An investigation into certain aspects of the ecology of Fenham flats and budle bay, Northumberland

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AN INVESTIGATION INTO CERTAIN ASPECTS OF THE ECOLOGY

OF FENHAM FLATS AND BUDLE BAY, NORTHUMBERLAND

BY

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INDEX

INTRODUCTION

The estuarine ecosystem, more than most ecosystems, may be considered an 'open' system in which energy flow and nutrient cycling are dependent to some degree upon allocthonous inputs, ie those originating outside the system.

In most ecosystems the ratio of primary to secondary production is fairly high, but in estuarine systems the ratio is smaller, as a result of lower primary production within the system rather than higher secondary production. An estuary is suitable for colonisation only by specialised plants and Benthic and Epiphytic autotrophs. Hence the secondary producers depend largely on organic detritus derived from outside the estuary.

Since primary (autotrophic) production far exceeds secondary production in most environments it is not surprising to find that the great bulk of organic detritus in estuaries is derived from autotrophs rather than heterotrophs. The sources of autotrophic material have been listed by Darnell (1968).

1 AUTOCHTHONOUS
- a) Phytoplankton (including Algae and Autotrophic Bacteria)
- b) Marginal submerged vegetation
- c) Diatoms and filamentous Algae of Mud Flats (especially Blue-Green Algae)
- d) Periphyton growing on stems of emergent plants and other surfaces

2 ALLOCHTHONOUS
- a) Marginal marsh vegetation
- b) River borne Phytoplankton and organic detritus
- c) Marginal swamp vegetation
- d) Beach and shore material washed in during storms and high water
- e) Windblown material especially leaves and pollen grains

2 ALLOCHTHONOUS f) Phytoplankton and other material originating in adjacent marine environments.

A comparable list of materials of animal origin could be drawn up. The only important addition would be a category for organic sewage derived from human settlements, up stream or adjacent to the estuary.

Quantitative data on the significance of each source of estuarine detritus has seldom been published, but estimates of relative importance clearly indicate that the sources differ in abundance from one estuary to another and that within an estuary they vary with season of the year and in relation to position on the salinity gradient.

Most rivers and streams entering estuaries carry a load of negatively charged organic particles which are precipitated by the positively charged metallic ions of the salt water (Day 1952). However, positively charged organic particles may not be precipitated in the same way, but may persist for some time suspended in salt water. Additionally, materials carried into the estuary by tidal currents are often precipitated within the estuary.

In no ecosystem does detritus play such an important role as that in the Food Chain of an estuary. The majority of estuarine animals are invertebrates and many of these feed on detritus. A wide range of morphological and behavioural adaptations have been evolved to obtain and ingest detritus in quantity. Even the most carnivorous invertebrates find it difficult not to ingest detritus in the procurement of their prey (Darnell 61, 58) and although its calorific importance is probably small, its contribution to the dietary diversity of the carnivore may be of significance.

The detrital material available to detritus feeders in estuarine sediments may be classified according to particle size (Fig I). This scheme also follows the pathway of biological decomposition of the original organic material.

The organic material entering the system includes the total gross primary and secondary production available to the system from both autochthonous and

allochthonous sources.

Organic Detritus

(Potential Energy Sources)

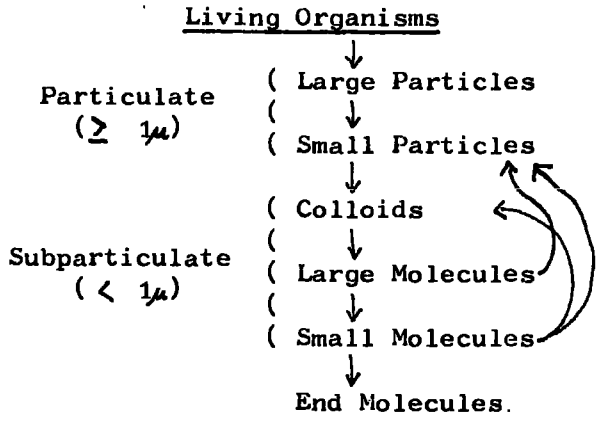


FIG I (Darnell 1967)

The particle sizes available to detritus feeders are of great importance as different species are adapted to feeding preferentially on different particle sizes. Therefore, the distribution of sediment types plays some part in determining the distribution of the animals. The relative importance of sediment type and quantity of detritus available as food, in determining the distribution of invertebrates, is a topic on which very little information is available and it was hoped that during the course of this project some information might be gained.

Degradation of particle size can be brought about in many different ways; mechanically, chemically and biologically. Perhaps the most important means of degradation is by bacteria and fungi. Undoubtedly, bacteria and fungi are responsible for most of the chemical breakdown of organic material in most aquatic systems.

When considering bacteria in their established roles of decomposers and mineralisers, numerous problems arise, not least their enumeration and classification. The role of bacteria in the trophic dynamic system may be considered from two points of view. Firstly, in their well-established roles as decomposers, in which they prevent the accumulation of organic material by degrading it and making the constituents available for reassimilation by primary producers. Secondly, they themselves are producers since they assimilate much of the organic material themselves, thus producing consumable proteins.

The role of bacteria as nitrogen fixers may be of paramount importance in some systems in which the nitrogen would otherwise be available in only very limited quantities. This is especially true of estuarine sediments, in which bacterial protein is a very important reservoir of available nitrogen for the detritus feeders.

The bacteria of major importance in this system are those physiological groups which metabolise organic materials which would otherwise be unavailable as a nutrient source to animals, since they are not readily assimilated.

The idea that bacteria may form an important part of the diets of many estuarine animals is not new. MacGinitie (1932) and Zobell and Feltham (1938) have shown that several marine invertebrates can exist indefinitely on a diet of bacteria. Recently, Russian workers have focused attention upon bacteria and detritus as food sources for aquatic invertebrates (Rodina 1963, Zhukova 1963).

The information available is limited however, due to the difficulty in distinguishing the assimilation of organic materials from detritus itself from the assimilation of bacterial organic matter. In addition, the difficulty in identification of gut contents and faeces of 'detritus feeders' renders accurate appraisal of the relative roles of bacteria and detritus extremely hard.

Recent work by Hargrave (1970) has shown that of all material available, invertebrates ingest fungi, bacteria, algae and diatoms most readily. These are also assimilated most efficiently. As a result feeding rate depends on the 'quality' of the ingested food. The higher the concentration of 'live' micro-organisms the slower the necessary rate of ingestion because of the higher over all assimilation efficiency. Hargrave found that lignin and cellulose were not assimilated by aquatic invertebrates. As these accounted for about fifty percent of the organic matter in the lake studied, it represents a large proportion of the detritus available to invertebrates.

It is left to the micro-organisms to degrade this into more digestible components or to assimilate it themselves. Hargrave states that the efficiency of assimilation of organic and protein material by invertebrates was usually below twenty percent for most sediments. (Surface sediment was higher than subsurface sediments.) But, epiphytic micro-organisms on Chara were used with an efficiency of seventy to eighty percent. It would thus seem possible that protein is assimilated more efficiently than total organic matter. Therefore, if protein content of sediments are proportional to bacterial numbers as Newell (1965) suggests, then bacteria should be assimilated more efficiently than other organic matter.

The distribution of detritus in an estuary is determined in part by the force of the tidal currents which pass over the sediments and not only by the points of entry. Detritus is usually small and light and easily carried by currents. At the edges of estuaries the currents are often slower and hence detritus tends to accumulate here as it is deposited.

In many British estuaries, large swards of Spartina anglica occur; the detritus available in these swards is noticeably higher than on the open mud and comes from several sources. Firstly, decaying matter from the Spartina itself and secondly, material carried into the Spartina by tidal currents and then deposited as the flow is slowed by passage through the Spartina. Hence the Spartina acts as a 'reservoir' of detritus.

Throughout the estuary the sedimentation patterns vary according to geographical location, affecting the distribution of animals, detritus and also the depth at which the boundary of the sulfide biome begins. All these factors together affect the distribution of invertebrates. No work has yet been done on the respective importance of each factor in determining distributions.

The distribution of sand particles also determines to a certain extent the distribution of bacteria as bacteria tend to collect on the surfaces of sand grains. Therefore, in areas where the sand grains have a higher surface

area per unit volume or unit weight, there should be more bacteria, if all other factors are constant. However, the distribution of bacteria is affected by many other factors such as exposure to tidal forces, food availability etc. (Anderson and Meadows 1969).

It is hoped that during this project some further information might be gained as to the relationships between bacteria and invertebrates, as well as to other aspects determining the distribution of both of them. The importance of this in the context of the estuary up at Lindisfarne is that the area is a National Nature Reserve and as such is so far protected from industrial exploitation, unlike similar estuarine areas further south. As a result of this, the area is used by ever increasing numbers of over-wintering wading birds, increasing due to the reclamation of their former feeding grounds elsewhere. If Lindisfarne is to be able to support these possibly much larger populations in the future, much more must be known, not only about the behaviour of the birds, but also about the ecology of the estuary on which they depend; in particular the ecology of the food chain on which the birds depend. Once more is known about the factors affecting the availability of the invertebrates, used as food by the birds, the management of the Reserve can be geared to support maximum production of this commodity whilst still retaining its essential aspects as a Reserve of all flora and fauna.

AIMS OF THE PROJECT

Three main aspects of the ecology of Budle Bay, Fenham Flats and Holy Island Sands in Northumberland were investigated: the freshwater inflows, the *Spartina* marshes and the open mudflats.

The areas named above have several streams running into them, carrying water from the surrounding farmland and villages. A high sewage content of these streams could contribute a significant proportion of the food available to detritus feeders. If so, any move on the part of the local Councils to decrease the sewage content of the outflows could decrease the productivity of the estuaries and hence might affect the overwintering population of wading birds which the estuaries could support.

The western edges of Holy Island Sands and the northern edge of Budle Bay have large swards of *Spartina anglica* growing along them. At present the contribution of *Spartina anglica* to the ecology of the estuary is not known. It was therefore decided to make bacterial counts and estimates of activity in and around these swards, to find what effect the sward had on the bacterial content of the surrounding sediments of the estuary. As bacteria may constitute an important part of the diets of several common estuarine invertebrates, the presence of *Spartina anglica* may contribute to the invertebrate productivity of the estuary.

At the same time as my project, invertebrate counts were made at specific points on the mudflats. It was therefore decided to combine the invertebrate counts with bacterial counts at some of the sites to determine if there were any correlations between the bacterial numbers in the upper sediment and the invertebrate populations below.

PART ONE

1,0 (Performed in APRIL)

Determination of Total and Coliform Bacteria in Stream Inflows

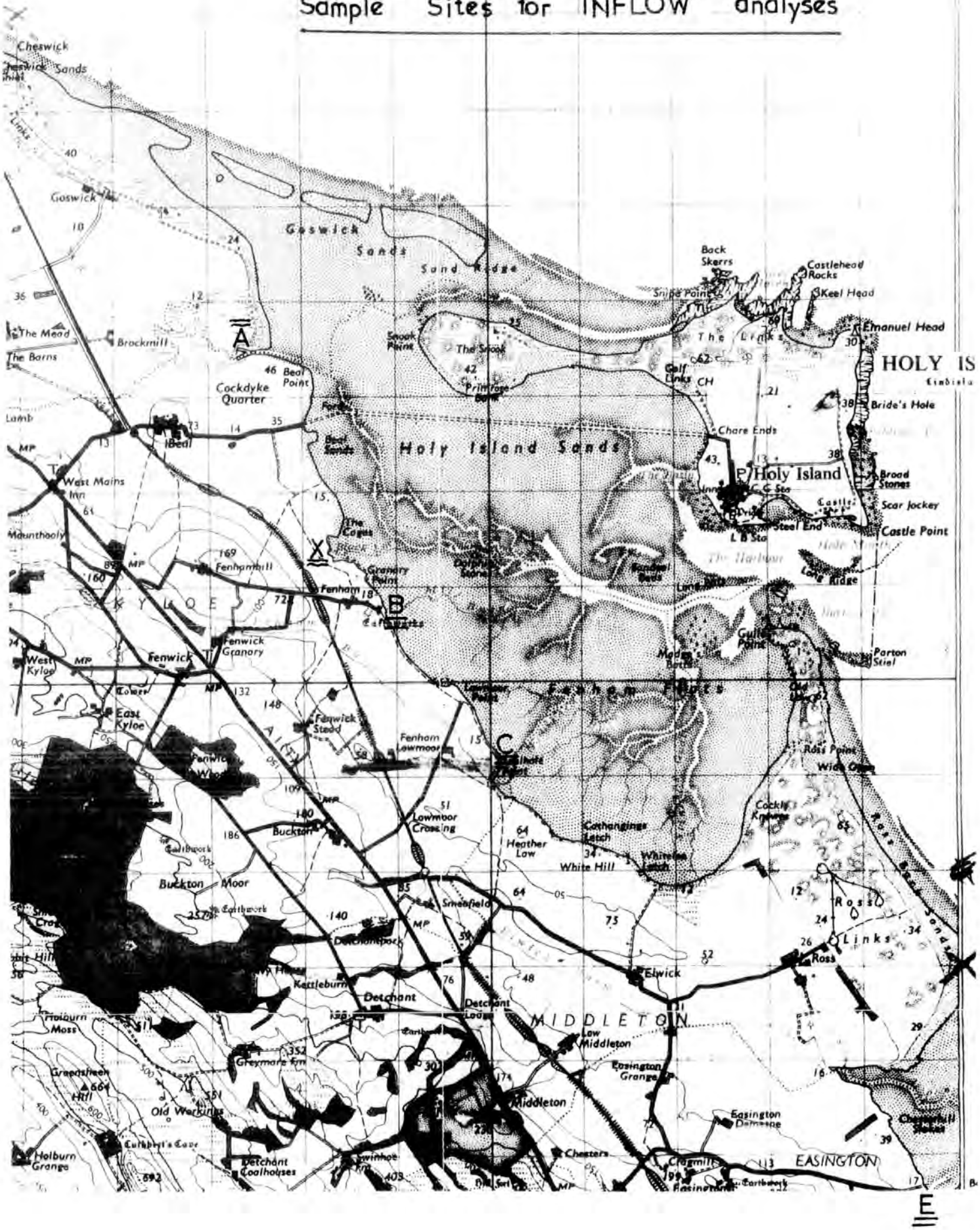
Water specimens were taken from the streams in bottles of 250 ml capacity fitted with screw tops. They were first sterilised by autoclaving at 121°C for twenty minutes. To obtain a water sample the screw top was carefully removed with one hand while with the other the bottle (held at its base) was inserted mouth downward, below the surface of the water (if possible, at least one foot below). The bottle was then turned so that the mouth was directed to the current so that water flowed in without coming into contact with the hand. When full the bottle was removed and the top replaced. For all samples, at least three hours elapsed before plating took place, so they were placed on ice immediately after collection and were kept in this state while transported back to Durham.

Two bacterial tests were performed on these samples.

a) A presumptive coliform count This was performed using MacConkeys fluid medium (a bile salt lactose peptone water with an indicator of acidity - See Appendix). Serial dilutions were made of the original water sample in the range 10^{-1} to 10^{-9}. One ml of each dilution was added to 10 mls of MacConkeys broth in a test tube with a Durham tube which had been sterilised at 121°C for 15 minutes. Triplicates were performed for each sample at each dilution. The tubes were then incubated at 37°C for eighteen to twenty-four hours. A positive reaction was indicated by both the production of gas in the Durham tube and a change of acidity (indicated by colour change in the indicator). A positive test indicates the presence of bacteria of a Coliform type, either human or animal in origin, and gives an indication of the amount of sewage present in the water. In crude sewage the numbers range from one million to a hundred million per ml.

b) An enumeration of viable bacteria known as the plate count, performed in duplicate with incubation at 37°C and 22°C respectively, using nutrient agar (See Appendix). The bacteria that grow at 37°C are associated with organic material of human or animal origin, whereas those growing at the lower temperature are mainly saprophytes, normally inhabiting water or derived from soil and vegetation. Incubation is for twenty-four hours in the case of the plates at 37°C and for three days for those at 20°C.

Sample Sites for 'INFLOW' analyses



PART TWO

2.0 (Performed in AUGUST)

Determination of Possible Origins of Coliform Bacteria in the Streams

For the analysis of the samples in this case it was not considered necessary to perform any viable counts, but only to ennumerate the respective numbers of Escherichia coli and Streptococcus faecalis.

Streptococcus faecalis occurs in the intestine as a commensal and dies after only a few days in water. It is never more abundant than Escherichia coli (type I), in the human intestine, but in other animals it is the more numerous. Therefore, if viable Streptococcus faecalis is found in a water sample, contamination must be fairly recent; if present in numbers greater than Escherichia coli, then the origin is not from human faeces.

For these samples a presumptive coliform count was first performed, as previously described. Then followed some differential coliform counts to find the relative numbers of Escherichia coli and Streptococcus faecalis.

a) From the 'positive' tubes in the presumptive coliform test, 1 ml quantities were transferred to 10 ml quantities of Brilliant Green Bile agar (found by Mackenzie et al (1948) to be superior to MacConkeys for detecting Escherichia coli) in test tubes which also contained Durham tubes. Incubation was at $44^{o}C \pm 0.5^{o}C$ for 18 to 24 hours. A positive reaction was shown by gas production and turbidity.

b) Isolation of Streptococcus faecalis was performed in a similar way using a Glucose Sodium Azide medium (See Appendix), as developed by the Metropolitan Water Board. Incubation was at $45^{o}C \pm 0.5^{o}C$ for 24 hours. A positive result was indicated by a change in acidity shown by a colour change in the Bromocresol purple indicator.

In all the tests performed, all the glassware used as well as all the distilled water, broths and agar media were sterilised in an autoclave at

121°C for 15 to 20 minutes. The pipettes used in the tests were only used for the transference of one sample and were fitted with cotton wool plugs at their mouths. The petri dishes used were of the sterile, disposable plastic type. All the test tubes had white caps fitted over their mouths.

RESULTS

1 PRESUMPTIVE COLIFORM

DILUTION	A	B	C	E
0	+ + +	+ - -	+ + +	+ + +
-1	+ + +	- - -	+ + +	+ + +
-2	+ - -	- - -	+ - -	- - -
-3	- - -	- - -	- - -	- - -
-4	- - -	- - -	- - -	- - -
-5	- - -	- - -	- - -	- - -
-6	- - -	- - -	- - -	- - -
-7	- - -	- - -	- - -	- - -
-8	- - -	- - -	- - -	- - -
-9	- - -	- - -	- - -	- - -
NUMBER OF COLIFORMS PER ML	APPROX 330	APPROX 3	APPROX 330	APPROX 100

+ = Positive Result

- = Negative Result

RESULTS

2 PLATE COUNTS

NB No growth at 37°C after twenty-four hours or even after three days, therefore dilutions were too high to give positive tests at 37°C.

20°C

DILUTION	A	B	C	E
-4	TOO HIGH	HIGH	TOO HIGH	TOO HIGH
-5	TOO HIGH	32	TOO HIGH	TOO HIGH
-6	15	15	TOO HIGH	TOO HIGH
-7	13	2	81	248
-8	2	0	0	151
VIABLE COUNT (x10-Dilution Factor) in plating	16.5×10^8 Per Ml	91×10^6 Per Ml	81×10^8 Per Ml	876×10^8 Per Ml

Too High indicates a density of colonies too high to count.

RESULTS

PRESUMPTIVE COLIFORM

SITE / DILUTION	A	X	B	C	E
-1	+ + +	+ - -	+ + -	+ + +	+ + +
-2	+ + -	- - -	- - -	+ + +	+ + +
-3	- - -	- - -	- - -	+ + -	+ + +
-4	- - -	- - -	- - -	- - -	+ + -
-5	- - -	- - -	- - -	- - -	- - -
-6	- - -	- - -	- - -	- - -	- - -
VIABLE COUNT (APPROX) PER ML	660 PER ML	3 PER ML	6 PER ML	6,600 PER ML	66,000 PER ML

A = Beal Point

X = Black Low

B = Mill Burn

C = Tealhole

E = Waren Mill

RESULTS - PRESUMPTIVE COLIFORM

ESCHERICHIA COLI

SITE / DILUTION	A	X	B	C	E
-1	+ + +	+	+ +	+ + +	+ + +
-2	+ +			+ + +	+ + +
-3				+ +	+ + +
-4					+ +
E coli per ml	660 ml	3 ml	6 ml	6,600 ml	66,000 ml

STREPTOCOCCUS FAECALIS

SITE / DILUTION	A	X	B	C	E
-1	+ + +	+	+	+ + +	+ + +
-2	+ +			+ + +	+ + +
-3				+ +	+ +
-4					
S faecalis per ml	660 ml	3 ml	3 ml	6,600 ml	6,600 ml

A = Beal Point
X = Black Low
B = Mill Burn
C = Tealhole
E = Waren Mill

CONCLUSIONS

The presumptive coliform test performed in April indicated that there was only a very small proportion of the total bacterial count which was composed of Coliform bacilli.

The highest counts were at A (Beal Point) and C (Tealhole) at approximately 330 Coliforms per ml. The waters obviously were not carrying any large amounts of sewage. This is borne out by the plate count, in which those plates incubated at 37°C showed no growth at the 10^{-4} dilution, meaning that those bacteria present in the water associated with human and animal sewage were at levels below 10,000 per ml.

In fact levels may have been well below this value, since no colonies at all were seen in 10^{-4} and higher dilutions. The saprophytic bacteria count was high, however, indicating perhaps that much of the water tested had drained off rich agricultural land, eg well fertilised pastures.

In the August samples, the presumptive coliform counts had changed markedly. All the sites had at least doubled their April counts and the Waren Mill count had increased by a factor of 660. The Escherichia coli and Streptococcus faecalis counts at the different sites were as follows.

At Beal Point, the sample was taken on the landward side of the sluice gates. The presence of equal numbers of Escherichia coli and Streptococcus faecalis indicates that the contamination was very recent and also that the source may well have been both human and other animal.

The site at Black Low had only very low counts, and again there were equal numbers of both Escherichia coli and Streptococcus faecalis, indicating recent contamination and a probability that animal sources are involved.

The site at Mill Burn doubled its presumptive coli count even though remaining very low. The higher numbers of Escherichia coli than Streptococcus faecalis indicate fairly recent contamination of mainly

human origin.

The Tealhole site showed an increase of twenty times in the presumptive coliform count and the results indicate recent contamination with animal sources as the main contributors.

As mentioned above, the Waren Mill site showed a vast increase. The Escherichia coli and Streptococcus faecalis results indicate a human rather than animal origin with contamination probably fairly recent. In fact a very densely packed camping site only a few hundred yards upstream is the probable cause. It would have been of interest to have taken a sample upstream of the site to measure exactly what effect it had.

The overall picture is of an increase in coliforms and therefore of sewage during the summer, although regular counts would have to be done to amplify this. At both Budle and Lindisfarne the low counts of the winter indicate a negligible inflow of sewage. In summer, however, the sewage discharged into Budle Bay was considerable and may even present a health hazard to bathers.

At no point on the Lindisfarne mudflats is the sewage bacterial contribution so high, but at Tealhole it may be of some importance. However, the sources of the sewage at Lindisfarne seem to be mainly of animal rather than human origin.

Thus it may be seen that any move to clean up the sewage effluents in the streams running into the Lindisfarne estuary would have only a minimal effect on the invertebrate populations.

However, if the high sewage content at the Waren Mill site were to persist then this could contribute appreciably to the detritus and bacteria available to the invertebrates, and any controls exerted on the quality of the river could well affect the invertebrate population.

PART THREE 3.0

In this section of the project bacterial counts were obtained from sediments at various sites on the Lindisfarne estuary and at Budle Bay to look for correlations with invertebrate densities, the depth of the anaerobic layer and positioning with respect to the Spartina swards. In addition all samples taken were subjected to a presumptive coliform test to ascertain whether sewage bacteria from the inflows were also present in the estuarine sediments.

The question arose as to whether total counts or viable counts should be used to express bacterial numbers when viewed through a microscope. It was far from easy to differentiate between bacteria and small particles of detritus in a substrate sample. Also many bacteria grew in aggregates or on the detritus and sand particles. It was decided therefore to perform viable counts only. These would not yield the total numbers of bacteria present in the sediments, but would give valid results for comparison of different sites.

Anaerobic counts were also made for comparative purposes. Finally, samples of seawater were taken to ascertain the numbers of bacteria available to those invertebrates which rely on filtration of water, rather than sediments, to obtain their food.

METHODS

Sediment samples were scraped carefully from the surface into a sterile glass container and stoppered. Only the top 0.5 cm of the sediment was used for analysis. After collection, both the water samples and sediment samples were placed on ice until such time as they could be plated out (usually four to five hours later.)

Two grams (wet weight) of sediment were suspended in 100 mls of sterilised de-ionised water (Anderson and Meadows 1969) and were shaken at the maximum rate for thirty minutes on a flask shaker. After this treatment the suspension was counted as a 10^{-2} dilution and further serial dilutions

were made from this in the range of 10^{-3} to 10^{-8}. The aerobic plate media were then inoculated with 0.1 ml of the respective dilutions, a sterile glass rod being used to spread the sample over the agar surface. The anaerobic tube was inoculated with 1 ml of each dilution.

Water samples were diluted in geometric series, one dilution differing from the next by a power of 10. The dilution series and the original sample were then added to the culture media (0.1 ml per plate and 1 ml per anaerobic tube).

For the culture of aerobic bacteria the media used was the 2216E medium of Zobell (1946) with the addition of 10 gram of nutrient agar per litre (See Appendix). This has been found by many workers to be suitable for the isolation of marine bacteria. The media was sterilised at 121°C for fifteen minutes and then poured into sterile petri dishes in 10 ml quantities and allowed to solidify.

For the isolation of anaerobic bacteria the same media was used with the addition of 8.4 grams per litre of D Glucose. Thirty ml quantities were allowed to solidify in test tubes. Before use these were heated to 100°C for one hour to drive off excess oxygen. They were then allowed to cool to just above the solidification point of agar and were then inoculated. A layer of liquid vaseline was poured on top of the agar to prevent further exchange of oxygen and to maintain anaerobic conditions. After inoculation the tubes were placed in cold water so as to expose the bacteria for as short a time as possible at the higher temperatures.

For each set of samples one sterile run was performed using only the water in the medical flasks (used to shake the samples) and the McCarthy bottles (each bottle containing 9 mls sterile water and used for serial dilutions) and plating this out on the aerobic media. All glassware and water used in the practical was previously sterilised at 121°C for fifteen minutes.

The incubation time was twenty-one days at a temperature of 20°C (Nedwell and Floodgate 1971). To ensure that the incubation time was adequate to obtain the maximum number of colonies which would appear, the first samples were examined at intervals up to twenty-one days. It would have been desirable to have had duplicate samples from each site to ascertain how reproducible were the results obtained. However, the time taken to plate out even one sediment sample was quite considerable, for all glassware and all water used in diluting the samples had to be sterile. The cleaning, preparation and sterilisation of equipment, as well as the preparation of the media imposed a limit on the number of samples which could be taken at any one time. In addition, it was not possible to leave any sediment samples overnight before plating as this delay might well have resulted in growth or death of bacteria in the later samples, leading to incorrect counts. It was therefore decided that while several duplicates were to be taken and tested during the sampling, priority should be given to obtaining results from as large a variety of sites as possible.

The counting of the colonies on each plate was performed by inverting the petri dish, with its lid on, on a black 'colony counter' with lights shining onto the black surface. This enhanced the distinctive character of the colonies, and made them easier to count. An average count was taken of all the plates from each sample, using the 10^{-5} dilution as the reference dilution.

The question of the units in which the bacterial counts are given is important. All the results are given as total viable numbers of bacteria obtained by counting the colonies on the plates. This gave values in the region of x X 10^{6}. However, if one wished to know the actual numbers of bacteria per gram wet weight of sediment, the following correction had to be applied.

If two grams of sediment were diluted in 100 mls of water, and this was the 10^{-2} dilution, then this would give a bacterial count of x X 10^{6} per 0.0002 grams. Therefore this gave a correction of 5x X 10^{9}, for the number of bacteria per gram wet weight of sediment.

RESULTS

3.0

A 24 May 1973

A transect was taken through a sward of Spartina anglica near Beal Point. Counts of the colonies were taken at intervals during the twenty-one days to obtain some form of growth curve to ensure that a twenty-one day incubation period was adequate. (See transect map)

AEROBIC BACTERIA (Counts x 10^6)

SAMPLE SITE	DAY $4\frac{1}{2}$	DAY 11	DAY 19	DAY 21
1	3.26	6.37	6.97	6.98
2	3.70	4.74	5.29	5.30
3	2.07	3.63	4.60	4.64
4	4.63	5.17	5.17	5.17
5	3.57	4.11	4.11	4.11
6	2.58	3.10	3.11	3.11
7	0.70	1.40	1.53	1.55

ANAEROBIC BACTERIA (Counts x 10^3)

SAMPLE SITE	DAY 6	DAY 11	DAY 21
1	61.5	181.5	200.0
2	86.0	181.0	192.0
3	35.5	82.5	84.0
4	72.5	113.0	113.0
5	80.0	140.0	150.0
6	45.0	85.0	95.0
7	41.6	76.8	82.0

A presumptive coliform test using MacConkeys broth was performed on the 10^{-2} dilution and the 10^{-3} dilution, using duplicate tubes for each sample at each dilution. All the results were negative after 18 hours except for sample site 3 which showed positive acid production but no gas after 24 hours. These were probably due to anaerobes.

B 1 June 1973

These samples were taken at sites being used for invertebrate counts on the Lindisfarne estuary. In addition anaerobic layer depths were recorded and duplicates were taken for the first three samples. (See transect map)

AEROBIC BACTERIA (Count x 10^6)

SAMPLE SITE	DAY 3	DAY 11	DAY 15	DAY 21	DEPTH OF ANAEROBIC LAYER (cm)
1A	0	4.05	4.5	4.5))	0.5
1B	0	3.95	4.3	4.35)	
2A	0.5	6.13	6.15	6.15))	1.5
2B	0	6.10	6.20	6.24)	
3A	0.5	1.18	1.18	2.18))	10 +
3B	0.7	1.10	1.4	2.30)	
4	3.0	4.62	4.63	4.63	0.1
5	0	1.19	1.69	2.15	2.0
6	0	4.45	4.45	4.6	5.0

ANAEROBIC BACTERIA (Count x 10^6)

SAMPLE SITE	DAY 12	DAY 16	DAY 21
1A	235	261	266.5
1B	230	230	250
2A	31	52	67.5
2B	37	58	72.0
3A	22.5	33.5	44.0
3B	28	38	49.0
4	90	107	107
5	28.5	27	32
6	10	10.5	10.5

The presumptive coliform count using similar methods as previously gave all negative results after 18 hours.

C 6 June 1973 Lindisfarne

Several samples were taken at a variety of sites.

a) Sites 1 to 5 were taken going down the river at Beal Point towards the sea. The Spartina increased as one travelled further downstream.

b) A sample of seawater (6).

c) Sites 7 to 11 were taken in a transect through a Spartina sward at Beal Point.

d) Sites 12 to 15 were taken in a transect on the north shore of the river at Beal Point where there was no Spartina sward. (See transect map)

		AEROBIC BACTERIA (Count x 10^6)	ANAEROBIC BACTERIA (Count x 10^6)	DEPTH OF ANAEROBIC LAYER (cms)
	SAMPLE SITE	DAY 21	DAY 21	
R	1	7.5	77	-
I	2	4.3	42	-
V	3	4.5	37	-
E	4	3.45	31.5	-
R	5	5.35	67.5	2
SEA	6	530 per ml	-	-
SPARTINA	7	4.2	38.5	10 +
	8	6.3	85	3.5
	9	6.6	90	0.5
	10	3.4	162	4.0
	11	2.1	62.5	9.0
NO SPARTINA	12	2.5	95.5	9.5
	13	0.85	73.0	10 +
	14	0.9	45	10 +
	15	0.75	35	10 +

As previously the presumptive coliform tests gave negative results apart from a few false positives in acid production due to anaerobes.

D 18 June 1973 Budle Bay

Two sets of samples were taken at Budle Bay on this occasion. One set was taken on a transect through a Spartina sward (A) and the other set taken outside (B) this sward, but near to it in an area where there was no Spartina. These samples were taken to parallel invertebrate sampling along the same transects, at the same sites. Anaerobic layer depth was also neasured. All sites were at thirty metre intervals. Duplicates were plated for A1 and A2. (See transect map)

AEROBIC BACTERIA (Count x 10^6)		ANAEROBIC BACTERIA (Count x 10^6)	DEPTH OF ANAEROBIC LAYER (cm)
SAMPLE SITE	DAY 21	DAY 21	
1a	1.15)	29	4.0
	) 1.20		
1b	1.25)		
2a	0.8)	46.5	3.0
	) 0.75		
2b	0.7)		
3	0 (Stone + clay)	30.5	-
4	3.1	57.0	8.0
5	4.8	85.5	2.5
6	1.25	68.0	8.0
7	3.1	26.5	1.0
8	0.65	42.0	10 +
9	5.8	33.5	1.5
10	0.7	58.5	10 +
11	0.75	14.5	10 +
12	1.3	26.0	9.0
13	1.1	24.5	10 +
14	0.9	20.5	10 +
15	0.95	18.0	10 +
16	1.85	36.0	5.0

The presumptive coliform count was performed as before and again all were negative after 18 hours.

E 27 June 1973 Budle Bay

These samples were taken near those taken on 18 June, but in an area where the Spartina sward was more continuous and less clumped. Invertebrate samples were also taken on this transect at the same sites. Anaerobic layer depth was measured. Duplicates were taken of some of the samples. (See transect map)

	SAMPLE SITE	AEROBIC BACTERIA (Count x 10^6) DAY 21		DEPTH OF ANAEROBIC LAYER (cms)
SPARTINA	2A	3.35		
			3.05	4.0
	2B	2.75		
	3A	6.75		
			6.45	0.2
	3B	6.15		
	4	4.0		0.8
	5A	4.75		
			4.52	1.0
	5B	4.30		
	6	6.0		0.5
	7	3.15		10 +
SEAWATER	8	1090 per ml		-
	9	2270 per ml		-
	10	3505 per ml		-

All presumptive coliform tests performed on the samples all gave negative results.

F 4 July 1973

These samples were taken on Holy Island Sands, Lindisfarne National Nature Reserve. The samples were taken in parallel with the invertebrate samples taken at the same sites, and for comparison with anaerobic layer depth. There was no Spartina in the vicinity. The first samples were taken at some distance from the shore, near a tidal drainage channel running into the estuary and which affected the sediment composition. Samples were also taken at sites used in Macoma growth studies by Ingvarsson. (See transect map)

AEROBIC BACTERIA (Counts x 10^6)		DEPTH OF ANAEROBIC LAYER
SAMPLE SITE	DAY 21	(cms)
1	3.35	6.0
2	5.2	4.0
3	1.2	8.0
4	0.65	7.0
5	0.75	7.5
6	0.95	9.0
7	1.7	5.0
8	1.2	9.0
9	5.6	1.0
10	0.7	8.0
11A	0.7)	
	) 0.75	8.0
11B	0.8)	

The presumptive coliform count gave all negative results.

G 19 July 1973

Samples were taken for comparison with nitrogen analyses performed on samples of sediment from these same sites.

*		
11	1	Mud and Fine Sand
10	2	Mud
8	3	Sand
7	4	Sandy
Site 2	5	Muddy
Tealhole	6	Mud and Sand
No 3	7	Sand and Mud

AEROBIC BACTERIA (Counts x 10^6)		N * % Dry Weight	SILT CONTENT % * (.08 cm)
SAMPLE SITE	DAY 21		
1	4.45	0.132	53.4
2A	2.1)		
	) 2.1	0.052	20.6
2B	2.1)		
3	1.3	0.034	3.22
4	0.75	0.015	0.3
5	4.05	0.290	73.2
6	1.15	0.146	59.4
7	0.7	0.052	28.3

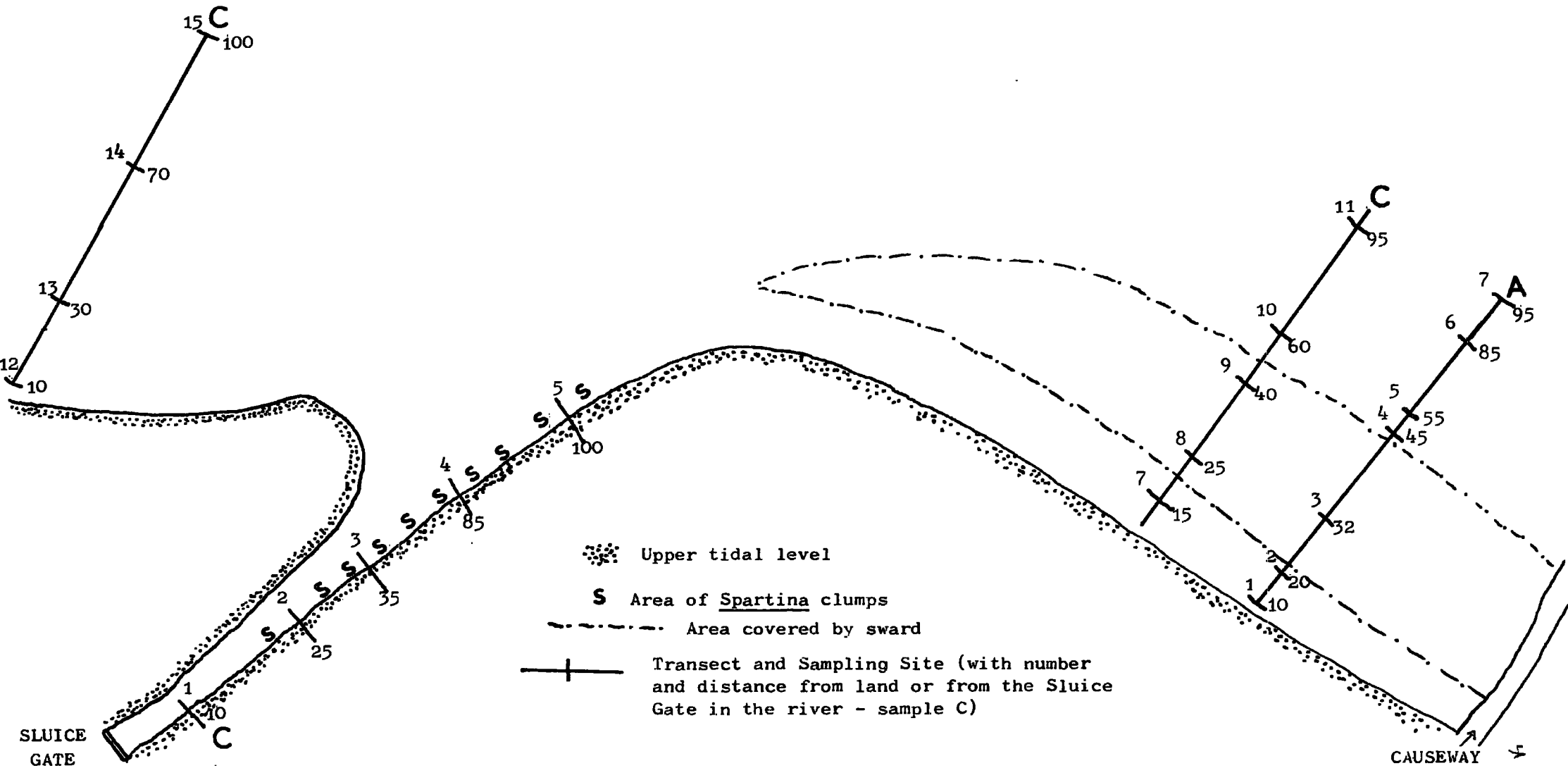
* Data from Ingvarsson.

No presumptive coliform counts performed.

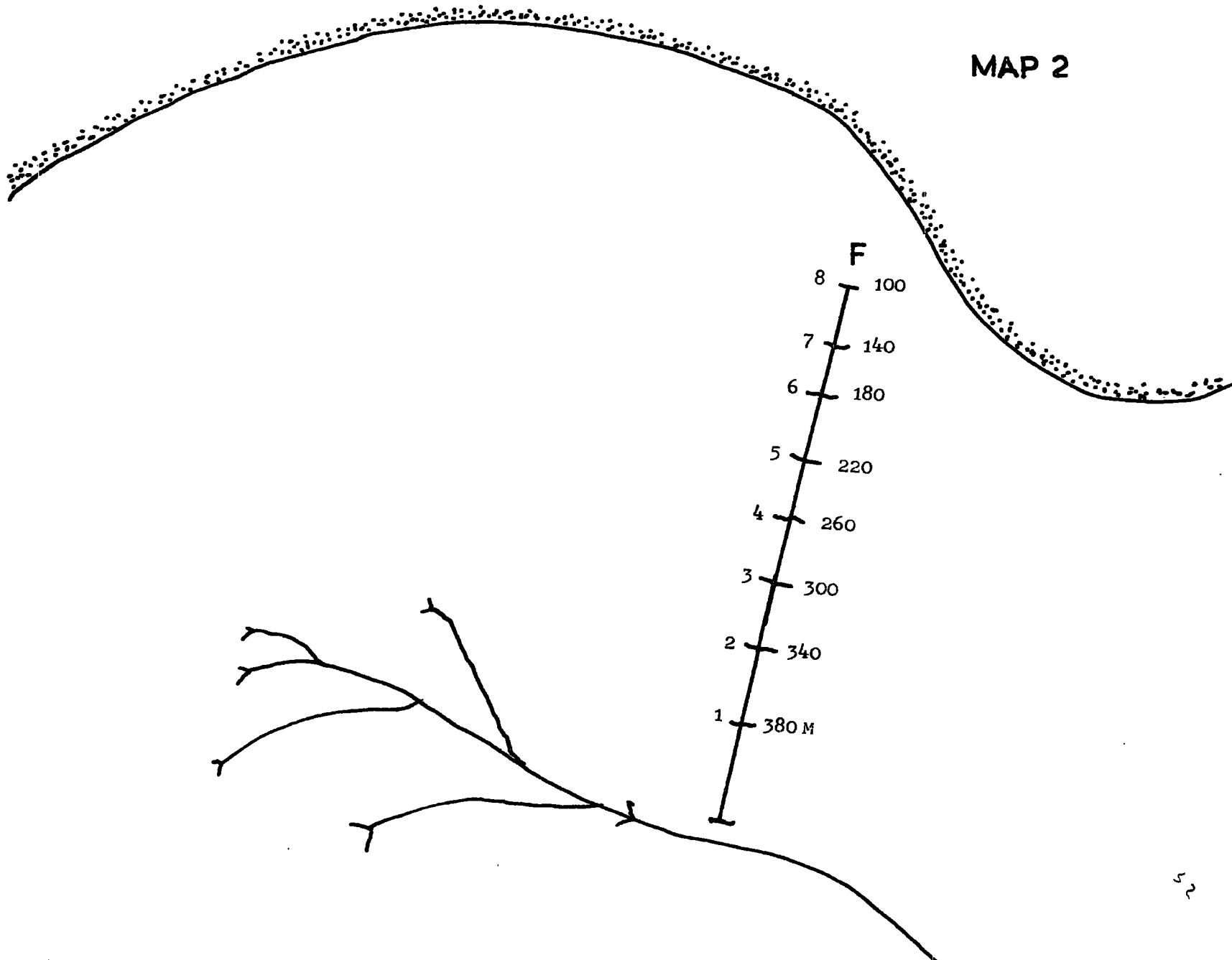
In none of the sterile runs was any colony growth found, showing that the equipment and technique was sufficient to avoid contamination.

DIAGRAM OF POSITION OF TRANSECTS A AND C AT BEAL POINT IN RELATION TO SPARTINA AND UPPER TIDAL LEVEL

MAP I

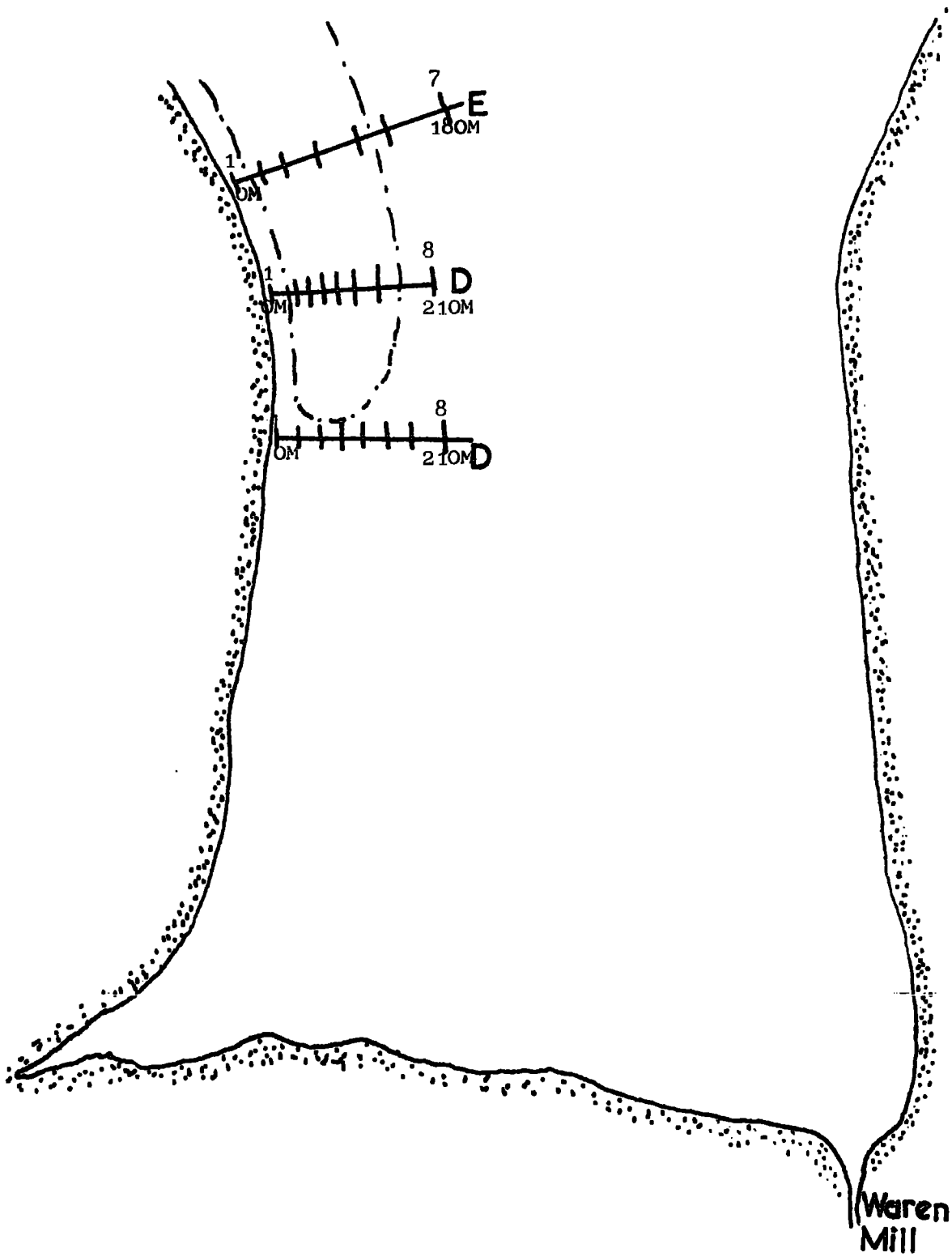


SAMPLE SITE F FROM HOLY ISLAND



POSITION OF TRANSECTS D AND E TAKEN AT BUDLE BAY

Map 3



INVERTEBRATE COUNT PER SQUARE METRE - 1 JUNE (B)

1 June Site	Littorina	Saxatilis	Abra tenuis	Macoma	Hydrobia	TOTAL-Molluscs	Ampharete grubeii	Eteone flava	Nereis diversicolor	Spionidae	Small Oligochetes	Polych scoloplos Armiger	Priaphulus	Sabellidae	Aerobic bacterial count	Corophium	Annelida TOTAL- minus small oligochetes
20 (1)	50			200	200	450	125	25			5550	250			4.5		400
21 (2)	25			150	300	475	50			75	4175	2050	25		6.15	25	2225
22 (3)			25	125		150	25			50	6400	700	25	50	2.18		850
23 (4)						0									4.63		0
24 (5)			25		50	75		25	475	400	75				2.15	725	1625
25 (6)			175	75	50	300	25			25	1120	575			4.6		625

Data from W F Miller and A Turk (unpublished)

INVERTEBRATE COUNTS PER SQUARE METRE - TAKEN ON HOLY ISLAND SITES ON 4 JULY (F)

SITE	Macoma	Abra nitidia	Tellina fabula	Tellina tenuis	Cockle	Corophium	Pallaemon	Bathyporeis	Nototroppies	Small Oligochetes	Aerobic bacterial Count x 10^6
1 (1)	100	100				25				4500	3.35
3 (2)	325	25	50				25			2500	5.2
5 (3)	175									7500	1.2
7 (4)	25			25		25		225		0	0.65
9 (5)										0	0.75
11 (6)								300	100	0	0.95
13 (7)								150		0	1.7
15 (8)					25			100		0	1.2

Data from D M Evans (unpublished)

INVERTEBRATE COUNTS PER SQUARE METRE - FROM BUDLE BAY ON 18 JUNE (D)

SITE	Hydrobia	Macoma	Corophium	Arenicola	Nereis	Scoloplos	Aerobic Bacteria Count x 10^6
A1	0		1100				1.2
A2	200		800				0.75
A3	2700						0
A4	1600		100				3.1
A5	3400						4.8
A6	900						1.25
A7	4400						3.1
A8	130	200	5500	200			0.65
B1	100	100	1600		100		5.8
B2	600	0	3800	700			0.7
B3	2700	0	1200	200			0.75
B4	1700		7200	600			1.3
B5	4100		200	100			1.1
B6	2400		3700		100		0.9
B7	1200		200	100			0.95
B8	2100	200	300	200		500	1.85

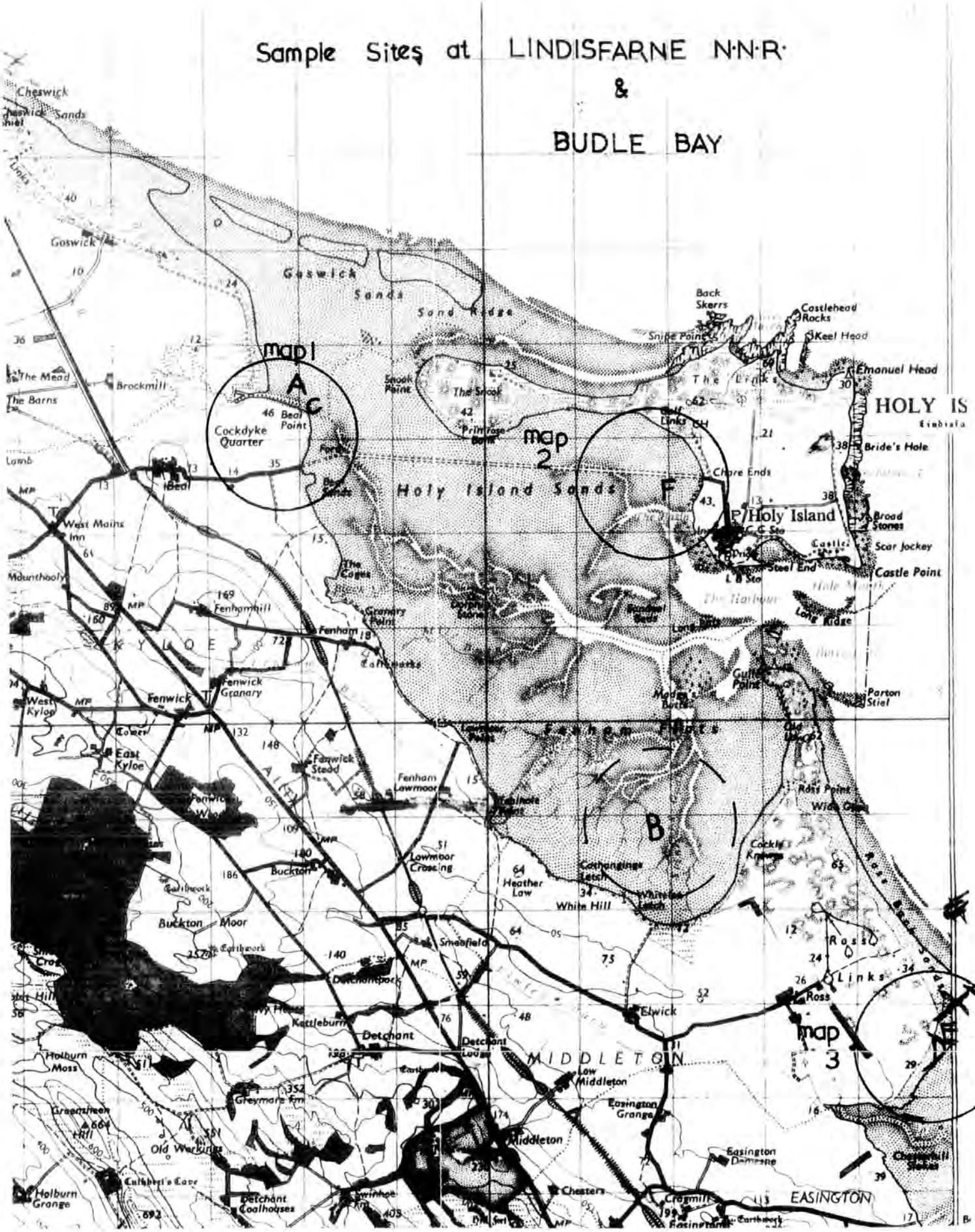
(Table continued)

INVERTEBRATE COUNTS FROM BUDLE BAY TAKEN ON 22 JUNE (E)

SITE	Hydrobia	Macoma	Corophium	Arenicola	Nereis	Scoloplos	Aerobic Bacteria Count x 10^6
27 JUNE OM	0		400				-
15M	300		100				3.05
30M	0						6.45
60M	100						4.0
90M	2000					400	4.52
144M	1700		100				3.15

Data from A V Millard (unpublished)

Sample Sites at LINDISFARNE N.N.R. & BUDLE BAY



3.1 Confirmation of adequate incubation time

By plotting the numbers of colonies formed against time, one can show the rates of colony appearance at different times after incubation commences. Hence, one can find the time beyond which it is not worth incubating the plates as the rate of colony appearance is negligible.

From FIG II and FIG III it may be seen that after twenty-one days, the rate of colony increase is so low that additional incubation time is unnecessary.

Fig II

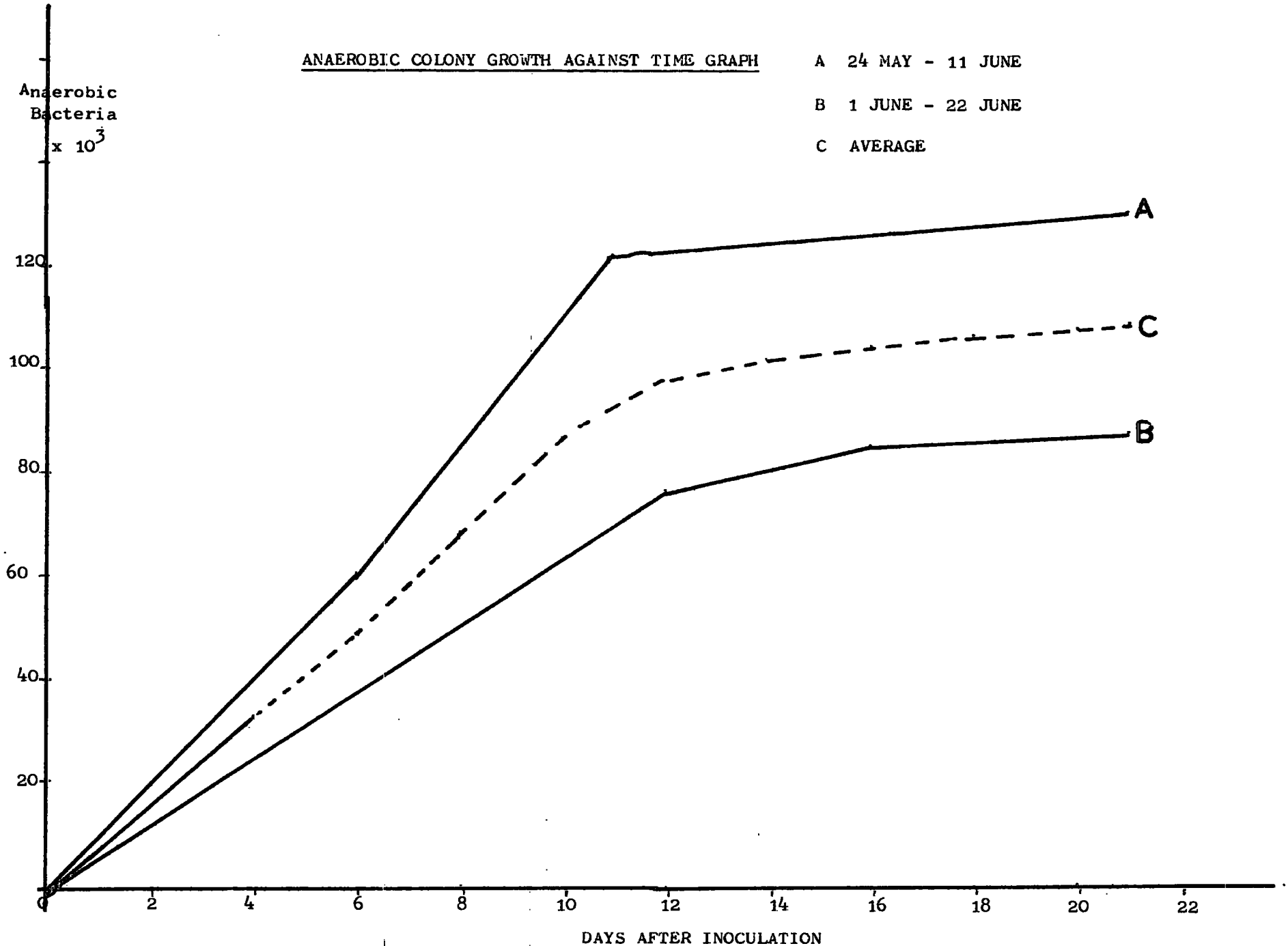

ANAEROBIC COLONY GROWTH AGAINST TIME GRAPH
A 24 MAY - 11 JUNE
B 1 JUNE - 22 JUNE
C AVERAGE
Anaerobic Bacteria x 10^3
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
22
DAYS AFTER INOCULATION
A
B
C

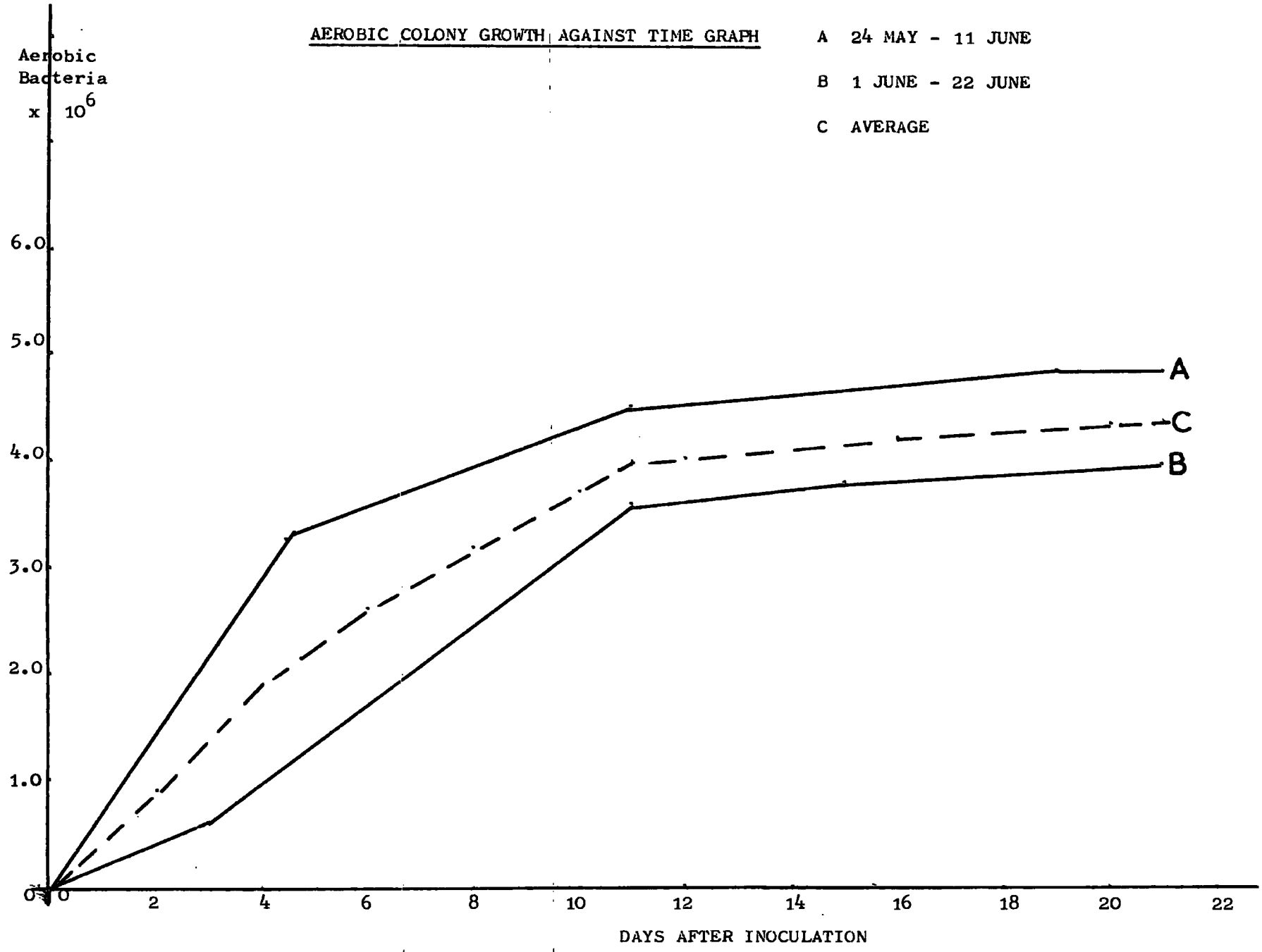
Fig III

AEROBIC COLONY GROWTH AGAINST TIME GRAPH

A 24 MAY - 11 JUNE

B 1 JUNE - 22 JUNE

C AVERAGE



3.2 Estimation of reliability of bacterial counts

Duplicate samples were taken from nine sampling sites and were used to estimate the reliability of single samples.

The results are shown below:

SAMPLING AREA	SITE	COUNTS OBTAINED		MEAN	COEFFICIENT OF VARIATION
B	1	a)	4.5	4.42	3.38%
		b)	4.35		
	2	a)	6.15	6.195	0.72%
		b)	6.24		
	3	a)	2.18	2.24	2.67%
		b)	2.30		
D	1	a)	1.15	1.2	4.16%
		b)	1.25		
	2	a)	0.80	0.75	6.6%
		b)	0.70		
E	2	a)	3.35	3.05	9.8%
		b)	2.75		
	3	a)	6.75	6.45	4.65%
		b)	6.15		
	5	a)	4.75	4.52	4.97%
		b)	4.30		
G	2	a)	2.1	2.1	0
		b)	2.1		
		MEAN COEFFICIENT OF VARIATION =			4.1%

CONCLUSION

From these results it can be seen that the duplicates do not always give identical results. Taking the average coefficient of variation as a guide one could say that the bacterial counts obtained are accurate to

$\pm \frac{\text{Mean C of V}}{2}\ \%$ or $\pm$ 2.05%.

3.3 Correlation between numbers of surface bacteria and depth of anaerobic layer

The bacterial populations in the top 0.5 cm of estuarine sediments are those most readily available for assimilation by filter feeders which live in the sediments. If, as has been reported, certain invertebrates depend to a large degree upon these bacteria for food, one would expect that increased bacterial content of the upper layer of sediment would be correlated with increased populations of these invertebrates below. However, the bacterial populations in the upper sediment might also have an adverse effect on the lower layers of sediment by deoxygenation, producing a situation of conflicting advantage and disadvantage to the invertebrates.

The reducing conditions found in the anaerobic layer of estuarine sediments are not favourable for certain invertebrate life and the more reduced these conditions become, the lower are the numbers of invertebrates which are able to withstand the conditions. (Fenchel and Riedl, 1970). Therefore, the nearer to the surface the anaerobic layer becomes the less the sediment depth is favourable for the invertebrates. (This is not always true, since some invertebrates, eg Corophium volutator in fact prefer slightly anaerobic conditions.)

The object of this part of the project was to correlate the numbers of aerobic bacteria in the top 0.5 cm with the depth of the anaerobic layer below, to determine whether the bacteria might exert beneficial or adverse conditions on the lower sediments. The results are presented in the form of a scatter diagram (FIG IV) from which it can be seen that the correlation coefficient is significantly different from zero ($p < 0.001$).

The value of the square of the correlation coefficient, r^2, (= 56.5%), is the proportion of the variation in the depth of the anaerobic layer that can be associated with variation in the number of aerobic bacteria.

It thus represents a major proportion of the variation. However, the part that sediment composition plays in this correlation is not known and as this may affect both bacterial numbers and anaerobic layer depth (by affecting the rate of the percolation of the oxygen-carrying seawater) the latter factors are not strictly independent.

In so far as the lower sediments are affected by the bacterial densities in the upper layer, this could be due to two main causes.

Firstly, bacteria in the upper layer use up some of the oxygen available to them; those below depend upon the oxygen passing through the upper sediments. Therefore, the more bacteria in the upper sediments, the less oxygen will be available to the bacteria in the lower sediments, resulting in more reduced conditions and growth of anaerobes.

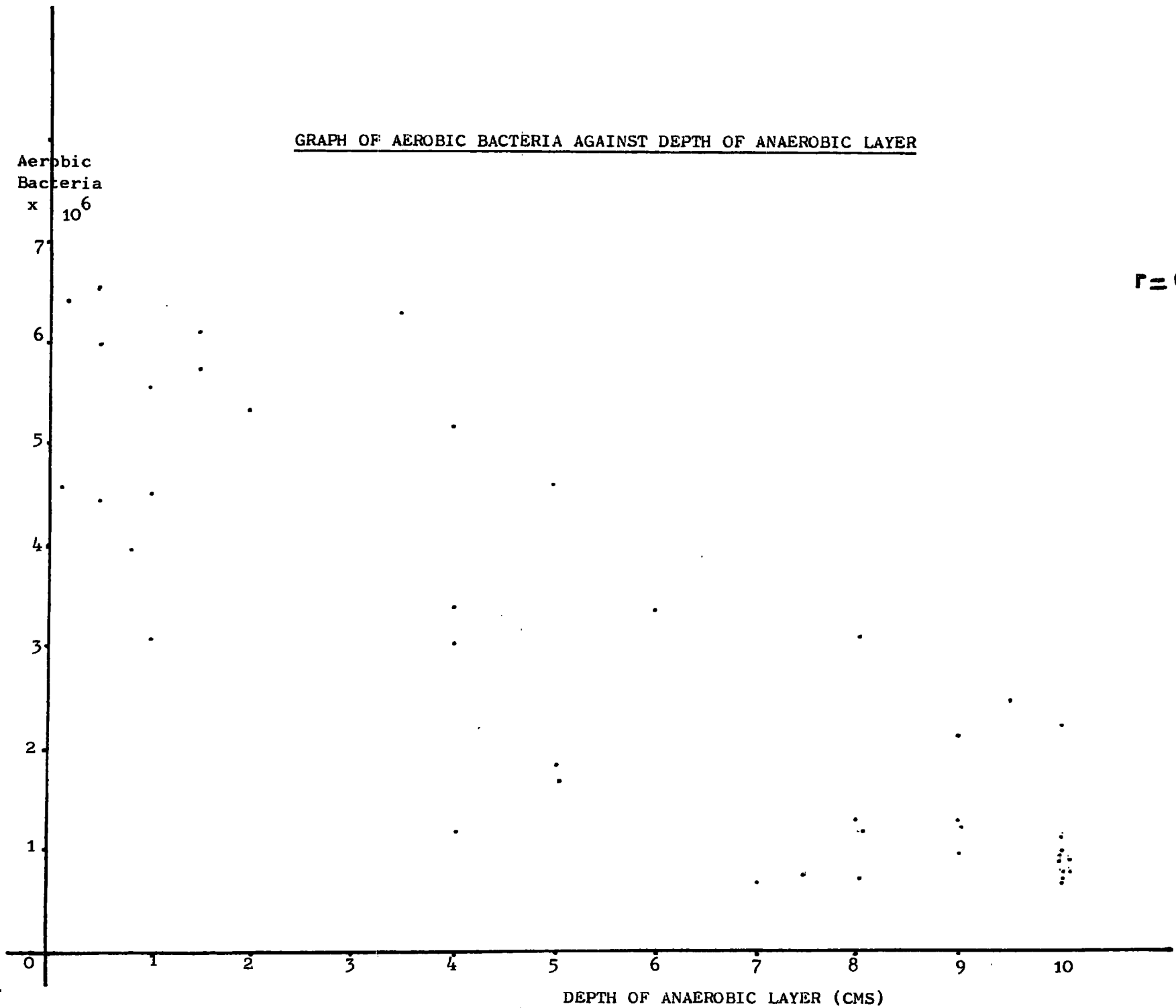
Secondly, much work has been done on the relationship between particle size in the sediment and the bacterial counts and it has been suggested that bacterial counts increase with decrease in particle size (ie increase in silt content) because this increases the surface area per unit weight for bacteria to colonise. However, this decrease in particle size and increase in silt might also result in a reduction in the rate at which water carrying oxygen percolates through the sediment. Therefore, the correlation could be due largely to changes in particle composition of the sediment rather than the bacterial content.

FIG IV shows that an increase in bacteria in the upper sediments does not necessarily lead to advantages to the invertebrates as it is accompanied by a reduction in the volume of suitable living space preferred by many invertebrate species. Hence a balance between bacterial content and available living space is probably the situation in which maximum invertebrate populations would exist or those exceptional cases of high bacteria and deep sulphide layer.

To see whether the idea that increased surface area in the sediments leads to increased bacterial densities, a correlation was performed

Fig IV

GRAPH OF AEROBIC BACTERIA AGAINST DEPTH OF ANAEROBIC LAYER



r = 0·7520

using silt content of some of the sediments from which bacterial counts had been made (from results taken on 19 July).

When the silt content (% silt) was correlated against bacterial content, a correlation coefficient of 0.660 was obtained. This is not significant, however, it does show a fair tendency for the bacterial numbers to rise with increased silt content. (FIG IX) h?

This could be for two reasons:

1 The increase in surface area

2 The increase in silt content may be largely composed of small particles of organic detritus and this would also lead to an increase in the numbers of bacteria present to degrade it.

3.4 Correlations between bacterial numbers and invertebrate densities

Invertebrates in estuarine sediments depend for their existence on the assimilation of material from those sediments or the waters above (eg. cockles). In the following attempts to correlate invertebrates with bacterial numbers, it must be remembered that it is almost impossible to separate the effects of viable bacteria from those of the detrital material found in the sediments. An increase in detritus inevitably leads to an increase in bacteria which assimilate it. Therefore, the existence of any significant correlations does not mean that bacteria alone determine the distribution of certain invertebrates, but rather that bacteria and detritus act together and that it is the availability of food which determines the distribution of those invertebrates. The lack of significant correlation could indicate that other factors such as sediment composition, or position on the salinity gradient may be more important.

For the correlations with the bacterial numbers, the invertebrates were treated as independent species. This was done for the reason that each species feeds in a different way. Not all the species found on the estuary are filter feeders or detritus feeders. Nereis diversicolor is a carnivore and therefore does not enter into this question. Within each Taxa the species use different modes of obtaining their food. In the Mollusca for instance, Macoma is a fairly stationary detrital feeder, whereas Hydrobia is probably more dependent on grazing of diatoms, blue-green algae and bacteria on detritus. In addition it is capable of moving large distances. Also, the relative sizes of the different species within each Taxa made it impossible to treat them as one unit. One Macoma is not equivalent to one Hydrobia. Therefore each was treated separately except where a very similar method of feeding and size was shared by two species within the same Taxa. In these cases they were treated both together and separately - eg. Abra tenuis and Macoma.

RESULTS

The following table represents the correlations obtained after analysis. Those correlations found to be significant have been plotted and are shown on their respective figures.

SPECIES	CORRELATION COEFFICIENT r	NUMBER OF SAMPLES	SIGNIFICANCE AT 0.05 LEVEL	SIGNIFICANCE OR NO
Macoma				
Fenham	.4796	8	.6319	NO
Budle	.2734	10	.5760	NO
Abra tenuis	0.99	3	.8783	YES
Abra tenuis and Macoma	.6302	9	.602	YES ?
Corophium				
Fenham	.2468	4	.8114	NO
Budle	.2630	12	.5131	NO
Hydrobia				
Fenham	.7795	4	.8114	NO
Budle *	.8280	6	.7067	YES
Budle **	.5238	8	.6314	NO
ALL	.1741	18	.4438	NO
Arenicola				
Budle	.1039	7	.6664	NO
Bathyporeis	.5625	4	.8114	NO

* Inside Spartina

** Outside Spartina

CONCLUSION

It is evident from these results that the bacterial content of the upper sediments is not the main factor determining the density of the invertebrates which have been examined. Although, Abra tenuis shows a significant

correlation, only three samples were involved and this is certainly not sufficient to show a definate reliance on the bacteria. The Hydrobia also shows a significant correlation inside the Spartina, but again this is not shown outside the Spartina and this result must be treated with care.

However, perhaps these results do show that the area inside the Spartina should be treated differently from that outside. In addition, the Budle Bay and Fenham Flat areas should also be treated separately. The Abra tenuis and Hydrobia correlations are shown on scatter diagrams in FIGS V and VI.

The other main factors which could be determining the density of the invertebrates include the position on the salinity gradient, the time for which the sites are covered by the tide, the sediment particle composition and the depth of the anaerobic layer. The latter is treated in the next section.

An interesting point of additional interest is the data obtained on 4 July (F), in an area with no Spartina. The results here show the usual low numbers of aerobic bacteria as one gets further from the shore, but then a very marked increase in the bacteria as one gets near the tidal drainage channel.

From FIG VIIb one can see that changes in the Macoma and Abra tenuis numbers correspond to changes in bacterial numbers and the depth of the anaerobic layer. Although, as has been shown, neither of these parameters seem to be a major factor in determining invertebrate densities. A change in sediment composition was noted as one neared the channel. The sediments became finer and muddier. It may well be this parameter which is determining the others.

Fig V

SCATTER DIAGRAM OF AEROBIC BACTERIA AGAINST DENSITY OF MACOMA AND ABRA TENUIS

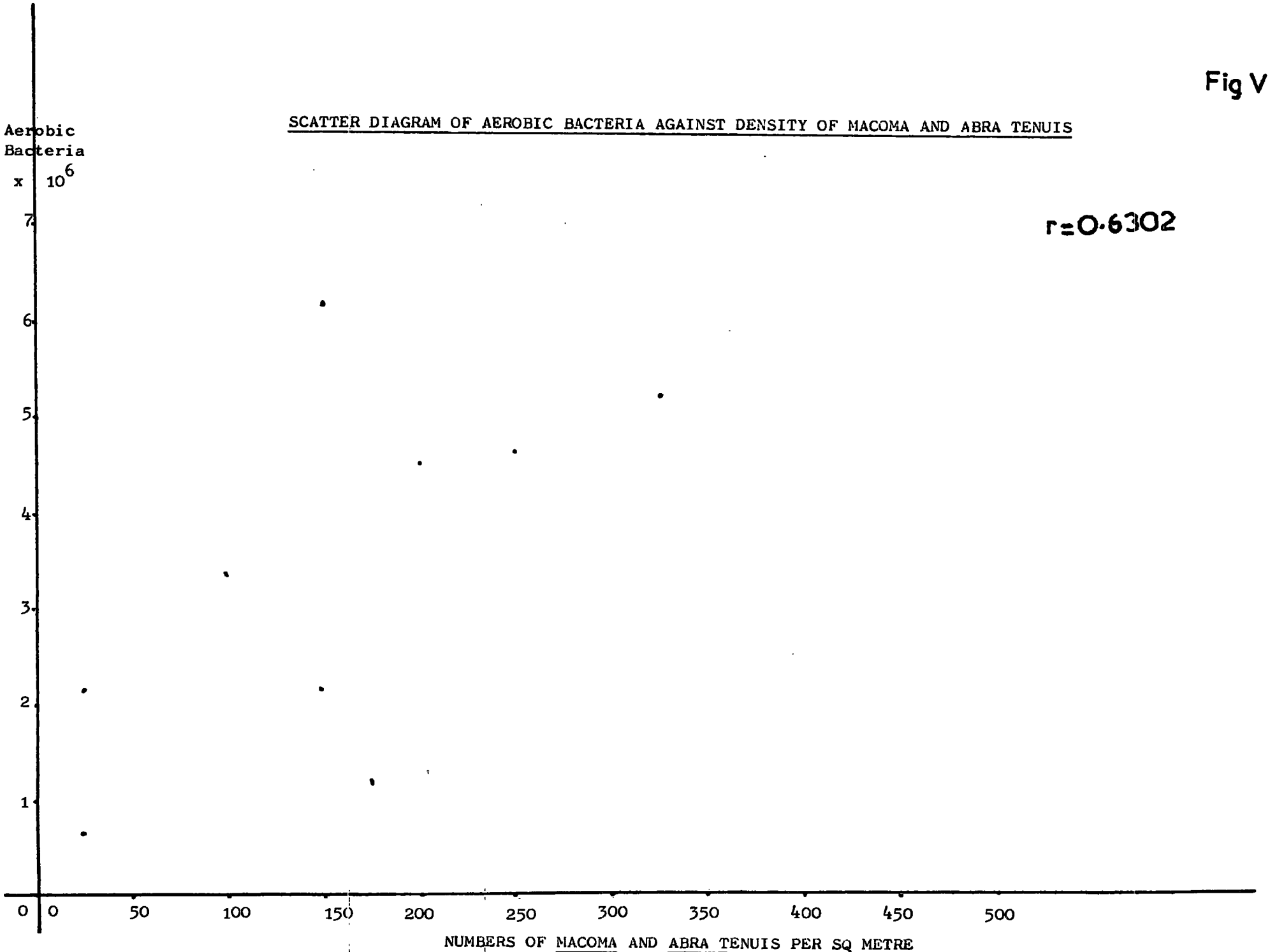


Fig VI

SCATTER DIAGRAM OF THE NUMBER OF HYDROBIA IN THE SECTION OF SPARTINA (BUDLE BAY)

AGAINST AEROBIC BACTERIA

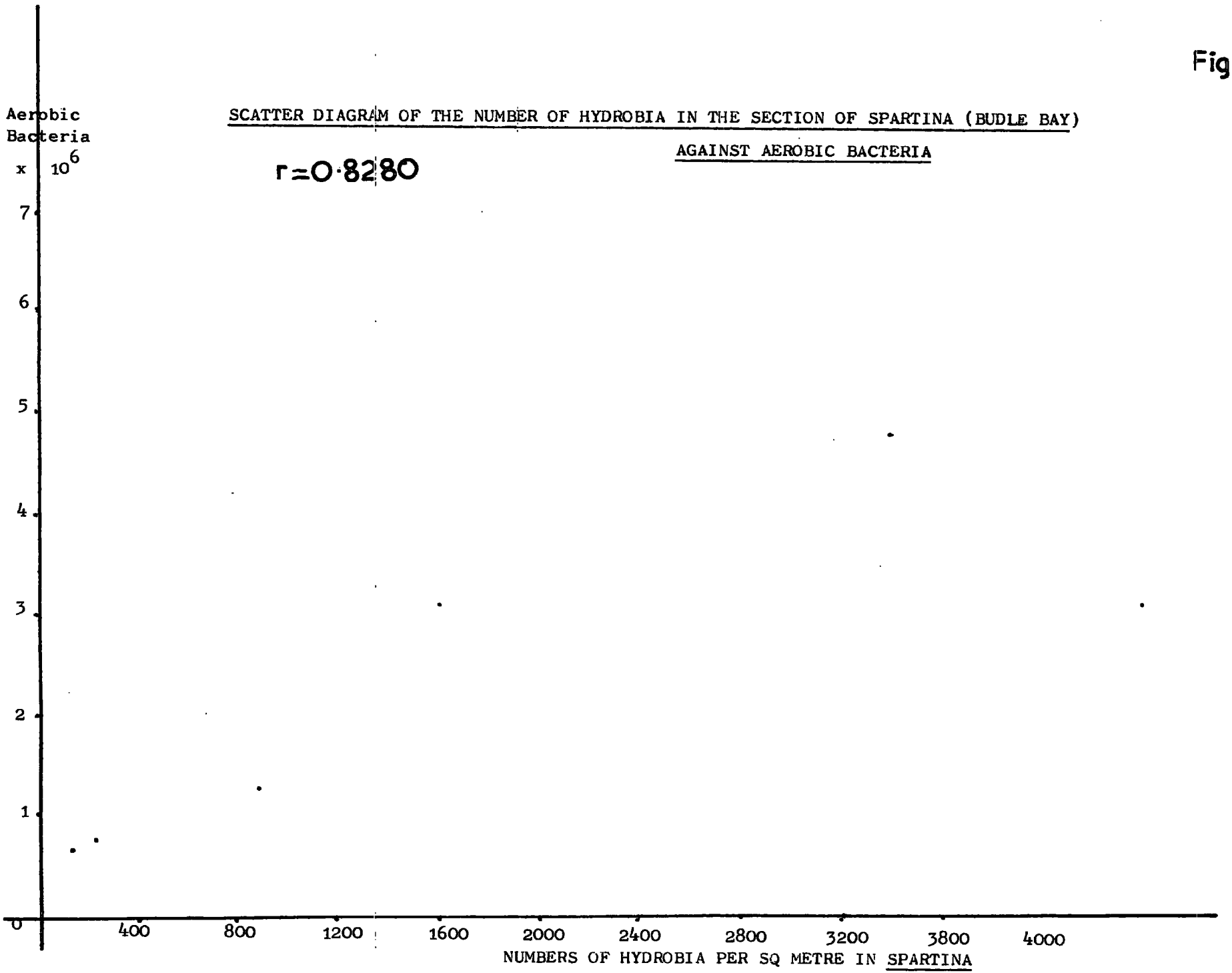
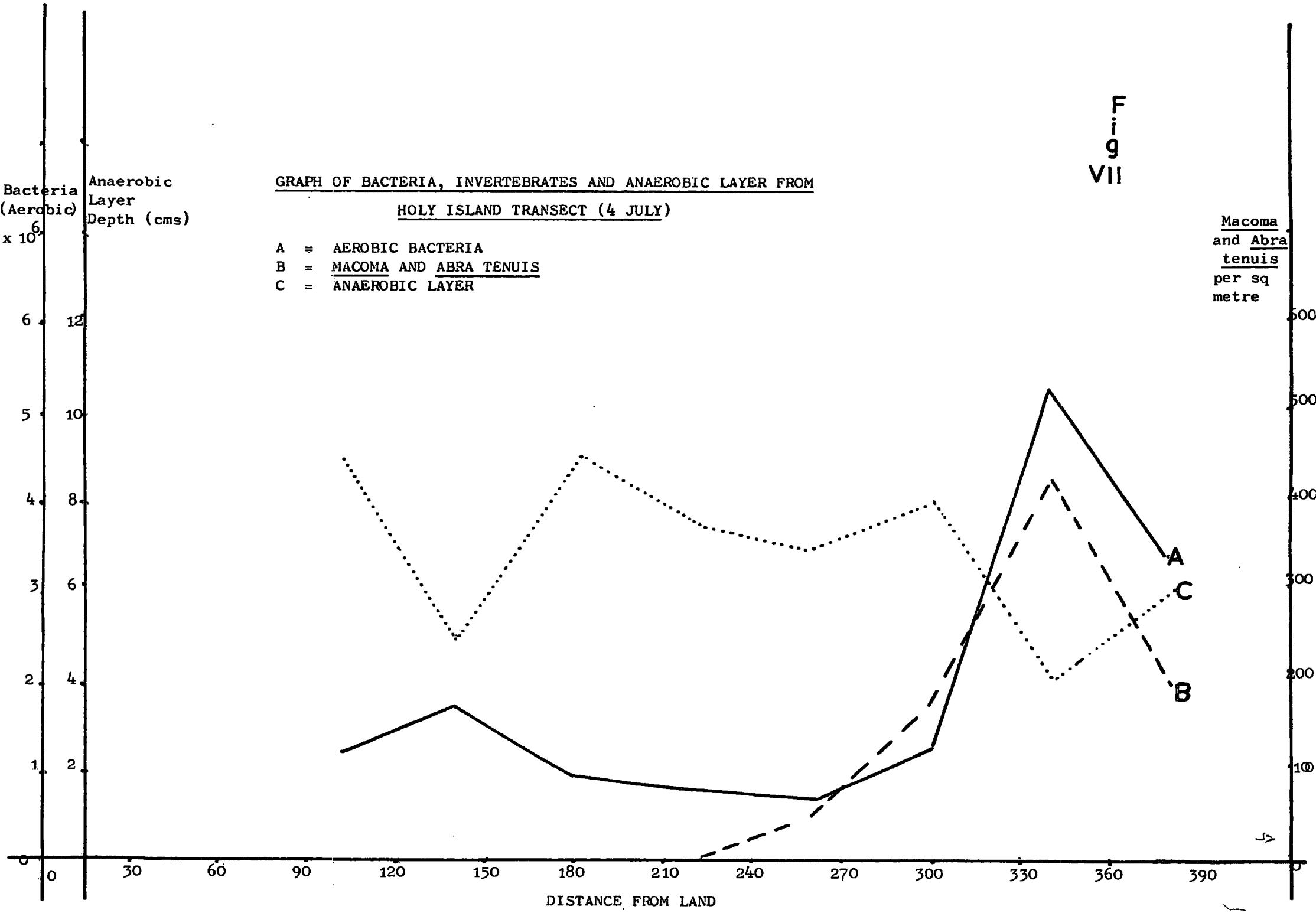


Fig VII

GRAPH OF BACTERIA, INVERTEBRATES AND ANAEROBIC LAYER FROM HOLY ISLAND TRANSECT (4 JULY)

A = AEROBIC BACTERIA
B = MACOMA AND ABRA TENUIS
C = ANAEROBIC LAYER



3.5 Correlation of Invertebrates with Anaerobic layer depth

The possible importance of the anaerobic layer in respect to invertebrate distribution has been discussed. So, it was decided to perform a correlation on invertebrate counts with the depth at which the anaerobic layer appears. This was done to see if the anaerobic layer was significantly important in some species in determining densities of that invertebrate.

Species	Correlation Coefficient r	Significant value .05% level	Number of Samples	Significance or No
Macoma				
Fenham	.3638	.6319	8	NO
Budle	.8180	.8783	3	NO
Fenham + Budle	.1247	.5529	11	NO
Abra tenuis	.1428	.8783	3	NO
Abra tenuis + Macoma				
Fenham	.0975	.602	9	NO
Budle	.8108	.8783	3	NO
Corophium				
Fenham	.5095	.8114	4	NO
Budle	.329	.5324	12	NO
Hydrobia				
Fenham	.6324	.8114	4	NO
Budle *	.7002	.7067	6	NO
Budle **	.4911	.6319	8	NO
ALL	.1739		14	NO
Arenicola				
Budle	.0729	.4973	7	NO
Bathyporeis	.2238	.8114	4	NO

* Inside Spartina ** Outside Spartina

CONCLUSION

Here again there does not seem to be any significant correlation between the depth at which the anaerobic layer begins and the density of the invertebrate population associated with it.

This does not necessarily mean that the depth of the anaerobic layer plays no part in determining the density of the invertebrates, but that the part it does play, if any, is very small.

To find the relative importances of the different parameters in determining invertebrate density one would have to measure these parameters and then perform a multiple regression on them.

3.6 Correlation of Nitrogen content of estuarine sediments with bacterial content of those sediments

As has been stated previously, it is possible that invertebrates in the estuary depend largely on bacteria in the sediments for their Nitrogen source. To find out if a significant correlation exists between nitrogen contents of the upper sediments and bacterial counts at the same sites, samples were taken on 19 July. Nitrogen determinations were performed by Ingvarsson using the semi-micro kjeldahl method, and bacterial counts by myself using methods already described.

The results were presented earlier - 19 July (G) - and are illustrated in FIG IIX

There is a significant correlation between the nitrogen content of the surface sediments and their bacterial content. The r^2 value of 50.1% shows that at least half the variation in nitrogen in the sediments is associated with variation in bacterial abundance. This represents a large degree of reliance upon bacteria as a reservoir of nitrogen. The remainder of the nitrogen may well be present in detrital matter in the sediments. However, the technique used for the bacterial counts detected only viable aerobic bacteria. Therefore it is possible that much of the missing nitrogen is present as non-viable bacteria in the detritus and in the anaerobic bacteria. In addition the amounts present in the algae and diatoms should not be forgotten.

At the same time, some silt analyses were done and these were used to perform a correlation between the percentage silt and bacterial count. The correlation coefficient obtained was found to be 0.6602 which was discovered to be significant at the 0.05% level. This gave an r^2 value of 43.6% showing that this percentage of the variation in bacterial numbers could be attributed to the silt content of the sediment. This could be for two main reasons.

Firstly, as already stated it could be due to the higher surface area per unit weight of sediment when the silt content is higher, or secondly, it could be attributed to the fact that 'silt' contains much organic material and this would result in an increase in the numbers of bacteria which are degrading it. For a scatter diagram of this correlation, see FIG IX.

Fig IIX ?

NITROGEN (% DRY WEIGHT) WITH BACTERIA AT FENHAM

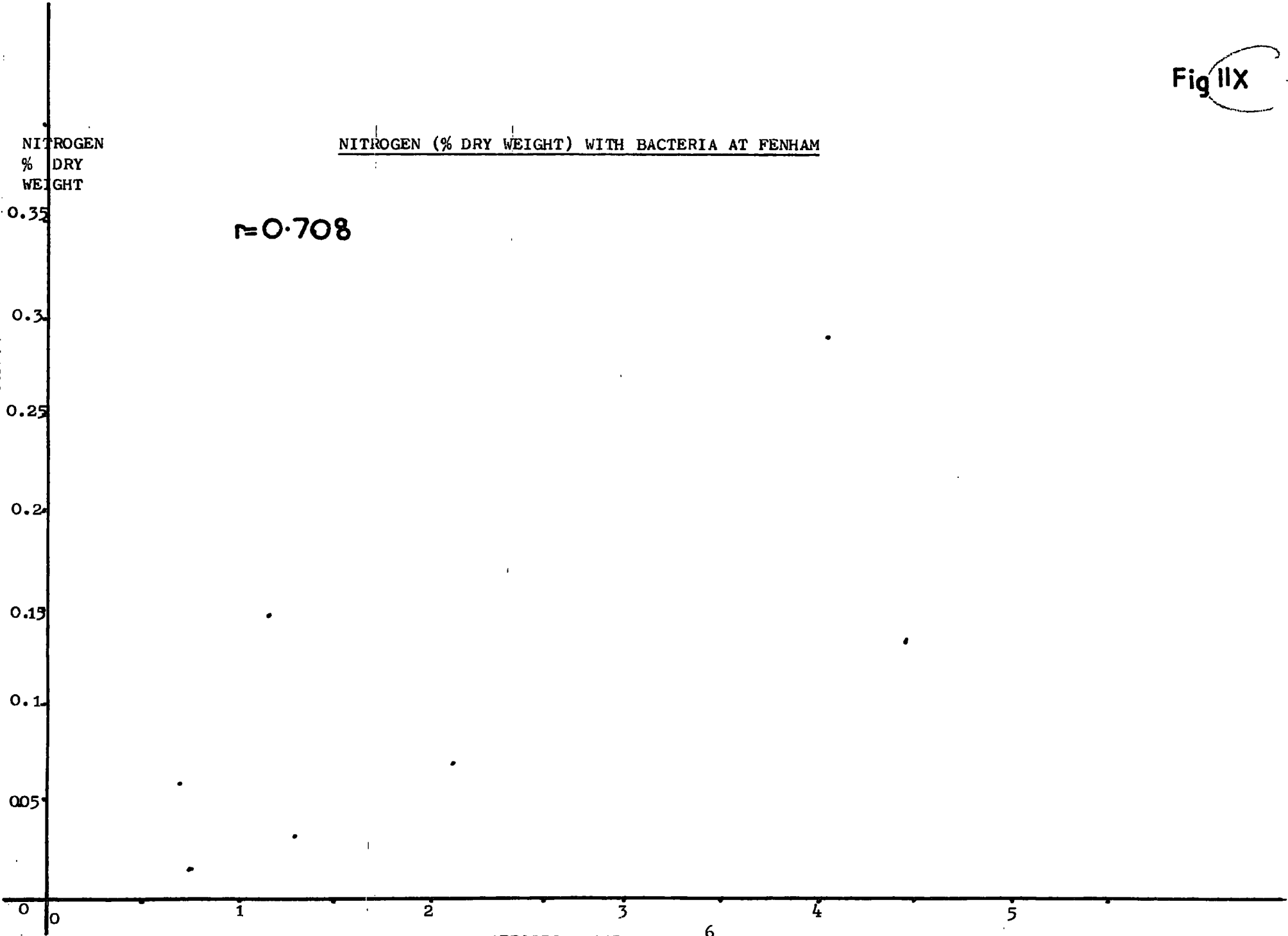
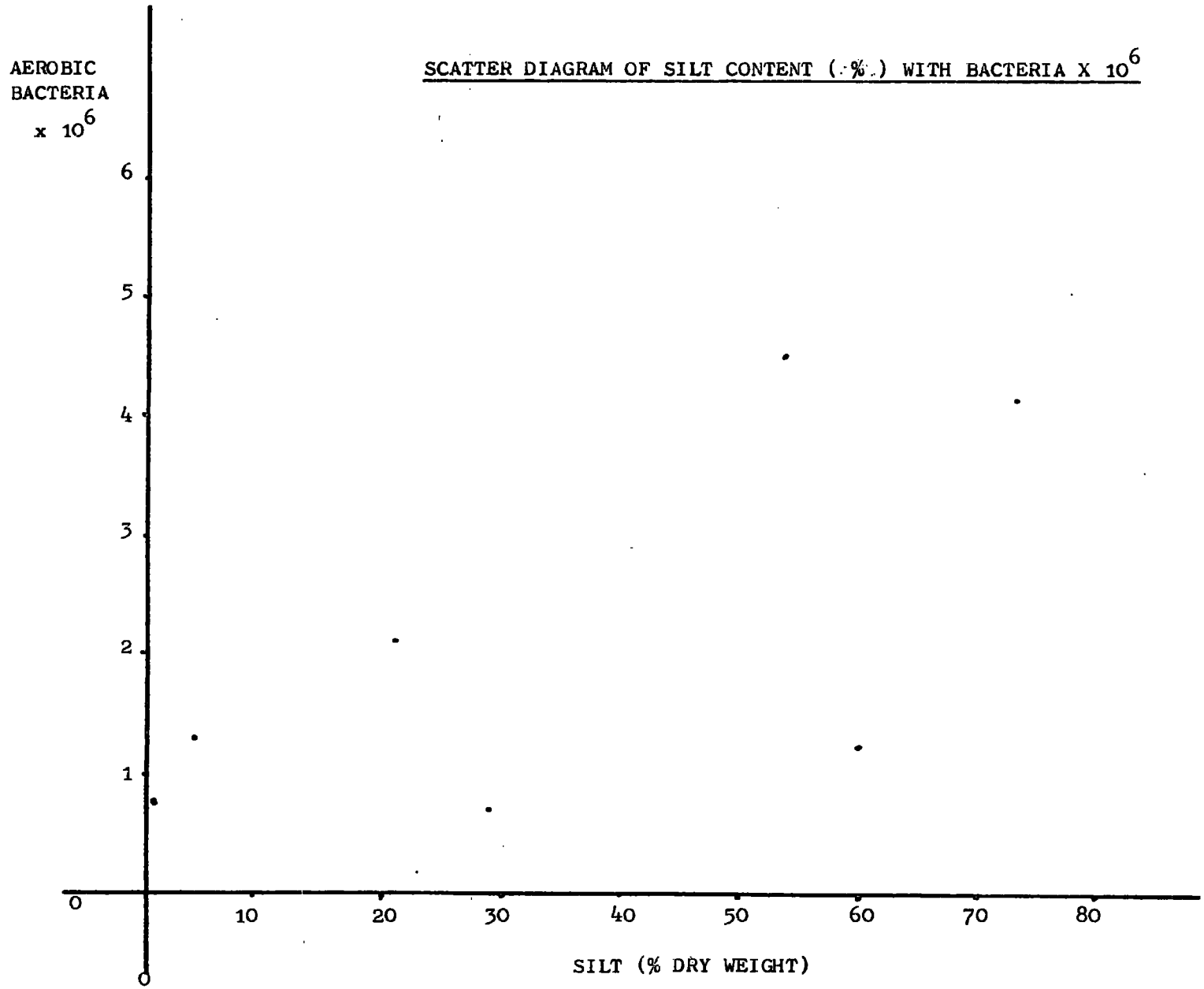


Fig IX



r = 0·6602

3.7 Effect of Spartina swards on the bacterial content of estuarine sediments

The mean particle size of the sediments within Spartina swards is smaller than of the sediments outside, as is evident even form a cursory glance at the two areas. The Spartina acts as block to tidal currents and slows them down, resulting in deposition of silt and detrital material within the sward. This, added to material produced by decaying Spartina itself results in a fine sediment which is probably (as) high in organic matter and therefore, very possibly, in bacteria. The rooting system of the Spartina however changes the sub-sediment evnironment, and some of those invertebrates which live outside the sward are apparently unable to live within it. Others however may be better adapted to live within this system and might therefore be at a competitive advantage, if any competition exists between the species on the open mud.

The object of this series of tests was to find out by how much the bacterial count increases within the Spartina sward and how far beyond the sward the effects of any increase in bacteria can be detected.

To investigate this, the bacterial counts taken in the Beal Point area on 24 May (A) and 6 June (C) were plotted against the distance from the upper tidal level. The same was done for samples taken on 18 June (D) and 27 June (E). The results are shown in FIGS X and XI respectively. It can be seen clearly that the number of aerobic bacteria in the surface sediments within the Spartina increases by a large amount over counts outside.

On the landward side of the Spartina sward the counts are very similar to those on the shore close to land where Spartina is absent. This is probably due to two factors. Firstly, the tidal currents are always slowed when close to land due to functional forces similar to those occurring within the sward. Secondly, run-off from the land carries with it much organic material and bacteria, thus increasing the count.

Fig X

BACTERIAL COUNTS AND DISTANCE FROM LAND AT BEAL POINT

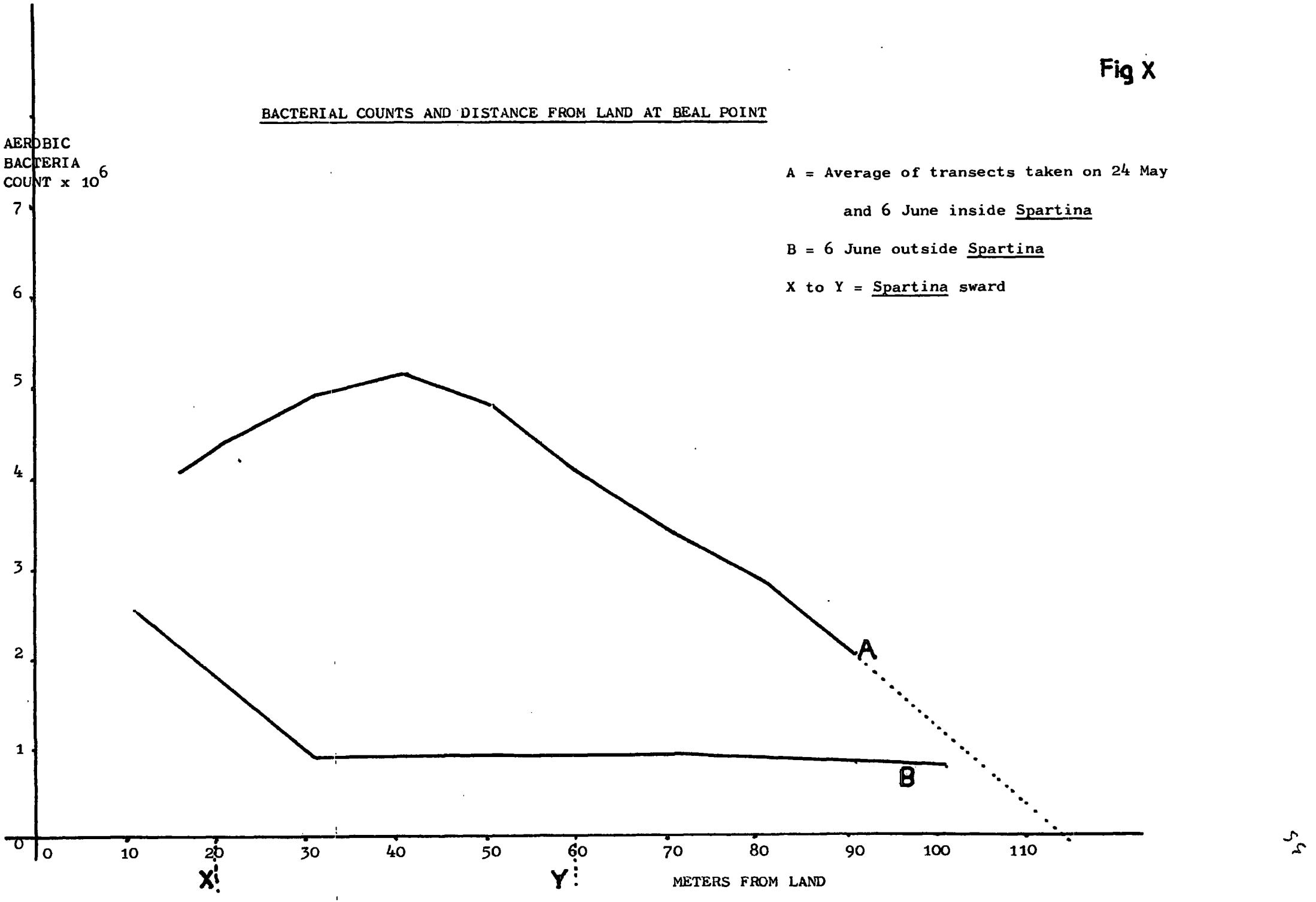
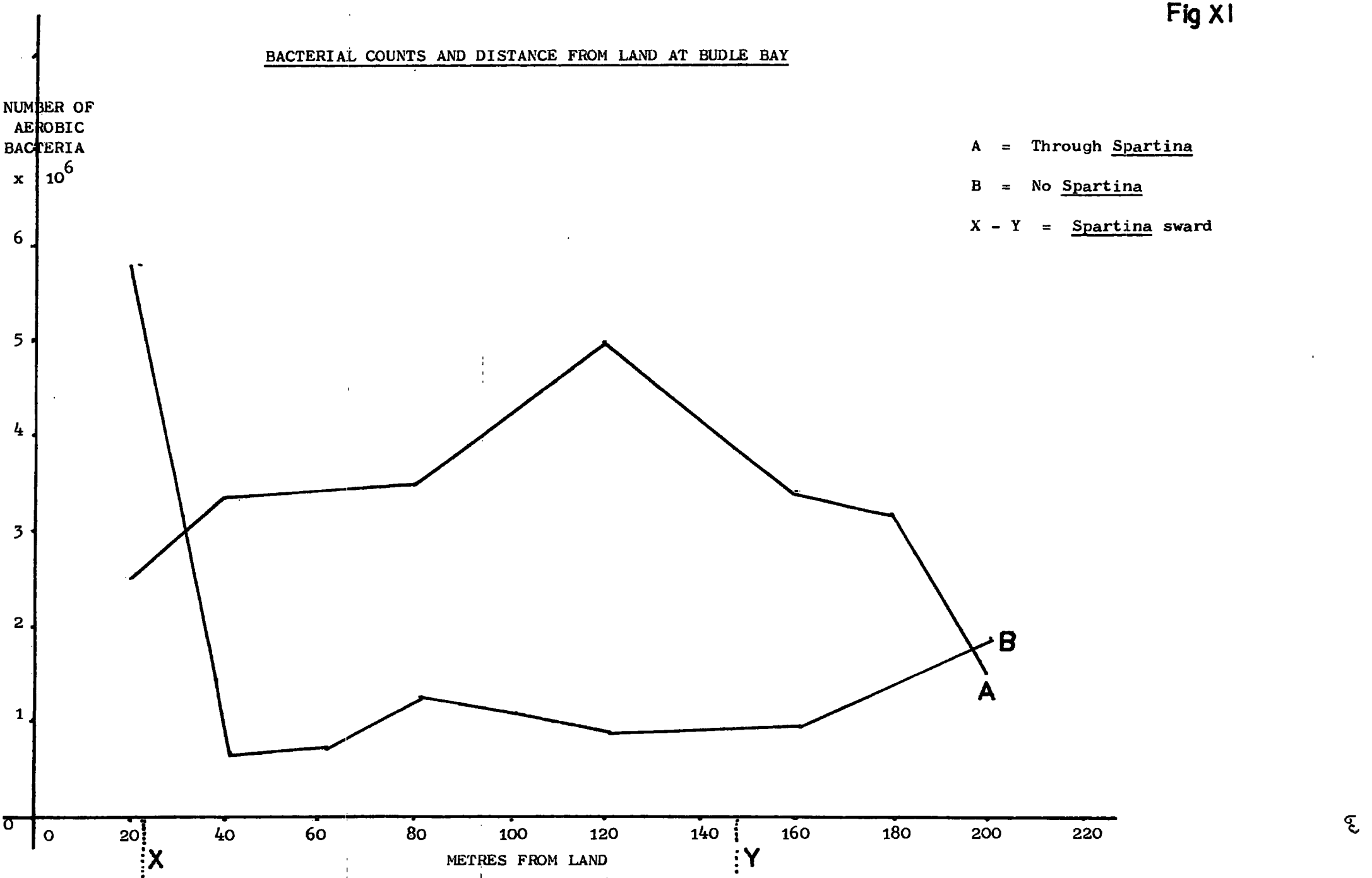


Fig XI

BACTERIAL COUNTS AND DISTANCE FROM LAND AT BUDLE BAY



Once within the sward the bacterial count rises but only by a small amount. However, on the seaward side of the Spartina the bacterial count drops very rapidly to low levels similar to those found throughout the transect in non-Spartina areas.

At Beal Point, the distance beyond the Spartina through which the bacterial count is still high is in the region of 40 metres (with small extrapolation on graph). At Budle Bay the distance is about 40 to 50 metres.

Thus, the effect of the increase of bacteria within the sward is not felt for very far once the sward has been left. Any gain which may be effected by the higher bacterial count in the sward being felt for only about 50 metres outside it, could well be nullified by the very presence of the Spartina causing adverse conditions within the sediments in which it grows, and thus perhaps excluding many invertebrates from living in these areas. In addition it has been found that wading birds, with the exception of Redshank (A V Millard, P C Smith, P R Evans - Department of Zoology, Durham) will not feed within the vegetation of the sward. Thus large areas of potentially productive estuary (in respect to the feeding of birds) are lost, with only a very small gain, if any, in the possible increase in productivity outside the sward.

3.8 The Effect of a Spartina sward on distribution of diatoms

It has been generally accepted that diatoms are a constituent of the diets of many of the invertebrates in an estuary; although work on the importance of diatoms in the diets has never been published. Therefore it was decided to measure what effect a Spartina sward might have on the distribution of the diatoms in the upper 0.5 cm of sediment.

The samples used were those taken on 6 June and the material left after bacterial plating was used to perform diatom counts.

METHOD

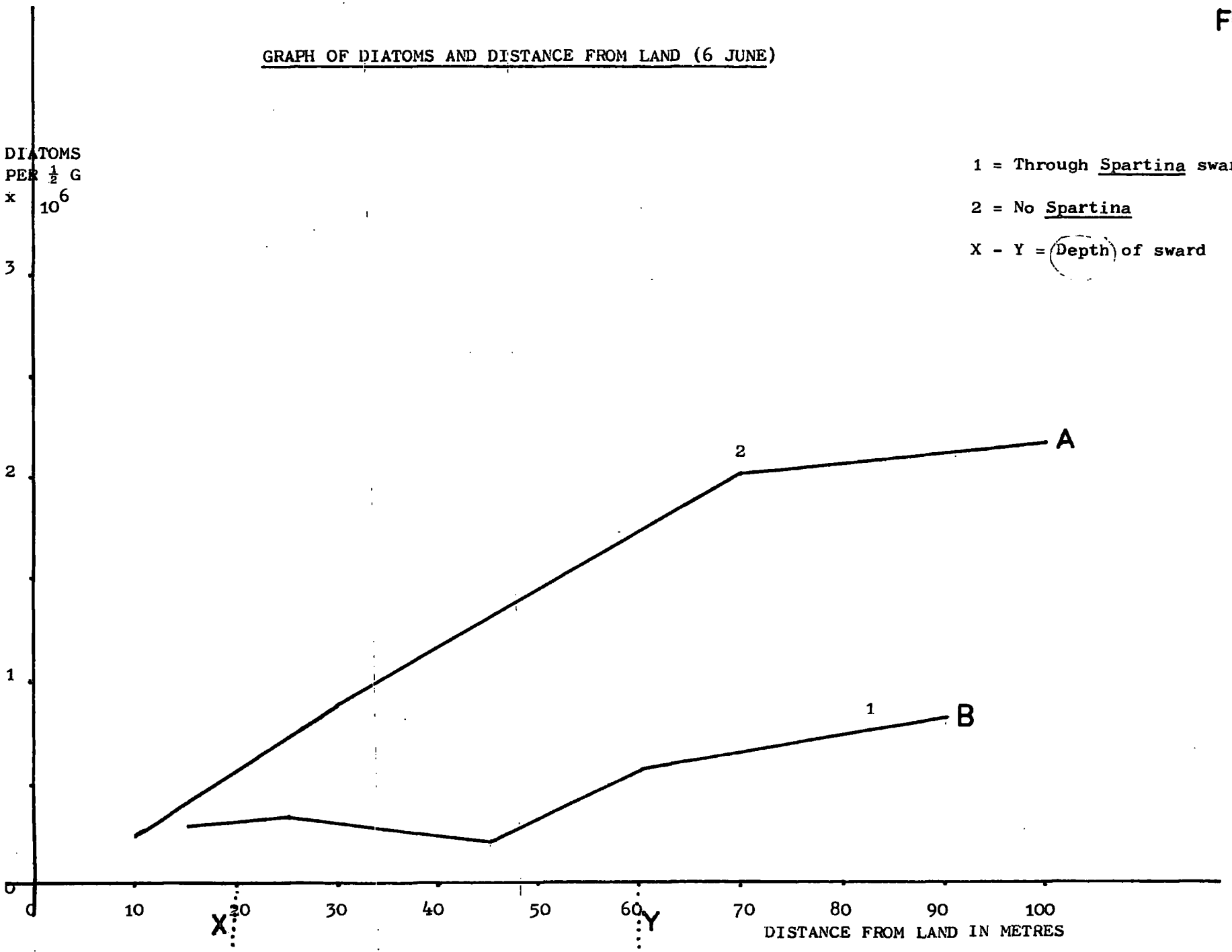
Half a gram of sediment was shaken in 5 mls of distilled water for a couple of minutes. As soon as shaking was stopped, a small sample of the water was removed and placed on the platform of a Haemocytometer and the glass slide was placed on top. When the space between platform and coverslip is filled with suspension, the volume of each small square is 0.1 x 1/400. The average number of diatoms per square is calculated from counts made in sufficient squares (eg 100). Once the average number of diatoms has been obtained the figure was multiplied by 4×10^6 per ml (obtained as area of each square is 0.0025 mm^2 and the depth of liquid is 0.1 mm).

SITE	DISTANCE FROM LAND	DIATOMS PER ML x 10^6
A1	15M	0.29
A2	25M	0.34
A3	45M	0.23
A4	60M	0.57
A5	90M	0.81
B1	10M	0.23
B2	30M	0.88
B3	70M	2.0
B4	100M	2.15

A = Through Spartina B = No Spartina

Fig XII

GRAPH OF DIATOMS AND DISTANCE FROM LAND (6 JUNE)



CONCLUSION

It would seem from these results that the Spartina sward decreases the density of diatoms and that more diatoms are avilable in areas in which there are lower bacterial counts. Therefore, even though the bacterial and detrital matter may decrease, the diatoms increase, thus perhaps providing another source of food for the invertebrates. It would be of great interest to know how important the diatoms are in the diets of the invertebrates and to know how efficiently they are assimilated, for they are present in some areas in very high numbers.

The reason for an increase in diatoms in areas away from the high bacterial (detritus) counts, could be that in these areas they are more able to obtain sufficient light, without being covered in small particles of detritus and bacteria which would obscure the light from them.

3.9 Comparison of bacterial and diatom counts with Biological Oxygen Demand of the sediments

Bacteria and diatoms should exert an oxygen demand upon the sediments in which they are living. By measuring the oxygen demand of the sediments one should obtain results which are similar to those obtained for the direct counts. This test was performed to test whether measurements of BOD could be used as a short method for comparing bacterial and diatom densities in different sites.

METHOD

Two transects were chosen from which samples were to be taken. Firstly, a transect through a Spartina sward and secondly, a transect in an area where there was no Spartina growth. Both these transects were taken at Beal Point so that the results could be compared with the bacterial and diatom counts obtained in this area.

Several grams of surface sediment were scraped into sterile glass containers, these were then placed on ice until they could be transported back to Durham.

On arrival at Durham, the samples were weighed into 3 gram (wet weight) quantities and were placed in 120 ml glass bottles with ground glass stoppers and labelled. This was done in triplicate for each sample. Then, to two of the three bottles, for each sample, was added 6 mg of Neomycin Sulphate and 6 mg of Streptomycin Sulphate. This gave a concentration of each antibiotic of 50 mg per litre (Hargrave 1971) which has been found to be sufficient to prevent bacterial growth. A control run using only seawater was also performed.

The bottles were then filled with fresh sea water at a temperature of 16.5°C and 98% saturation of oxygen. The stoppers were then placed back into the bottles, ensuring that there were no air bubbles trapped underneath.

One set of bottles (one from each sample) containing the antibiotic was then left in the light and the rest, one set with antibiotic and one set without antibiotic, were placed in the dark. All the bottles were then left for five days at a temperature of 16.8°C.

The samples were assayed for dissolved oxygen by the Winkler Titration and the following results were calculated. (See Appendix for details of the technique.)

RESULTS - Through Spartina sward

SITE	LIGHT AND ANTIBIOTIC PPM OXYGEN DEMAND	DARK AND ANTIBIOTIC PPM OXYGEN DEMAND	DARK PPM OXYGEN DEMAND	TOTAL DEMAND PER SITE	DISTANCE FROM SHORE IN METRES
1	8.13	6.93	8.53	23.6	5
2	7.73	8.53	8.53	24.8	15 (5M inside Spartina)
3	7.73	8.00	8.13	23.8	45 (5M from outside Spartina)
4	4.93	9.3	9.3	23.5	60 (10M from Spartina)
5	7.33	6.93	8.93	23.2	80 (30M from Spartina)
TOTAL AT EACH TREATMENT	35.8	39.7	43.4		
% INCREASE		10.8	8.6		

(Table continued)

RESULTS - No Spartina

SITE	LIGHT AND ANTIBIOTIC PPM OXYGEN DEMAND	DARK AND ANTIBIOTIC PPM OXYGEN DEMAND	DARK PPM OXYGEN DEMAND	TOTAL DEMAND PER SITE	DISTANCE FROM SHORE IN METRES
1A	2.93	4.13	4.53	11.6	10
2A	1.33	4.13	7.33	12.8	30
3A	2.53	2.93	(7.0)	13.5	80
TOTAL AT EACH TREATMENT	6.79	11.2	18.86		
% INCREASE		39.4	40.7		
CONTROL SEAWATER ONLY	0.2	0.2	0.3	0.7	-

CONCLUSION

Each set of differently treated bottles should show some aspect of the respiration of the sediments.

Those bottles left in the light with antibiotic should have acquired additional oxygen due to photosynthesis of the diatoms and any algae that may be present. They should not have any oxygen demand, unless the antibiotic was not totally effective. Those bottles in the dark with antibiotic should have lower oxygen concentrations than the previous set due to the demand from respiration of the algae and diatoms. The third set of bottles left in the dark with no addition of antibiotic should have the highest oxygen demand as a result of respiration of bacteria as well as diatoms and algae. From the histogram (FIG XIII) it can be seen that if each treatment from all the sites on each transect are added together, the trend of oxygen demand does follow this pattern, although individual sites do not always do so.

Fig XIII

HISTOGRAM OF OXYGEN DEMAND PER TREATMENT INSIDE AND OUTSIDE SPARTINA

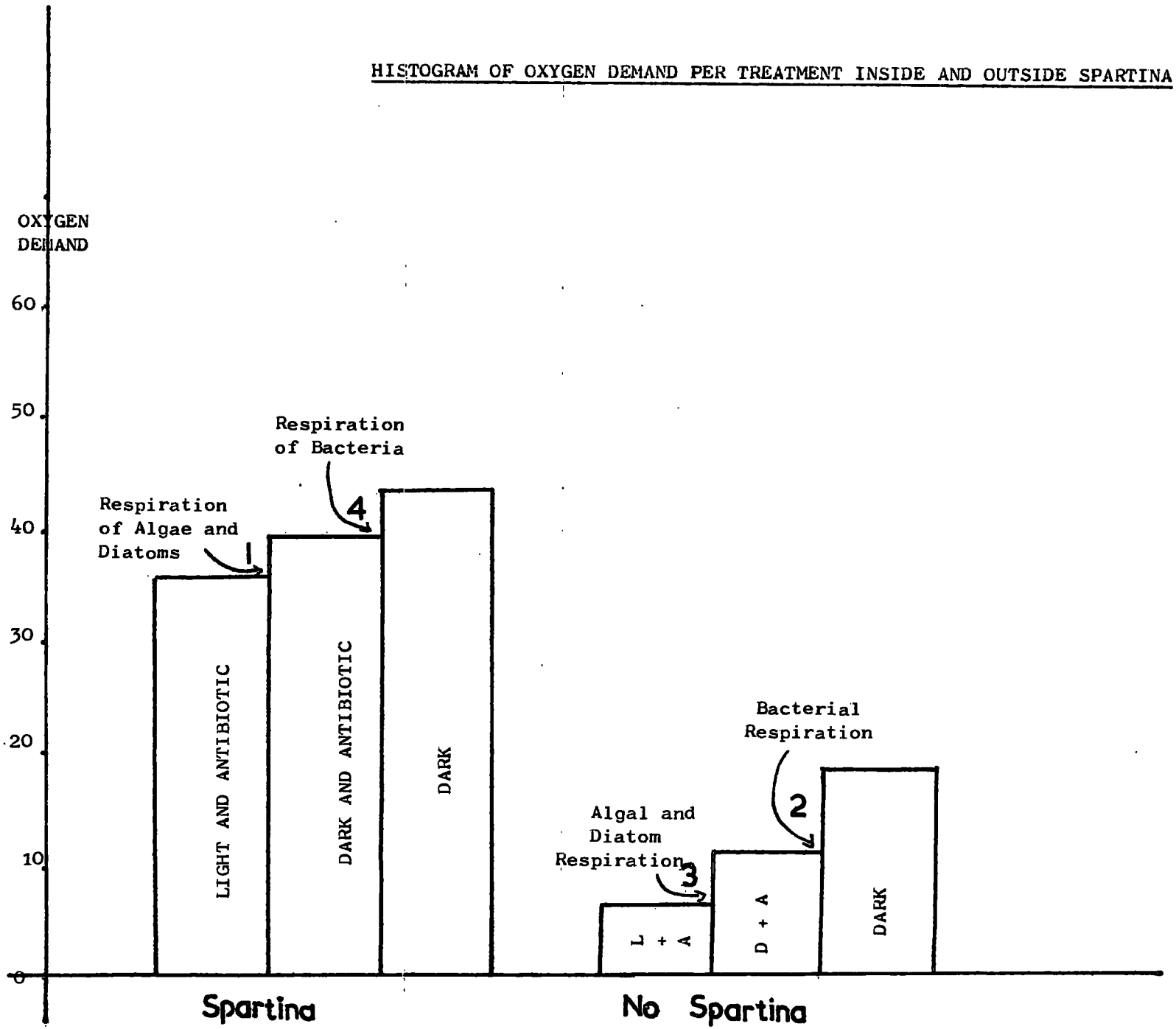
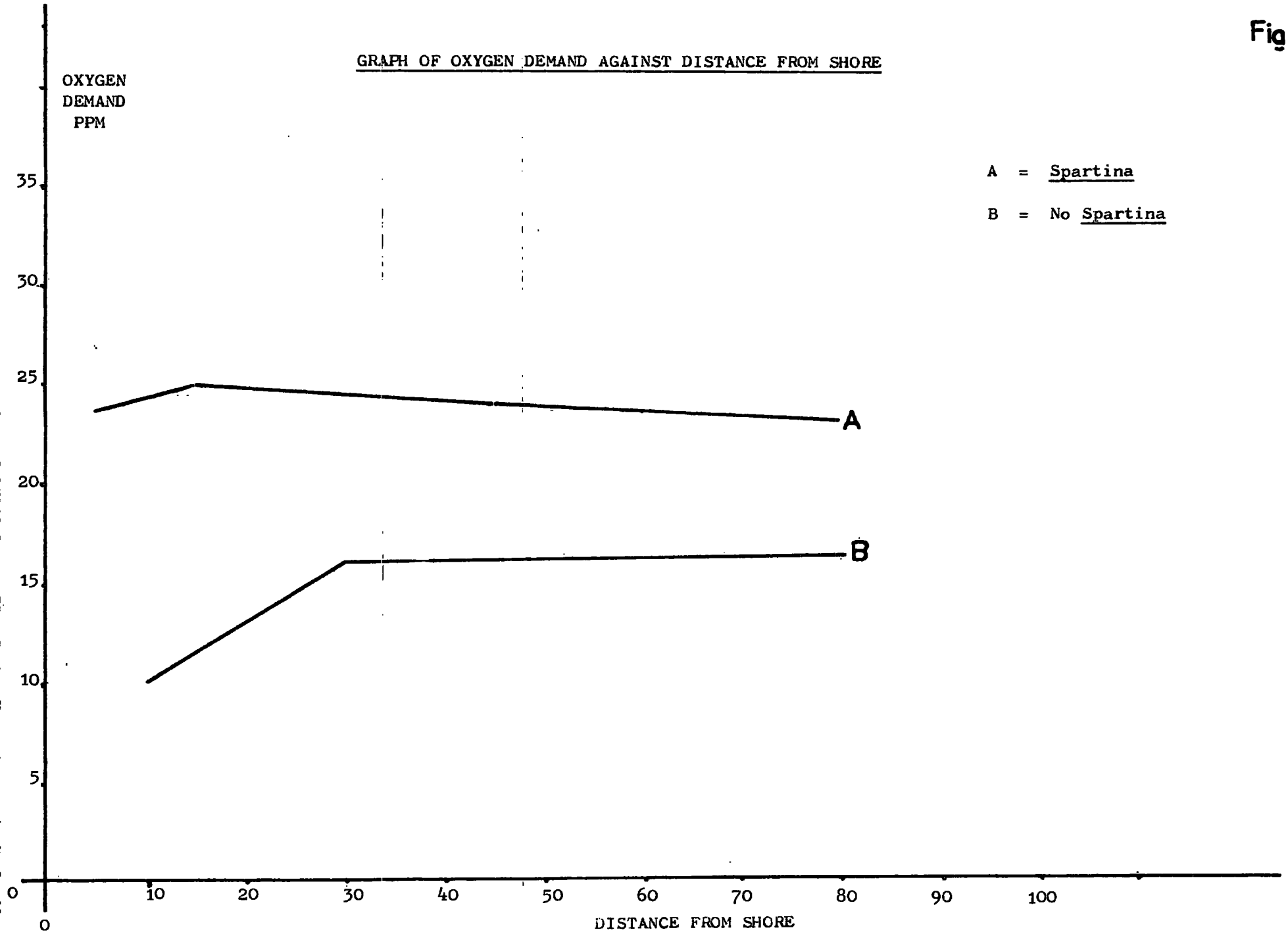


Fig XIV

GRAPH OF OXYGEN DEMAND AGAINST DISTANCE FROM SHORE



Perhaps the most striking aspect of these results is the marked difference in oxygen demand between those samples from the Spartina transect and those from the area where there is no Spartina (FIG XIV). Also, there is a trend for the oxygen demand along the Spartina transect to decrease with distance from land, although this decrease is not as pronounced as one would have expected from the bacterial counts.

Thus the attempt to relate oxygen demand to the bacterial and diatom results was reasonably satisfactory, although more attempts would have to be made to obtain more reliable results.

The fact that the antibiotics do not seem to have been totally successful is not surprising, since the samples contained many millions of bacteria, many of which are aggregated or absorbed onto sand and detrital matter. Hence even though 90% of the bacteria might have been destroyed or unable to synthesise new material, if there were ten million bacteria present originally, this leaves a million still viable. These would exert a considerable oxygen demand and could divide and increase by a large amount. One is not able to shake the sample over much as this could well affect the bacteria, algae and diatoms and result in an even greater error. Hence direct counts of bacteria and diatoms are preferable to BOD measurements which are subject to greater inaccuracies.

3.10 An investigation into the relative importance of detritus and and bacteria in the diet of Corophium volutator

The object of this experiment was to assess how important detritus and bacteria were in the diet of Corophium. This was to be done by feeding Corophium with combinations of the two foods in otherwise controlled conditions. After several days the Corophium were removed and their calorific values measured after various treatments.

METHOD

The Corophium were obtained from Beal Point at Lindisfarne. They were present in large numbers in the sandy areas between Spartina clumps. Sand samples were sieved and the Corophium picked out and placed in bottles containing a layer of fine sand and a layer of seawater. Nine hundred and fifty Corophium were collected in this way and transported back to Durham where they were transferred to 16 small tanks. These were given eight different treatments with two tanks for each treatment.

The tanks contained the following:

A1 + 2 - Sterile sand and sterile seawater

B1 + 2 - Sterile sand and non-sterile seawater

C1 + 2 - Sterile sand and sterile seawater

D1 + 2 - Sterile sand and sterile seawater

E1 + 2 - Sterile sand and non-sterile seawater

F1 + 2 - Sterile sand and non-sterile seawater

G1 + 2 - Non-sterile sand and non-sterile seawater

H1 + 2 - Non-sterile sand and sterile seawater

The sand covered the bottom of the tanks to a depth of 4 cm and the water to a depth of 6 cm above the sand. The tanks were kept continuously aerated by a system of rubber tubes running into them.

The Corophium were divided up into sets of fifty individuals and one set of fifty was put into each tank. An effort was made to try to avoid picking all the largest in one set and as near an equal size distribution as

possible was attempted.

Sterile sand refers to sand which has been washed thoroughly and then sterilised in the autoclave for 45 minutes at 130°C, then rewashed and again sterilised at 130°C for 45 minutes. This washes out most of the organic matter present and kills any bacteria. Sterile water refers to seawater which has been sterilised at 121°C for twenty minutes. Thus, by using these methods one can control the detritus and bacteria available to the Corophium as closely as possible.

The Corophium were fed every two days with either detritus (or obtained from the floor of a wood), sterile detritus (treated at 121°C for twenty minutes) or live or dead bacteria. The bacteria were killed by autoclaving for twenty minutes at 121°C.

The tanks were fed as follows:

A - None (Control)

B - None (Control)

C - Sterile detritus

D - Sterile detritus and dead bacteria

E - Non-sterile detritus and live bacteria

F - Live bacteria

G - Sterile detritus

H - None

The detritus was added in 4 gm (dry weight) quantities and the bacteria in 5 ml quantities. Both the live and dead bacterial solutions contained about 6.75×10^6 bacteria per ml.

The original intention had been to leave the experiment for about 21 days before removing the Corophium and finding their calorific values. However, after only 12 days it was noticed that the Corophium activity in some of the tanks had either ceased altogether or decreased so the tanks were carefully emptied and the remaining living Corophium were removed.

Many of the tanks had no living Corophium in them, so the dead Corophium and parts of them were also removed. However, the number of whole animals was low and the pieces may have come from several animals so it was considered not worth determining their calorific contents since different parts of a Corophium may have different calorific values. Calorific contents were measured in a Ballistic Bomb Calorimeter. (See Appendix for Method and Calibration Curve.)

RESULTS

SET NUMBER	NUMBER OF COROPHIUM REMOVED	GALVONOMETER READING	CALS.(FROM CALIBRATION) SEE APPENDIX	DRY WEIGHT BURNT GMS.	CALS.PER GRAM.DRY WEIGHT
1	26	2.9	0.187	.0257	7.2762
2	62	4.7	0.3375	.0836	4.037
3	29	2.8	0.1775	.0426	4.166
4	0	-	-	-	-
5	5	-	-	-	-
6	2	-	-	-	-
7	81	5.7	0.4325	.1152	3.750
8	74	5.0	0.3725	.1516	2.457

CONCLUSIONS

From the table it can be seen that three of the treatments yielded such low numbers of Corophium that it was not possible to determine their calorific values. These three were the only sets to have been treated with additional bacteria, either live or dead, which apparently had some adverse effect on the Corophium. Since the dead bacteria had the same effect as the live it is probable that the Corophium were killed because the bacterial cultures used (which were in seawater) had been allowed to grow until they became toxic. Possibly the populations had reached the death phase of the growth curve and as a result were becoming toxic

due to lysis of dead bacteria and the production of toxins by the living bacteria. The media had been enriched with nutrient agar and this may have increased the production of toxins.

The calorific values per gram obtained in the remainder of the cultures are difficult to understand. The quantities burnt were too small to give accurate results but the highest calorific value per gram is obtained in the treatment in which not only was no food added, but also the sand and water were sterile.

The results obtained are related to the size of sample. The samples with the lowest number of Corophium gave the highest apparent calorific value per gram and vice versa. Therefore, it would seem that the determinations based on the highest numbers of Corophium are the most accurate and those with only small numbers, very inaccurate.

If this experiment were to be repeated there are two alterations which should be made to the technique.

a) The use of much larger numbers of Corophium and

b) the addition of bacteria which had not reach the 'death' phase, but were in the 'growth' or the start of the 'stationary' phase and had therefore not begun to build up toxic compounds in the medium in which they were living.

3.11 The numbers of bacteria in seawater

Many invertebrates depend almost entirely on the filtration of seawater for obtaining their food. Therefore, it is of interest to know how much bacterial food is available in seawater. This section reports results from samples of seawater which were plated to find approximate numbers of bacteria present. So few samples were taken that it is not possible to generalize from the results; however, they should be accurate and certain trends can be gained from them.

The first sample was taken on 6 June from a depth of about 30 cm in a total water depth of about 60 cms. This gave a count of 530 bacteria per ml.

The second set were taken on 27 June at three different sites: firstly, at the surface of the seawater, giving a count of 1090 bacteria per ml; secondly, just above the surface of the sediments, at a depth of about 60 cms and this gave a count of 2270 bacteria per ml. The third sample was taken at the front of the incoming tidal current and this gave a count of 3505 bacteria per ml.

Thus, seawater can contain up to 3,500 bacteria per ml in calm conditions. Waters in which wave action is more fierce will possibly contain more due to the abrasion by the sea washing the bacteria off particulate matter.

There seems to be an increase in bacterial numbers nearer to the sediments. Whether this gradient is related to the bacterial content of the sediment over which it was taken cannot be established. It would seem from these results that the bacterial content of seawater is relatively low and large volumes of water would need to be filtered before enough were obtained to supply sufficient energy to support an invertebrate.

These results correspond fairly well to results obtained by W E Krumbein (1971) and the results obtained for seawater were well within the range of values quoted in the literature.

3.12 The importance of sewage bacteria on the estuaries

Throughout the bacterial counts on the sediments, the sediments were continuously tested for any coliforms which may have been present in them. All these tests proved negative, thus showing that the numbers of coliforms present in the sediments were very low and unimportant in relation to the large viable counts obtained.

This perhaps was not entirely unexpected since the inflows to the estuaries contained only very low numbers of coliforms. However, the August coliform count at Waren Mill was high (See Part Two) and it may have been expected that at least some of these might have appeared on the sediments. The reason for their lack of appearance could be that all the sediments examined at Budle Bay were taken some distance from the path of the stream as it flowed through the bay. Coliforms might have become more numerous nearer to this stream, in which case, they might have played an important role in the nutrition of some invertebrates in some parts of the estuary.

It is possible that the flow of the Waren Mill stream is not high enough to overcome the very high dilution factor involved when it reaches Budle Bay when the tide is in. The high dilution experienced by the stream may result in dispersal of the coliforms to such an extent that they are present only in very low numbers in the seawater. It would have been of interest to have performed a presumptive coliform test on the sea-water.

It must be remembered that the coliforms do not live for more than one day or perhaps two in saline conditions. Therefore, non-detection may be due to their having died or become non-viable, even though they may be present in fairly high numbers, they would therefore not be detected. Perhaps it would be possible to detect the dilution factor and the dispersal patterns in the bay using a non-coliform bacteria, present in the inflow in high numbers, which was able to live for some time in the

saline conditions. Differential tests could then be made in the sediments and the seawater for this bacteria. As long as the bacteria was not present in seawater, nor a normal constituent of the sediments, one could find the likelihood of the coliforms being present but non-viable.

The use of radio active tracers would not be possible in these circumstances, due to the large area involved and the large doses which would have to be used.

However, on present evidence it would seem that the sewage does not play a very important role in the food chain at Lindisfarne, although its importance may be higher at Budle Bay.

3.13 Additional point of interest

During the calculation of bacterial numbers for samples taken on 6 June, it was noticed that the first sample taken (1) had much higher numbers of bacteria present than any other samples. It was decided to examine if there were any differences between the growth rates of the bacteria from this first sample and from the other samples taken in the river. All the samples had been counted at intervals over the incubation period of 21 days.

RESULTS

The counts after 8 days were taken and the average of the counts at sites 2, 3, 4 and 5 was calculated.

SITE	8 days x 10^6 (Average)	21 days x 10^6 (Average)	% GROWTH after 8 days	% GROWTH for next 13
1	6.95	7.5	92.7%	7.3%
2, 3, 4, 5	2.77	4.4	63.0%	37.0%

The percentage of the total growth after eight days was then calculated, as well as the percentage of total growth for the remaining thirteen days. This showed a marked difference in the rates of growth. The sample from Site 1 had a growth rate far in excess of those of the remaining samples over the first eight days, whereas in the next thirteen days, the position was reversed.

CONCLUSION

This indicates that the bacteria at Site 1 were of a different type to those in the remining sites. This could well be due to a high number of soil bacteria and other saprophytic bacteria which have a higher growth rate than those normally found in seawater and the sediments associated with it. Therefore, one can conclude that perhaps the tidal seawater does not reach Site 1 and as a result a large number of bacteria which would be killed in seawater are able to survive and hence the larger number of bacteria which are present.

4.1 DISCUSSION

Many of the problems and points of interest encountered in this project have been discussed in the foregoing sections. One point which has not been mentioned however, concerns anaerobic bacterial counts.

The results obtained for the anaerobes show that in most cases they correspond in numbers fairly well with the aerobic counts at the same sites. However, I have not included the anaerobes in the discussions for two main reasons. Firstly, the method I used for the isolation of anaerobes, although certainly not allowing any facultative aerobes to grow, certainly did not stop the growth of micro aerophiles. This was evident from the increase in the number of colonies which formed near the top of the agar column in the test tube, where there would have been more oxygen. Therefore in calculating the anaerobic counts I would have included large numbers of bacteria which may have already been counted in the aerobic isolation, thus giving a false picture of the numbers of anaerobes present.

The second reason for not using the anaerobe counts is that after the tubes had been incubated for some time, the colonies growing in the agar began to produce gases (showing that at least some anaerobes were present). These gases resulted in the agar being broken up into small chunks, full of bubbles. This made it quite impossible to count the number of bacterial colonies inside the agar as they were obscured by the air bubbles. This led to very erratic differences in the counts of the same tube from one occasion to the next.

Due to these large inaccuracies I decided not to use the anaerobes in the calculations.

Another parameter which I have ignored in my correlations is the tidal level at which the sampling sites were situated. This would be of importance in deciding for how long the invertebrates, especially the

Mollusca and Annelida, would be able to feed in twenty-four hours. This factor should not be overlooked when discussing the viability of the results.

As has already been mentioned more work should have been done on the sediment composition of the estuary and this should have been correlated with the other factors. It would have been especially interesting to have seen how closely the bacterial numbers correlated with the finer sediment particles and their abundance in different sediments.

The bacterial counts obtained and used in the analyses show only a very small absolute range from the low values to the high ones eg. in the range 1.0×10^6 to 6.5×10^6. However, if the number per gram is calculated, (see Method), one obtains readings in the range 5.0×10^9 to 32.5×10^9 which would suggest a more realistic difference between the sediments.

4.2 SUMMARY

1 The influx of sewage material into the Lindisfarne estuary is fairly low all the year round, although there is an increase during the summer. Much of the sewage that does enter seems to be of animal as well as human origin. The part this plays in the ecology of the estuary is probably fairly small. At Budle Bay, however, the influx is much higher, especially during the summer and it is possible that this plays an important role in the ecology of certain areas of the estuary, though not as viable bacterial food for the invertebrates.

2 The depth at which the anaerobic biome begins is correlated with the number of bacteria in the upper sediments.

3 The bacterial content of the upper sediments does not seem to be a major factor in determining the densities of invertebrates in the sediments below, although its importance may differ from one species to another.

4 The depth of the anaerobic layer does not seem to play a major role in determination of invertebrate densities.

5 About 50% of the nitrogen in the sediments is derived from viable aerobic bacteria.

6 The raised bacterial content of the sediments found within Spartina swards continues for about 50 metres outside the sward.

7 Spartina detracts from the productivity of the estuary by covering large expanses of the inter tidal zone, thus decreasing the productivity of these areas, as far as wading birds feeding is concerned, and having little effect for very far outside the sward in respect to increasing available food for the invertebrates.

8 Diatom numbers are very high in the estuary and have been largely overlooked as a source of nutrition for the invertebrates.

9 There would seem to be an increase in the bacterial content of seawater as one nears the bottom sediment.

4.3 APPENDIX

1 Zobells 2216E Medium

5 g Bacto peptone

1 g Yeast extract

0.01 g $FePO_4$ x $4H_2O$

15 g Bacto agar

750 ml aged seawater

250 ml distilled water

pH 7.6 - sterilised at 121°C for 15 minutes

For anaerobic culture add 8.4 gm per litre D Glucose

2 MacConkey Bile-Salt Lactose Peptone Medium

used for detecting coliforms in water

Sodium taurocholate	5 g
Peptone	20 g
Sodium chloride (NaCl)	5 g
Lactose	10 g
Bromocresol purple 1% Solution in Ethanol	1 ml
Water	1 litre

(Obtained commercially)

APPENDIX

3 Brilliant Green Bile Broth

used to isolate Escherichia coli

Ox bile: 20 g of dehydrated Ox bile in 200 mls water, adjusted to pH 7.0 to 7.5

Preparation

Peptone	10 g
Ox bile	200 ml
Lactose	10 g
Brilliant green 0.1% aqueous solution	13 ml
Water to	1 litre

(Obtained commercially)

4 Sodium Azide Medium

used for the isolation of Streptococcus faecalis

Peptone	10 g
Sodium chloride (NaCl)	5 g
Dipotassium hydrogen phosphate (K_2HPO_4)	5 g
Potassium dihydrogen phosphate (KH_2PO_4)	2 g
Glucose	5 g
Yeast extract	3 g
Sodium azide (NaN_3)	0.25 g
Bromocresol purple 1.6% solution in ethanol	2 ml
Water to	1 litre

APPENDIX

INSTRUCTIONS FOR USING THE BALLISTIC BOMB CALORIMETER

1 Make pellet (about 0.3 - 0.4 gm) and weigh accurately.

2 Put pellet in crucible on end of standard 2 inch length of cotton, which passes to hole in ignition wire.

3 Check that rubber washer is seated correctly on the bomb base.

4 Screw on bomb carefully till hand tight (avoid damaging rubber seating ring).

5 Close oxygen control on bomb unit.

6 Open oxygen cylinder.

7 Open oxygen control on bomb unit slowly to flush the bomb through with oxygen.

8 Close gas escape valve on bomb base to let pressure rise slowly in bomb (to 26 atmosphere).

9 Close oxygen control valve on bomb unit and check that pressure in bomb holds steady at 25 - 26 atmospheres.

10 Plug thermocouple into top of bomb casing.

11 Switch on mains switch on bomb unit. Unclamp galvanometer and zero it.

12 Press Fire button: stand back.

13 Read maximum galvanometer deflection and record this value.

14 Clamp galvanometer; turn off mains switch on bomb unit.

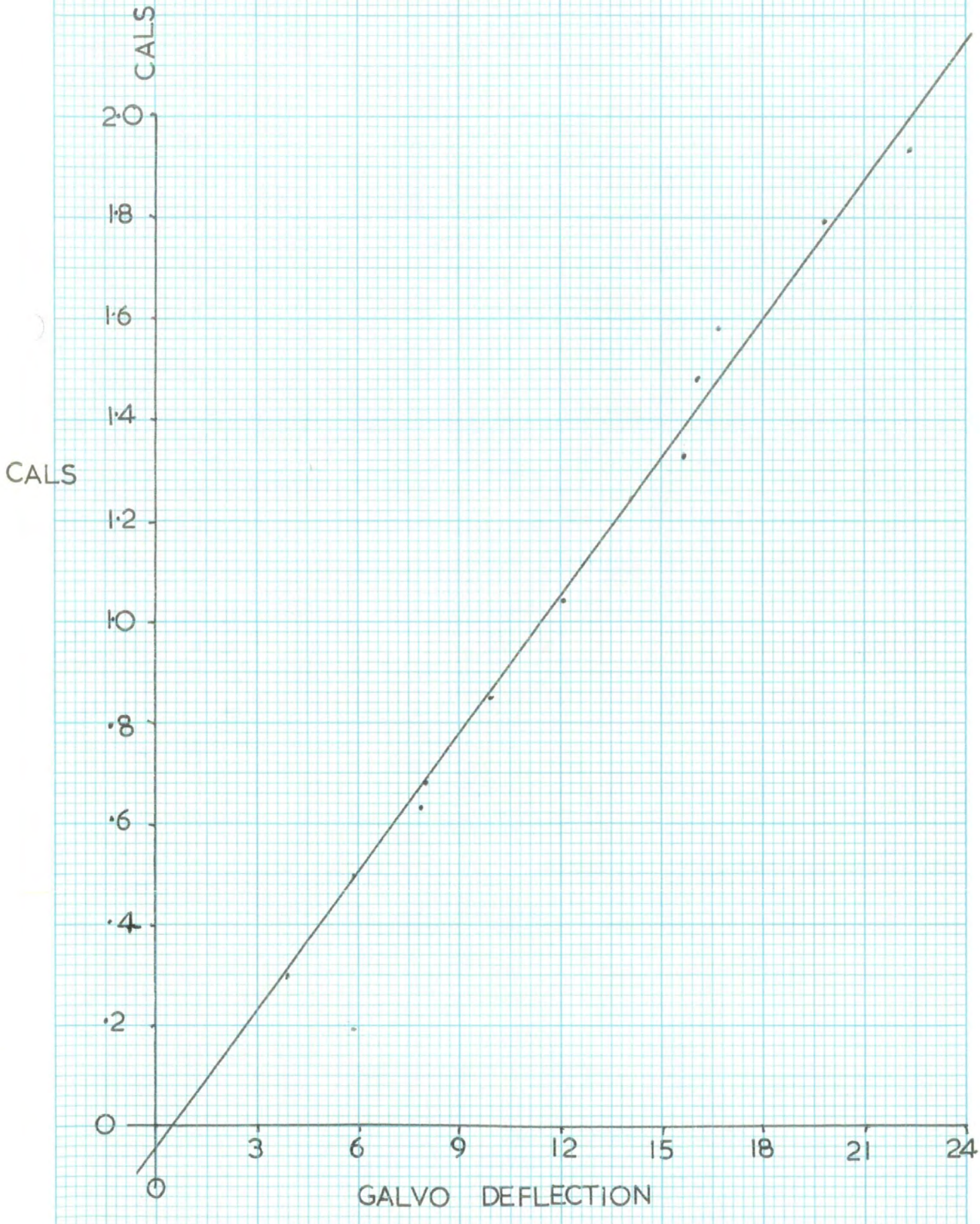
15 Open gas escape valve and release pressure in bomb.

16 Unscrew bomb and rinse with cold water: allow to drain dry or wipe with filter paper.

At the end of a set of determinations of calorific values turn off the oxygen cylinder.

CALIBRATION FOR THE BOMB CALORIMETER

Fig XV



APPENDIX

The Winkler Method for the Analysis of Dissolved Oxygen

Manganese Sulphate Solution: Dissolve 480 gms $MnSO_4.4H_2O$ in distilled water and dilute to 1 litre.

Alkali-Iodide-Azide reagent 'POISON': Dissolve 500 gms NaOH

135 gms NaI

in distilled water and dilute to 1 litre.

Add 10 gms Sodium azide (NaN_3)

Concentrated Sulphuric Acid: 36N H_2SO_4

Starch Solution: Disperse 1 gm of starch in 100 mls of distilled water and warm to 80^o to 90^oC. Stir well, allow to cool and add 0.1 gms salicylic acid.

Stock Sodium Thiosulphate solution (0.10N): Dissolve 24.82 gms sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in distilled water and dilute to 1 litre. Add 1 gm NaOH to preserve.

Standard Sodium Thiosulphate Solution (0.0250N): Dilute 250 mls of the stock solution to 1 litre, or dissolve 6.205 gms sodium thiosulphate in distilled water and dilute to 1 litre.

Method

To the sample as collected in a 120 ml bottle, add 1 ml manganese sulphate solution followed by 1 ml Alkali-Iodide-Azide reagent, well below the surface of the bottle. Stopper with care to avoid air bubbles and mix by inverting the bottle several times. Let the precipitate settle and wait for a further ten minutes, then shake again. When settling has produced more than 75 mls of clear supernate, remove the stopper and immediately add 1 ml concentrated sulphuric acid by allowing the acid to run down the neck of the bottle. Restopper and mix by gentle inversion until dissolution is complete. The iodine should be evenly distributed throughout the bottle before pipetting the amount needed for titration.

Pipette 100 mls of the resulting solution into a conical flask and titrate with 0.025 sodium thiosulphate to a pale straw colour. Add 1 ml of freshly prepared starch solution and continue the titration until the blue colour is just discharged.

Calculation

In the titration 100 mls of resulting solution are taken, not the 100 mls samples. Thus the actual volume of the sample must be calculated. Now, 2 mls of reagent are added to the bottles which originally contain 120 mls of sample. Thus the volume left is 120 - 3 = 117 mls. Therefore 120 mls solution contains 117 mls sample and also, therefore, 100 mls contains 97.49 mls sample.

If X mls of YN sodium thiosulphate are used in the titration and since 1 ml of YN sodium thiosulphate ≡ 8Y mgms dissolved oxygen,

Then, 97.49 mls sample contain 8XY mgm oxygen

$$100 \text{ mls of sample contain } \frac{8XY}{97.49} \times 100 \text{ mgms oxygen} = 8.20(XY) \text{ mgm Oxygen (PPM)}$$

From this calculation the oxygen demand of each bottle could be calculated.

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