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WSG-MP 71-5

BIG BEEF ESTUARY  
PHYTOPLANKTON AND BACTERIA STUDIES  
1968-1969

By Fred Palmer, Jon Heller,  
John Gatjens, and Ruth Hung

December 1971

DIVISION OF MARINE RESOURCES  
UNIVERSITY OF WASHINGTON 98195

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## INTRODUCTION

This report is intended primarily for investigators engaged in studies at Big Beef estuary. We have emphasized 1) estimates of phytoplankton populations, 2) bacteria of shellfish sanitation significance, 3) estimates of total food for shellfish, and 4) indicators of ecological change. The data may be useful in comparing Big Beef with other areas and as a reference line for following relatively long-term changes at Big Beef.

Figure 1 is a rough map of the estuary at Big Beef showing the locations of Stations E, 1, 3 and 9. The stream (Station S) was sampled about one mile above the laboratory. The estuary fills and empties with each tidal cycle; Station 9 is above water more of the time than it is covered; Station 3 is submerged more than Station 9 and has few shellfish; Station 1 is similar to Station 3, but has dense shellfish beds. Samples were collected near high tide and were drawn from the middle depths, when possible, to avoid the fresh water surface layer from the creek and muddy water from stirred sediments near the bottom. The 1968 samples were obtained by lowering a polyethylene bottle and allowing it to fill at the desired depth. Beginning in 1969 a submersible pump, powered by two 6-volt "Hot Shot" batteries, was used.

Phytoplankton samples were collected both in the form of unconcentrated water samples and in the form of concentrated samples by putting known volumes of water through a net, retaining particles larger than about  $100\mu$ . Net samples were preserved in iodine-potassium iodide solution and the others in 5% neutralized formalin. Organisms in the stored samples were identified and counted with the aid of an inverted microscope (loaned to us by Dr. Kelshaw Bonham) and settling chambers.

Water for bacteriological analysis was collected aseptically in sterile bottles. Dilution and inoculation of appropriate media were often performed immediately after collection of the samples. When this was impractical, samples were stored in ice for the few hours required to transport them to the University.

Coliform numbers were estimated, using Lauryl tryptose broth with confirmation on EMB agar plates (APHA 1965). Total numbers of aerobic, heterotrophic bacteria were estimated by MPN in a medium consisting of peptone, 0.1%; yeast extract, 0.05%; and starch, 0.4% in sea water. Bacteria from the positive tubes were subcultured in gelatin medium for estimation of numbers of gelatin liquifiers as representing proteolytic organisms, and on starch agar plates for estimation of numbers of starch digesters.

Analyses for oxidizable carbon and Kjeldahl nitrogen were done on particulates nominally larger than  $1.0\mu$ , by procedures recommended by Strickland, Parsons (1965). Chlorophyll analyses were begun in 1969 on particles held on  $0.45\mu$  filters (UNESCO 1966).

Salinity measurements were obtained for the sample water with the use of hydrographic hydrometers. Surface water temperatures were recorded.

#### RESULTS AND DISCUSSION

Figures 2 - 6 show the total numbers of some of the more common genera of plankton for each station on the dates sampled. Tables 1 through 5 present similar information and data on other genera.

##### Algal Abundance

Naked flagellates predominate numerically in most of the samples, except possibly those from the stream. Due to their small size, the proportion of the biomass which they represent may be much smaller than their numbers would indicate. They are generally considered to be good food for shellfish.

Chlorophyll a values for 1969 are included in Figures 2 through 6 and in Table 6. The relationship of chlorophyll a to numbers of phytoplankters is unclear in some cases, especially Stations E and 1 on August 5, 1969

It would be tempting to assign the discrepancies between chlorophyll a and numbers of organisms to error in the chlorophyll analyses for August 5, 1969. However, these pigment values continue a downward trend which began earlier.

In general, chlorophyll a should be a good index of food available to shellfish. Although as a measure of biomass it may be biased to give higher results with smaller organisms, it is these which are thought to be especially good feed for juvenile shellfish.

Total phytoplankton numbers at each station are compared in Figure 7. Stations E (Hood Canal) and S (Big Beef Creek) are often quite different in their populations, as would be expected. Samples taken at the other stations will usually be either predominantly Hood Canal or creek water, without very much mixing, depending upon the season and the depth of sampling, among other factors. This is confirmed by the salinities plotted in Figures 8 - 12. The fresh water layer is much thicker during the rainy months when the stream volume is high.

It is obvious that although the area is small, the sampling problem is complex. Patchiness in Hood Canal populations will be reflected in the estuary and modified by mixing with the stream and stratification. Averaging of values for Stations 1, 3 and 9 may be justified on the basis that the characteristics of the water and the populations in contact with the shellfish vary throughout the tidal cycle. The bottom animals are exposed to fresh water plankton when the tide is low and to sea water plankton as the water deepens.

It is believed that even such a limited sampling program is of some value in describing the conditions in the estuary and in detecting changes.



It may be worthwhile to substitute additional chlorophyll sampling for plankton enumeration and classification, which are very time-consuming.

#### Bacterial Abundance

The bacteriological sampling has the two aims of using bacterial numbers as indicators of changes in the estuarine ecology and of monitoring the sanitary quality of the water. Figures 8 - 11 and Table 7 plot data for total aerobic heterotrophs, starch digesters, and gelatin liquifiers. Long-term increases in any of these three categories would probably mean that substances available to bacteria as food were increasing. Cultures obtained in the sampling are tested for ability to hydrolyze starch and to liquify gelatin, to determine whether these characteristics contribute information beyond that obtained from total numbers of aerobic heterotrophs.

Significant numbers of coliforms frequently occur in Big Beef Creek, at the Hood Canal sampling Station (E) and, from both of these sources, in the estuary. The U. S. Public Health Service (1965) states that in approved shellfish-growing areas, coliform MPN may not exceed 330 per 100 ml during the most unfavorable pollution and hydrographic conditions, unless detailed studies show that the coliforms are not of fecal origin and do not indicate a public health hazard. Results of sampling in 1968 and 1969 (Figure 12) indicate that, occasionally, coliform counts exceeded the U. S. Public Health Service standards.

#### Chemical Estimations of Shellfish Food Abundance

Table 8 provides results of analyses of particulate material in water samples for oxidizable carbon and Kjeldahl nitrogen. It is suspected, on

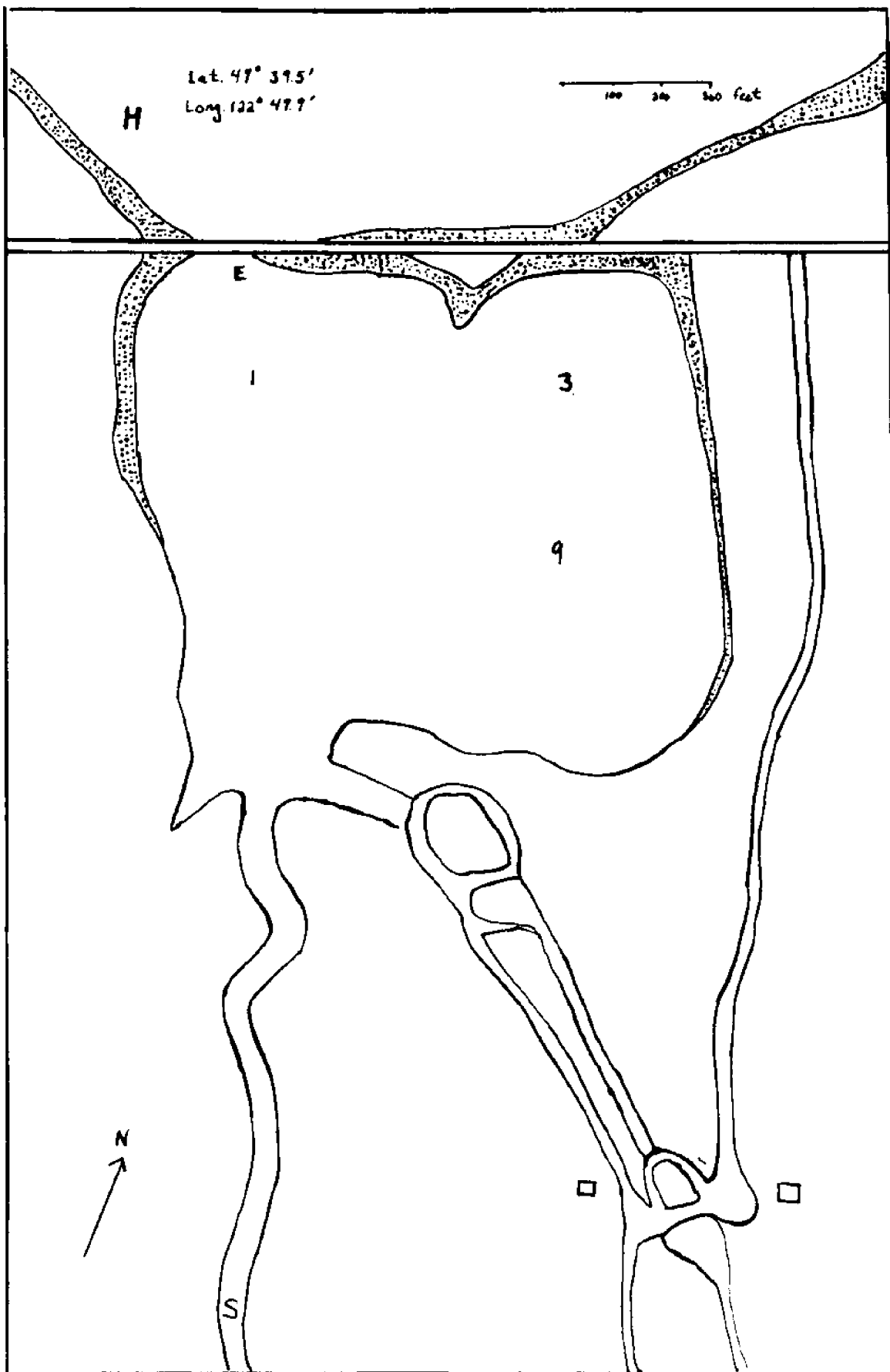
the basis of the low C:N ratios, that the oxidizable carbon values tend to be too low. Whether the fault is due to technique or to a method which may not be suitable for samples containing sediment is not known. The Kjeldahl nitrogen values are thought to be the more reliable of the two. These analyses could provide useful information on potential shellfish food if the reliability of the results was confirmed. However, rather than trying to prove out the methodology, we will concentrate on other techniques.

During 1970 the number of field trips was greatly reduced and the data gathered were limited to salinity, temperature, pigments and bacteria (including coliforms). Phytoplankton samples were collected, but have not been examined.

The 1971 sampling program was similar to the program described herein.

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- Strickland, J. D. H. and Parsons, T. R. 1965. A Manual of Sea Water Analysis. Fisheries Research Board of Canada Bull. 125, 2nd Ed. Ottawa.
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-



BIG BEEF ESTUARY AREA

Figure 1

FIGURE 2

PIRANOMY COUNTS  
B16 SHEEP  
STATION E

- TOTAL COUNTS
- NAMED FLAGELLUMS
- △—△ NAVICULUM
- ×—× MITZSCHIA
- CILIOZOOPHYLL A

NO/ml

400

350

300

200

150

100

50

10

1968

1969

1970

1971

1972

1973

1974

1975

1976

1977

1978

1979

1980

1981

1982

1983

1984

1985

1986

1987

1988

1989

1990

1991

1992

1993

1994

1995

1996

1997

1998

1999

2000

2001

2002

2003

2004

2005

4.0

3.0

2.0

1.0

0.0

1968

1969

1970

1971

1972

1973

1974

1975

1976

1977

1978

1979

1980

1981

1982

1983

1984

1985

1986

1987

1988

1989

1990

1991

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1993

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1997

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1999

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2002

2003

2004

2005

2006

2007

2008

CHLORO-

PHYLLA

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1971

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2002

2003

2004

2005

2006

2007

2008

2009

2010

2011

SEPTEMBER

AUGUST

JULY

JUNE

MAY

APRIL

MARCH

FEBRUARY

JANUARY

DECEMBER

NOVEMBER

OCTOBER

1968

1969

1970

1971

1972

1973

1974

1975

1976

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210

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290

300

310

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330

340

350

360

370

380

390

400

410

420

430

440

450

460

470

480

490

500

510

520

530

540

550

560

570

580

590

600

610

620

630

640

650

660

670

680

690

700

710

720

730

740

750

760

770

780

790

800

810

820

830

840

850

860

870

880

890

900

910

920

930

940

950

960

970

980

990

1000

1010

1020

1030

1040

1050

1060

1070

1080

1090

1100

1110

1120

1130

1140

1150

1160

117

FIGURE 3

PLANTATION COUNT  
BIG BEEP  
STATION I

- TOTAL COUNT
- △ INFECTION PRESSURES
- BRYOPHYTES
- × WATERWE
- CHLORO-PHYLL - A

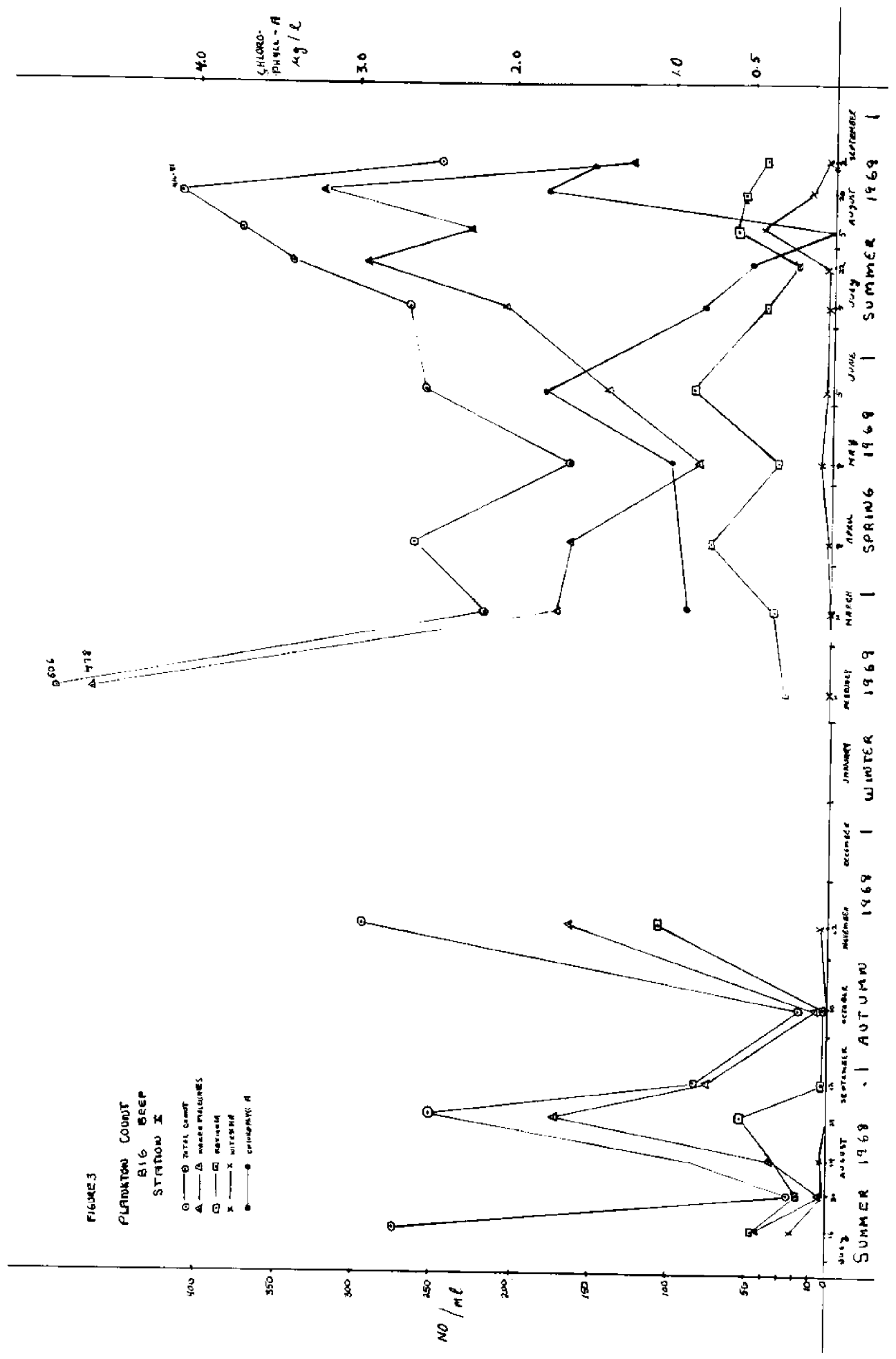
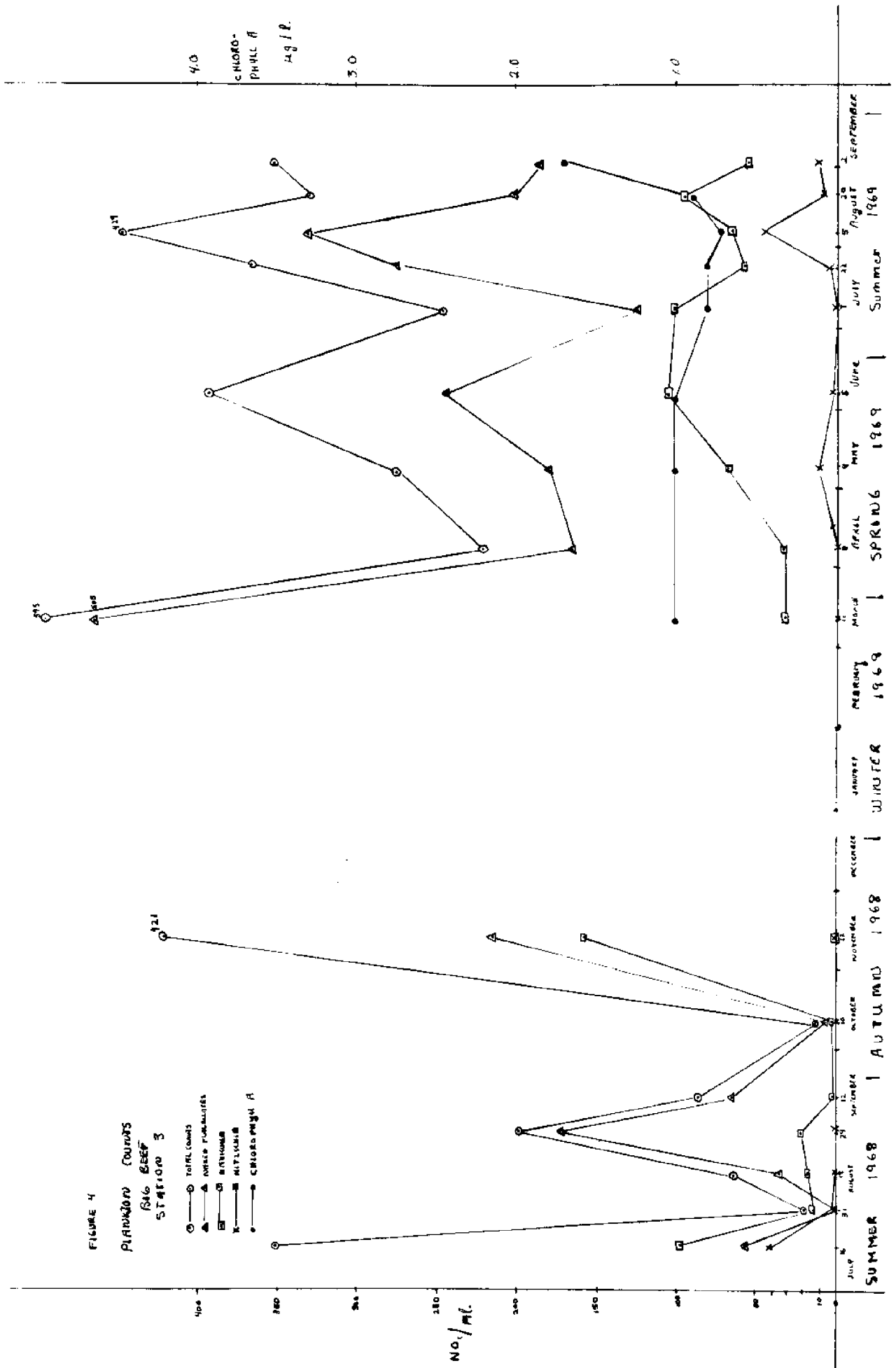


FIGURE 4

PLANKTON COUNTS  
BAG BEEF  
STATION 3

- TOTAL COUNTS
- △ DIATOM PLANKTON
- ROTIFEROA
- × MYSIDAE
- CHLOROPHYLL A



4.0  
CHLORO-  
PHYLL A  
µg/l.

3.0  
2.0  
1.0

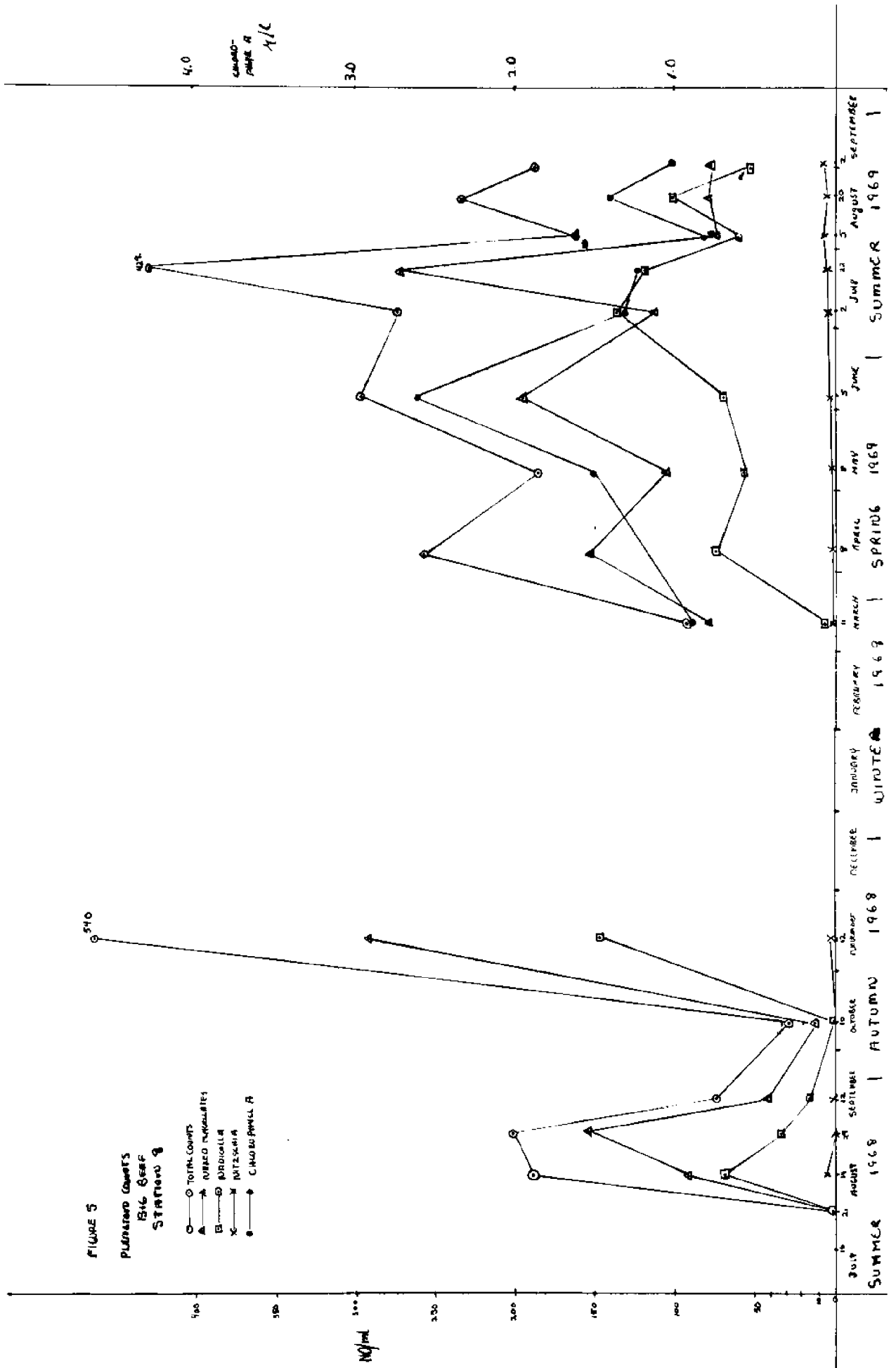
SEPTEMBER  
AUGUST  
JULY  
JUNE  
MAY  
APRIL  
MARCH  
FEBRUARY  
JANUARY  
WINTER  
1968  
AUTUMN  
SUMMER  
1969

NO./ML.

FIGURE 5

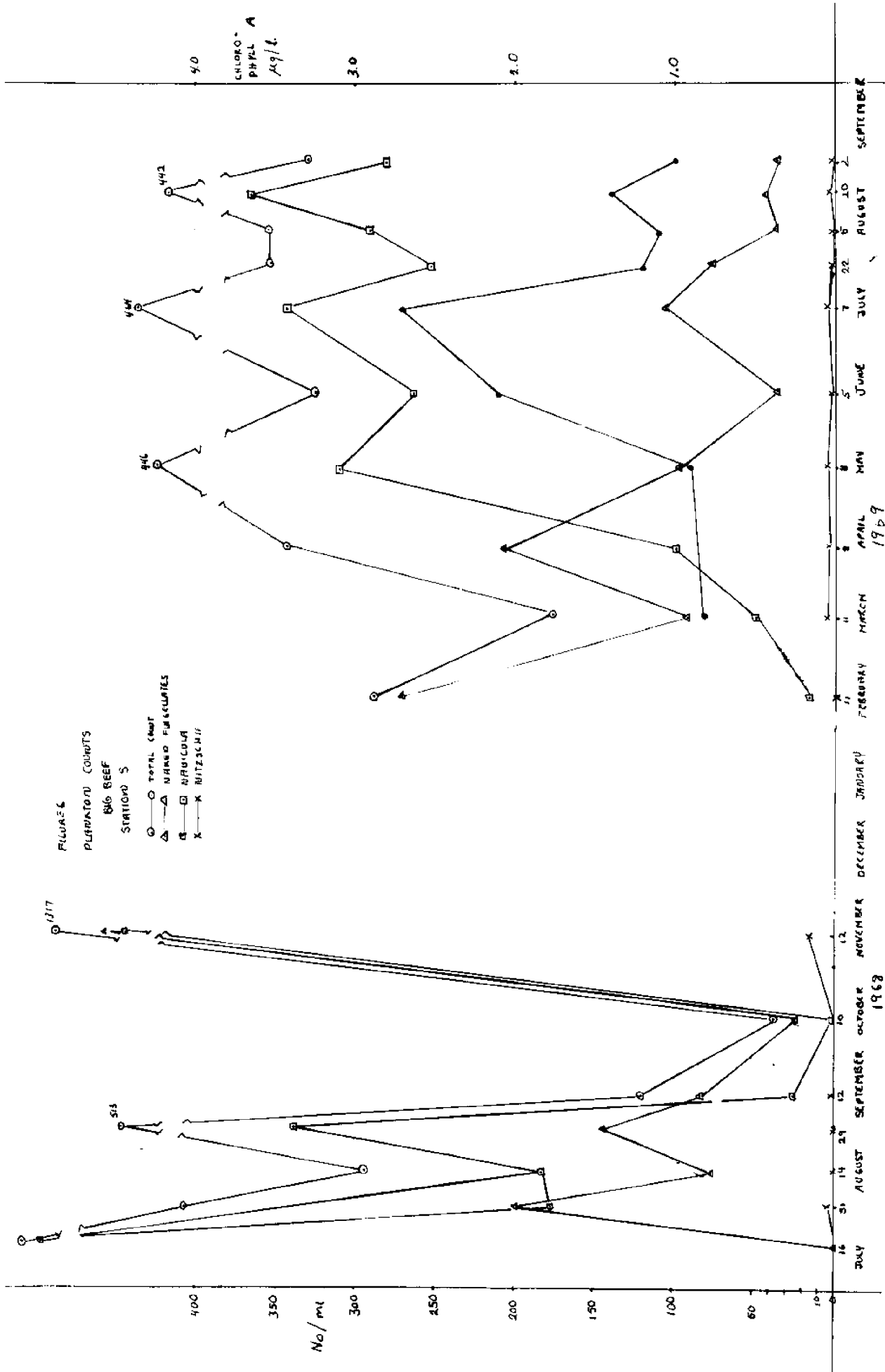
PLEISTOCENE COUNTS  
BIG BEEF  
STATION 9

- TOTAL COUNTS
- △ UREDO TYPICALS
- URIDICALS
- × BATESIANA
- C. H. B. SPINELL A



CANADIAN  
PAGE 2  
4/6





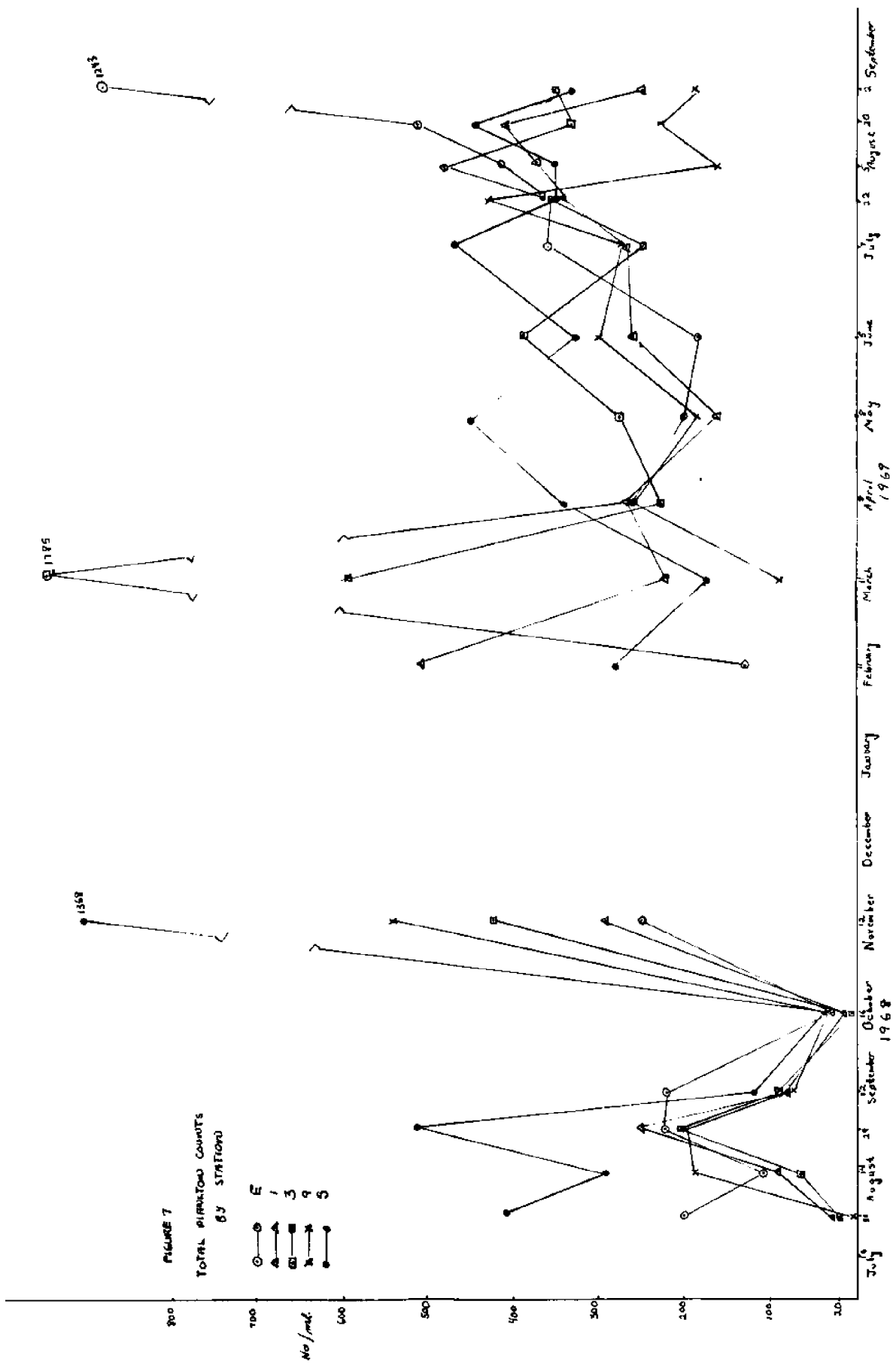
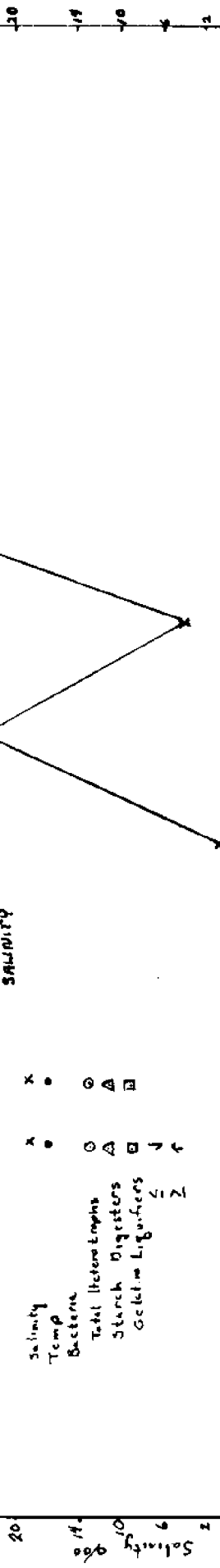


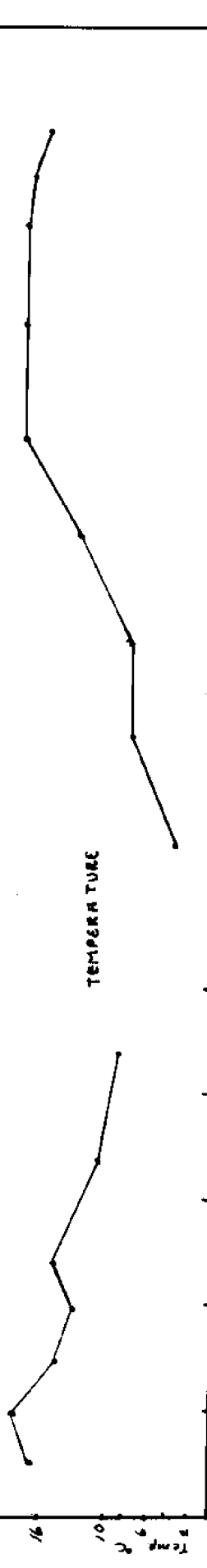
FIGURE 3  
STATION E

Salinity  
Temp  
Bacteria  
Total Heterotrophs  
Starch Digestors  
Gelatin Liquefiers  
E  
Z

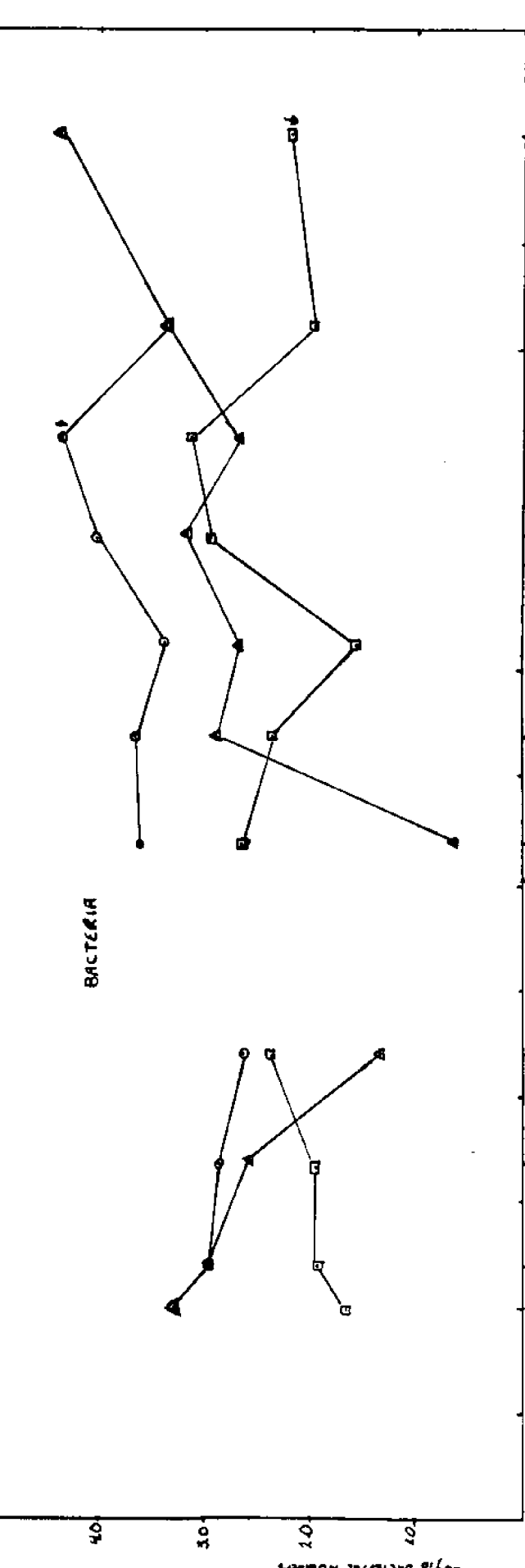
SALINITY



TEMPERATURE



BACTERIA



20  
15  
10  
5  
2

20  
15  
10  
5  
2

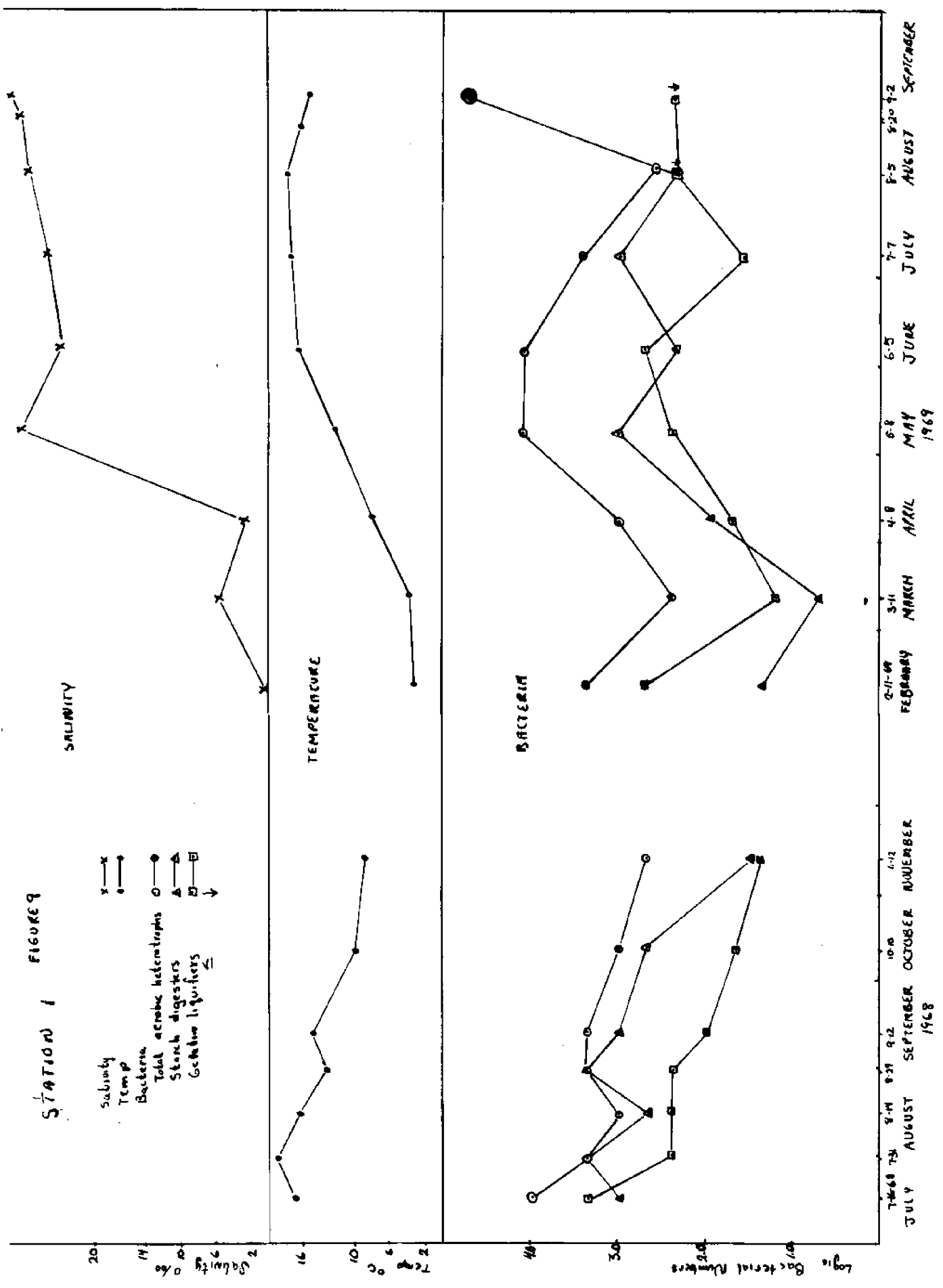
40  
30  
20  
10

7-4 7-11 7-18 7-25 8-1 8-8 8-15 8-22 8-29 9-5 9-12 9-19 9-26 10-3 10-10 10-17 10-24 10-31 11-7 11-14 11-21 11-28 12-5 12-12 12-19 12-26 1-2

JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER  
1968

STATION 1  
FIGURE 9

Salinity X-X  
Temp O-O  
Bacteria  
Total aerobic heterotrophs O  
Starch digesters A  
Gelatin liquefers □



STATION 3  
FIGURE 10

Salinity  
Temp  
Bacteria  
Total aerobic heterotrophs  
Starch digesters  
Gelatin liquefiers  
Z  
N

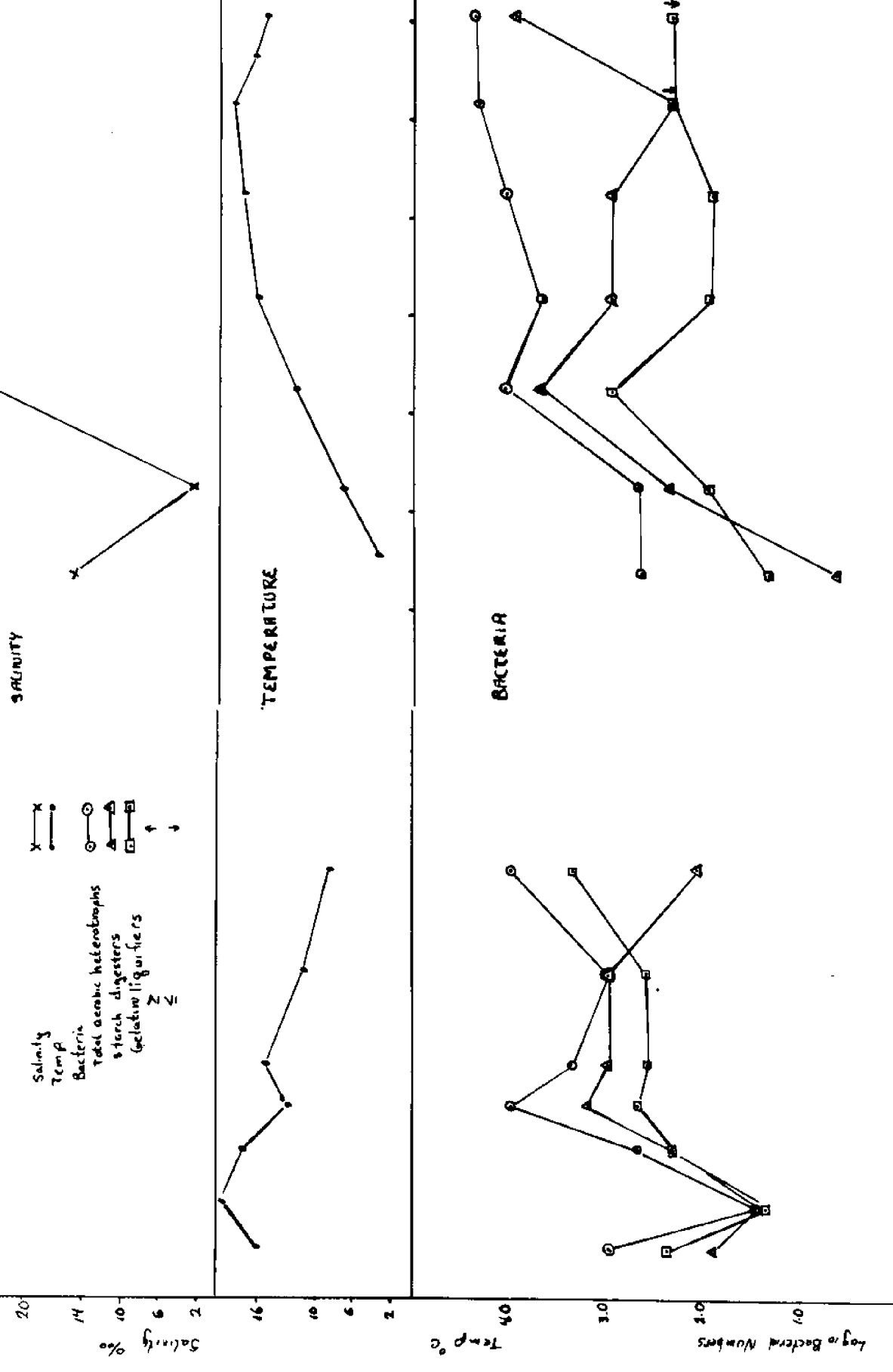


FIGURE 11

STATION 9

- Salinity
- Temp
- Bacteria
- Total aerobic heterotrophs
- Starch digestors
- Gelatin liquefiers
- Z
- S

SALINITY

- x
- o
- o
- △
- 
- ↑
- ↓

TEMPERATURE

BACTERIA

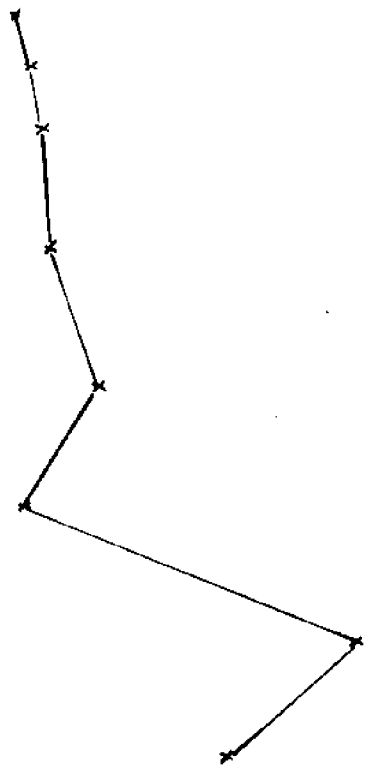


FIGURE 12.  
COLIFORM COUNTS and STREAM TEMPERATURE

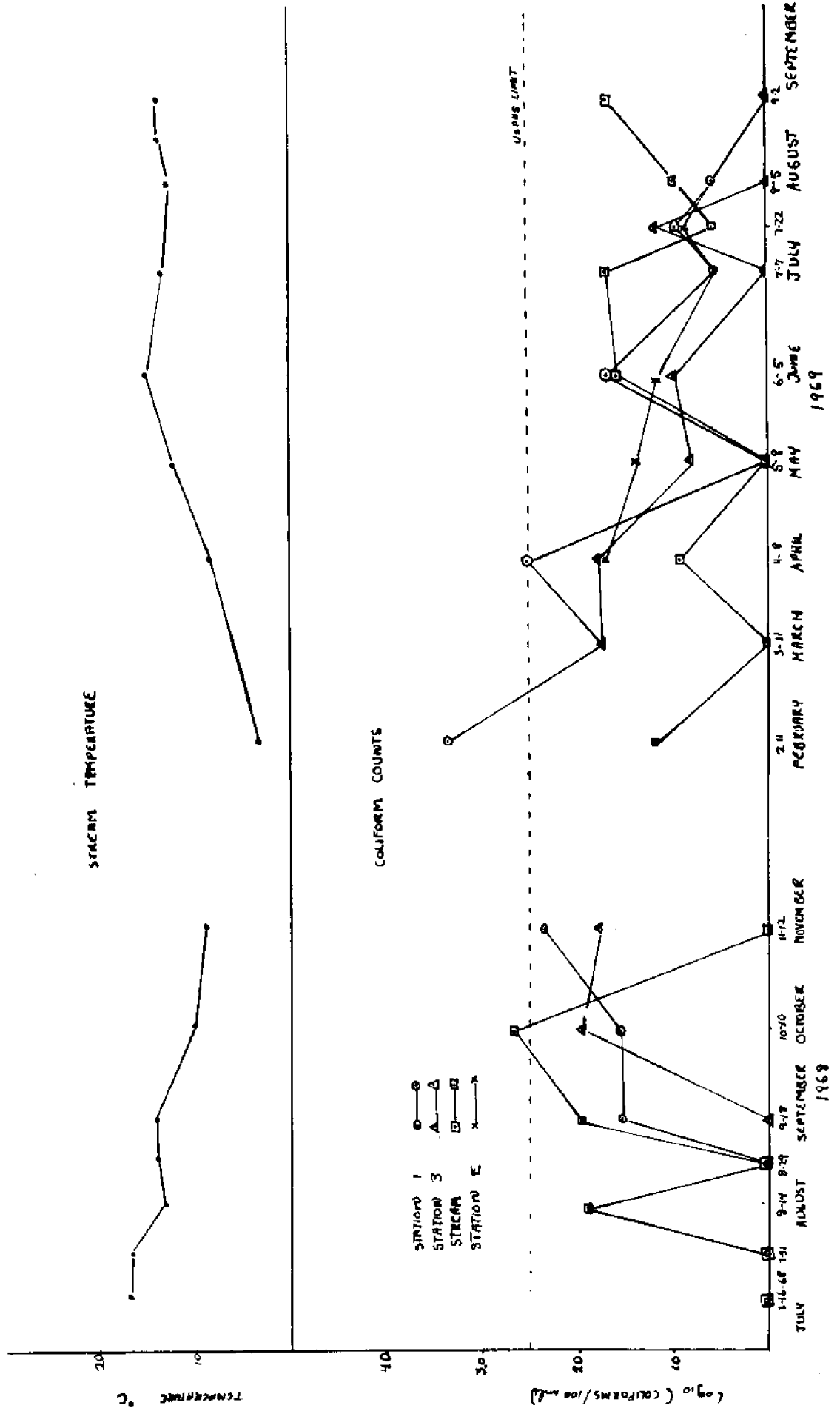


TABLE 1  
PLANKTON COUNTS #/ ml

STATION 2

Taxa	STATION 2																
	2 7-31-68 44.9	3 8-14 62.4	4 8-29 156.0	5 9-12 160.5	6 10-10 20.7	7 11-12 141.1	8 2-11-69 126.1	9 3-11 1763.6	10 4-8 151.9	11 5-8 112.895	12 6-5 154.2	13 7-7 237.8	14 7-22 301.05	15 8-5 287.6	16 8-20 447.7	17 9-2 435.7	
GENUS	120.8	31.2	40.8	29.3	1.4	84.3	2.8	11.4	87.2	41.54	5.8	69.0	30.71	48.1	39.7	92.4	
Mated flagellates	44.9	62.4	156.0	160.5	20.7	141.1	126.1	1763.6	151.9	112.895	154.2	237.8	301.05	287.6	447.7	435.7	
Navicula																	
Nitzschia																	
Melosira	0.1	2.9	1.4		0.7	1.4		0.04	0.03	15.7	2.9	3.6	4.195	45.3	5.8	23.9	
Cocconeidiscus	4.2	2.2		7.2	0.7	15.7	0.2	1.40	2.765	4.3	4.4	10.2	6.975	7.1	2.9	263.0	
Pleurosigma		2.9			0.04				1.4								
Skatocoma					0.4				2.7	0.2			5.6	5.7	1.3	256.0	
Chaetoceros	0.01	0.01	1.4		0.2	0.4			9.9	2.9	2.2					11.4	
Rhizolenia		0.01			1.4				1.36							7.0	
Fragilaria									0.03				0.05	5.7		5.8	
Thalassionema												7.2	1.4			2.9	
Thalassiosira								4.2								53.2	
Thalassiothrix			2.8	5.5													
Licmophora																	
Biddulphia																	
Rucampia																	
Achnanthes																	
Asterionella																	
Bisterionanus																	
Dinophysis																	
Cymodinium				8.31													
Peridinium				1.4													
Miscellaneous	312	2.90	18.2	10.1	2.8	7.1	1.4	4.2	18.0	14.1	13.1	25.4	19.4	15.7	15.9	91.4	
Total Plankton	201.1	108.8	223.4	220.9	28.3	254.1	130.5	1784.9	259.4	198.6	184.9	360.9	358.3	416.8	515.2	1243.3	
COPEPODS	2.8	0.16			2.1	0.01		0.09	0.01		1.4	0.26			0.01		











TABLE 6

## Plant Pigments 1969

		μg/l				
		K	1	3	9	
a	- Chlorophyll a					
b	- Chlorophyll b					
car.	- Carotenoids					
March 11, 1969	a	1.5	0.9	1.0	0.9	0.8
	b	0	0	0	0	0
	car.	1.2	0.7	1.2	0.8	0.8
May 8, 1969	a	1.0	1.0	1.0	1.5	0.9
	b	0.5	0.5	0.5	0.3	0
	car.	1.6	1.4	1.2	1.5	0.6
June 5, 1969	a	2.2	1.8	1.0	2.6	2.1
	b	0.5	0	0.5	0.5	0
	car.	1.7	1.6	1.2	1.8	1.4
July 7, 1969	a	1.3	0.8	0.8	1.3	2.7
	b	0	0	0.7	1.5	1.1
	car.	1.7	1.6	1.6	2.0	3.6
July 22, 1969	a	1.0	0.5	0.8	1.2	1.2
	b	0	1.0	1.2	0	1.7
	car.	1.4	1.2	2.0	2.4	1.0
August 5, 1969	a	0	0	0.7	0.8	1.1
	b	0	0	0	0.5	0
	car.	0.7	0.7	1.3	0.5	2.1
August 20, 1969	a	2.6	1.8	0.9	1.4	1.4
	b	1.2	1.2	0	0.5	0
	car.	2.0	1.3	1.2	1.6	1.6
September 2, 1969	a	6.4	1.5	1.7	1.0	1.0
	b	0.5	0.6	0.5	0	0
	car.	4.8	1.7	2.2	1.1	1.6

Pigment values less than 0.5 μg/l are reported as 0.

TABLE 7

## BACTERIA

Trip	Date	Station	Coliforms per 100 ml	Total per ml	Starch Digesters per ml	Gelatin Liquefiers per ml
1	7-17-68	1	0	9300	930	2100
		3	0	930	75	230
		Stream	0			
2	7-31-68	1	0	2300	2300	230
		3	0	43	43	23
		9		9300	750	230
		Stream	0			
3	8-14-68	1	0	930	430	230
		3	0	430	230	230
		9	73	9300	9300	9300
		Stream				
4	8-29-68	E		2100	2100	43
		1	0	2300	2300	210
		3	0	9300	1500	430
		9		4300	4300	430
		Stream				
5	9-12-68	E		930	930	93
		1	36	2300	930	93
		3	0	2300	930	230
		9		2300	930	230
		Stream				
		91				

TABLE 7 (continued)

Trip	Date	Station	Coliforms per 100 ml	Total per ml	Starch digesters per ml	Gelatin liquifiers per ml
6	10-10-68	E	36	750	390	93
		1	91	930	430	75
		3	930	930	930	230
		9	430	11,100	4300	430
		Stream				
7	11-12-68	E	230	430	23	230
		1	73	430	28	430
		3		9300	120	2300
		9	0	930	150	430
		Stream				
8	2-11-69	E	1500	4300	43	430
		1		2300	23	430
		3				
		9	15			
		Stream				
9	3-11-69	E	53	4600	750	150
		1	53	230	43	15
		3		430	43	23
		9	0	1500	75	93
		Stream				

TABLE 7 (continued)

Trip	Date	Station	Coliforms per 100 ml	Total per ml	Starch Digesters per ml	Gelatin Liquifiers per ml
10	4-8-69	E	43	2400	430	39
		1	290	930	75	43
		3	53	430	150	93
		9		11,000	11,000	430
		Stream	7.3			
11	5-8-69	E	20.4	11,000	1,500	930
		1	0	11,000	930	230
		3	6.2	11,000	4,600	930
		9		> 11,000	> 11,000	1500
		Stream	0			
12	6-5-69	E	15	> 11,000	430	1500
		1	43	11,000	210	430
		3	9.1	4,600	936	91
		9		> 11,000	> 11,000	430
		Stream	39			
13	7-7-69	E	56	2,400	2,400	91
		1	3.6	2,400	930	36
		3	0	11,000	930	91
		Stream	43			



TABLE 7 (continued)

Trip	Date	Station	Coliforms per 100 ml	Total per ml	Starch digesters per ml	Gelatin liquifiers per ml
14	7-22-69	E	7.3			
		1	9.1			
		3	15			
		Stream	3.6			
15	8-5-69	E				
		1	3.6	360	< 230	< 230
		3	0	2300	< 230	< 230
		9		< 230	< 230	< 230
15	9-2-69	Stream	9.1			
		E	0	24,000	24,000	< 230
		1	0	46,000	46,000	< 230
		3	0	24,000	9,300	< 230
		9		24,000	24,000	< 230
Stream		43				

TABLE 8

Carbon and Nitrogen in Big Beef Estuary  
(Oxidizable carbon in  $\mu\text{g}/\text{l}$  and nitrogen in  $\mu\text{g}/\text{l}$  by Kjeldahl)

Trip	Date		STATION				Stream
			E	1	3	9	
3	8-14-68	C	79	135	285	210	116
		N	50	31	79	47	29
4	8-29-68	C	218	83	174	151	144
		N	76	75	70	72	79
5	9-12-68	C	367	305	281	305	141
		N	48	31	38	36	25
6	10-10-68	C	47	136	70	89	0
		N	58	42	55	54	52
9	3-11-69	C	143	222	28	18	0
10	4-8-69	C	102	177	126	99	139
		N	77	79	116	138	118
11	5-8-69	C	166	167	165	229	158
		N	143	88	62	9.1	69
12	6-5-69	C	218	153	232	164	27
		N	32	143	140	140	119
14	7-22-69	C	330	99	123	150	-
		N	82	72	62	92	47
15	8-5-69	C	175	127	144	215	119
		N	20	21	45	57	35
16	8-20-69	C	400	400	538	648	-
		N	106	95	77	76	80
17	9-2	C	590	228	331	278	181
		N	52	42	53	52	120