype 2 should be excluded from consideration as V. parahaemolyticus. They also showed that biotype 2 was infrequently isolated from patients, but often isolated from fish, shellfish, and utensils used in their preparation (22).

Sakazaki (58) restudied 100 cultures of each subgroup and, after a numerical taxonomic study on these and other marine bacteria, came to the same conclusion. Biotype 2 isolates fermented sucrose, produced acetoin, and grew in 10% NaCl while biotype 1 isolates did not. Biotype 2 was named Vibrio alginolyticus, in 1965, by Sakazaki (22). The species V. parahaemolyticus was first described in detail and redefined by Sakazaki et al. (22).

Baumann, et al. (9) conducted an extensive study of 145 isolates of marine origin in 1971. They concluded that V. parahaemolyticus should be placed in the genus Beneckea and proposed the designation Beneckea parahaemolytica (9).

In 1974, Fujino et al. (30) redesignated Strain EB 101 (ATCC 17802) as the type strain of V. parahaemolyticus. There now appear to be two types of V. parahaemolyticus as a result of epidemiological and pathogenicity studies in recent years. The difference in isolates from human gastroenteritis cases and those isolated from the marine environment has been centered on the "Kanagawa reaction" or phenomenon (22). The Kanagawa reaction involves a thermostable hemolysin and is demonstrated on a special high-salt and human erythrocyte medium, "Wagatsuma agar". Those from patients are positive (show hemolysis) for the Kanagawa reaction; whereas, those cultures from the environment are predominantly negative.

Fujino et al. (30) concurred with this view after comparing 200 different isolates of V. parahaemolyticus. Of 58 characteristics examined, the only definitive difference in the 100 cultures from marine sources and 100 cultures from human sources reported was the Kanagawa reaction. Ninety-eight percent of the latter and none of the former were positive for this characteristic.

Data compiled by Sakazaki et al. (22) showed 96% of 3,370 isolates of V. parahaemolyticus from human cases gave a positive Kanagawa reaction on Wagatsuma medium and only

1% of those isolated from marine sources gave positive results.

Distribution. The distribution of V. parahaemolyticus, once thought to be limited to Southeast Asia, has been found to be much more extensive in recent years. It has been isolated in Hong Kong, Taiwan, Singapore, The Philippines, Hawaii, and Germany (58). Barrow and Miller (6) reported isolating V. parahaemolyticus from fresh fish and shellfish in Britain in 1972. It has been reported in Calcutta by Chatterjee and Sen (18) and Sakazaki et al. (22). Other geographical areas where isolations have been reported included: The Black Sea (1), Indonesia (33), the Netherlands (34), Panama Canal Zone (42), Vietnam (57), Italy (63), Spain (66), Israel (67), Canadian Atlantic Coast (75, 76), China (47), and Bangladesh and Toga (4), Java (47) and Japan (22).

Ward is credited with the first report of V. parahaemolyticus in the United States in 1968 (22). Baross and Liston (5) reported isolating V. parahaemolyticus from Puget Sound and Washington Coast sediments during the same year. Krantz et al. (43) isolated the pathogenic halophile from moribund blue crabs from Chesapeake Bay. In 1970, Fishbein (28) reported isolating V. parahaemolyticus from Chesapeake Bay blue crabs. Estuarine water and oysters in New Hampshire were found to harbor V. parahaemolyticus by Bartley and Slanetz (7) in 1971. Kaneko and Colwell (35) reported isolations of the organism from the Rhode River Estuary.

Salt springs in Florida of non-marine origin were reported to contain the vibrio by Koburger and Lazarus (41). V. parahaemolyticus was found in low numbers in southern coastal regions of Alaska by Vasconcelos et al. (87) in three seafood processing areas. McEachern (49) reported recovering the pathogen from soft-shell clams in Massachusetts in 1973. Earle and Crisley (34) reported isolating the organism from soft-shell clams from 10 different areas on Cape Cod during July and August 1972, and in 1975, Watkins, et al. (90) recovered V. parahaemolyticus from the water column in Narragansett Bay using a new membrane filtration procedure in 1974.

The widespread occurrence of this organism is apparent. However, there appears to be a question of its existence in South American and non-Mediterranean shores of Africa. It is thought that this gap in its distribution can be attributed to the lack of investigation in these areas and will be proven to be found there also (22).

APPENDIX C

SURVIVAL OF ENTERIC BACTERIA IN SEAWATER

Information on the fate of enteric bacteria in seawater is essential to any attempted evaluation of the public health significance of sewage disposal by marine outfalls. The factors that influence the survival or removal of enteric microorganisms in seawater can be organized under four different categories. These categories are: biological factors, dissolved organic matter, dissolved inorganic matter, and physicochemical parameters. There has been considerable disagreement as to the relative importance of these categories to the survival of enteric bacteria in an aqueous environment.

One of the earliest studies indicating a biological factor contributing to the bactericidal activity of seawater was that of de Giaxa in 1889 (60). In his study of the Bay of Naples, he found that, in heat-sterilized seawater, Salmonella typhi and Vibrio comma persisted for 25 and 36 days, respectively. However, in raw seawater S. typhi survived for only 9 and V. comma for 4 days. Further study by de Giaxa (60) revealed that there was an inverse relationship between total bacterial count and survival time. He was able to isolate a number of marine bacteria which possessed definite antagonistic characteristics to the cholera vibrio. Thus, the role of indigenous marine bacteria in the bactericidal activity of seawater was suggested at this early date.

In 1950, Vaccaro et al. (83) concluded that the results of various treatments of seawater, such as storage, autoclaving, boiling, adding organic matter, pasteurization, and chlorination, indicated that the influence of native marine microflora remained to be evaluated. More recent work was reported by Kim and ZoBell (40). They found that at 1 atm pressure E. coli cells survived longer in seawater at 4 C than 8.5 C and 25 C. Chiang and Kim (20) in 1974 found that Salmonella typhimurium and E. coli were inactivated at 1000 atm at 4 C in 7 days.

Seasonal variations in the bactericidal property of seawater have been noted by several investigators over the years (32, 60, 83). These variations in the bactericidal property of seawater were generally attributed to fluctuations in the biological and physicochemical factors in the environment. Vaccaro et al. (83) observed the highest survival in May, the lowest in July. Survival in November was slightly less than in May. They concluded that changes in water temperature had the greatest effect on survival and suggested that water temperature was the most important factor in predicting fecal coliform survival.

Biological factors are generally considered by most investigators to be the most important factor in the inactivation of enteric bacteria in seawater. These factors included indigenous marine bacteria, bacteriophage (16), Bdellovibrio (26), zooplankton, phytoplankton (52), protozoan predators (26), filterfeeding animals (such as shellfish

and tunicates) (59), and conditions of the bacterial cells themselves (antibiotic resistance (70), metabolic activity (53), and origin (91).

The influence of dissolved organic matter is difficult to isolate from the biological agents because many of the substances are thought to be products of these agents or, at least, influenced by them. The elements considered as dissolved organic matter included: nutrients (14), antibiotics (15) (biogenic compounds, antibacterial substances, bactericidal macromolecules, heat-labile substances), and sewage fractions (10). Organic nutrients were considered to have a negative effect in low concentration on the survival of enteric bacteria in seawater. The existence of specific concentrations of nutrients which increase the bactericidal properties of seawater has been postulated and suggested as an important factor in the death of enteric bacteria in seawater. In relatively high concentrations, organic nutrients have been reported to enhance survival (14).

Dissolved inorganic matter has been considered one of the most potentially toxic elements in natural seawater or polluted waters. Industrial wastes (62), detergents (10), heavy metal ions (62), specific toxic ion (14), and trace metal (62) ions have been implicated in the reduction of the survival of sewage bacteria in sewage and in seawater. Inorganic nutrients have, however, been found to favor the survival of these organisms in seawater (14).

The influence of the physicochemical parameters of seawater on the survival and removal of enteric bacteria from marine waters has been discussed. Many authors considered these parameters to be of secondary importance in the bactericidal effects of seawater. Depending on environmental conditions, the relative importance of these factors in the bactericidal effects, removal, or resuspension of bacteria in marine waters may become highly significant. Those physicochemical parameters considered as contributing to the antibacterial characteristics of seawater or the physical distribution of members of Enterobacteriaceae in the sea are: salinity (14), temperature (27), pH (14), surface area (55), adsorption (12), sedimentation (12), dilution (39), tidal mixing (39), hydrostatic pressure (40), wind (83), aeration (39) and sunlight (32).

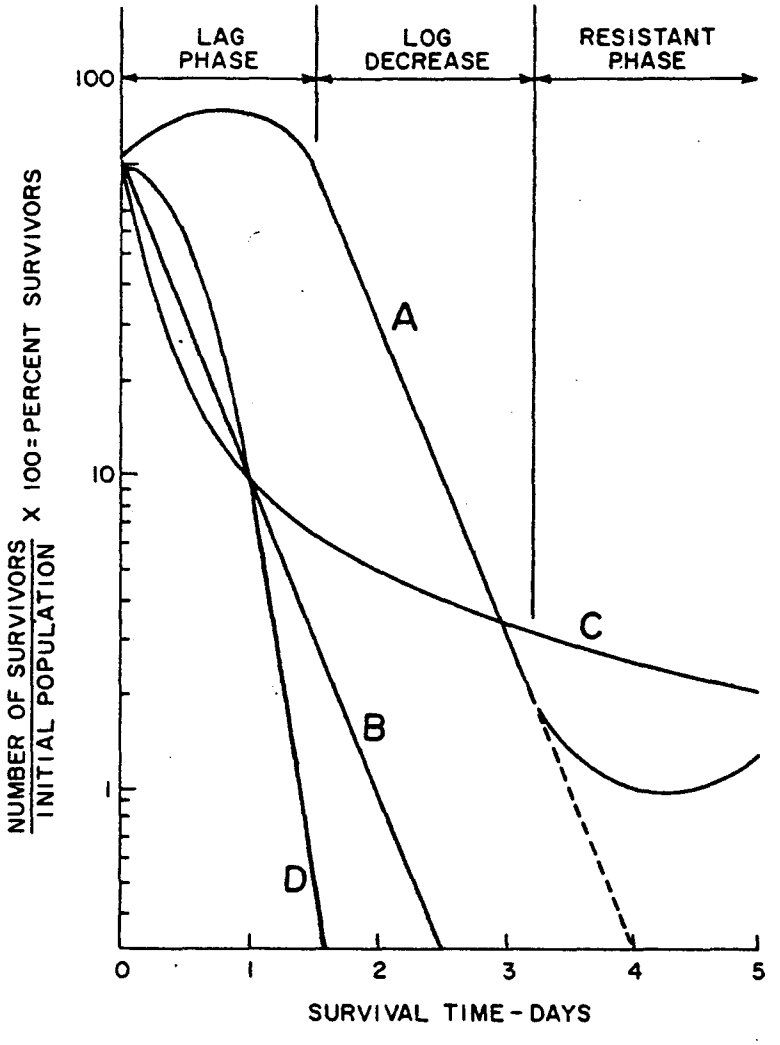
Shown in figure 12 are typical survival curves of enteric bacteria in the aqueous environment.

FIG. 12. Typical survival curves for enteric bacteria in seawater. (After Orlob, 1956). Survivors are expressed as follows:

$$\frac{\text{Number of survivors}}{\text{Initial population}} \times 100 = \text{Percent survival}$$

Survival time is expressed in days.

FIG. 12


LAG PHASE
LOG DECREASE
RESISTANT PHASE
100
10
1
A
B
C
D
0
1
2
3
4
5
SURVIVAL TIME - DAYS
NUMBER OF SURVIVORS / INITIAL POPULATION X 100 = PERCENT SURVIVORS

METHODS AND MEDIA

Test organisms. The test organism used in this study included an Escherichia coli culture, ATCC 11775 (Roche Diagnostics, Nutley, N.J.) and a Salmonella enteriditis, serotype Group C (freshly isolated from New Bedford sewage) designated T59/21a. Biochemical characteristics of the Salmonella spp. are shown in Table 6, page 49.

Standard inocula. Standard inocula of E. coli ATCC 11775 and S. enteriditis T59/21a were prepared routinely by suspending growth from 18-24 hour nutrient agar (NA) slants of the test organisms in sterile normal saline. The cells were collected by centrifugation at 9,000 rpm for 10 minutes in a Beckman Model J-21 Centrifuge (Beckman, Palo Alto, Calif.) and washed with normal saline three times. The final cell suspension was adjusted with normal saline to an absorbance of 0.07 (transmittance 0.85) with a Perkin-Elmer Double-Beam Grating Spectrophometer, Model 124 (Fisher Scientific, Medford, Ma.), equal to 10^8 viable cells per ml. One ml of this suspension was added to 99 ml filter-sterilized seawater (FSW). Seawater was filtered through 0.20 micron pore diameter plain membrane filter units (Nalge, Sybron Corp., Rochester, N.Y.).

Dialysis sac assemblies. Dialysis sac assemblies were prepared from Dialyzer Tubing with a flat width of 3.3 cm, a 0.025 mm wall thickness, and an average pore diameter of 4.8 millimicrons (Arthur H. Thomas, Co., Philadelphia, Pa.).

Lengths of ca. 40 cm were rinsed thoroughly to remove the 25% by weight of glycerin from the cellulose tubing. Screw-top test tubes were cut approximately 1.5 inches from the top. The cut edge was then fire-polished and the dialysis tubing tied securely to the screw-top neck leaving the fire-polished end exposed. The assemblies were filled with and immersed in deionized water, covered and autoclaved for 15 minutes at 121 C, 15 psi. Vacutainer rubber stoppers (VWR Scientific, Boston, Ma.) were autoclaved separately and inserted into the fire-polished end of the tube assembly after addition of the FSW and inoculum, providing a leak-proof unit. After preparation, the units were placed into a nylon bag which was suspended in seawater at Station 3 (Fig. 9), Clarks Point. A net float and no. 2 fishing weight maintained the sacs at a depth of ca. 25 cm. Samples were removed at intervals by the use of sterile 5 ml syringes. After inverting each assembly four times for mixing, the needle was inserted through the rubber stopper and ca. 2 ml was removed. The stopper was disinfected with 70% ethanol and dried with sterile gauze prior to drawing the sample. After removing the needle from the syringe, the contents were deposited into a sterile test tube on ice for transport to the laboratory. Transport time was generally 45 minutes, and at no time over 90 minutes.

Media. Initially three different media were tested for suitability for the enumeration of E. coli ATCC 11775: Tergitol 7 agar (Difco), Levine EMB agar (Difco), and Endo

agar (Difco). Three different media were tested for enumeration of the S. enteriditis T59/21a: Salmonella-Shigella agar (Difco), Levine EMB agar, and Endo agar. Levine EMB agar was employed in all subsequent experiments for the enumeration of both E. coli ATCC 11775 and S. Enteriditis T59/21a. All dilutions were performed using phosphate-buffered dilution water as suggested by the APHA (2, 3) and Thatcher and Clark (73). Viable counts were determined by spreading 0.1 ml of appropriate dilutions on the respective agar surfaces. Seawater samples were collected at the same sampling intervals for determination of environmental parameters.

Physicochemical parameters of the seawater. Catch samples were collected and processed for salinity, pH, DO BOD_5, DON, nitrites (NO_2), nitrates (NO_3), and phosphates (PO_4) as shown on page 8.

Microbiological parameters of the seawater. Total and fecal coliform counts and PV and PVP counts were determined for catch samples as previously described (page 12).

Procedure. Samples were drawn from the dialysis units at intervals over 72-hour periods for the determination of viable counts of E. coli ATCC 11775 and S. enteriditis T59/21a. Samples of the seawater were collected simultaneously for analysis of physicochemical and microbiological parameters (page 8).

RESULTS AND DISCUSSION

The limited recovery of Salmonella from the New Bedford marine environment prompted a study to examine the *in situ* survival of this organism in New Bedford marine waters. A defined culture of *E. coli* was included as a comparison of the response of an indicator organism to the marine environment to that of a known pathogen. In this way, some indication of the validity of the fecal coliform count as a measure of water quality in this specific marine area could be determined. The dialysis assembly provided a relatively inexpensive, reproducible method of determining the response of the bacteria to the environmental parameters of the marine water. The results of this study are shown in figures 13 and 14, beginning on page 101.

The disparity between the survival of a *Salmonella sp.* and the survival of *E. coli* has been noted by others (68). It appears that *E. coli* ATCC 11775 is more sensitive to unfavorable conditions and slower to recover than the pathogen, *S. enteriditis* T59/21a. The survival of the two test organisms in New Bedford seawater is affected by several environmental parameters, as reflected by the fluctuations in the survival curves during the *in situ* studies. These parameters will be discussed briefly in relation to bacterial survival.

Water temperature (Figures 13C and 14C), salinity (Figures 13D and 14E), and pH (Figures 13E and 14F) showed relatively small changes throughout both the July and September studies. The observed results, however, were an

expected inverse relationship of these factors to the survival of the test bacteria that has been reported by others (14, 27, 56, 60).

Dissolved oxygen (DO) (Figures 13H) exhibited an inverse relationship to the survival of the test bacteria. This result is in close agreement with data reported by Hanes et al. (27). They reported a longer survival time for E. coli when the average DO was low (0.4 mg/liter) and considerably more rapid die-off at high DO levels in the range of 7.8 to 38.0 mg/liter. Die-off remained relatively similar within this range of values. Dissolved oxygen values obtained during the present study ranged from ca. 7.0 mg/liter to ca. 11.0 mg/liter. As DO increased, a decrease in survival was seen. Faust et al. (27) found contradictory results in some survival experiments. The number of viable cells of E. coli MC-6 declined from 10^8 to 10^4 cells/ml below 4 mg/liter after three days in estuarine water. When DO was higher (10.0) the decline of viable cell numbers was very small during the same period of time. Further examination of DO in relation to the survival of enterics in seawater is needed.

Dissolved nutrients showed an inverse relationship to the survival of the test organisms. (Figures 13J,K,L,M; 14G,H,I). This result was unexpected because most investigators generally agree that increased nutrients enhance the survival capabilities of enterics in seawater (12, 14, 56, 60, 83). It has been suggested that a level of nutrients

in seawater too high to restrict the bacterial cells to the cellular nutrient pool and, yet, too low to maintain a higher metabolic rate, would result in a more rapid inactivation (53, 54). If this assumption is correct, a similar mechanism may have been in operation during the survival studies, thus partly explaining the observed inverse relationship of dissolved nutrients to bacterial survival.

Total coliform counts (TC) and fecal coliform counts (FC) (Figures 13F and 14J) demonstrated a delayed inverse relationship to the survival of E. coli and S. enteriditis. This relationship is probably not due to any direct effect of the TC and FC on the survival of the test bacteria, but only indicates the composition of the sewage and seawater mixture or the degree of pollution in the water column.

Presumptive vibrio (PV) and presumptive Vibrio parahaemolyticus (PVP) counts exhibited a positive correlation with the survival of the test bacteria (Figures 13G and 14K). This is contrary to the accepted opinion that increases in marine bacteria are accompanied by reduced survival of enterics in seawater (32, 52, 55, 56, 83). Competition for limited nutrients and production of antibacterial substances by indigenous marine bacteria contribute to the die-off of enteric bacteria in seawater. It is very difficult to determine the precise cause of the observed relationship from the information available. It might be speculated, however, that an undetermined factor was present that was unfavorable to both the marine and enteric bacteria.

It is apparent that the survival and distribution of the large numbers of enteric bacteria entering the sea by marine outfalls is dependent on the sum total of possible combinations of the positive and negative influences of the parameters discussed. The problem of correlating the results of laboratory and *in situ* studies can be appreciated. Restricting the variations of factors in the laboratory limits the applicability of the results to the environment. Whereas, *in situ* studies present difficulties in determining the contribution of each factor to the death or survival of enterics in seawater. The interpretation of the interaction of sewage and the marine environment is of the utmost importance in evaluating the public health hazards.

FIG. 13 A. July *in situ* survival of *E. coli* ATCC 11775 (○) and *S. enteriditis* T59/21a (△) in seawater expressed as instantaneous percent of original population.

FIG. 13 B. Tidal cycle calculated from the National Ocean Survey Tide Tables (79).

FIG. 13 C. Air temperature (○) and water temperature (△) recorded in degrees Centigrade.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 13

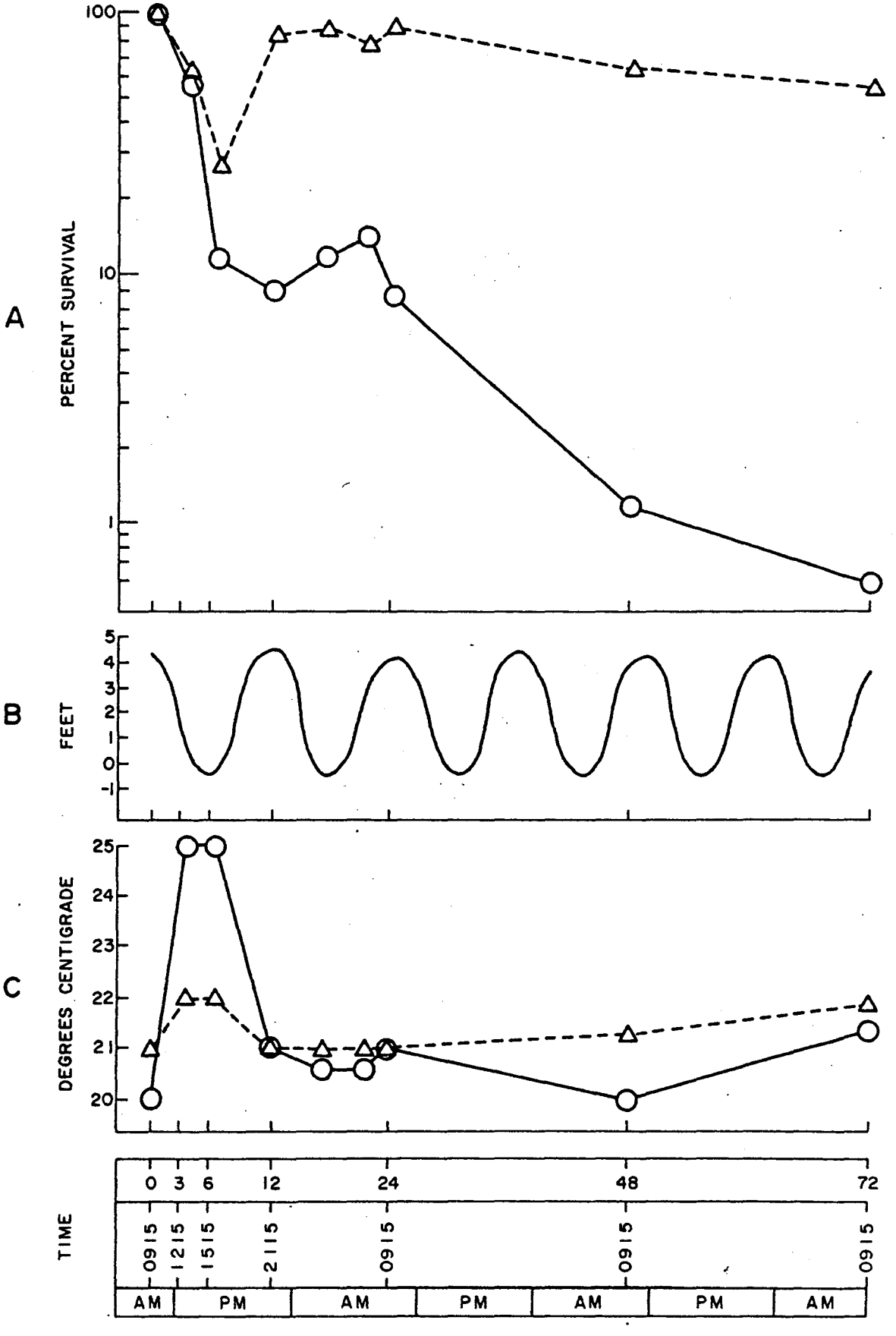

A
PERCENT SURVIVAL
100
10
1
B
FEET
5
4
3
2
1
0
-1
C
DEGREES CENTIGRADE
25
24
23
22
21
20
TIME
0
3
6
12
24
48
72
0915
1215
1515
2115
0915
0915
0915
AM
PM
AM
PM
AM
PM
AM

FIG. 13 D. Salinity recorded in parts per thousand during the July survival study.

FIG. 13 E. pH recorded concurrently.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 13

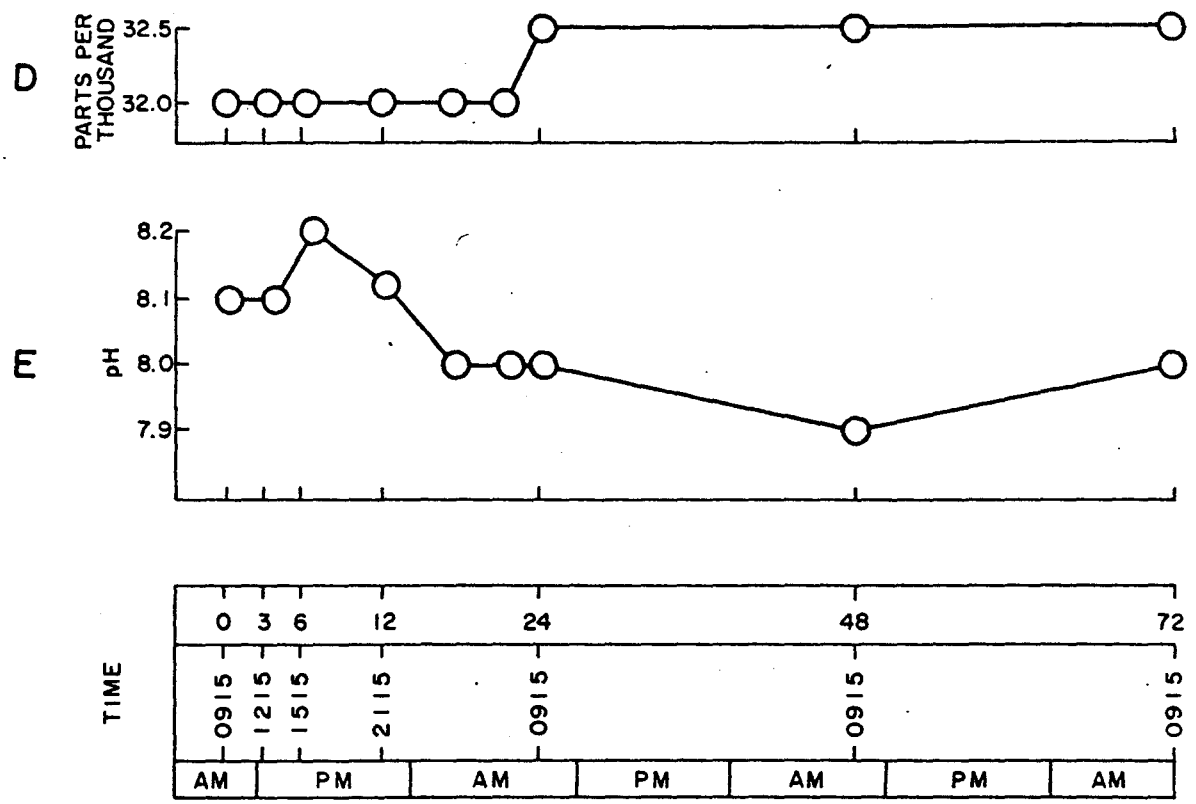

D
PARTS PER THOUSAND
32.5
32.0
E
pH
8.2
8.1
8.0
7.9
TIME
0
3
6
12
24
48
72
0915
1215
1515
2115
0915
0915
0915
AM
PM
AM
PM
AM
PM
AM

FIG. 13 F. Total coliforms (○) and fecal coliforms (△) per 100 ml seawater during the July survival study.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 13

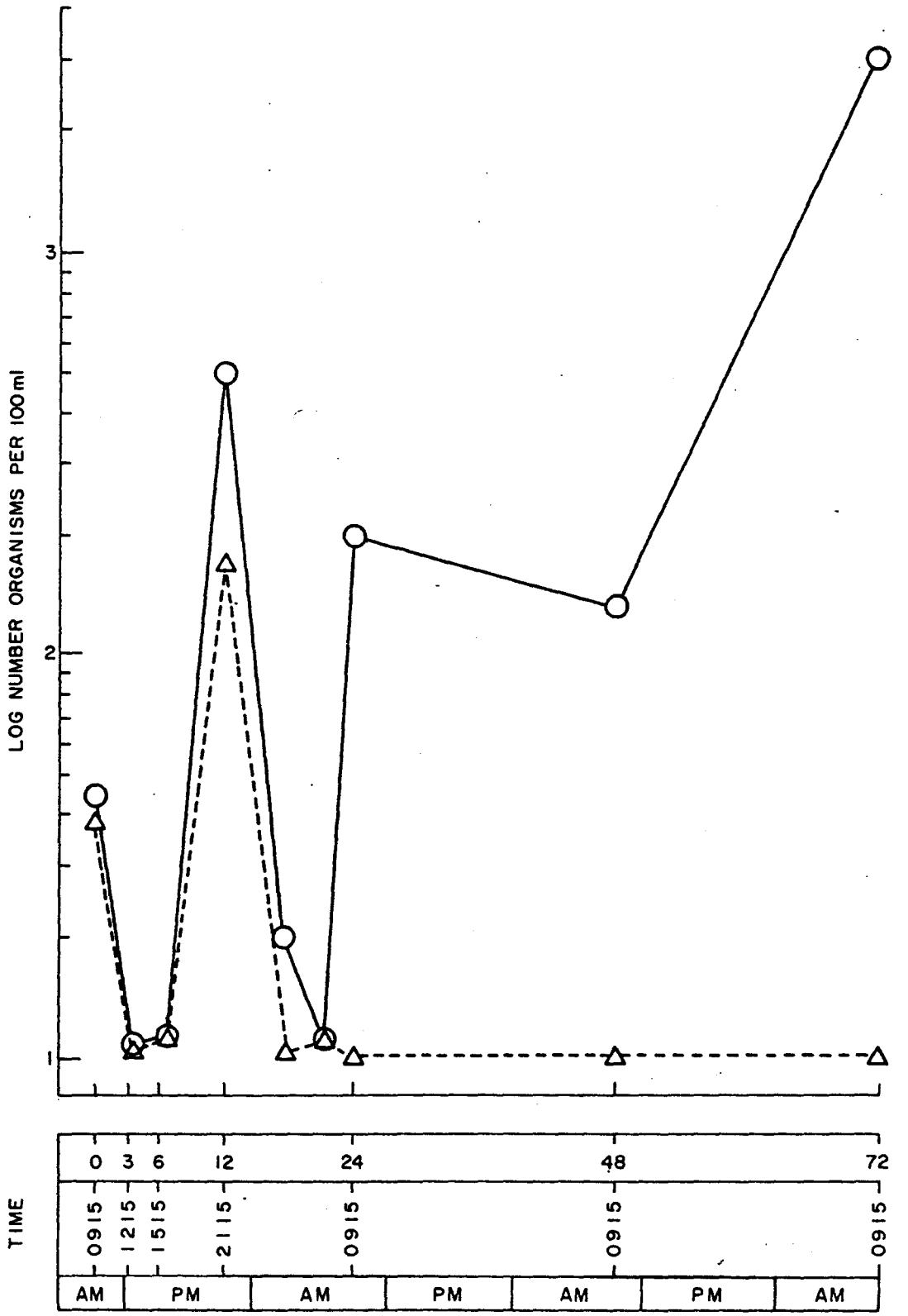

F
LOG NUMBER ORGANISMS PER 100 ml
3
2
1
TIME
0
3
6
12
24
48
72
0915
1215
1515
2115
0915
0915
0915
AM
PM
AM
PM
AM
PM
AM

FIG. 13 G. Presumptive vibrios (○) and presumptive Vibrio parahaemolyticus (△) plotted as log number organisms per 100 ml seawater during the July study.

FIG. 13 H. Dissolved ozygen recorded as mg 0_2/liter.

FIG. 13 I. Biochemical oxygen demand shown as mg 0_2/liter.

FIG. 13 J. Dissolved organic nitrogen recorded as microgram-atoms N/liter.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 13

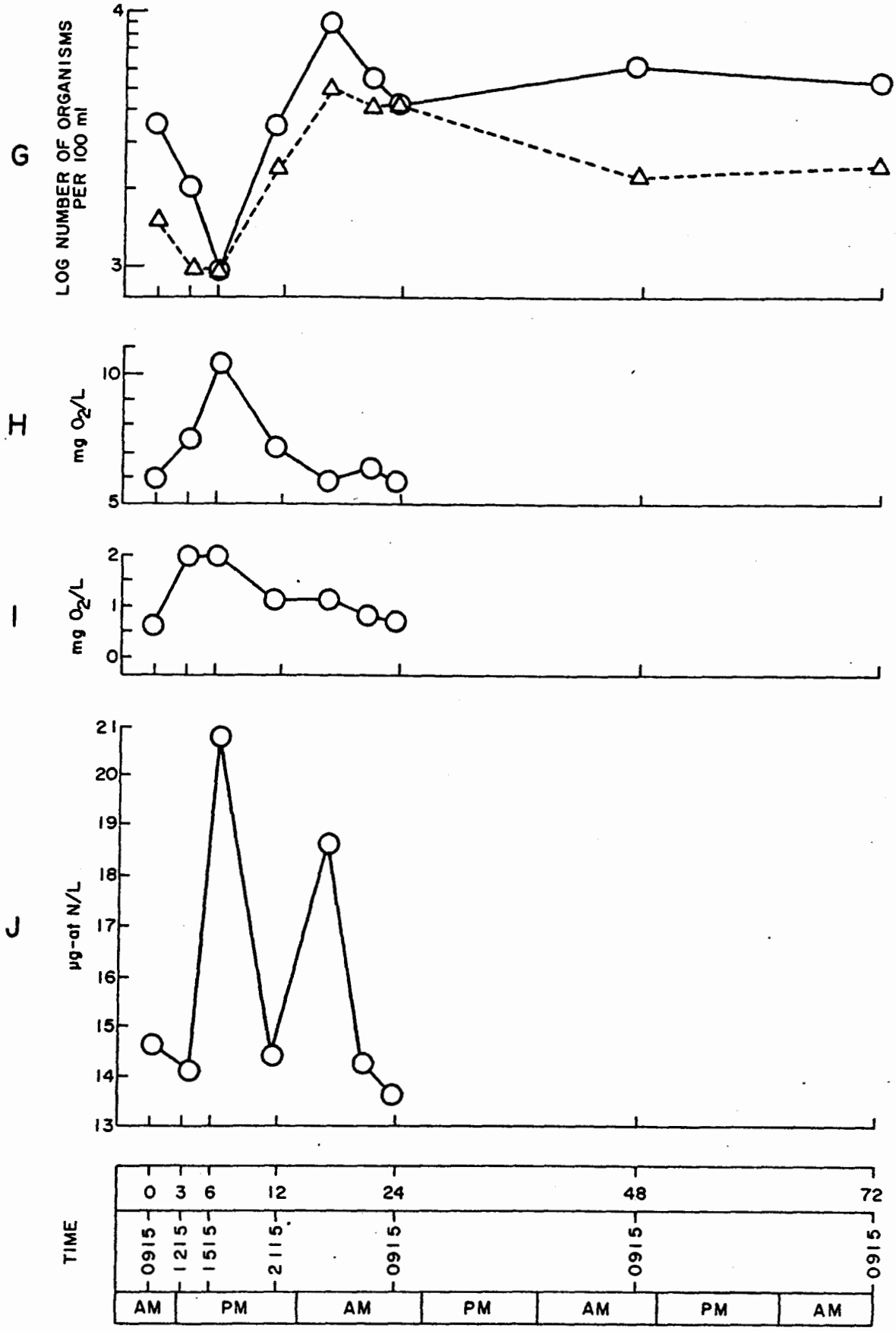

G
LOG NUMBER OF ORGANISMS PER 100 ml
4
3
H
mg O2/L
10
5
I
mg O2/L
2
1
0
J
µg-at N/L
21
20
19
18
17
16
15
14
13
TIME
0
3
6
12
24
48
72
0915
1215
1515
2115
0915
0915
0915
AM
PM
AM
PM
AM
PM
AM

FIG. 13 K. Nitrites as microgram-atoms NO_2-N/liter.

FIG. 13 L. Nitrates as microgram-atoms NO_3-N/liter.

FIG. 13 M. Orthophosphates as microgram-atoms PO_4-P/liter.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 13

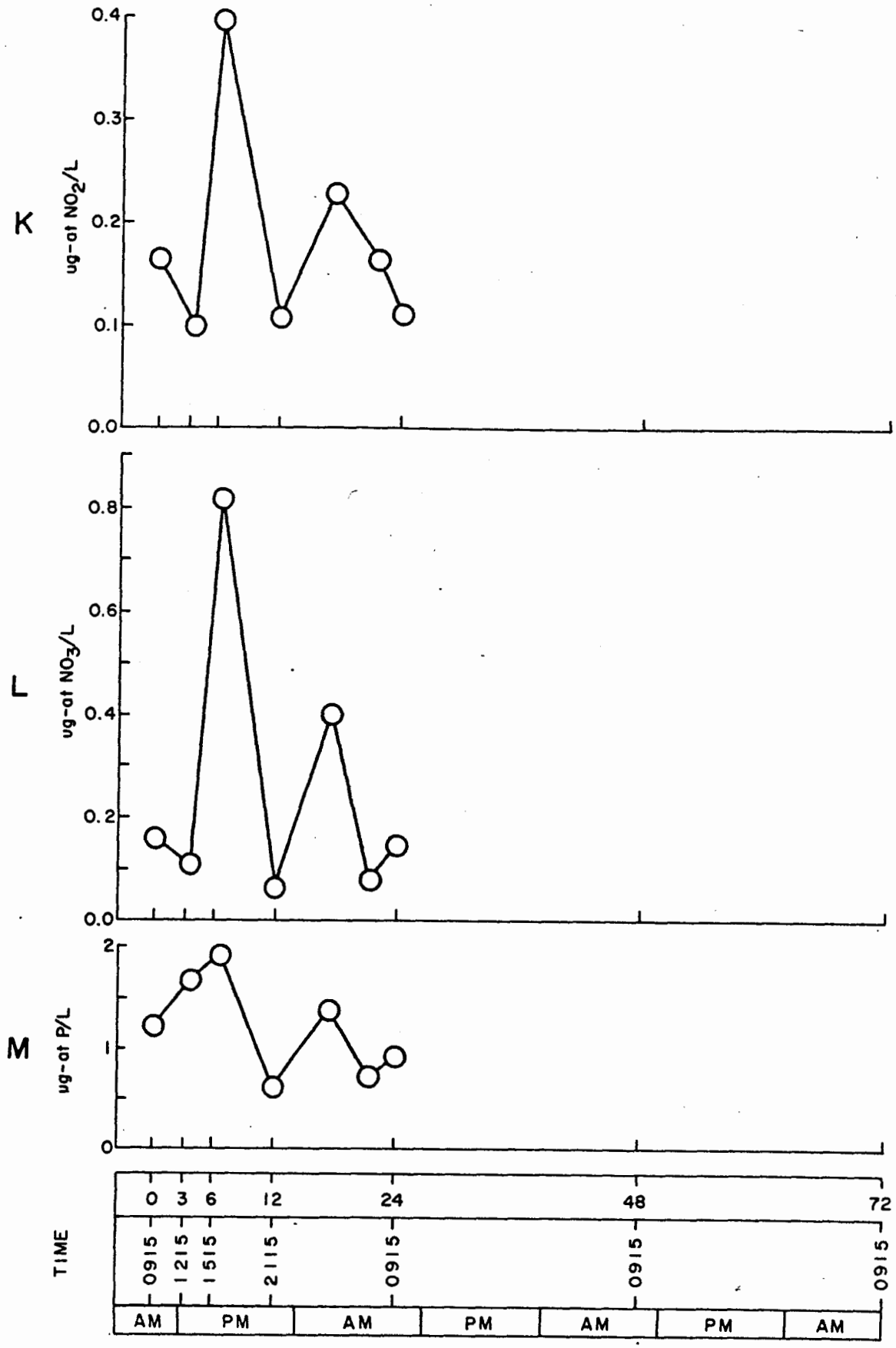

K
ug-at NO_2/L
0.4
0.3
0.2
0.1
0.0
L
ug-at NO_3/L
0.8
0.6
0.4
0.2
0.0
M
ug-at P/L
2
1
0
TIME
0
3
6
12
24
48
72
0915
1215
1515
2115
0915
0915
0915
AM
PM
AM
PM
AM
PM
AM

FIG. 14 A. September *in situ* survival of *E. coli* ATCC 11775 (○) and *S. enteriditis* T59/21a (△) in seawater.

FIG. 14 B. Tidal cycle calculated from the National Ocean Survey Tide Tables (79).

FIG. 14 C. Water temperature recorded as degrees Centigrade.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 14

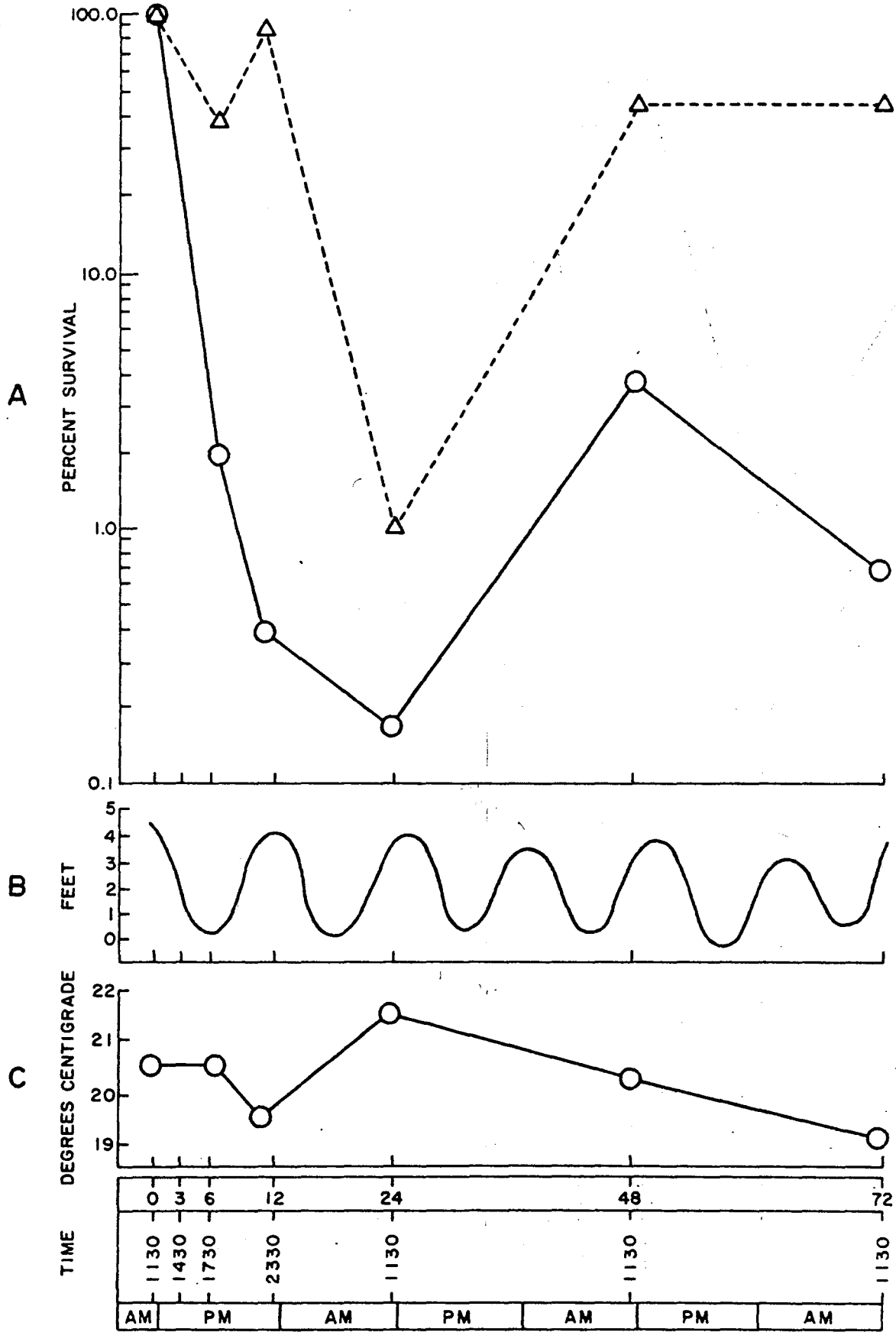

A
PERCENT SURVIVAL
100.0
10.0
1.0
0.1
B
FEET
5
4
3
2
1
0
C
DEGREES CENTIGRADE
22
21
20
19
TIME
0
3
6
12
24
48
72
1130
1430
1730
2330
1130
1130
1130
AM
PM
AM
PM
AM
PM
AM

FIG. 14 D. Air temperature recorded as degrees Centigrade during the September survival study.

FIG. 14 E. Salinity recorded as parts per thousand.

FIG. 14 F. pH concurrently recorded.

FIG. 14 G. Nitrites reported as microgram-atoms NO_2-N/liter.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 14

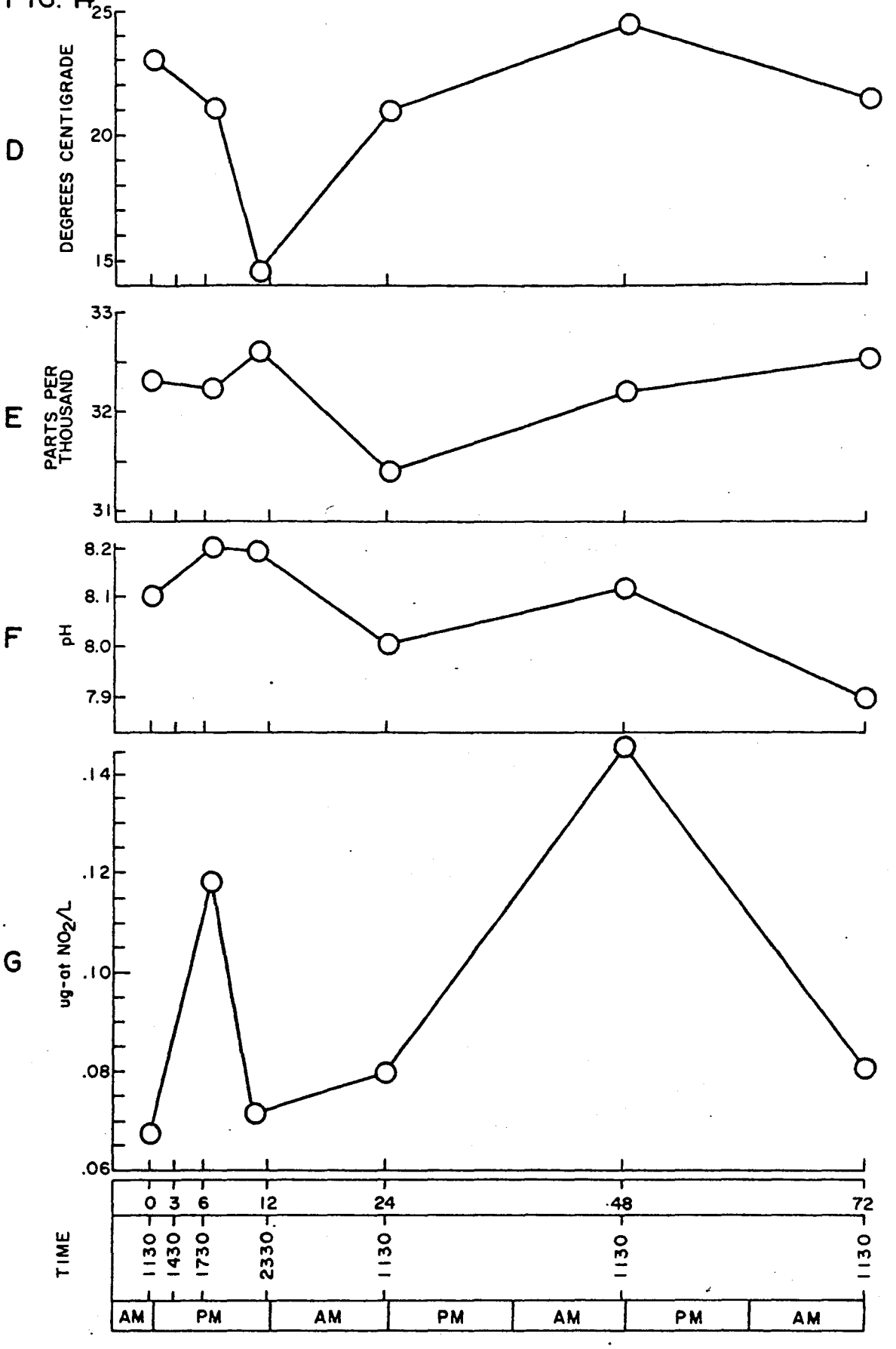

D
DEGREES CENTIGRADE
25
20
15
E
PARTS PER THOUSAND
33
32
31
F
pH
8.2
8.1
8.0
7.9
G
ug-at NO2/L
.14
.12
.10
.08
.06
TIME
0 3 6 12 24 48 72
1130 1430 1730 2330 1130 1130 1130
AM PM AM PM AM PM AM

FIG. 14 H. Nitrates reported as microgram-atoms NO_3-N/liter during the September study.

FIG. 14 I. Orthophosphates recorded as microgram-atoms PO_4-P/liter.

FIG. 14 J. Total coliforms (○) and fecal coliforms (△) per 100 ml seawater.

Time. The top set of numbers indicates time in hours; the lower set of numbers indicates time of day.

FIG. 14

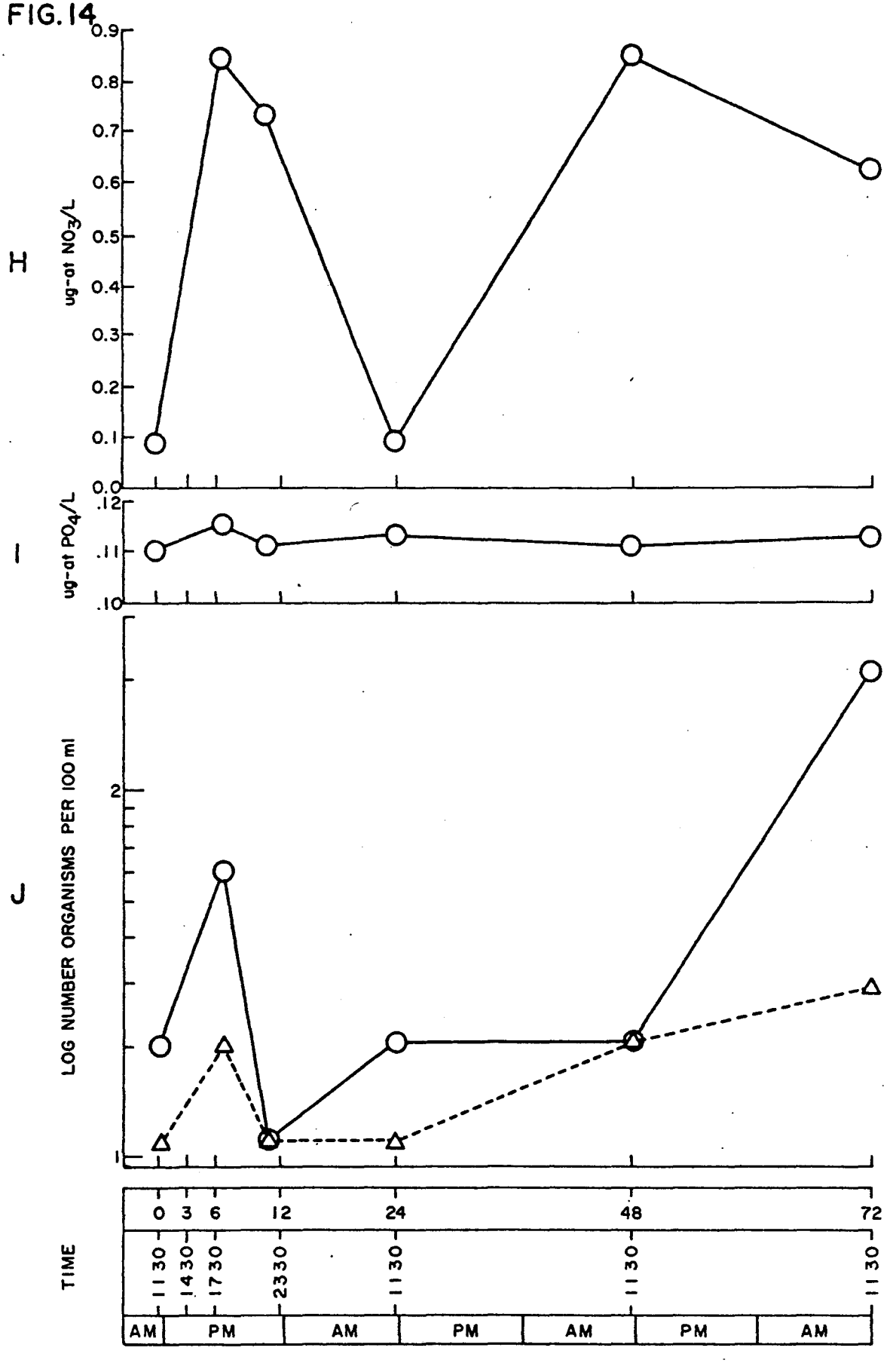
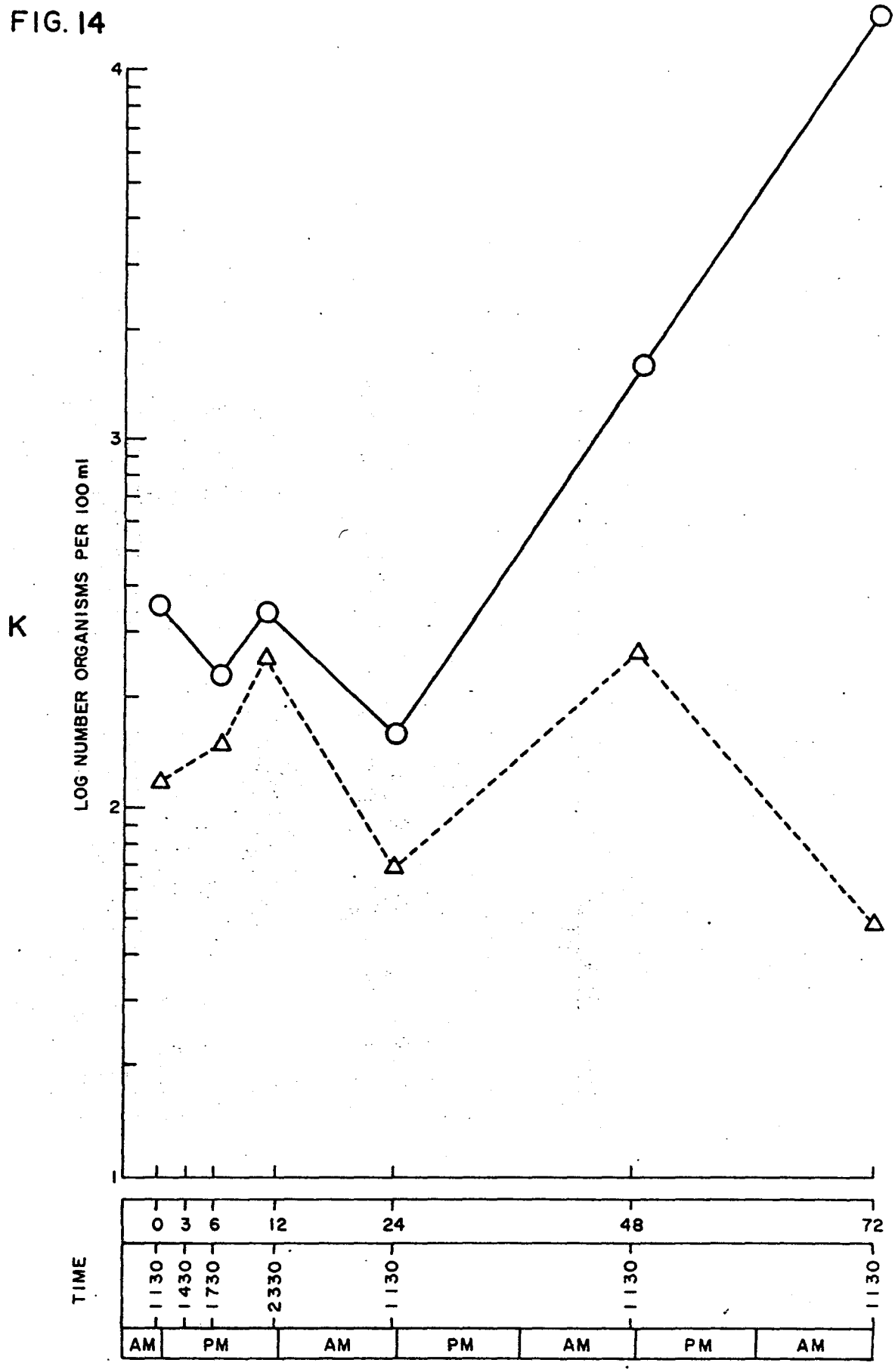

H
ug-at NO_3/L
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
I
ug-at PO_4/L
.12
.11
.10
J
LOG NUMBER ORGANISMS PER 100 ml
2
1
TIME
0 3 6 12 24 48 72
11 30
14 30
17 30
23 30
11 30
11 30
11 30
AM PM AM PM AM PM AM

FIG. 14 K. Presumptive vibrios (○) and presumptive Vibrio parahaemolyticus (△) plotted as log number organisms per 100 ml seawater during the September survival study.

FIG. 14


K
LOG NUMBER ORGANISMS PER 100 ml
4
3
2
1
0 3 6 12 24 48 72
TIME
1130
1430
1730
2330
1130
1130
1130
AM
PM
AM
PM
AM
PM
AM

APPENDIX D

GROWTH OF *VIBRIO PARAHAEMOLYTICUS* IN FILTER-STERILIZED SEAWATER

Standard inocula. The inocula was prepared from a 4.5 hour TSSB 3% culture incubated at 37 C. The absorbance determined with a Double-Beam Grating Spectrophometer, Model 124 (Fisher Scientific, Medford, Ma.), at 620 nm was 0.375 (Transmittance 42%). This was equivalent to *ca*. 10^4 viable cells per ml.

Seawater. The seawater was freshly collected from Clarks Cove, Station 1 (Figure 9), by the procedures outlined by the APHA (2, 3), with the exception that it was maintained at the temperature at which it was collected (21.5 C) until inoculated. Physicochemical parameters and total and fecal coliform counts were determined as previously described. The seawater was filter-sterilized and dispensed in 99 ml aliquots in 250 ml flasks.

Media. Dilutions were prepared using PBDW 3% (phosphate-buffered dilution water with 3% NaCl-pH 7.0). Both Thiosulphate Citrate Bile Salts agar and starch-galactose agar were used to estimate viable counts.

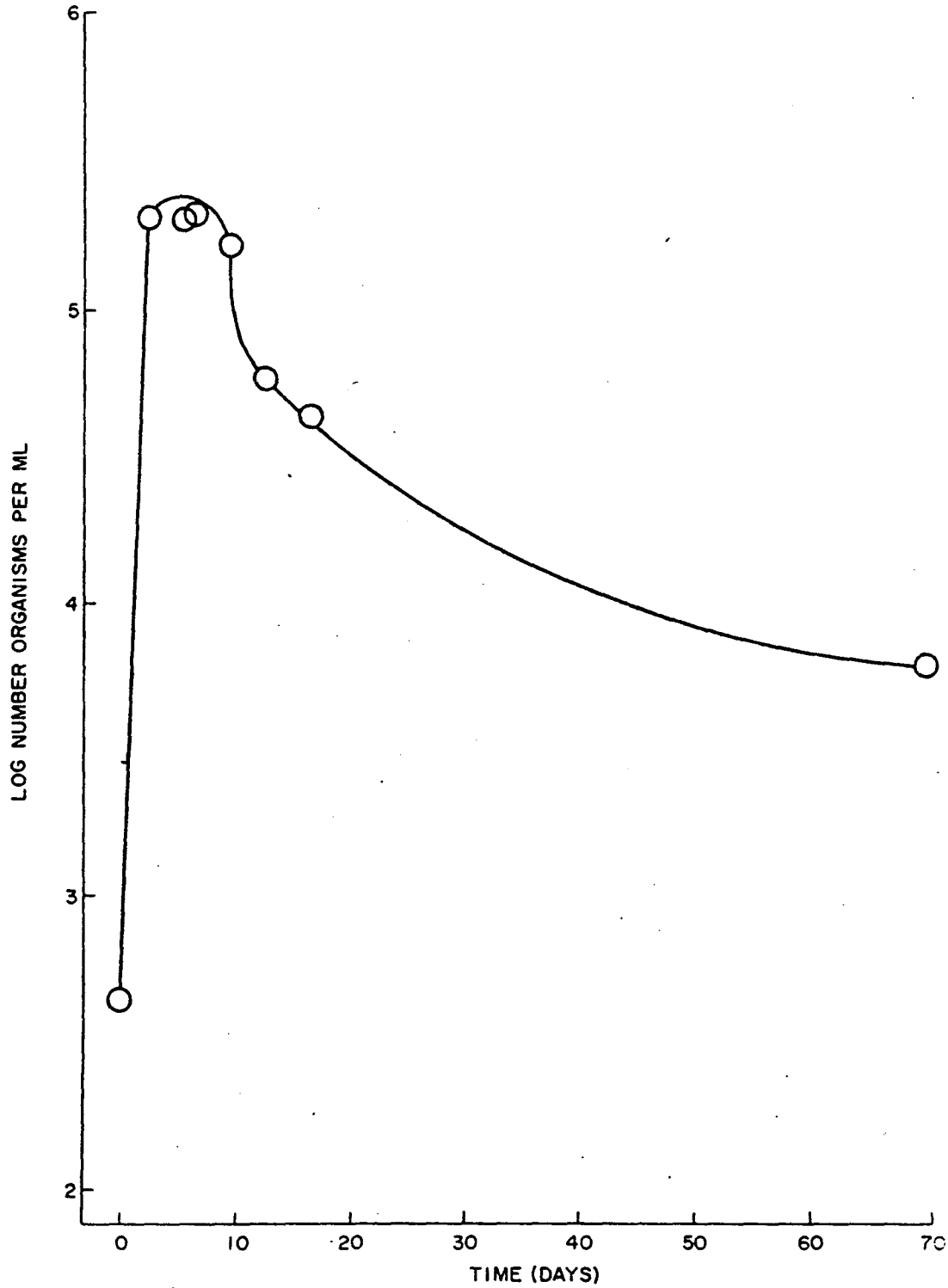
Procedure. One ml of inocula was introduced into each of three flasks containing 99 ml of the filter-sterilized seawater. The flasks were incubated at 20 C (near the collection temperature of 21.5 C) in an Environ-Shaker 3597 (Lab-Line Instruments, Instruments, Inc., Melrose Park, Ill.).

The shaker was set for 150 rpm. Aliquots of 0.1 ml of each dilution were spread on triplicate plates to determine viable counts. Samples were taken for plate counts at 0, 3, 6, 7, 10, 13, 17, and 70 days.

Results. Shown in Figure 15 is the growth of V. parahaemolyticus in filter-sterilized seawater collected from Clarks Cove. Shown in Table 7, page 53, is a comparison of TCBS and Starch-Galactose agars.

FIG. 15. Growth of Vibrio parahaemolyticus in filter-sterilized seawater collected from Clarks Cove.

FIG. 15


6
5
4
3
2
LOG NUMBER ORGANISMS PER ML
0
10
20
30
40
50
60
70
TIME (DAYS)

GROWTH OF VIBRIO PARAHAEMOLYTICUS IN TRYPTIC SOY BROTH

Test organism. A culture of Vibrio parahaemolyticus was kindly provided by William Watkins of the Environmental Protection Agency, Water Supply Research Laboratory, West Kingston, R.I.

Media. Tryptic Soy Broth (Difco) was prepared with 0.85, 3.0, and 7.0% NaCl added and distributed into 300 ml Nephelo culture flasks (Bellco Glass Inc., Vineland, N.J.). The flasks containing the broths were autoclaved (121 C, 15 psi) for 15 minutes and allowed to cool to room temperature.

Procedure. One ml of inoculum was introduced to each flask. The flasks were incubated at 37 C or 41 C in a shaker bath set at ca. 120 rpm. Absorbance was read with a Spectronic 20 (Bausch and Lomb, Inc., Rochester, N.Y.) at 620 nm wavelength.

Results. The results are shown in Figures 16, 17, 18.

FIG. 16. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in TSSB with 0.85% sodium chloride.

Symbols

○ - Growth of V. parahaemolyticus at 37 C

△ - Growth of V. parahaemolyticus at 41 C

FIG. 16

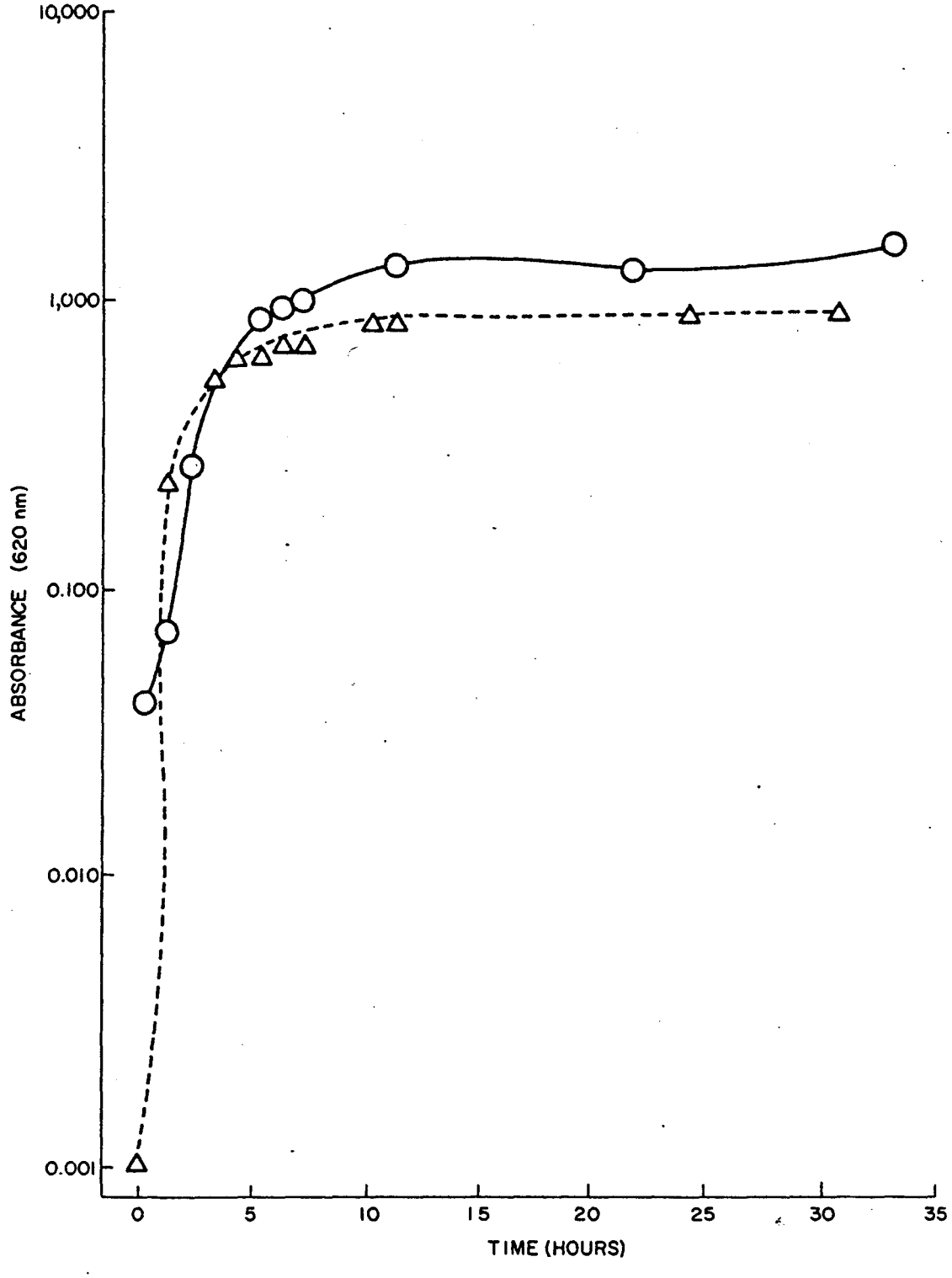

10,000
1,000
0.100
0.010
0.001
ABSORBANCE (620 nm)
0
5
10
15
20
25
30
35
TIME (HOURS)

FIG. 17. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in TSSB with 3.0% sodium chloride.

Symbols

○ - Growth of V. parahaemolyticus at 37 C

△ - Growth of V. parahaemolyticus at 41 C

FIG. 17

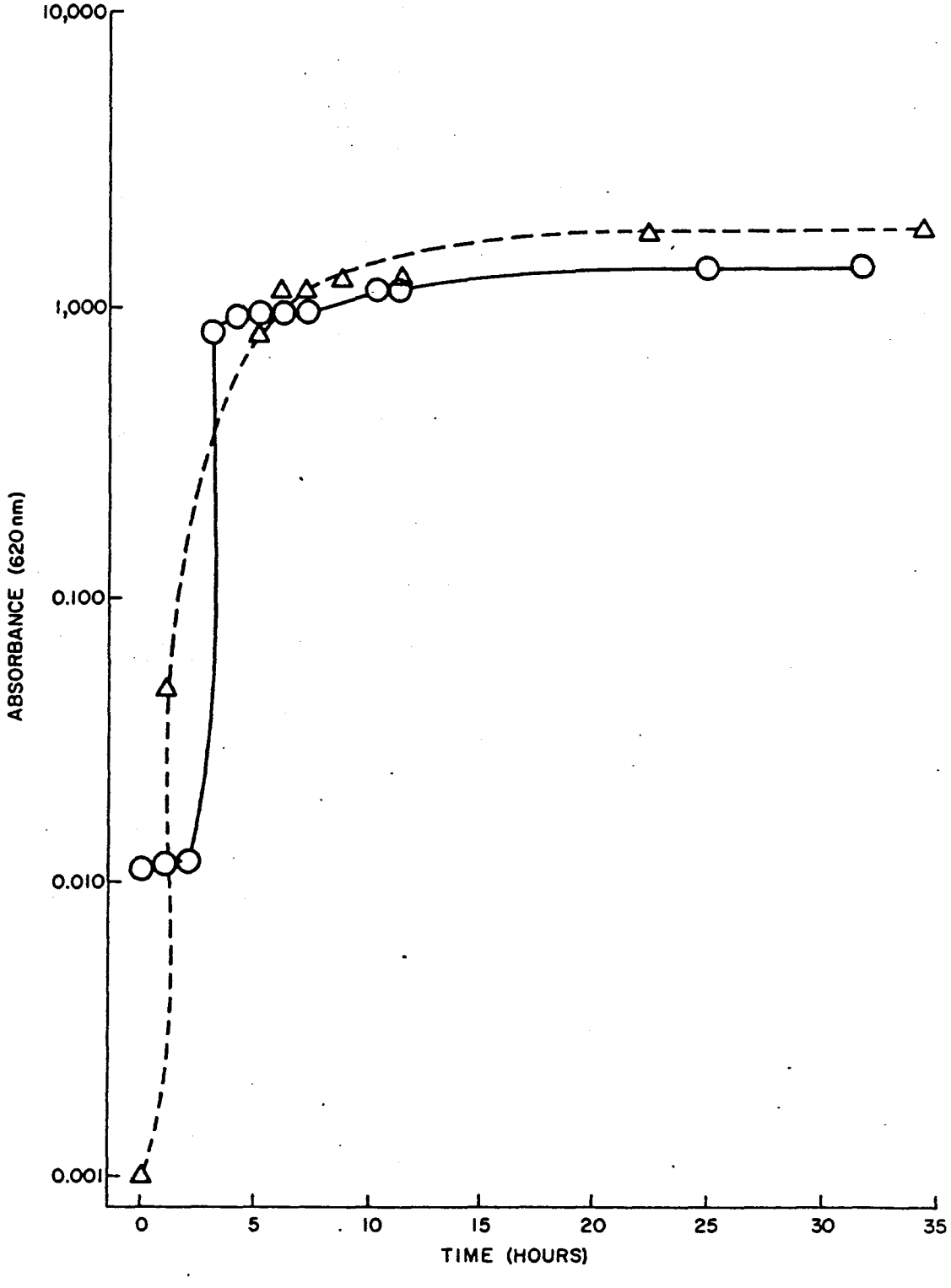

10,000
1,000
0.100
0.010
0.001
ABSORBANCE (620 nm)
0
5
10
15
20
25
30
35
TIME (HOURS)

FIG. 18. Effect of two different temperatures on the growth of Vibrio parahaemolyticus in TSSB with 7.0% sodium chloride.

Symbols

○ - Growth of V. parahaemolyticus at 37 C

△ - Growth of V. parahaemolyticus at 41 C

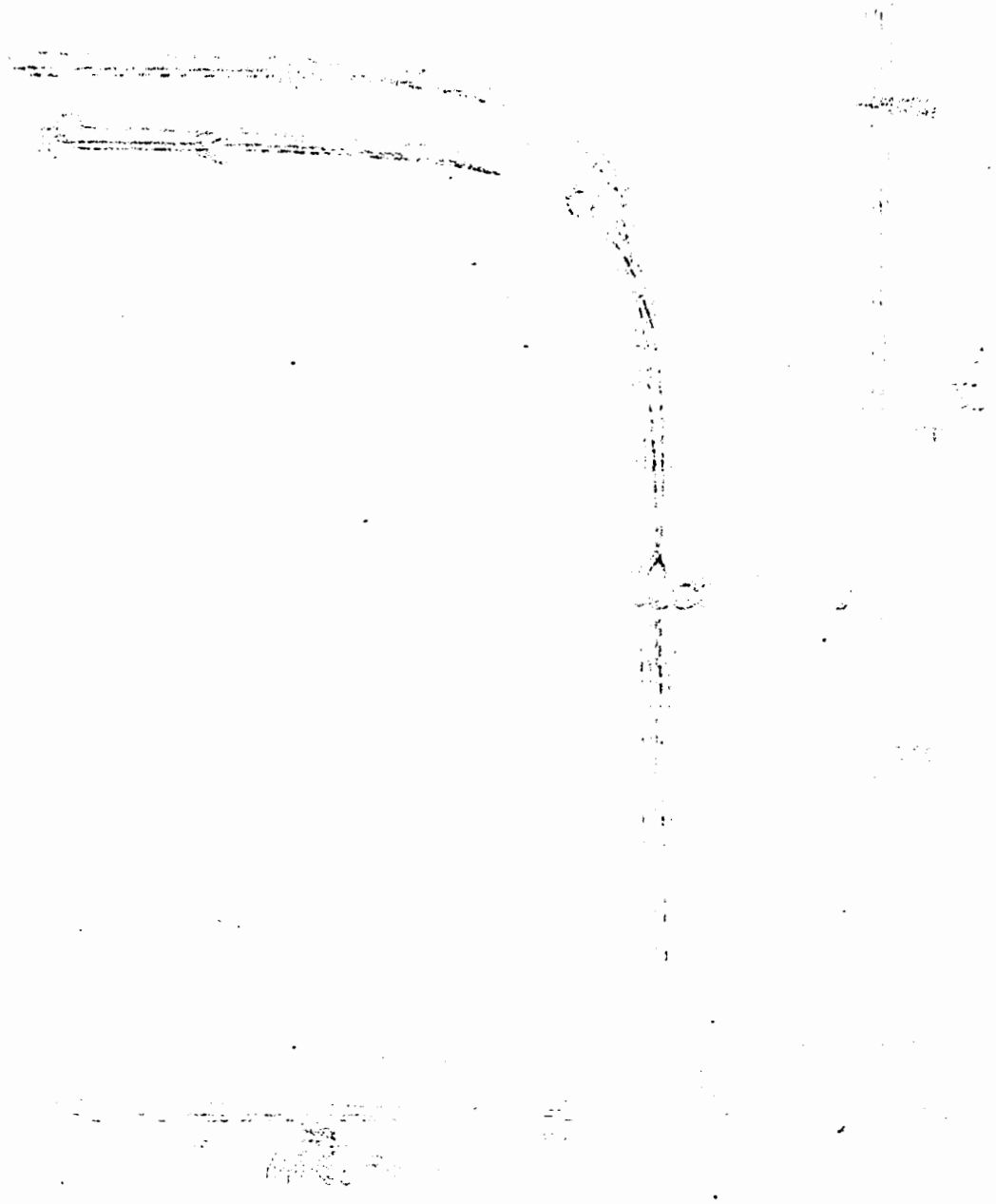
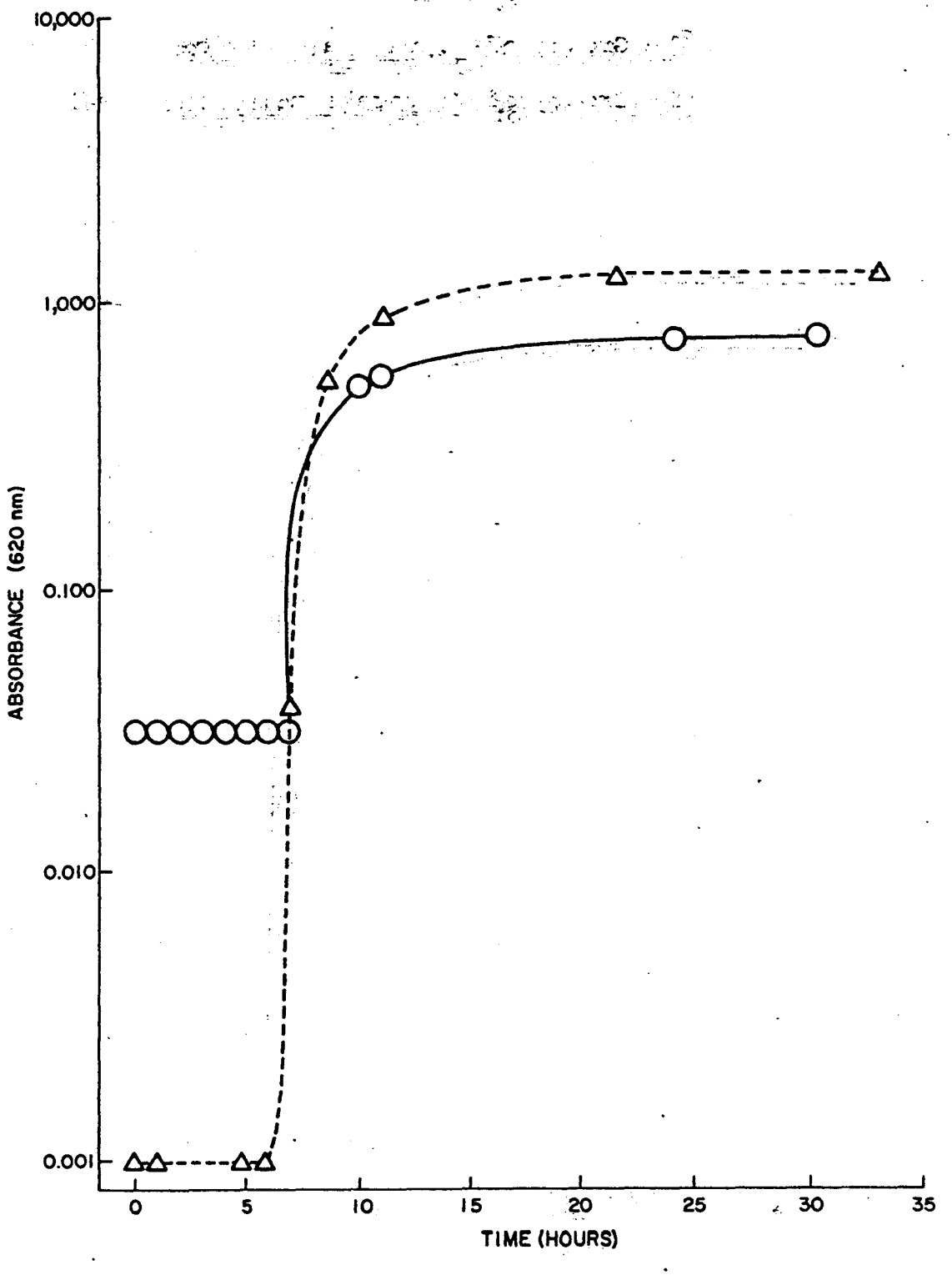


FIG. 18


10,000
1,000
0.100
0.010
0.001
ABSORBANCE (620 nm)
0
5
10
15
20
25
30
35
TIME (HOURS)