A study of heavy metal contamination in two intertidal invertebrates

Sarah Priest

A Study of Heavy Metal Contamination in Two Intertidal Invertebrates

SARAH PRIEST

Submitted as part of the requirements for the degree of Master of Science (Advanced Course in Ecology) at the University of Durham

1975.

CONTENTS

INTRODUCTION

Although trace amounts of many metals are essential to life and growth of organisms, at high concentration they may become toxic. From an ecological standpoint it is necessary to know not only the levels which are lethal, but also those resulting in sub-lethal effects such as changes in morphology, growth rate, and behaviour. Since marine invertebrates are exposed to pollutants by a variety of routes, and are also of importance to Man as food, it has been suggested that they should be used to monitor Heavy Metal contaminants in the marine environment.

Problems arise in deciding which organism or parts of organisms to choose for analysis, so that contamination levels may be compared amongst different geographical locations. Further problems concern the number and type of animals which should be sampled at each location to obtain reliable indications of contamination levels, and the position of the sampling sites in the intertidal or sub-littoral zone. Some of these problems are considered in this dissertation.

Aquatic organisms may acquire Heavy Metals by absorption from solution through skin, gills and digestive tract, from particulate matter (ingested particles of food or the sediment in which they live), or by both routes. Thus for monitoring purposes, species should be chosen with regard to their feeding habits: filter feeders such as the lamellibranchs e.g. Mytilus spp must be submerged to feed and therefore have more opportunity to absorb metals from solution than particulate feeders which often live at higher tidal levels where they can feed even when the tide has receded. Generalist feeders such as the polychaete Nereis diversicolor may absorb heavy

metals from the sediment either through their cuticle or from ingested particulate matter.

A number of factors other than concentrations in the sea and sediment affect heavy metal uptake and accumulation in a marine invertebrate: the quantities acquired and concentrations reached may vary with body size and hence age, as found by Boyden (1974) for Mytilus and Mackay et al (1975) for Crassostrea commercialis. The position of an animal in the intertidal zone affects not only the period of immersion, but also the filtration rate as found by Segal et al (1953). Mytilus taken from sub-littoral sites filtered faster than those from higher intertidal levels. Rates of water transport also varied between populations of the same species taken from different latitudes. Obviously the heavy metal concentrations in the surrounding medium will determine the potential maximum levels in the tissues. However, the concentrations of some metals appear to be regulated in some aquatic animals by the regulation of the rates of absorption or of excretion. Bryan and Hummerstane (1973) present results which suggest that the concentration of Zinc can be regulated by Nereis diversicolor whilst that of Cadmium cannot. Of course the level of contamination of the surrounding aquatic medium may not be constant with time, or for a species which is mobile. Seasonal alterations in the rate of growth may result in differing concentrations of a given metal in the same animal within a short time; in particular a sudden spurt of growth could cause a reduction in tissue concentrations.

In monitoring heavy metal contamination, previous studies have tended to overlook the effect of these variables, but unless they are taken into account, comparisons between areas or dates may be meaningless. In most published papers, sizes or ages of animals

analysed have not been quoted, nor limits of variability estimated. Again, it is often not clear whether single animals or pooled samples have been analysed. This raises the problem of choosing representative samples from each study area. Variation within a population as a result of factors such as those outlined above makes it desirable for samples to be taken from different tidal levels and different age groups, at each site, for comparison with other sites.

The aims of this study were threefold: First, to make rigorous and detailed comparisons between Heavy Metal concentrations in animals from a polluted and a much less polluted site. This required (i) determination of appropriate sample sizes to give accurate estimates of mean contamination levels at each site, and (ii) investigation of the influences of their location in the intertidal zone on levels of metals accumulated. The animals chosen were Mytilus edulis, a sessile filter feeder, and Nereis diversicolor, a more mobile scavenger and predator, and chiefly a feeder on particulate matter. Second, to investigate morphological characteristics and growth in Mytilus edulis at different sites, since comparison of contamination levels in animals from different areas could be made on the basis either of animals of the same age but different sizes and weight, or of the same weight but different ages. Third, to carry out preliminary transplantation experiments with Nereis between polluted and relatively unpolluted sites, to look for any indication of the development of resistance to heavy metal toxicity in animals from the polluted site.

The study involved collection of animals from three areas:

Fig 1a

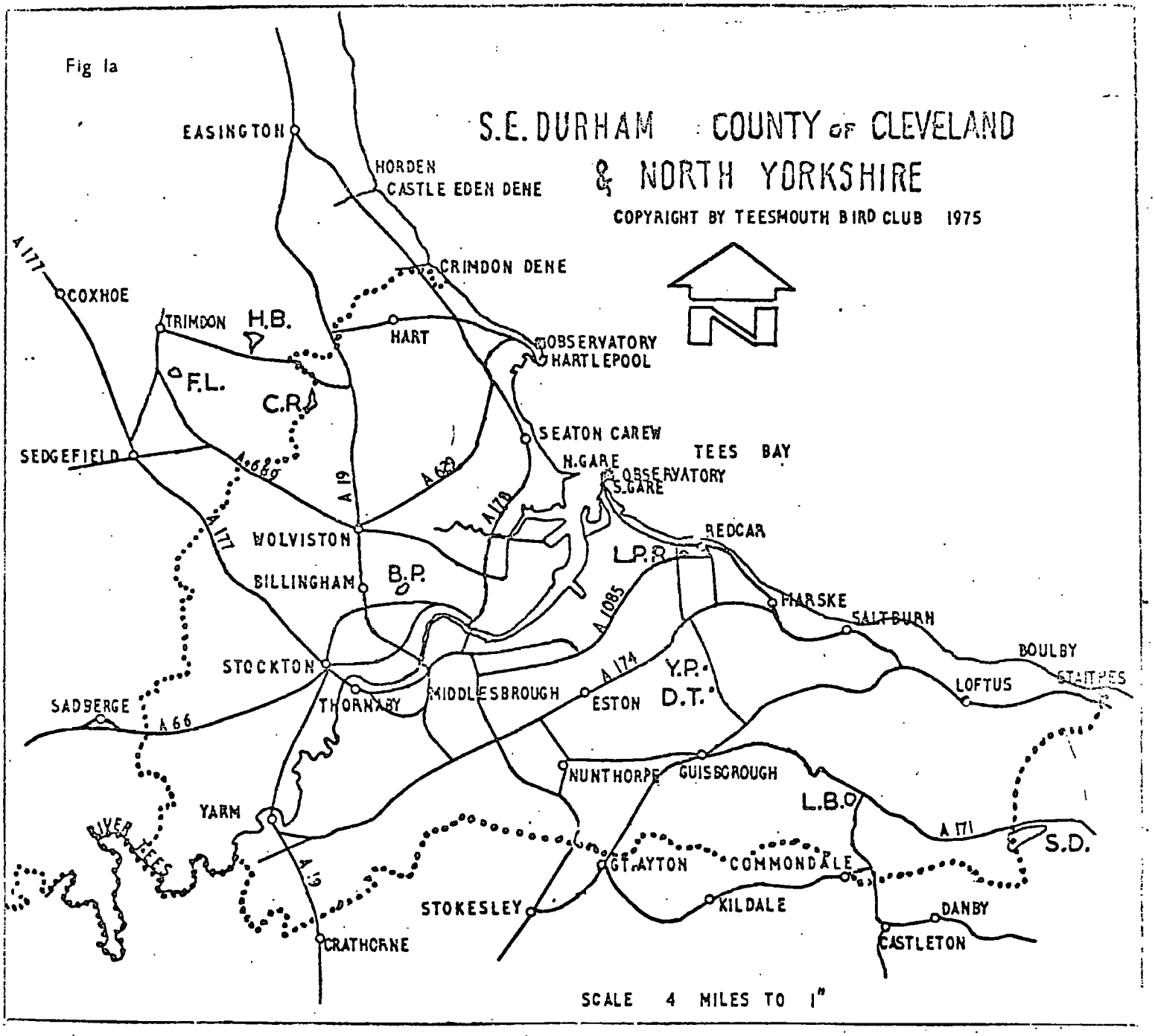

S.E. DURHAM & COUNTY OF CLEVELAND
NORTH YORKSHIRE
COPYRIGHT BY TEESMOUTH BIRD CLUB 1975
EASINGTON
HORDEN
CASTLE EDEN DENE
CRIMDON DENE
A 177
COXHOE
TRIMDON
H.B.
HART
OBSERVATORY
HARTLEPOOL
F.L.
C.R.
SEATON CAREW
TEES BAY
SEDGEFIELD
A 689
A 19
A 689
H.GARE
OBSERVATORY
S.GARE
A 178
A 177
WOLVISTON
REDCAR
L.P.B.
BILLINGHAM
B.P.
A 1085
HARSKE
SALTBURN
BOULBY
STOCKTON
A 174
Y.P.
D.T.
LOFTUS
SADBERGE
A 66
THORNABY
MIDDLESBROUGH
ESTON
NUNTHORPE
GUISBOROUGH
L.B.O.
YARM
A 171
S.D.
RIVER TEES
A 19
GT. AYTON
COMMONDALE
STOKESLEY
KILDALE
DANBY
CASTLETON
CRATHORNE
SCALE 4 MILES TO 1"

Fig 1b

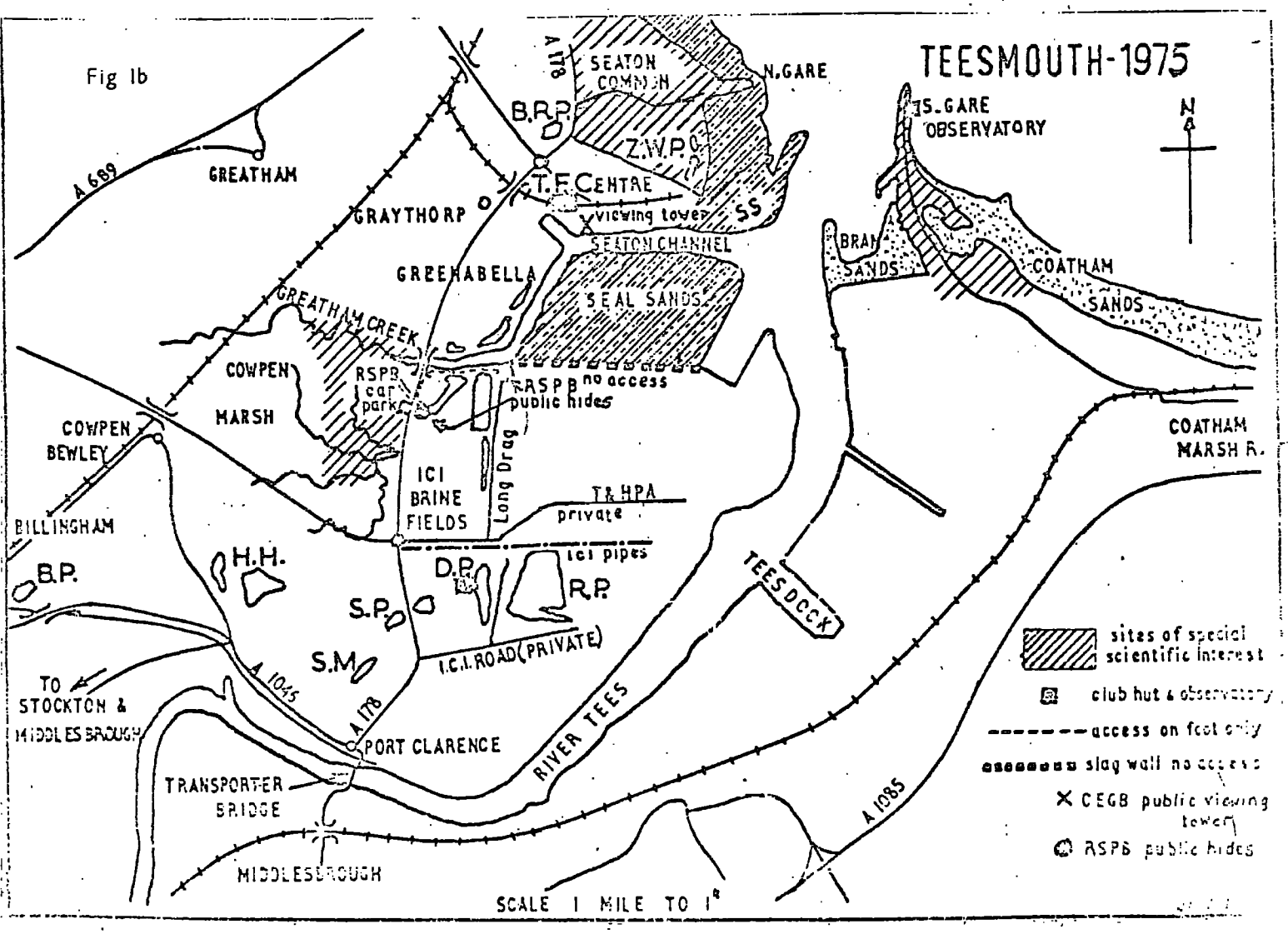
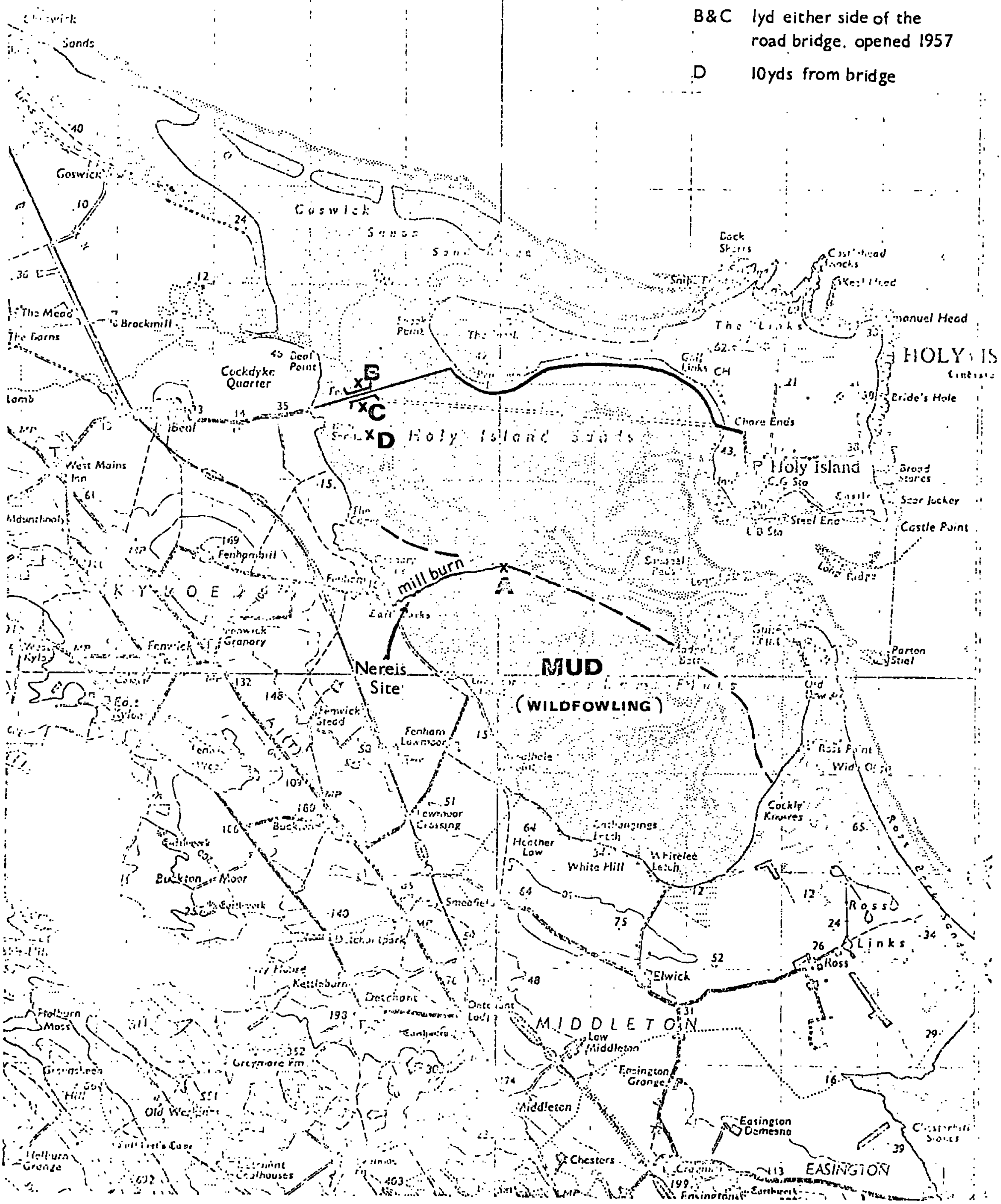

TEESMOUTH-1975
N
A 178
SEATON COMMON
N.GARE
S.GARE OBSERVATORY
B.R.P.
Z.W.P.C.
T.F. CENTRE
viewing tower
SS
A 689
GREATHAM
GRAYTHORP
SEATON CHANNEL
GREENABELLA
SEAL SANDS
BRAN SANDS
COATHAM SANDS
GREATHAM CREEK
COWPEN MARSH
RSPB car park
RSPB no access public hides
COWPEN BEWLEY
ICI BRINE FIELDS
Long Drag
T & HPA private
COATHAM MARSH R.
BILLINGHAM
B.P.
H.H.
D.P.
ici pipes
R.P.
S.P.
S.M.
TEES DOCK
I.C.I. ROAD (PRIVATE)
A 1046
A 178
TO STOCKTON & MIDDLESBROUGH
PORT CLARENCE
RIVER TEES
TRANSPORTER BRIDGE
A 1085
MIDDLESBROUGH
sites of special scientific interest
club hut & observatory
access on foot only
slag wall no access
CEGB public viewing tower
RSPB public hides
SCALE 1 MILE TO 1"

Fig Ic

Mytilus Sites: A

B&C lyd either side of the road bridge, opened 1957

D 10yds from bridge



1. Seaton Carew (Fig. 1a)

This small holiday resort on the Durham coast lies about three miles south of Hartlepool and two miles North of the Mouth of the River Tees. It is subjected to considerable levels of pollution, both atmospheric from the steel and petrochemical industries, and aquatic from industrial discharges into the river. The beach is broad and sandy, with two rocky scars supporting mussel beds. The more southerly of these was used in this investigation, and an old breakwater served as a useful transect from High to Low water marks.

2. Seal Sands (Fig. 1b)

This is an area of mud flats in the Tees estuary in process of reclamation. The particular site for the collection of Nereis was between the Seaton channel and a small stream, the resulting reduction in salinity being attractive to these animals. Possible sources of pollution on Teeside include industries such as battery manufacture, steel and galvanizing works, and production of pigments for paints.

3. Holy Island (Fig. 1c)

Fenham Flats and Holy Island Sands are tidal, and lie to the North of Ross links, Northumberland, between Holy Island and the mainland. The substrate in the Northern part of the area, Holy Island Sands, is mainly clean sand and firm sandy mud; Fenham Flats is softer and more muddy. The four sites A, B, C and D where Mytilus was collected are shown on the map (Fig. 2). A is in the middle of the flats, alongside Mill Burn, a popular wildfowling area; B and C lie one yard either side of the road bridge opened in 1957; D lies about ten yards downstream from this bridge.

The site at which Nereis was collected is also marked. It lies close to the shore near the Mill Burn and again is in an area where

wildfowling has been customary for many years.

Possible sources of pollution here are domestic wastes, drainage from road surfaces and agricultural land and lead pellets from shotguns. Effluents from metal industries on the North East coast probably do not exert any major influence as far north as Holy Island.

CHAPTER 1:

Growth and Population Age Structure of Mytilus edulis

Introduction

The effect of environmental conditions on the Common Mussel Mytilus edulis may be measured by studying its growth. Similarly the favourability of different localities may be inferred from comparisons of morphological characters. Using growth curves of shell length and Ring Number, Seed (1968) was able to show that in M. edulis growth rate slows with increased age, and changes with habitat. He also demonstrated changes in shell shape according to age and locality.

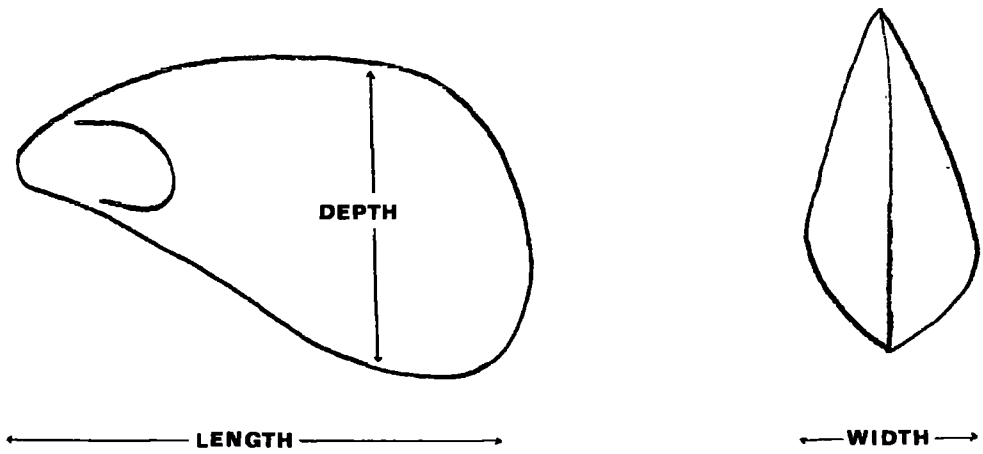
To compare heavy metal contents of animals from different areas, it may be necessary to choose specimens of different size and weight if the same age is to be obtained. Thus in addition to investigating differences in Heavy Metal concentration in animals from sites of rather different environmental conditions, morphological and size-distribution data were collected.

Measurement of Growth

Growth in molluscs may be measured either by an increase in dimensions, or by the number of Growth Rings on the shell. Such rings are characteristic of many kinds of mollusc and are indicative of diminished or arrested growth during the winter, possibly depending on water temperature and abundance of foodstuffs. Mossop (1922) and Mateeva (1948) showed these rings to be annual in M. edulis, but Coe and Fox (1942) could find no correlation between numbers of rings and age in M. californicus. Weymouth (1923) and Orton (1926) did not regard this a good method for age determination in M. edulis since it is possible for several rings to be produced each year as a result

of erratic seasonal changes in food and perhaps temperature. Seed (1968) found that in a normal year 90% of the total annual growth occurred between April and September, resulting in one major growth ring each year. He does acknowledge however that detection of such rings may be difficult as a result of shell erosion.

In the present study, the growth rings were obscure, possibly because the animals were taken from exposed shores. Instead, an index of growth was chosen, based on shell dimensions: the approximate area (2 x length x depth) of the shell was thought to be a more accurate measure of growth than length above, since rates of increase of width and depth also change with age. Area is more likely to be proportional to weight of shell than length, if shell of the same thickness is added each year.



1.1 Size Distribution of animals at different sites

(i) Seaton Carew

Originally twelve tidal levels were sampled, these being evenly spaced along a transect between the Low Tide Mark (site 1) and the High Tide Mark (site 12). All the mussels within a randomly-chosen

area of the rock were collected to give a representative sample for that tidal level. The size of the area sampled was chosen to give a sufficiently large sample. Each animal was measured with Vernier calipers whilst fresh, and the frequency distribution of shell area was plotted for each site, (Fig. 2). Most shells were less than 1 cm^2 in area. The accuracy with which these could be measured, counted and effectively collected was low. Thus further sampling was carried out, this time from four tidal levels; only mussels with shell areas greater than 1 cm^2 were collected, (Fig. 3).

When probability paper was used (Cassie 1954), no clear class separation could be achieved for any of the population sampled at tidal levels 1-12 or a-d.

Discussion

The size distributions at the twelve tidal levels originally chosen showed no marked differences. Changes in size distribution up the shore may have been obscured by the uneven yearly spatfall and/or survival rate, this being particularly characteristic of polluted environments on the Durham and Northumberland coast (Jones 1970). This is one situation in which complete year classes can be missing so that the size classes cannot be picked out on probability paper, nor the area index be related to age.

As mentioned above, both numbers and measurements of shells less than 1 cm^2 in area were unreliable, and so in a further analysis were ignored (Fig. 3). Site a, at the Low Tide Mark, shows a peak for the smallest size class measured (1-1.5 cm^2), the frequency falling roughly exponentially to the larger shell sizes. At the High Tide Mark (site d) the trend has changed, the numbers there being more evenly spread up to 3.5 cm^2, with a peak at 2.5-3 cm^2. The largest mussels, between 9 and 9.5 cm^2 were found at site b.

Fig. 2 Distribution of mussels, according to area, at Seaton Carew sites 1 (Low Water Mark) to 12 (High Water Mark). Each sample comprises about 100 individuals, and the frequency is expressed as a percentage.

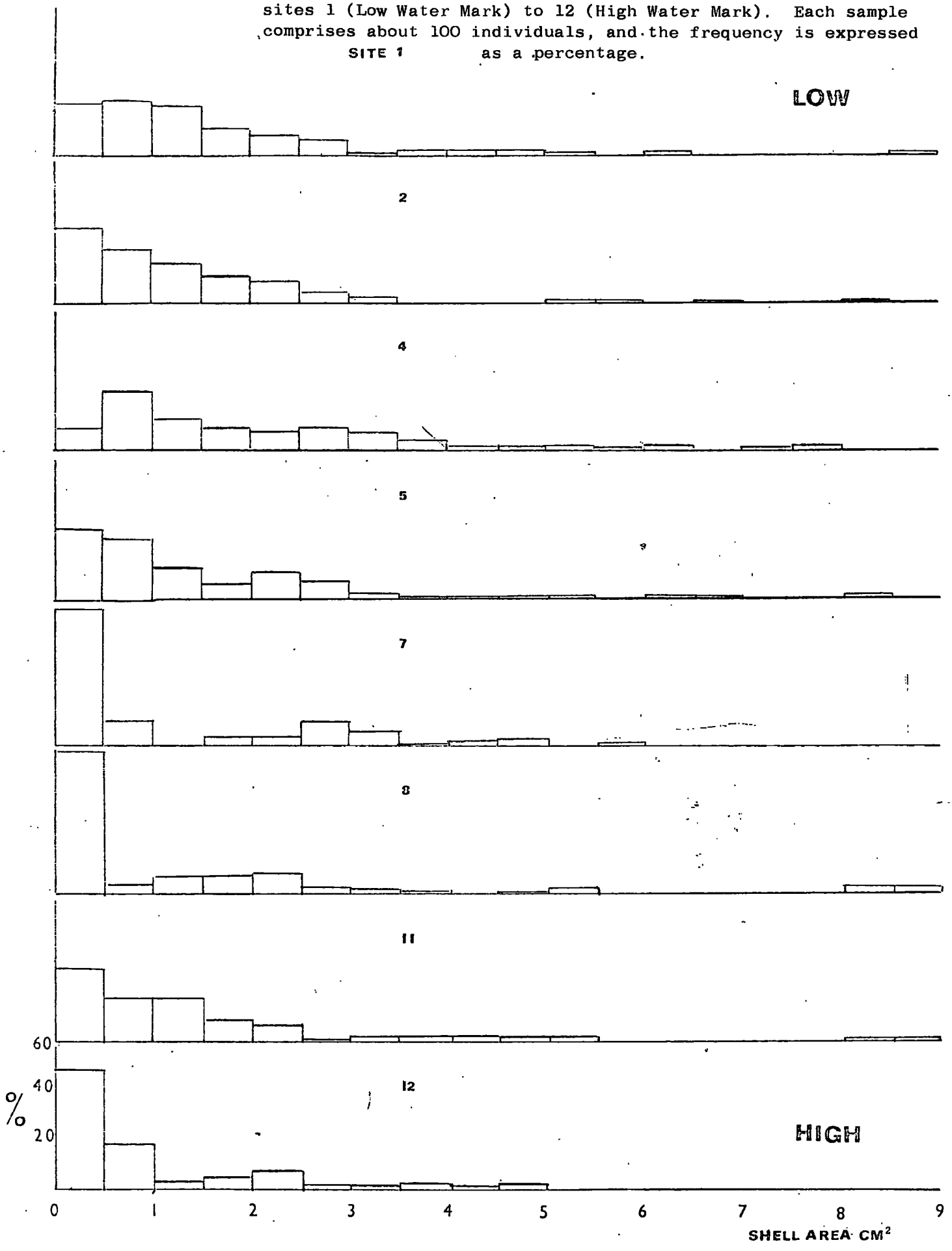
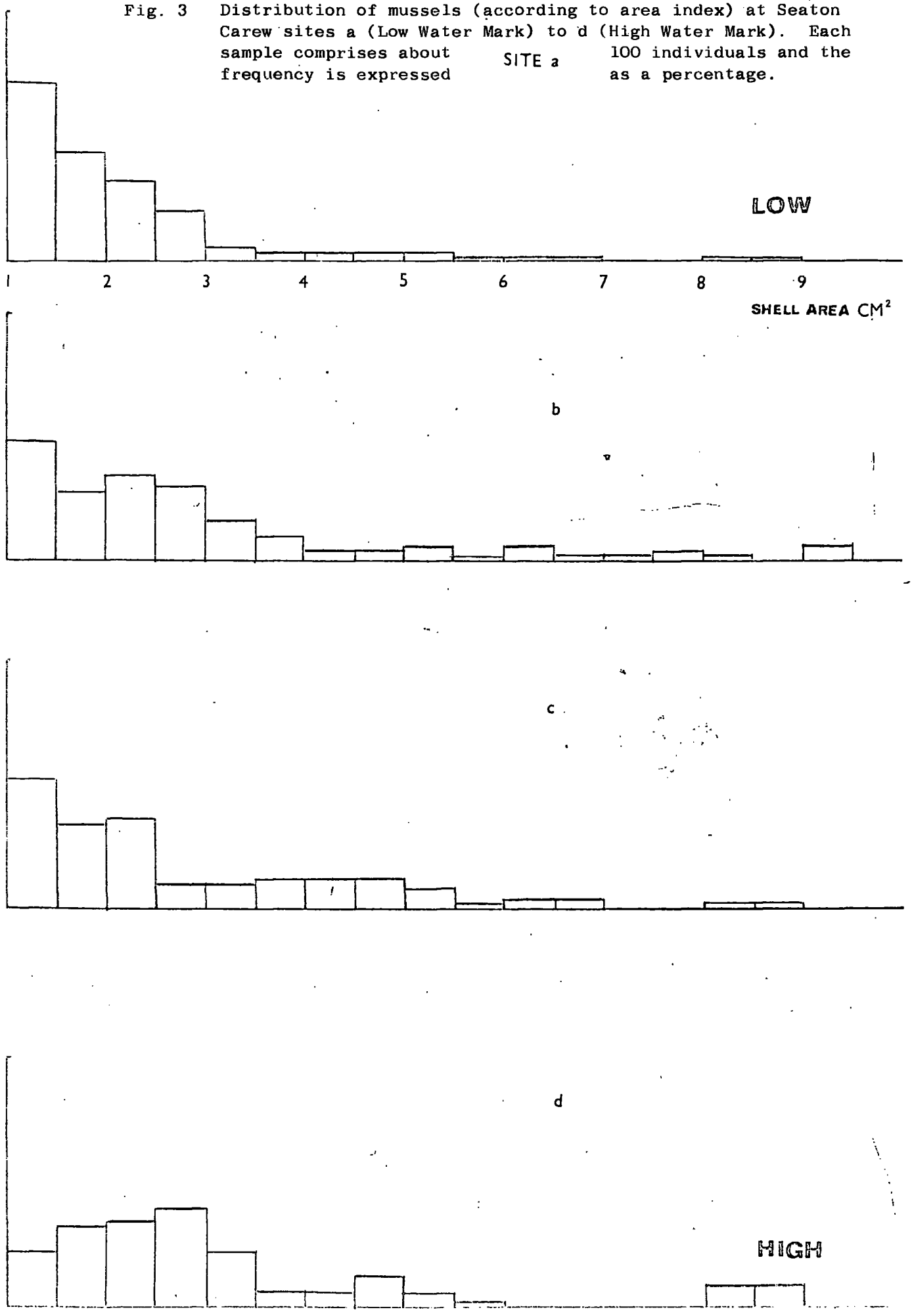


Fig. 3 Distribution of mussels (according to area index) at Seaton Carew sites a (Low Water Mark) to d (High Water Mark). Each sample comprises about 100 individuals and the frequency is expressed as a percentage.



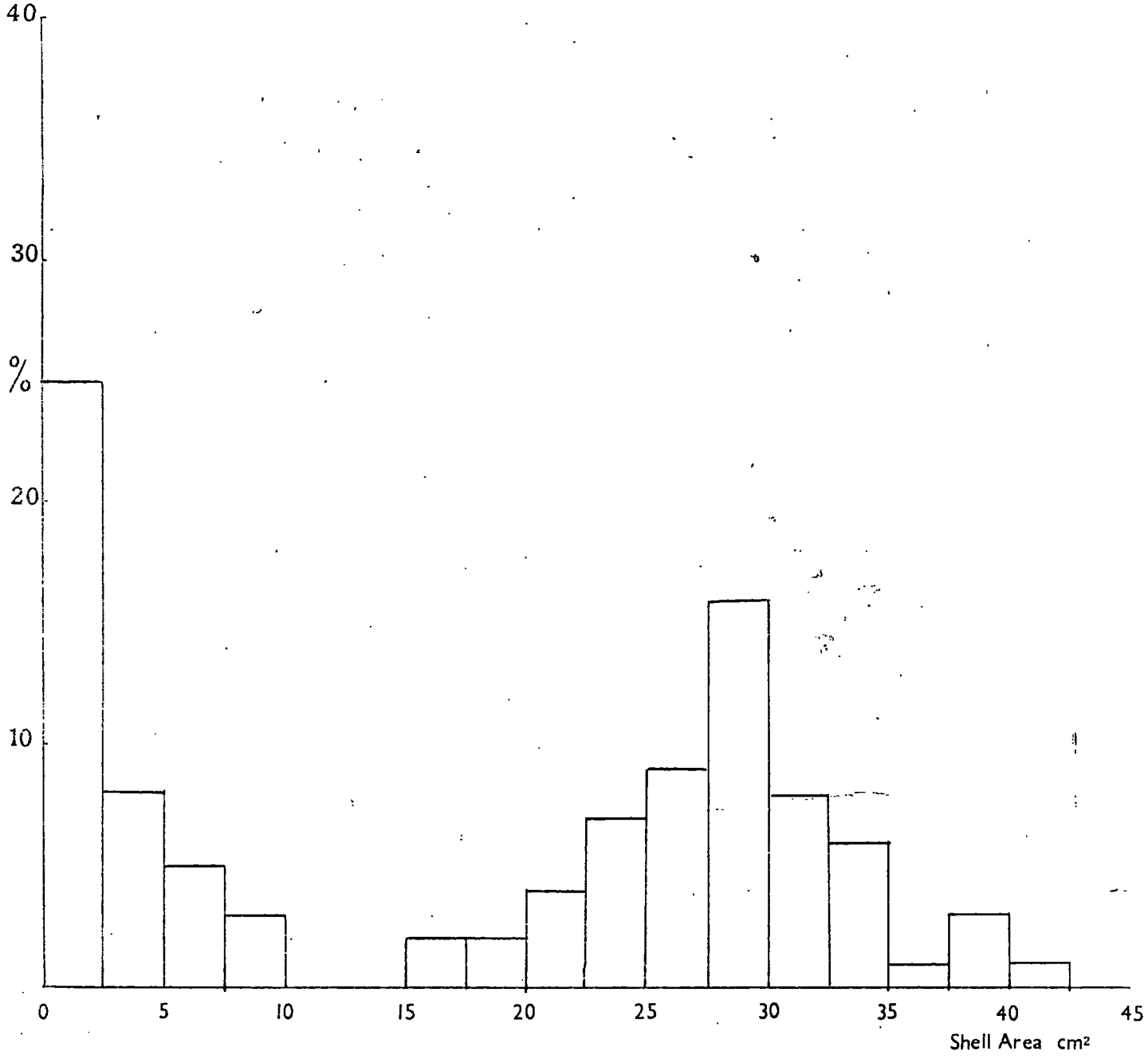
Predators such as Asterias rubens, Carcinus maenas and Cancer pagurus are more abundant low in the intertidal zone, this being one factor which may account for the observed size frequencies: fewer predators high on the beach would perhaps mean a more even distribution between the age classes. Smaller animals would be more susceptible to dehydration or lack of food if a series of low tides failed to cover them, and this too would select for the larger individuals at the High Tide Mark. Another factor which may be involved is wave action, which is less severe at high tidal levels.

(ii) Holy Island

A random sample of animals was collected from site A, close to the low-water level for neap tides. As before they were divided into size classes on the basis of an area estimate, and the results expressed in the form of a histogram (Fig. 4). The animals from this site were much larger, so the size classes were correspondingly expanded to cover ranges of 2.5 instead of 0.5 cm^2. Probability paper was used to separate sub-populations in the manner discussed by Cassie (1954). In this method, Percent Cumulative Frequency (PCF) is plotted against the mid-point of each size class, and points of inflection in the resulting curve used to determine boundaries between sub-populations. A point of inflection occurred at 41% PCF (Fig. 5) implying that the smallest group comprises 41% of the population. Multiplication of each PCF value (x) below this point of inflection by $\frac{100}{41}$, and those between 41 and 100 by $(x - 41) \times \frac{100}{(100 - 41)}$ resulted in the straight lines shown, these representing the two sub-populations of the total.

The mean for each is found using the 50% PCF value from the straight lines, and the Standard Deviation by subtracting the mean value from the size at 84.13%. (Table 1).

Fig. 4 Distribution of mussels, (according to area index) at Holy Island site A, from a sample of 112 individuals. (The frequency is expressed as a percentage).



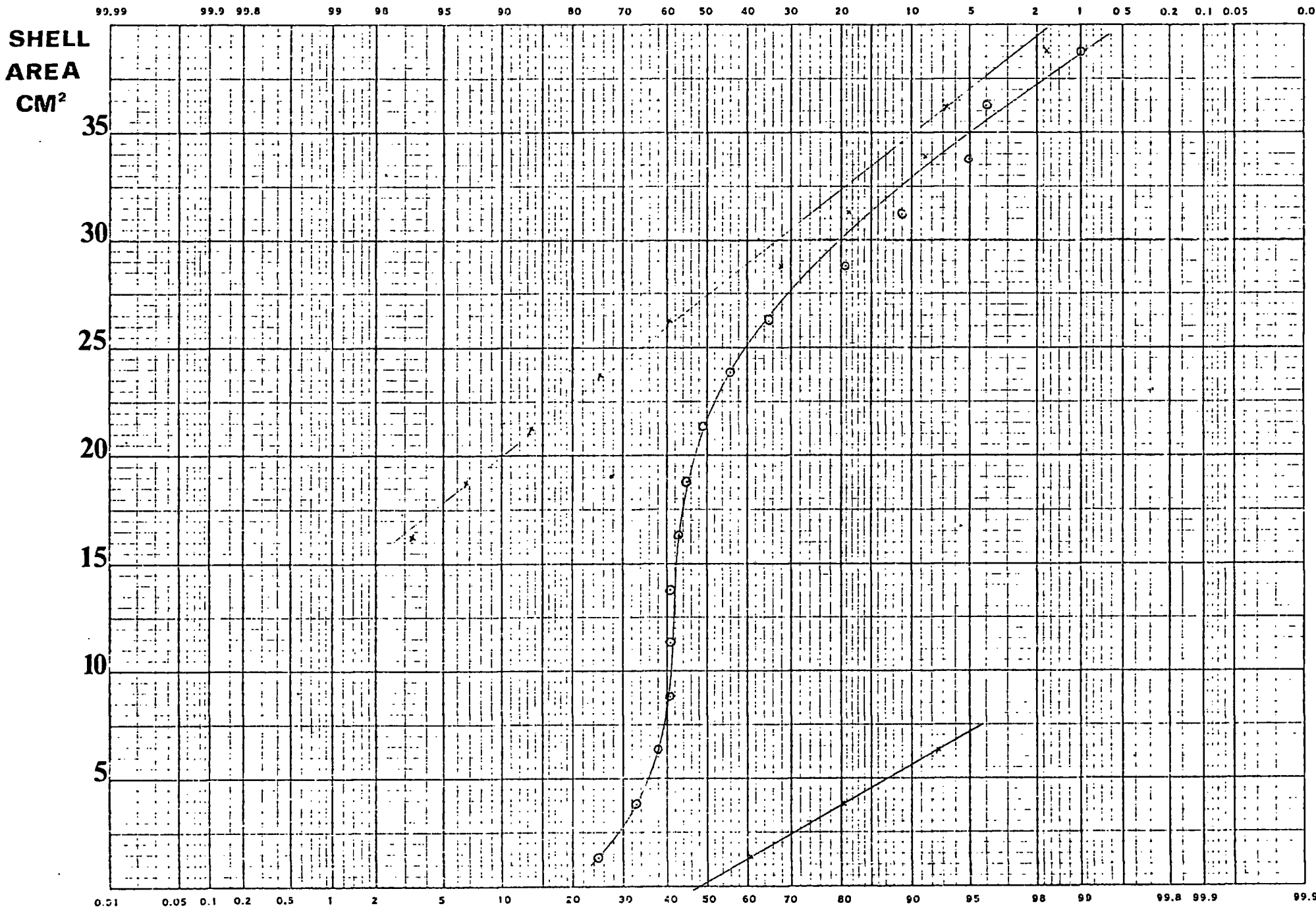


Fig. 5

The distribution (according to area index) of M. edulis from Holy Island site A plotted on probability paper, showing point of inflection, and calculated straight-line plots.

Table 1:

Characteristics of two sub-populations of M. edulis at Holy Island site A

	Shell area (cm^2)	Mean	Standard Deviation	% of Total
Sub. population 1	0-15	0.2	4.1	41
Sub. population 2	15-45	27.2	5.8	59

Discussion

This random collection of mussels may therefore be separated into two sub-populations representing different size classes. These may be treated independently to indicate morphological differences between individuals of different size (age) groups.

Probability paper is used to calculate the parameters only of normally distributed populations such as sub-population 2. Since sub-population 1 is clearly a truncated distribution (Fig. 4) the mean and Standard Deviation are not reliable estimates. The truncation of the distribution in this way may be a consequence of the pattern of spatfall. If a prolonged period of spatfall occurs several times a year, the smallest size classes will be supplemented continually whilst fewer animals reach larger classes.

1.2 Changes in shell shape with age

Coe and Fox (1942) showed that in M. californicus the depth: length ratio decreased with increasing length and age, but width: depth increased. Field (1922) found that shell proportions changed with increase in size also in M. edulis. More recently, Seed (1968) also working with M. edulis, found ratios of length:width and depth: width decreasing, whilst length:depth increased with increasing shell

length, i.e. as found previously in M. californicus. In older mussels, increase in width is greater than increase in depth, so animals become progressively wider in relation to their depth and length. Shell weight and volume also continue to increase when increase in length and depth has almost stopped. None of these studies has considered changes in weight of soft parts with age.

Method and Results

Data from site A on Holy Island lend themselves to a study of change in shell shape with age, since probability paper analysis enables two sub-populations of different age groups to be separated from within the randomly-collected population. Ratios of shell dimensions were calculated, and their means compared using the Student's T-test, to give results as shown in Table 2.

Table 2:

Comparison of ratios of shell dimensions in M. edulis from two sub-populations at site A on Holy Island

Ratio	Younger Sub-population 1 (Mean of 41 shells ± S.E.)	Older Sub-population 2 (Mean of 59 shells ± S.E.)	% Change	p values
Length:width	2.46 ± 0.02	2.34 ± 0.02	-4.9	p<.001
Depth:width	1.53 ± 0.02	1.18 ± 0.01	-22.9	p<.001
Length:depth	1.61 ± 0.01	1.99 ± 0.01	+23.6	p<.001

Discussion

The ratios length:width and depth:width decrease significantly with increasing area, and hence age, of mussel. The ratio length:depth

increases with area. This implies an increase of width, and to a lesser extent length as the mussel becomes older, and agrees completely with the results of Seed (1968).

1.3 Changes in Shell Dimensions with Locality or Habitat

Some of the great variety of shell form in the mussel may be interpreted in terms of environmental conditions. Coe and Fox (1942, 1943) found that Mytilus californicus had deeper, less wide, and thinner shells in those collected from the shelter of pier supports than those from exposed shores. Williamson (1907) had found mussels from wave-swept shores to be rounder and thicker, but not so deep as faster-growing mussels. Seed (1968), in examining the effects of density on shell shape by physical compression pointed out that owing to their orientation in the mussel beds, increase in shell depth would be more restricted than increase in length in dense populations. He concluded, however, that density is not the only important factor in determining shell form, which can be modified greatly by growth rate.

This study examines both shell dimensions and weights of shell and soft parts according to locality and environment.

Method

Samples of mussels of approximately 25 cm^2 in area (about 5 cm long) were taken from three sites in Seaton Carew: the neap tide Low and High Water Marks, and a site intermediate between these two. Four sites A, B, C, D on Holy Island; A close to the Low Water Mark for Neap Tides, the stress at half-tide. Three of them, B, C and D were surrounded by a flow of fresh to brackish water at low tide, but the mussels were not submerged then. As before, shell dimensions were measured with vernier calipers, and the dry weight of both shell and soft parts found after the animals had been dried in a vacuum oven at

Table 3:

Morphology of *Mytilus edulis* from three sites at Seaton Carew and four at Holy Island

	Seaton LTM	Seaton Mid	Seaton HTM	Holy Is. A	Holy Is. B	Holy Is. C	Holy Is. D
Shell length / Shell width	2.33 ±.02	2.21 ±.08	2.19 ±.01	2.23 ±.02	2.25 ±.02	2.26 ±.02	2.25 ±.03
Shell depth / Shell width	1.14 ±.02		1.15 ±.02	1.13 ±.01	1.12 ±.01	1.12 ±.01	1.11 ±.01
Shell length / Shell depth	2.05 ±.02		2.03 ±.02	1.94 ±.06	2.02 ±.02	2.02 ±.02	2.03 ±.02
Shell weight / Weight of soft parts	20.9 ±0.8	20.6 ±0.6	16.3 ±0.6	14.1 ±0.6	15.6 ±0.6	16.9 ±0.7	21.0 ±0.9
Shell weight / Shell length	1.22 ±.07	1.19 ±.01	0.75 ±.10	1.72 ±.06	2.30 ±.08	2.01 ±.06	2.34 ±.06
Log shell weight / Log shell length	1.14 ±.02		1.01 ±.02	1.35 ±.07	1.47 ±.15	1.40 ±.02	1.53 ±.04
Weight of soft parts / Shell length	0.0597 ±.0021	0.0575 ±.0022	0.0649 ±.0029	0.127 ±.006	0.155 ±.029	0.121 ±.007	0.110 ±.004
Shell weight / Area	0.286 ±.016	0.276 ±.007	0.242 ±.006	0.357 ±.006	0.394 ±.011	0.372 ±.01	0.424 ±.009
Regression x=area y=shell weight	0.79	0.59	0.70	0.85	0.76	0.88	0.66

(Each result is the mean of 40 individuals, shown with Standard Error)

TABLE 4:

T-tests to compare the morphology of *M. edulis* from six of the sites recorded in Table 3. Statistically significant values of p are shown; others are not significant (NS).

SL = Seaton LTM
SH = Seaton HTM
A, B, C, D = Holy Island Sites

	SL	SH	A	B	C
SL					
SH	NS			shell length	
A	.001<p<.002	NS		shell width	
B	.002<p<.01	NS	NS		
C	.01<p<.02	NS	NS	NS	
D	.02<p<.05	NS	NS	NS	NS

	SL	SH	A	B	C
SL					
SH	NS			shell depth	
A	NS	NS		shell width	
B	NS	NS	NS		
C	NS	NS	NS	NS	
D	NS	NS	NS	NS	NS

	SL	SH	A	B	C
SL					
SH	NS			shell length	
A	NS	NS		shell depth	
B	NS	NS	NS		
C	NS	NS	NS	NS	
D	NS	NS	NS	NS	NS

	SL	SH	A	B	C
SL					
SH	p<.001			shell weight	
A	p<.001	.01<p<.02		wt. soft parts	
B	p<.001	NS	NS		
C	p<.001	NS	.002<p<.01	NS	
D		p<.001	.002<p<.01	p<.001	.001<p<.002

	SL	SH	A	B	C
SL					
SH	.001<p<.002			shell weight	
A	p<.001	p<.001		shell length	
B	p<.001	p<.001	p<.001		
C	p<.001	p<.001	.001<p<.002	.002<p<.01	
D	p<.001	p<.001	p<.001	NS	p<.001

	SL	SH	A	B	C
SL					
SH	p<.001			log shell weight	
A	.002<p<.01	p<.001		log shell length	
B	.02<p<.05	p<.001	NS		
C	p<.001	p<.001	NS	NS	
D	p<.001	p<.001	.02<p<.05	NS	.002<p<.01

	SL	SH	A	B	C
SL					
SH	NS			wt. soft parts	
A	p<.001	p<.001		shell length	
B	p<.001	p<.001	NS		
C	p<.001	p<.001	NS	NS	
D	p<.001	p<.001	.02<p<.05	NS	NS

	SL	SH	A	B	C
SL					
SH	.002<p<.01			shell weight	
A	p<.001	p<.001		shell area	
B	p<.001	p<.001	.002<p<.01		
C	p<.001	p<.001	NS	NS	
D	p<.001	p<.001	p<.001	.02<p<.05	p<.001

50°C for 72 hours. Various ratios were calculated for purposes of comparison as shown in Table 3.

Discussion

By using data from mussels of similar length and area, the aim was to eliminate any variability in morphological ratios with age, and to look for differences resulting from different environmental conditions in the seven sites. However this ignores the possibility of differing growth rates in differing conditions, so that the mussels of the same length may not in fact be of a similar age. This means that the results presented in Table 3 cannot be interpreted solely in the light of environmental differences.

T-tests on ratios between the dimensions length, width and depth of shell (Table 4) show significant differences only in the case of length:width which for the Low Tide Mark at Seaton is higher than all the Holy Island sites. Such a high result, according to Coe and Fox (1942) is characteristic of a sheltered site.

The ratio weight of shell:length of shell is larger at each of the four Holy Island sites than at Seaton. Since, as discussed above, their other dimensions are largely similar, this indicates that mussel shells at Lindisfarne are thicker, or possibly denser. More evidence for this comes from the shell weight:area also being larger at each of the four Holy Island sites than at Seaton. Similarly the ratios of the three linear dimensions at Seaton LTM, mid-tide and HTM do not differ significantly, so the higher shell weight:length ratio for the two lower tidal levels indicates thicker shells there, although not as thick as those at Holy Island.

The ratio log shell weight:log shell length was also calculated since weight is unlikely to depend linearly on length, and so a significant difference could be obtained between ratios of weight:length

by using slightly different size classes of individuals. This log ratio confirms the significant differences between the Holy Island and Seaton Carew sites.

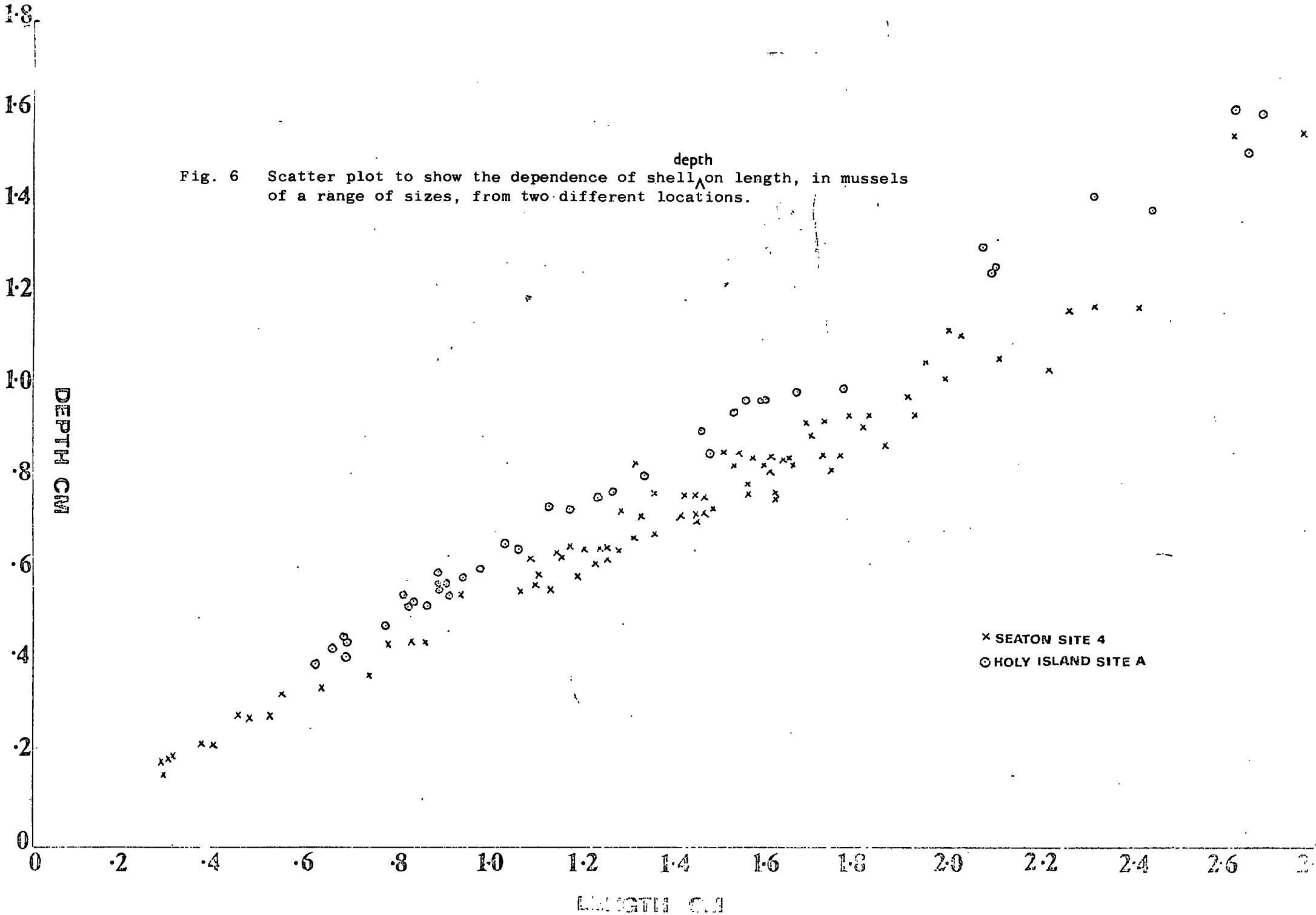
Ratios involving dry weight of soft parts show that these are heavier both for a given shell length, and for a given shell weight on Holy Island. Since there are already indications that Holy Island shells are thicker, this must mean the soft parts are considerably heavier. In weight of soft parts:shell length, samples from different Holy Island sites do not show any differences (apart from A being higher than D at the 95% level), nor is there any between the two Seaton sites.

The general characteristics of mussels from Holy Island as distinct from Seaton Carew are that they grow to be larger, and so are either older or faster-growing; their shells are thicker for a given length or area; their soft parts are heavier than those of mussels of a comparable size from Seaton. The thicker shells might have been expected at a more exposed site, to withstand harsh conditions. This agrees with the point made earlier that the length:width ratio at Seaton was characteristic of a more sheltered site.

Differences in shell thickness could perhaps be attributed to the proportion of Calcium in ingested particles. Certainly the available food will also differ between the two sites: much of the detritus at Seaton will be organic matter from sewage, whilst that at Lindisfarne will be marine organic detritus. The salinity of the water may also affect the shell thickness. Ingvarsson (1973) found shell thickness in Macoma balthica was greater at a marine than a brackish site. In the present study too the shell weight:area ratio is lowest on Holy Island at site A near Mill Burn where salinity will be lowest.

The main limitation to interpretation of these results is that

Fig. 6 Scatter plot to show the dependence of shell depth on length, in mussels of a range of sizes, from two different locations.



morphological variation can, at least in part, be attributed to age differences in mussels of a similar size as a result of differing rates of growth. Seed (1968) found that animals live longer in areas of slow growth, e.g. the high littoral, as had Savilov (1953). Also, since with increasing age, growth in depth becomes progressively less relative to increase in length, mussels from areas of slow growth will have a characteristic morphology of wide dorsally-rounded shells. The observation of Warren (1936) that mussels periodically exposed to air had thicker and heavier shells than mussels lower on the shore could also then be explained in terms of age differences, as could all the results above. Seed's idea is that an upper size limit is imposed by environmental conditions, since his transplantation experiments show that a transfer of very old animals to more favourable conditions stimulates renewed linear growth. Fast-growing animals may attain the limit imposed by environmental conditions quickly, but in areas where growth is slow, only a few very old animals may reach that limit.

Fig. 6 is a scatter plot of depth against length for mussel shells of a range of sizes from two different locations: one Seaton site, and one Holy Island site. This shows clearly that the dependence of shell depth on length differs in the two habitats.

CHAPTER 2:

Heavy Metal Analysis

Heavy metal analysis was carried out on:

(i) Mytilus edulis from High and Low tide marks at Seaton Carew, and from the four sites at Holy Island marked on Fig. 2.

(ii) Nereis diversicolor from sites at Holy Island and Seal Sands, and from a number of transplantation experiments.

2.1 Levels of contamination in Mytilus edulis

(a) Development of reliable analytical methods

A wet-ashing technique was used (Shenton, unpublished) followed by Atomic Absorption analysis for Lead and Zinc. Preliminary investigations of cadmium levels indicated very low concentrations, so further analyses were not made.

Mussels were washed, and any attached barnacles or weed removed before analysis. One sample of animals was treated with filtered seawater for 48 hours before drying, to allow them to empty the gut of any metal-containing particles. As will be shown later (Table 5), neither Heavy Metal levels, nor the percentage by weight of the residue from filtration after ashing differed significantly from those obtained from animals dried without prior treatment, and so this was discontinued. (The animals were starved while they emptied their guts but the reduction in tissue weight as a result was probably very slight for large animals in this short time, so should not have affected tissue concentrations of the metals).

Soft parts were removed from the shells whilst fresh, and both parts of the animal dried in cones of aluminium foil in a vacuum oven at 50°C for 72 hours. Dried samples of about 0.5 g of total soft parts of individual animals were weighed into silica crucibles and 1 ml 20%

Aristar H_2SO_4 added. The crucibles were heated with their lids in position, and stirred occasionally with a clean glass rod for 5-6 hours at 100°C until charred. The lids were then removed and the temperature increased for ½ hour to allow the sample to be reduced in volume before heating at 500°C in a muffle furnace for 15-18 hours. 2 ml Aristar HCl were added to dissolve the cold ash as completely as possible, and the samples again heated for about an hour to facilitate this process. Any residue was then removed by filtration through Whatman Number 1 paper into 25 ml volumetric flasks. The addition of 1 drop of thymol blue indicator allowed the pH to be adjusted, using 3N NH_4OH and 3N HCl also added dropwise. The volume of the sample was then made up to exactly 25 ml with deionised water and samples were stored in the cold in polythene bottles. Bottles were washed with 20% HNO_3 before use, as was all glassware, and control solutions of HCl stored in them showed no contamination.

Trial volumes of a few mls of these solutions were prepared and read in the Atomic Absorption Spectrophotometer (AAS) until a sample volume was found which gave a good scale deflection within the range of concentrations which the machine had been adjusted to measure. Obviously this volume differed between populations of mussels with different levels of heavy metal contamination, and the range of concentrations for the standard curve was chosen in order that the volume used should be always large enough to be measured accurately. For each reading, this chosen volume of solution was pipetted into volumetric flasks, and after readjusting the pH, was shaken with 2 ml of a 10% solution of Diethylammonium diethyldithiocarbamate (DDDC) in Methyl Iso-Butyl Ketone (MIBK) for 50 secs to extract the heavy metals into the organic phase. Deionised water was added to bring the MIBK solution into the neck of the flask so that the organic phase could be aspirated directly into the AAS and a

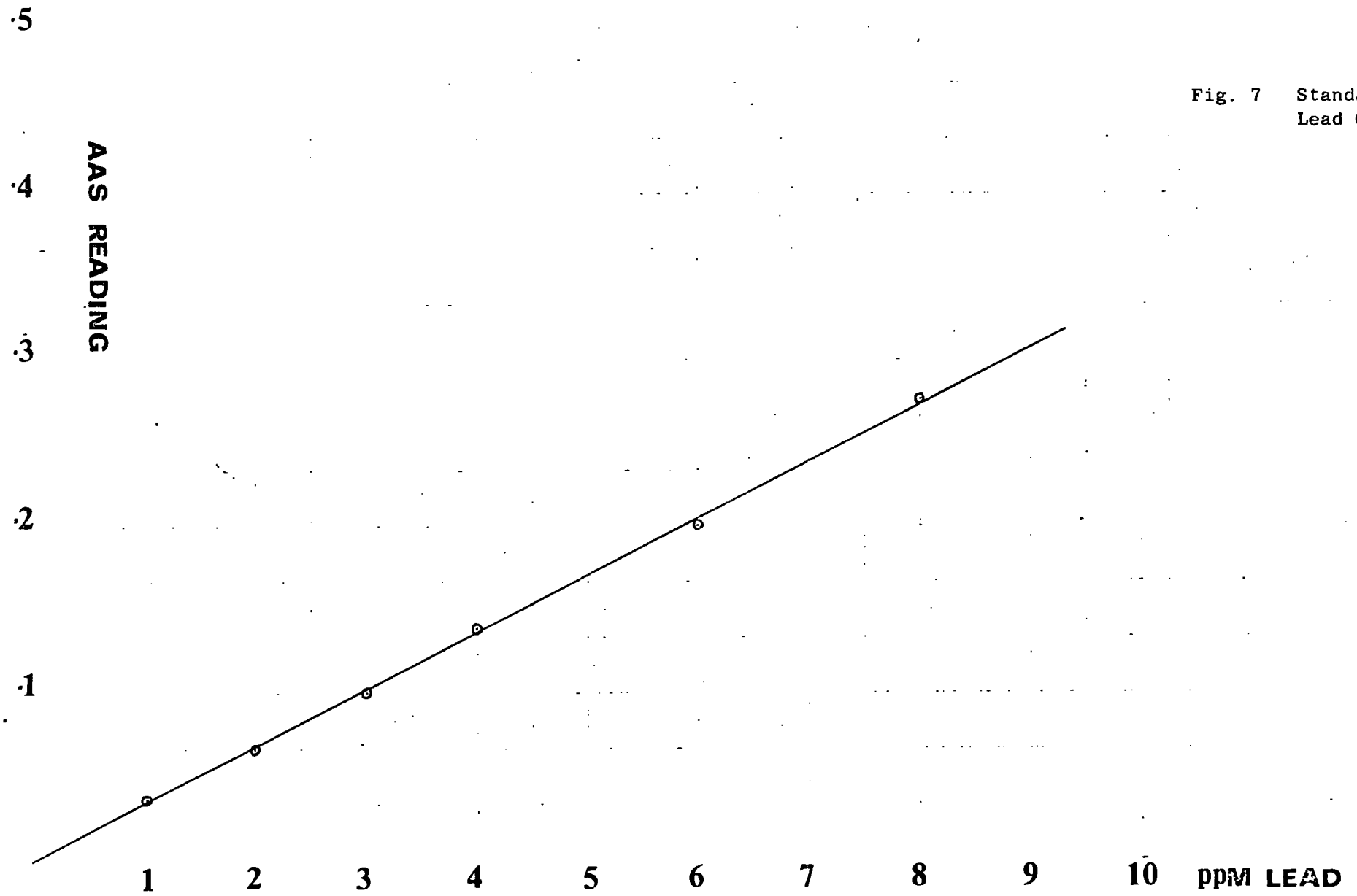


Fig. 7 Standard curve for Lead Concentrations.

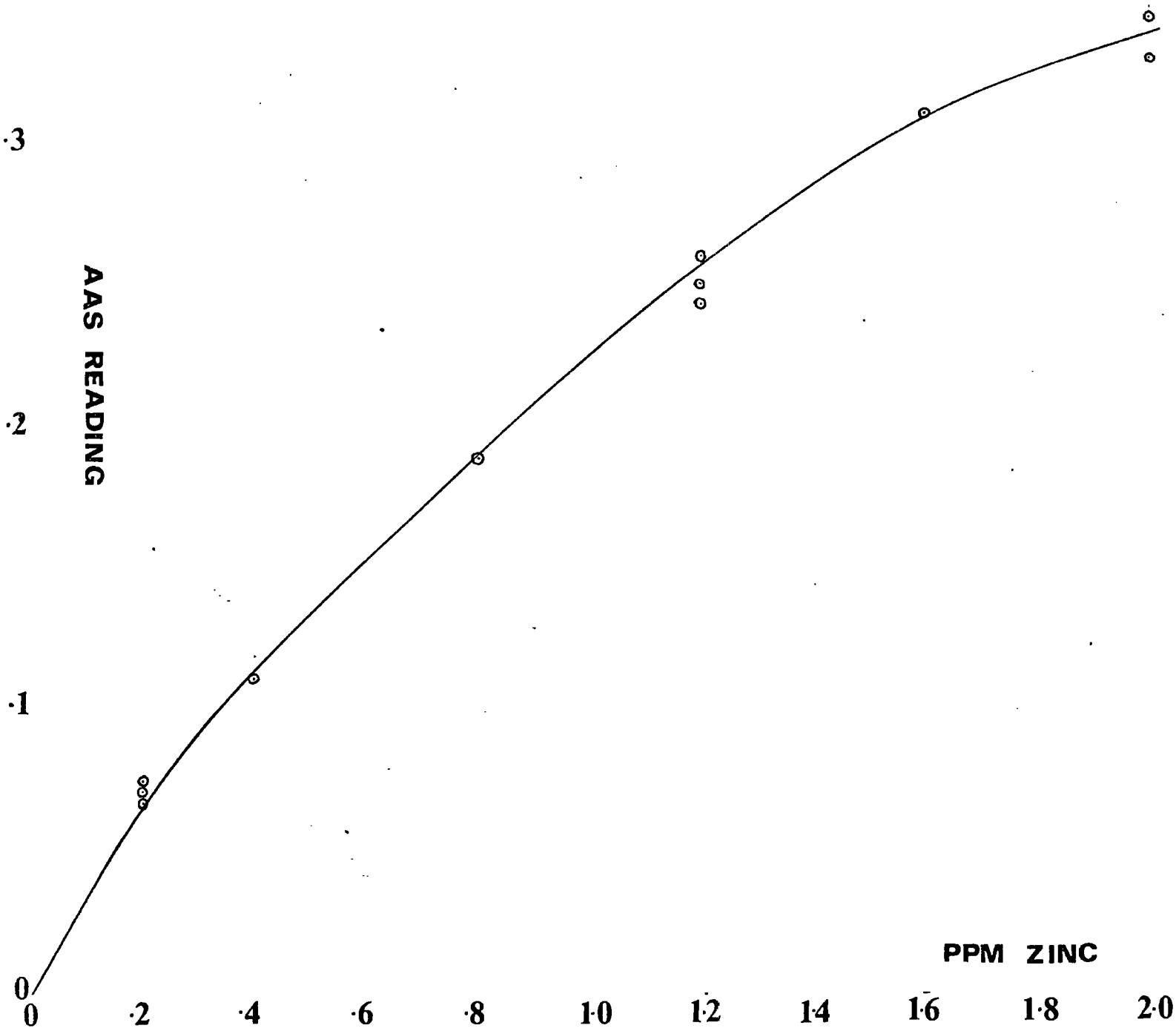


Fig. 8 Standard curve for zinc cencentrations.

reading taken. This reading was converted to ppm of heavy metal using standard curves, and this measured concentration adjusted according to sample volume, volume of MIBK used, and dry weight of soft parts, to give ppm of metal in the dry weight of soft parts. The standard curves are plots of AAS readings for various concentrations of lead and zinc, prepared by dilution from 1000 ppm solutions containing Analar lead acetate $Pb(CH_3COO)_2$ and Analar zinc sulphate $ZnSO_4.7H_2O$ respectively (Figs. 7 and 8). Minimum levels of detection are about 0.025 ppm zinc in MIBK (about 0.1 ppm dry weight of sample) and 0.2 ppm lead (about 0.8 ppm dry weight of sample).

Treatment of shell samples by wet ashing with H_2SO_4 yielded unsatisfactory results, giving low recovery rates for shells 'spiked' with known amounts of heavy metals before analysis (Williamson, unpublished). One problem involved was the formation of a large insoluble residue of calcium sulphate (from calcium in the shell) which was difficult to wash thoroughly on filtration. Oxidation with HNO_3 was therefore investigated since calcium nitrate is soluble. Shells were heated with, according to their weight, either 2 or 5 ml Aristar concentrated HNO_3 until dissolved. The solution was then evaporated to dryness, and a further small volume of acid added. Heating and evaporation were then repeated until the dry residue was white. This was then dissolved as completely as possible in dilute HCl and after filtration made up to 25 ml as before. Volumes of these solutions were extracted with MIBK as described above. This method gave good recovery percentages of known amounts of metal salts added to the shells before treatment.

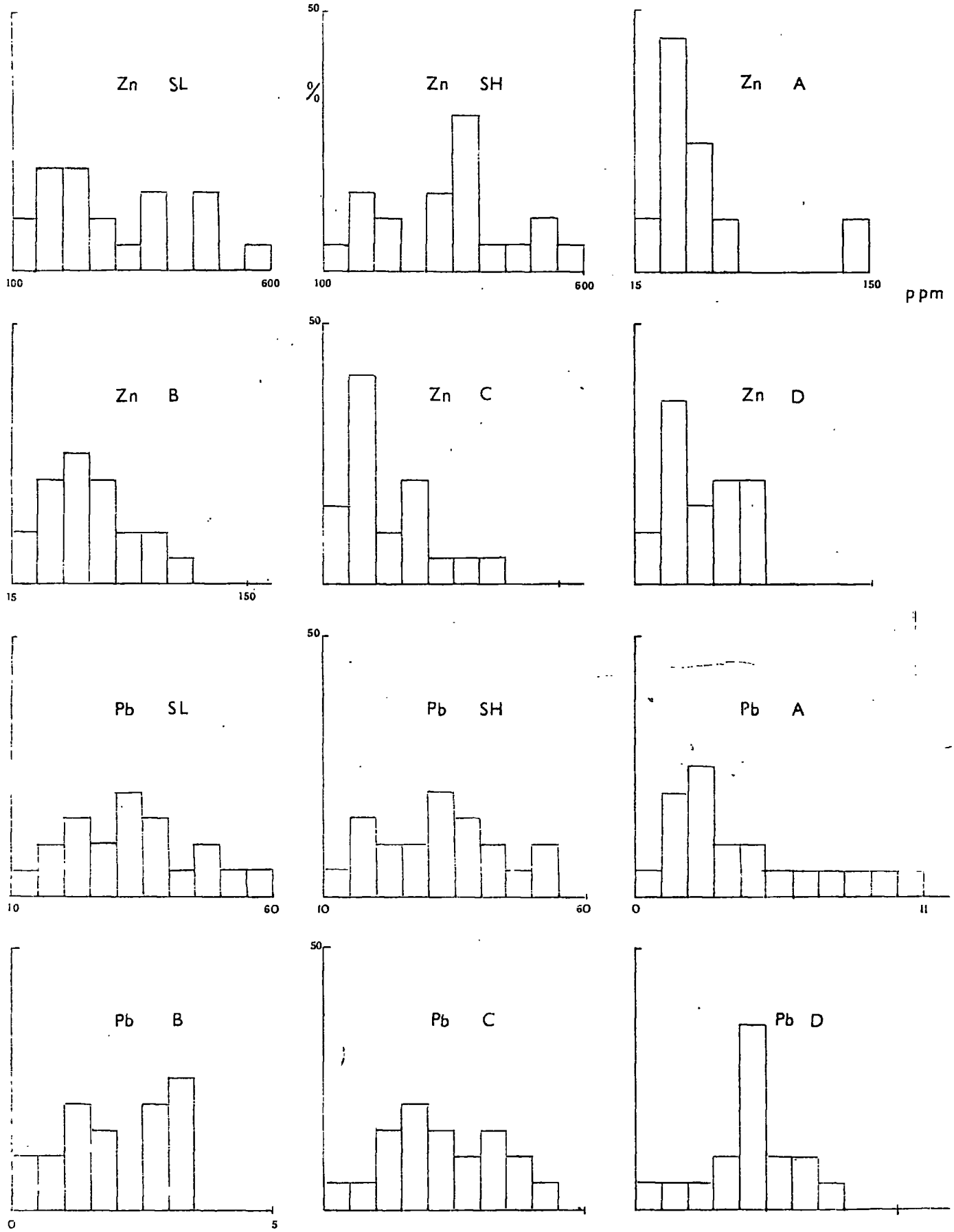
(b) Establishing a meaningful sample size

Since one object of this study was to compare Heavy Metal contamination of molluscs from polluted and less-polluted sites, it was

Fig. 9 Histograms to show that the percentage frequency distribution of individual concentrations of Heavy Metals in samples of 20 mussels approximates to the Normal Distribution.

Seaton Carew sites : SL and SH

Holy Island sites : A, B, C, D



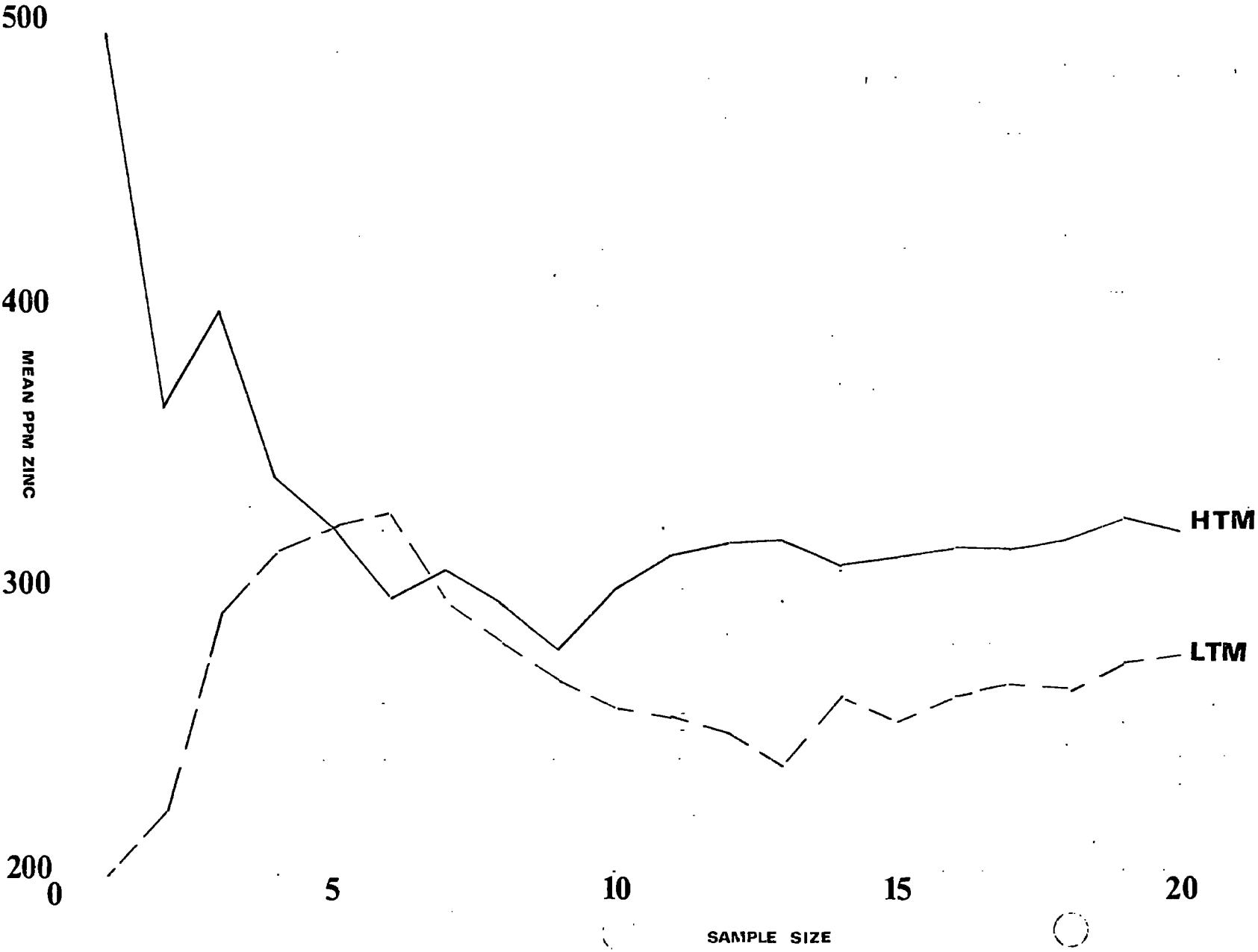


Fig. 10 Running Mean values for Zinc concentrations in large mussels from two sites (High and Low Tide Marks) at Seaton Carew.

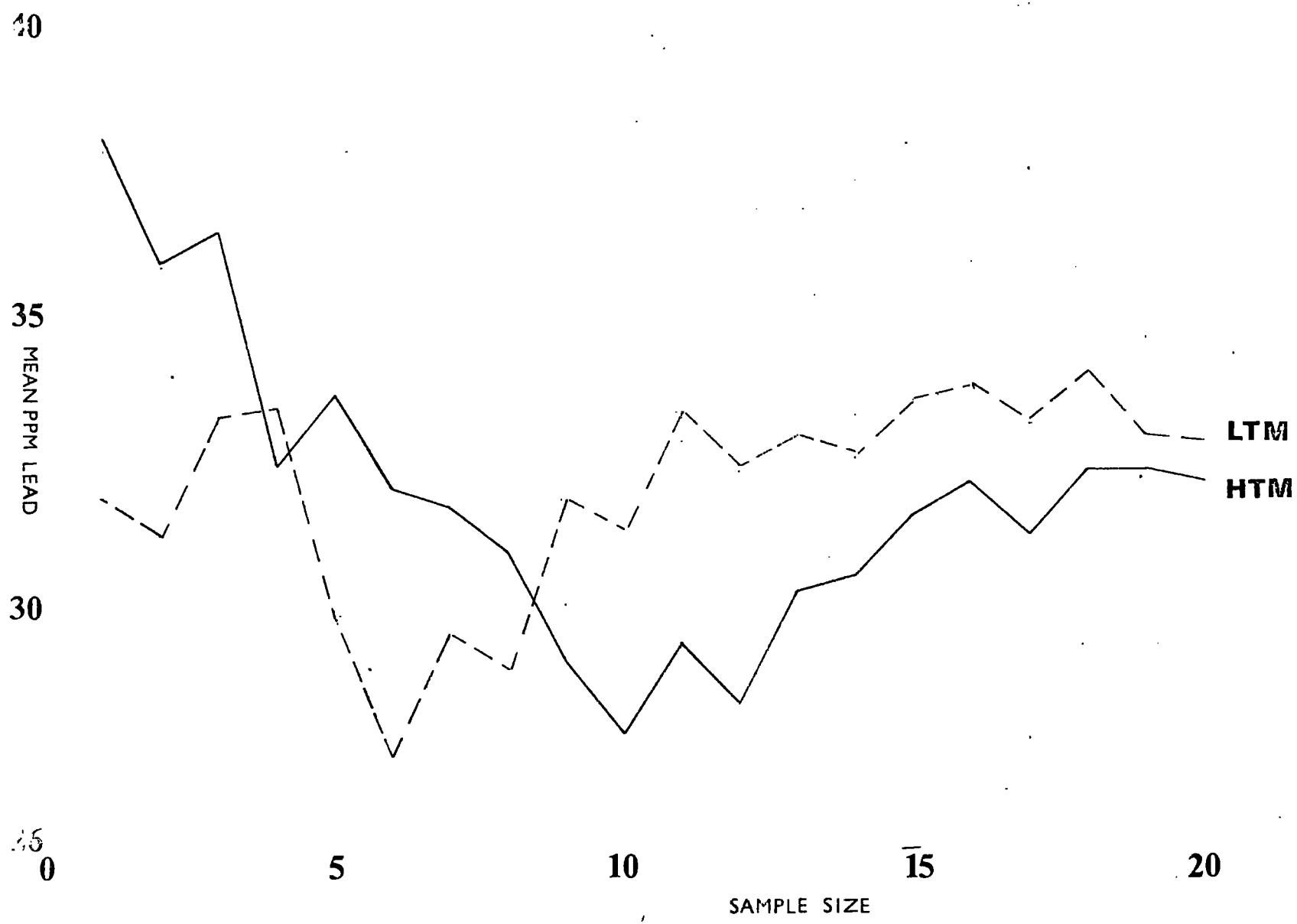


Fig. 11 Running Mean values for lead concentrations in large mussels from two sites (High and Low Tide Marks) at Seaton Carew.

necessary to establish appropriate sizes for representative samples. The largest animals found at Seaton Carew were chosen for individual analysis. These had shell areas of approximately 20 cm^2 and contained at least 0.25 g of soft parts, enough to allow accurate analysis from a consideration of the lowest levels detectable. Since age determinations as discussed earlier, were unsuccessful, animals of the same size were collected from Holy Island for comparison, in the hope of dealing with the same age class, or at least similar weights of body tissues.

Heavy metal concentrations in individual animals are plotted as frequency distributions for each sample of 20 (Fig. 9). Most histograms approximated to Normal distributions, as would be expected when dealing with a single age class. Running Mean values were also plotted for samples of increasing size between 1 and 20 (Figs. 10 and 11). Random Numbers were used to choose the individual measurements added successively to increase the sample size by one. As shown, the instability in the mean value was reduced to less than $\pm 5\%$ in samples of size 15-20, so a sample size of 20 was chosen for further investigations. As will be shown later (Table 5) the size of Standard Errors would recommend use of a larger sample size, but unfortunately time was a limiting factor in this study, and a larger sample size was forfeited so that several sites could be investigated. An improvement in estimation of the true population mean can be made by increasing the sample size to 30. This combines the samples of large filtered and large unfiltered mussels from the low Tide Mark at Seaton Carew. Between these two groups, no statistical difference in metal concentrations could be found.

In comparisons between sites, t-tests give reasonably reliable estimates of the degree of significance of differences, even for somewhat skewed distributions, so they have been used in preference to

non-parametric methods which are less powerful.

Individual analyses of the soft parts of 'large' animals (about 20 cm^2 in area) from each site (Seaton Low Tide Mark (L) and High Tide Mark (H) and Holy Island A, B, C, D) were carried out. Since age could not be calculated, animals of approximately the same shell size were used instead. Samples comprising the combined soft parts of 20 'small' individuals (about 1 cm^2 in area) from one Seaton site (L) were analysed, as also were 'large' shells from each site there, and a combined sample of the 'small' shells. Sediments were analysed in the same way as the tissue samples, and water was analysed by shaking 25 ml with 2 ml MIBK to read as before.

Results

TABLE 5:

Heavy Metal Analysis of *Mytilus edulis*

	Site	Sample Type	Sample Size	Zn ppm Mean ± SE	Pb ppm Mean ± SE
SOFT PARTS *	Seaton H	large	20	345.35 ± 28	32.29 ± 2.5
	Seaton L	large	20	297.25 ± 29	32.98 ± 2.7
	Seaton L	filtered large	10	278.4 ± 59.5	33.3 ± 3.3
	Seaton L	large	30	284.3 ± 20.8	33.09 ± 2.6
SOFT PARTS **	Seaton L	large; low weight	10	329.8 ± 40.9	35.8
SOFT PARTS **	Seaton L	large; high wt.	10	265.0 ± 76.6	30.2
	Seaton L	combined small	10	61.29 ± 6.3	14.03 ± 1.24
	Holy Is A	large	20	55.94 ± 8.0	4.17 ± 0.69
	B	large	20	60.82 ± 5.32	2.01 ± 0.23
	C	large	20	50.73 ± 5.72	2.22 ± 0.22
	D	large	20	53.45 ± 4.47	2.605 ± 0.31
SHELLS	Seaton L	large	5	1.0 ± 0.2	1.12 ± 0.3
	Seaton H	large	5	1.69 ± 0.3	0.96 ± 0.4
	Seaton L	combined small	10	8.49 ± 1.56	7.32 ± 0.96

'large' about - 20 cm^2 in area; 'small' about - 1 cm^2 in area

* by combining data from 20 large unfiltered and 10 large filtered.
** by dividing data from 20 large unfiltered, according to weight of soft parts.

TABLE 6:

T-tests to compare Heavy Metal concentrations in the total soft parts of 'large' mussels. Significant values of p are shown.

		SL	SH	A	B	C
Zn	SL					
	SH	NS				
	A	p<.001	p<.001			
	B	p<.001	p<.001	NS		
	C	p<.001	p<.001	NS	NS	
	D	p<.001	p<.001	NS	NS	NS
		SL	SH	A	B	C
Pb	SL					
	SH	NS				
	A	p<.001	p<.001			
	B	p<.001	p<.001	.002<p<.01		
	C	p<.001	p<.001	.002<p<.01	NS	
	D	p<.001	p<.001	.02<p<.05	NS	NS

2.2 Heavy Metal Analysis of Nereis diversicolor

Method

The procedure described earlier for the drying and wet-ashing of M. edulis was used also for Nereis since ragworms contain little calcium. Before drying, animals were kept for 48 hours in clean filtered seawater and acid-washed sand to enable them to empty their guts (Bryan and Hummerstone 1973). This length of time was chosen so that, although the gut would probably be clear of metal-containing particles, starvation would not have caused a severe loss of weight.

For the transplantation experiments, Nereis were collected from near the Mill Burn on Fenham flats, Lindisfarne, and from Seal Sands. Animals could be kept alive at 15°C in jars with loosely-fitting lids, $\frac{3}{4}$ filled with mud, and covered with a layer of seawater. Jars, each containing 10 animals, were maintained for one week. Mud and interstitial seawater from Seal Sands or Holy Island were added in each of the eight possible combinations.

Results

TABLE 7:

Heavy Metal Analysis of Nereis diversicolor maintained on different substrates and interstitial water

Substate	origin of animals	No. of replicates	Zn ppm dry wt. Mean ± SE	Pb ppm dry wt. Mean ± SE
1. Seal Sands mud) Seal Sands H_2O)	Seal Sands	3	106.92 ± 8.34	4.873 ± 0.7
2. Seal Sands mud) Holy Is. H_2O)	Seal Sands	3	82.63 ± 6.53	3.02 ± 1.0
3. Seal Sands mud) Seal Sands H_2O)	Holy Island	3	74.2 ± 6.2	2.26 ± 0.74
4. Seal Sands mud) Holy Is. H_2O)	Holy Island	3	52.55 ± 6.6	1.53 ± 0.3
5. Holy Is. mud) Seal Sands H_2O)	Holy Island	3	33.07 ± 2.9	0.47 ± .26
6. Holy Is. mud) Holy Is. H_2O)	Holy Island	3	30.31 ± 2.66	0.58 ± 0.5
7. Holy Is. mud) Seal Sands H_2O)	Seal Sands	All died within 48 hrs. (deoxygenation).		
8. Holy Is. mud) Holy Is. H_2O)	Seal Sands			

TABLE 8:

Heavy Metal Analyses of Sediments and Seawater

	Zn ppm dry wt.	Pb ppm dry wt.
Seal Sands mud	355	90
Holy Island mud	30	20
Seal Sands H_2O	0.4	<0.016
Holy Island H_2O	<0.002	<0.016
Seaton Carew H_2O	0.19	<0.016

TABLE 9:

Heavy Metal Analysis of Nereis diversicolor maintained on different substrates

	Substrate	origin of animals	No. of replicates	Zn ppm	Pb ppm
1+2	Seal Sands mud	Seal Sands	6	94.77 ± 8.8	3.95 ± 1.5
3+4	Seal Sands mud	Holy Island	6	63.37 ± 7.6	1.89 ± 0.54
5+6	Holy Island mud	Holy Island	6	31.69 ± 1.8	0.53 ± 0.3

TABLE 10:

T-tests showing significant p values for differences between zinc and lead levels in Nereis from Table 9.

Zn

	1+2	3+4
1+2		
3+4	.02<p<.05	
5+6	p<.001	.002<p<.01

Pb

	1+2	3+4
1+2		
3+4	NS	
5+6	.02<p<.05	NS

TABLE 11:

Correlations between lead and zinc leads in the soft parts of 'large' mussels from Seaton Carew.

Low TideMark :

Concentrations of Zn and Pb r = 0.44

Total weight (μg) Zn and Pb r = 0.50

High TideMark :

Concentrations of Zn and Pb r = 0.39

Total weight (μg) Zn and Pb r = 0.56

(For 18 degrees of freedom, a value of 0.45 is significant at 95% level).

DISCUSSION

It is known that some marine molluscs concentrate the Heavy Metals from seawater e.g. Brooks & Rumsby (1965), Segar et al (1971). The results from the present study support this, since measured concentrations of zinc and lead exceed by four to five orders of magnitude those to be found in the coastal seawater of the British Isles as quoted by Bryan (1971) and Preston et al (1972). This characteristic of Heavy Metal accumulation is not shared by Teleost fish (Eustace 1974), so the monitoring of commercial fish species would provide a much less sensitive index of pollution than would say the mussel.

For a monitoring study, questions arise as to sample size, animal size and position on the shore from which they should be collected. Setting aside the problems of choice of animal size and location, the size of sample is a basic consideration. As explained above, in the present study, samples of 20 individual mussel soft parts were used. This restricted the accuracy somewhat, since the Standard Errors were of the order of 10% of the mean value (Table 5). However, in the limited time available, it was preferred to use smaller samples from a wider variety of sites. When the sample size was increased to 30 by combining the filtered sample from Seaton Low Water Mark with the 20 untreated animals from the same site, the Standard Error of the mean was reduced to about 7% of the mean value.

Since age determination was difficult, animals of a similar size were chosen for comparison. The Heavy Metals absorbed by Mytilus must be derived almost entirely from the water in which they are immersed, by respiration or in food. It was hoped that since mussels are sessile, samples of animals from different tidal levels might show some differences in lead or zinc concentration, and hence give an

indication of the main source of contamination. The mean values of heavy metal concentrations found in large mussels at high and low littoral sites at Seaton did not differ significantly, even when the sample size for the low site was increased to 30. However, as well as the mean zinc level being considerably higher, if running means are plotted (Figs. 10 and 11) these show that the zinc levels at the high littoral site are consistently higher than those for the lower one, whilst for lead there appears to be no particular trend. This would possibly indicate either different sources or different chemical form for the zinc and lead contamination - only the zinc depending on the tidal level, and hence period of immersion. Further investigation would certainly be worthwhile using a larger sample size to determine whether this apparent 20% difference in zinc concentration with tidal level is a real one. The knowledge that differences of this order can exist between sampling sites is very important when monitoring areas for pollution.

When monitoring animals from different areas, care must be taken also that any differences are not just a result of sampling different size or age groups. In a recent paper Mackay et al (1975) have found evidence that metal concentrations in cultivated oysters decrease with increased age and weight of the animal. My results, presented in Table 5, show lower concentrations in small than large mussels from the same tidal zone by a factor of 5 for zinc and by a factor of $2\frac{1}{2}$ for lead. There are two possible explanations for differing concentrations in animals of different age: either a dose of heavy metals may be diluted steadily by tissue growth, or a gradual accumulation takes place. In either case the time factor is of prime importance, although the opportunity to absorb contaminants may vary, for example from seawater at different tidal zones. It would seem that comparisons are needed primarily on an age or time-dependent basis

rather than on one dependent on size. From this it would be expected that within a given shell size class, larger animals would have smaller concentrations than small animals, since despite having had approximately the same time to accumulate about the same amount of metal, this would be diluted in a larger weight of soft parts. The sample of 20 large mussels of one size class from Seaton LTM may be divided into two groups of 10 according to weight of soft parts. As shown in Table 5, although the difference is not statistically significant, it appears that the group with heavier bodies have lower concentrations of heavy metals.

There is some degree of correlation between zinc and lead uptake in animals of the same size at the same tidal level, as indicated by the coefficients presented in Table 11. Clearer evidence for correlation is found in total heavy metal contents rather than in concentrations, this lending weight to the argument above that the concentrations found in individuals within a size class depends on the particular weight of their soft parts.

The analysis of shells was carried out by HNO_3 digestion on a few samples only, since this method was tested only at a late stage of the project. Concentrations of heavy metals in shells are only about $\frac{1}{2}$-1% for zinc, and 3% for lead, of those found in the soft parts. There is no significant change in concentration of either metal with tidal level at Seaton. Analysis of shell samples for the small animals shows both metals to be present at concentrations about eight times greater than in the individually-analysed large shells. These results for shells can be regarded as minimum values, since on filtration it is difficult to wash the residue sufficiently thoroughly to be sure that all heavy metals are in solution. Segar (1971) however found rather similar values of 0.05% for zinc and 4% for lead for concentration

in shells compared with those in total soft parts, indicating the method is probably reliable.

The work by Mackay et al (1975) on the oyster is the only study of which I am aware in which animals were analysed individually for comparative purposes. It is a severe limitation of the present study that heavy metal determinations had to be carried out on samples of similarly-sized animals, and not a particular age group as Mackay has done. This is because the length of time over which accumulation is possible appears to be the important factor. Since rate of growth has been found to differ with environmental conditions, a comparison of contamination may involve choosing animals of different sizes to obtain the same age class, and with this population, determination of age was unsuccessful.

There is no significant difference between zinc contamination of large mussels from each of the Holy Island sites A, B, C, D, but animals from site A have twice as much lead per unit dry weight of soft parts as the other three sites. This discrepancy between lead levels could be explained in terms of longer exposure as a result of slower growth, or exposure to higher levels. Site A lies within the wildfowling area of Fenham Flats, so that the accumulation of lead shot in the sediments is a possible cause for higher levels of contamination. Sites for mussel collection were chosen adjacent to the road bridge, since it is known that lead from organo lead anti-knock compounds in petrol exhaust fumes contaminates organisms on roadside verges in a manner dependent on distance from the road. (Quarles et al 1974). Animals from sites B and C close to and either side of the road bridge show no difference in their concentrations of either lead or zinc. Site D, being 10 yards away from the bridge may perhaps have been expected to have lower lead levels, but there is no evidence that

this is the case.

Tables 5 and 6 show that the concentrations of zinc in the total soft parts of Mytilus at Seaton are roughly six times as great as those at Holy Island, whilst lead concentrations are as much as fifteen times greater. Table 6 shows these differences to be statistically significant at the 99.9% level. Values at Seaton Carew are particularly high, being three times greater than results quoted for M. edulis in the Irish Sea (Segar et al, 1971). As discussed in the previous chapter, Mytilus from Holy Island grow larger, have thicker shells, and heavier soft parts than those at Seaton from equivalent tidal levels. This of course may not be dependent on the degree of pollution, although the heavy metal analyses show there to be strikingly less contamination by both lead and zinc at Holy Island. Mussels at Seaton do not reach the large sizes they do at Holy Island, and it is certainly tempting to seek some explanation for their shorter lifespan or slower growth in the degree of pollution suffered there, although other environmental factors must not be discounted.

It is possible that Mytilus may regulate the uptake of heavy metals, as Nereis has been reported to do for zinc (Bryan and Hummerstone 1973). The results have shown that zinc, but not lead concentrations probably differ between the two Seaton sites so possibly lead is regulated. One type of experiment which increases our knowledge of heavy metal accumulation by molluscs is that carried out in the laboratory by keeping animals in fixed concentrations of metal salts, often radioisotopically-labelled. Such have been done with lead salts by Schutz-Baldes (1972, 1974) using M. edulis. He found no regulation, there being a constant rate of lead uptake linearly dependent on the lead concentration of the medium. Rates of uptake and loss in large

mussels were found to be less than those in small mussels.

In the case of Nereis, position in the littoral zone should be less important in affecting contamination levels since these animals may move. Both the sediment in which they burrow, and its interstitial water may provide a source of heavy metals. Thus in the transplantation experiments described above, each of these variables was altered. It is clear from Table 7 that both zinc and lead concentrations are highest in Nereis from Seal Sands maintained on their native substrate, and with either type of interstitial water. Also that the concentrations have increased in Holy Island Nereis transferred to Seal Sands substrate. Since only live animals were used for analysis, and the gut had been cleared, this increase must be due to an uptake of heavy metals into the body of the animal from the substrate over the period of a week.

Unfortunately only 3 replicates were possible for each sample and so student's T-tests between experiments would not be meaningful. However, since the effect of the interstitial water itself appears to be small, probably depending largely on dissolution of metals from the mud, results for animals and mud of the same origins have been combined in Table 9. The increased sample size enables T-tests to be carried out and reveals significant differences, shown in Table 10. The zinc concentration differs between all three sets of animals maintained differently, but the lead only differs significantly between Seal Sands animals and Holy Island animals each kept on their native substrate.

The concentrations of zinc found in Nereis from Holy Island is very similar to that found in the sediment there, indicating that no regulation is taking place. However zinc in Nereis at Seal Sands is less than that in the sediment there, so possibly regulation can take

place as reported by Bryan & Hummerstone (1973), but only comes into operation at higher contamination levels. Lead found in Nereis at each site is very much less than that in the sediment, so the possibility of regulation of lead levels also arises.

As shown in Table 7, when Seal Sands animals were transferred to Holy Island mud, they died within 48 hours. It would be tempting to speculate that Nereis had become adapted to the higher concentration of metals in the Seal Sands substrate (Table 8) and were unable to survive in the cleaner substrate. (Bryan and Hummerstone 1971). However it seems more likely here that the rather clayey mud from Holy Island was more anaerobic than the sandier medium from Seal Sands to which they were used, and it was this which made them unable to survive.

SUMMARY

1. A reliable analytical method was developed for a detailed comparison between heavy metal concentrations in animals from polluted sites at Seaton Carew and Seal Sands, and much less polluted areas on Holy Island. Atomic Absorption Spectrophotometric analyses for lead and zinc were carried out on individual soft parts and shells of Mytilus edulis, and bodies of Nereis diversicolor. Heavy metal levels in mussel soft parts were significantly lower at the Holy Island site; concentrations in shells were very much lower than those in soft parts.

2. Morphological characteristics of M. edulis were investigated in the hope of making a comparison of contamination levels on the basis of age. Since age determination was not possible, animals of the same size were used for comparison between sites.

Analysis of animals of different sizes from the same site show smaller animals to have lower concentrations of both lead and zinc in the soft parts, but higher concentrations in the shells then have large animals.

3. In choosing sampling sites, the influence of intertidal zone was considered. Although there is insufficient statistical support for this, it appears that zinc, but not lead, is higher in mussels from the higher region of the intertidal zone. This could have considerable significance in studies monitoring the difference in contamination between locations.

4. Transplantation experiments were carried out with Nereis diversicolor of different origins maintained on foreign substrates. Holy Island Nereis have increased zinc concentrations when kept on Seal Sands mud for

a week. There is some evidence for regulation of both zinc and lead in *Nereis*.

ACKNOWLEDGEMENTS

I should like to thank my Supervisor, Dr. Peter Evans, for his advice throughout my project, and in particular for his constructive criticisms in the preparation of this manuscript.

I am also extremely grateful to Dr. Phillip Williamson for all his help, and for his patience both with me and with temperamental machines.

My thanks are due to the NERC for their financial support this year, without which this project would not have been possible.

REFERENCES

BOYDEN, C. R. (1974) Nature 251, 311-314.

BROOKS, R. R. and M. G. RUMSBY (1965) Limnol. Oceanogr. 10, 521-7.

BRYAN, G. W. (1971) Proc. Roy. Soc. Lond. B. 177, 389-410.

BRYAN, G. W. and L. G. HUMMERSTONE (1971) J. Mar. Biol. Ass. 51, 845-63.

BRYAN, G. W. and L. G. HUMMERSTONE (1973) J. Mar. Biol. Ass. 53, 839.

CASSIE, R. M. (1954) Aust. J. Mar. Freshw. Res. 5, 513-22.

COE, W. R. and D. L. FOX (1942) J. Exp. Zool. 90, 1-30.

COE, W. R. and D. L. FOX (1943) J. Exp. Zool. 93, 205-49.

COULTHARD, H. S. (1929) Contr. Can. Biol. Fish. 4, 121-36.

EUSTACE, I. J. (1974) Aust. J. Mar. Freshw. Res. 25, 209-20.

FIELD, I. A. (1922) Bull. Bur. Fish. Wash. 38, 127-259.

INGVARSSON, S. (1973) M.Sc. dissertation, University of Durham.

JONES, D. J. (1970) Ph.D. Thesis, University of Durham.

MACKAY, N. J. et al (1975) Aust. J. Mar. Freshw. Res. 26, 31-46.

MATEEVA, T. A. (1948) Trudy Murmansk, Biol. Inst. 1, 215-41.

MOSSOP, B. K. E. (1922) Trans. Can. Inst. 14, 3-22.

ORTON, J. H. (1926) J. Mar. Ass. UK. 14, 239-79.

PRESTON, A. et al (1972) Environ. Pollut. 3, 69-82.

QUARLES III, H. D. et al (1974) J. Appl. Ecol. 11 (3).

SAVILOV, A. I. (1953) Trudy Inst. Onkol. 7 (198).

SCHULZ-BALDES, M. (1972) Mar. Biol. (Berl) 16 (3), 226-229.
(1974) Mar. Biol. (Barl) 25 (3), 177-193.

SEED, R. (1968) J. Mar. Biol. Ass. 48, 561.

SEGAL, E., K. P. RAO and T. W. JAMES (1953) Nature 172, 1108-9.

SEGAR, D. A., J. D. COLLINS and J. P. RILEY (1971) J. Mar. Biol. Ass. UK. 51, 131-36.

WARREN, A. E. (1936) J. Biol. Bd. Can. 2, 89-94.

WEYMOUTH, F. W. (1923) Bull. Dep. Fish. Game. St. Calif. No. 7.

WILLIAMSON, H. C. (1907) Scient. Invest. Fishery Bd. Scotl. 25th Annual Report, p. 221-55.