Some studies on macama balthica l

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Some studies on *Macoma balthica* L.

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CONTENTS

LIST OF TABLES IN THE TEXT

LIST OF FIGURES IN THE TEXT

CHAPTER 1

Introduction

The bivalve Macoma balthica is a common littoral inhabitant of both coastal and estuarine waters around the British Isles.

On intertidal mud-flats where Macoma often reaches high population densities this species may be ecologically important as a food for many shore birds and flatfish.

The distribution of Macoma has been investigated by many workers, e.g. Beanland, 1940; Rees, 1940; Brady, 1943; Holme, 1949; and Newell, 1965. Most of them have claimed that substrate nature is an important factor affecting distribution, but there are many different views about the kind of substrate which is the most favourable. One of the most recent investigations is that by Newell (1965) who also studied the diet of Macoma in the Thames estuary. Newell found that its population density increased as the substrate became finer. This he explained by showing that Macoma fed mainly on living microorganisms in his study area and that the population density of microorganisms is much higher in muddy substrate than in sandy substrate since there is a higher surface area to weight ratio in fine sediments, and surface area affects bacterial density.

Because the river Thames runs through areas with very dense human settlements the inflow of faecal organic matter and bacteria is high. The present study is concerned with the presence of Macoma in an unpolluted area to find out if its

distribution on the Lindisfarne Nature Reserve, Northumberland followed the same principles as in the Thames estuary.

Inflow of faecal bacteria into this area is very low, the main sources of bacteria being sea water and Spartina beds (A. Meyer, pers. comm.).

Previous investigations of the invertebrate fauna at Fenham Flats (P. R. Evans, unpublished) had shown that Macoma balthica is the most abundant bivalve species in this area, so this investigation was thought to be feasible.

Studies were also carried out on size and age distributions, dispersion in an area of high density, dry weight of flesh and shells, calorific content, and concentration of two heavy metals in the flesh of the animal.

The results of these studies are presented in the following chapters with a discussion at the end of each chapter.

Study Area

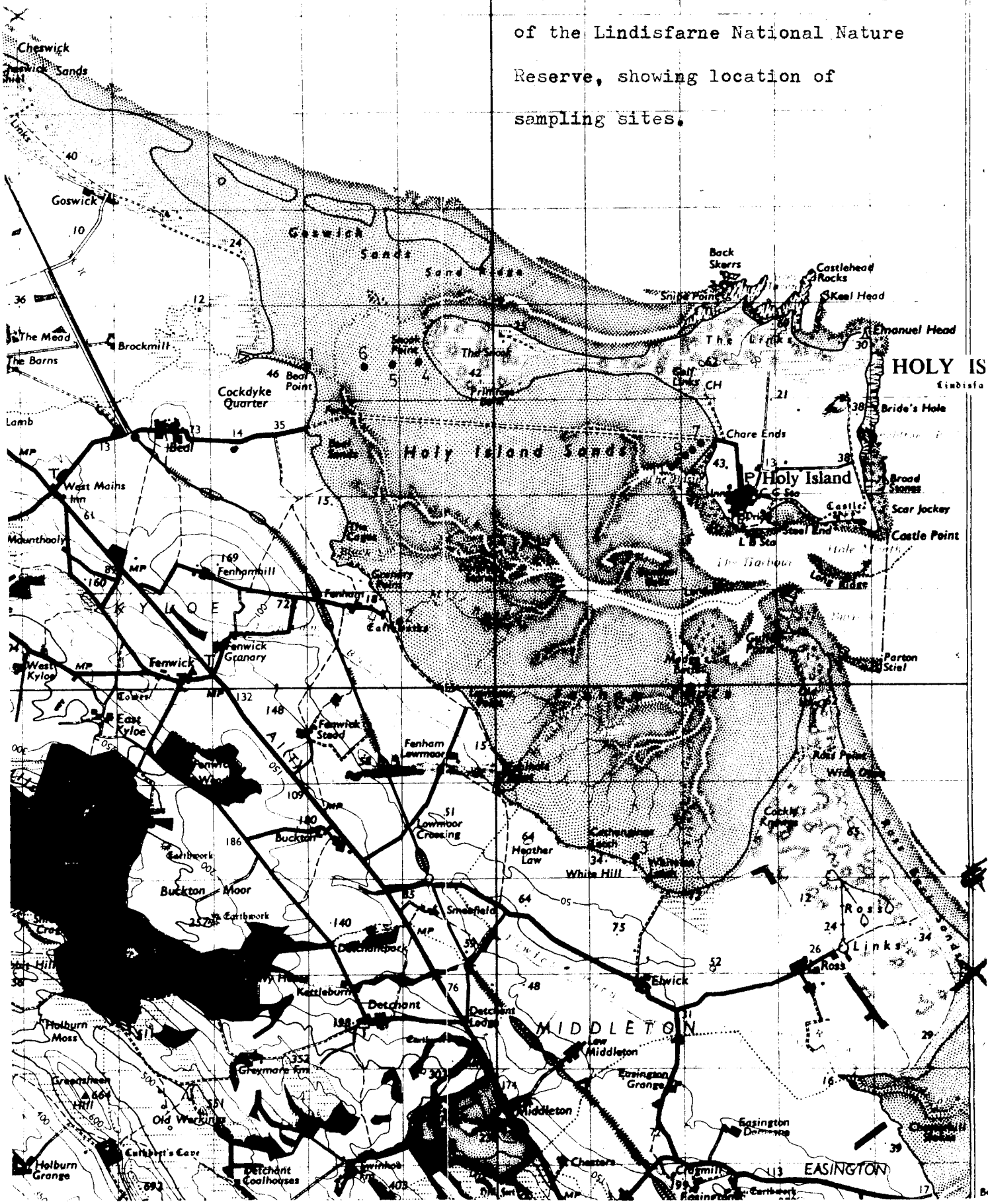
Fenham Flats and Holy Island Sands, Lindisfarne National Nature Reserve

These tidal flats (Fig. 1) lie to the north of Ross Links, Northumberland, between Holy Island and the mainland.

The tide comes in from the south because the strong southerly tidal drift along this part of the coast has formed a sand ridge at the northern end of the flats.

The substrate in the northern part of the area (Holy Island Sands) is mainly clean sand and firm sandy mud.

Figure 1. Map of the northern part of the Lindisfarne National Nature Reserve, showing location of sampling sites.



However, the southern part is very muddy so that sampling was difficult except at high tidal levels.

On the mainland side of the flats the highest tidal levels are occupied by the cord-grass, Spartina anglica. Zostera marina, the eel-grass, also occurs at slightly lower tidal levels.

Choice of sampling sites

Due to the extreme softness of the substrate in the southern part of Fenham Flats it was decided to sample only at high tidal levels which are accessible with safety.

Beanland (1940), studying the invertebrate fauna in the Dovey estuary, concluded that the distribution of Macoma balthica depended possibly on two factors, namely (a) "quality and quantity of available food supply, correlated with type of soil", and (b) "available feeding time, correlated with distance below high-tide mark".

If these conclusions are correct, then examination of stations at the same tidal level should show up any differences between sites in available food in the substrate by differences in density of Macoma.

Accordingly, for one part of the project, seven stations were chosen, all at similar tidal levels. The tidal level was checked by watching an incoming tide on 13 August. The height of this tide was an average one for this area, 11.8m (this equals 2.6m over Newlyn Datum). The time for which the animals were covered by the tide at the seven sites ranged from 2 hours 50min to 3 hours 30min.

To gain information about the distribution of Macoma at different tidal levels, a transect was taken down the shore from the end of the Causeway at Holy Island (site 12 on Fig. 1). This area was chosen for the transect because previous investigations of the invertebrate fauna of the Lindisfarne Reserve had shown high densities of Macoma there at the lower tidal levels, and also because at this site the distance from high water mark to low water mark is short.

CHAPTER 2

Substrate sampling and analysis

At each station where the density of the *Macoma* population was estimated, three other variables were also measured. These were the percentages of (i) silt and clay, (ii) organic carbon, and (iii) nitrogen in the deposits.

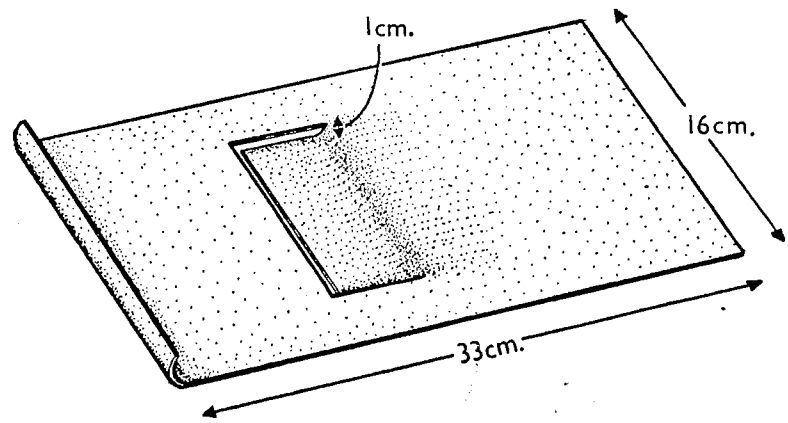
The technique used for substrate sampling was a mud-sledge similar to that designed by Capstick (1957) and illustrated in Fig. 2. By using this mud-sledge only the top 1cm of the substrate is sampled. *Macoma* is known to feed mainly from the surface deposits, so this technique should give relevant information about conditions in the animals' feeding area. The mud samples were transferred to polythene bags and brought to the laboratory. There a proportion of each sample was removed for organic analysis and oven dried at 105°C for 48 hours.

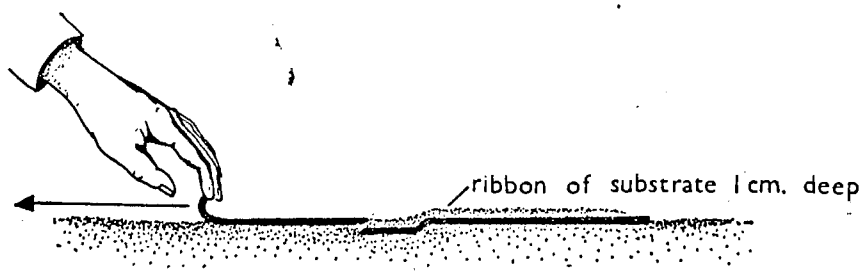
Percentage of silt and clay

The percentage by weight of silt and clay in the deposits was estimated by dry sieving using an automatic sieving machine (Endecott Sieve Shaker). The mesh aperture of the sieve that was used was 0.08mm. To avoid coherence of the finer particles of the substrate it was found necessary to wash the mud samples several times with industrial methylated spirit before drying, as suggested by Newell (1965).

Figure 2. Mud sledge (Capstick, 1957) and mode of use (from Dunn, 1967).

CAPSTICK MUD SLEDGE





After washing, the samples were dried in an oven at 105°C for 2 hours and then cooled in a desiccator. After this sieving could take place, and the percentage of silt and clay were calculated for each substrate as the proportion by weight of material that passed through the 0.08mm (200 mesh) sieve.

Estimation of organic carbon

To estimate the percentage of organic carbon the Walkley Black wet oxidation technique was used. This method is described in Appendix 1. The method has limitations in that the percentage recovery varies according to the condition of the organic carbon, but usually about 75% of the organic carbon present in the sample is detected (Morgans, 1956; Newell, 1965). Three determinations were made for each sample and the mean value calculated.

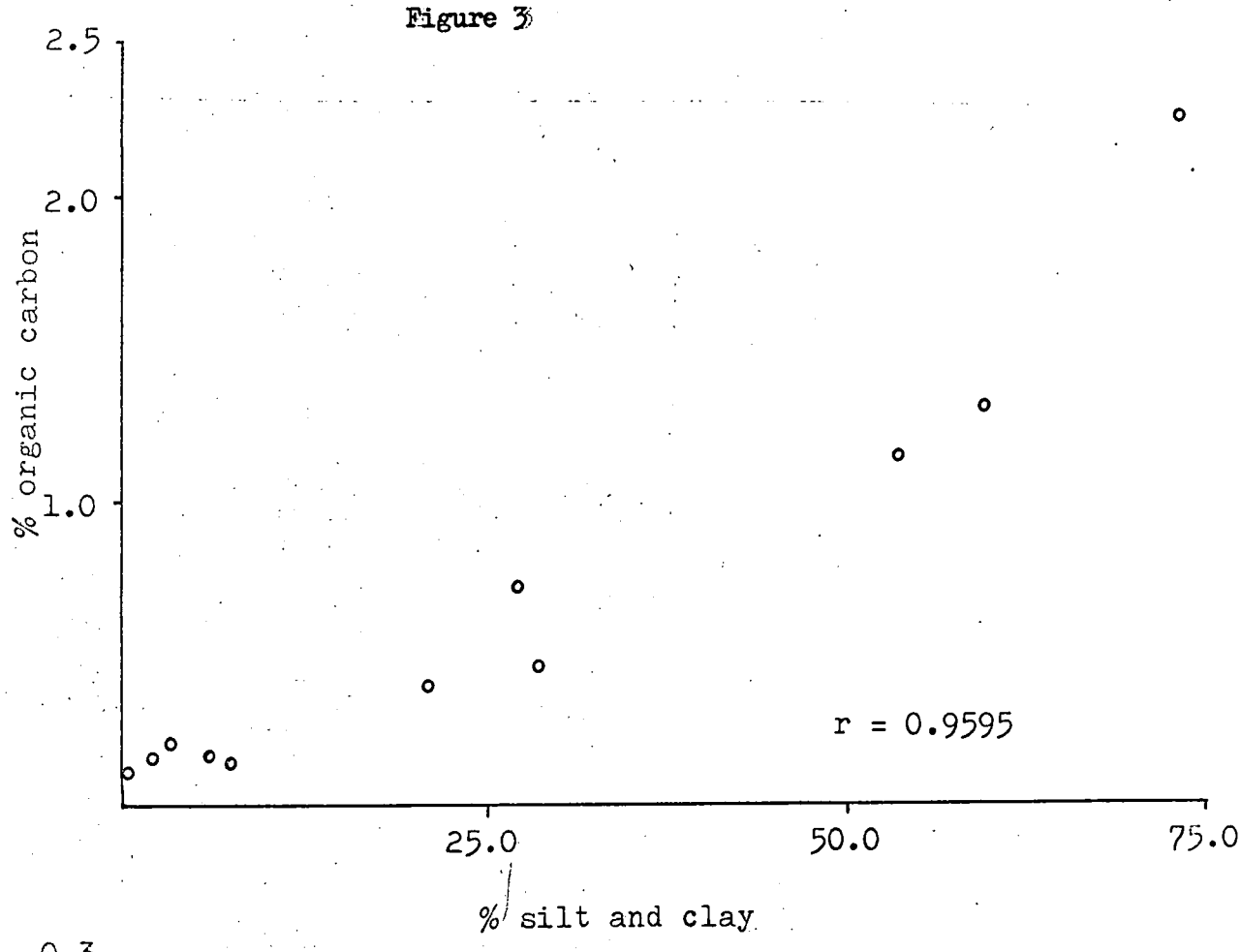
Estimation of organic nitrogen

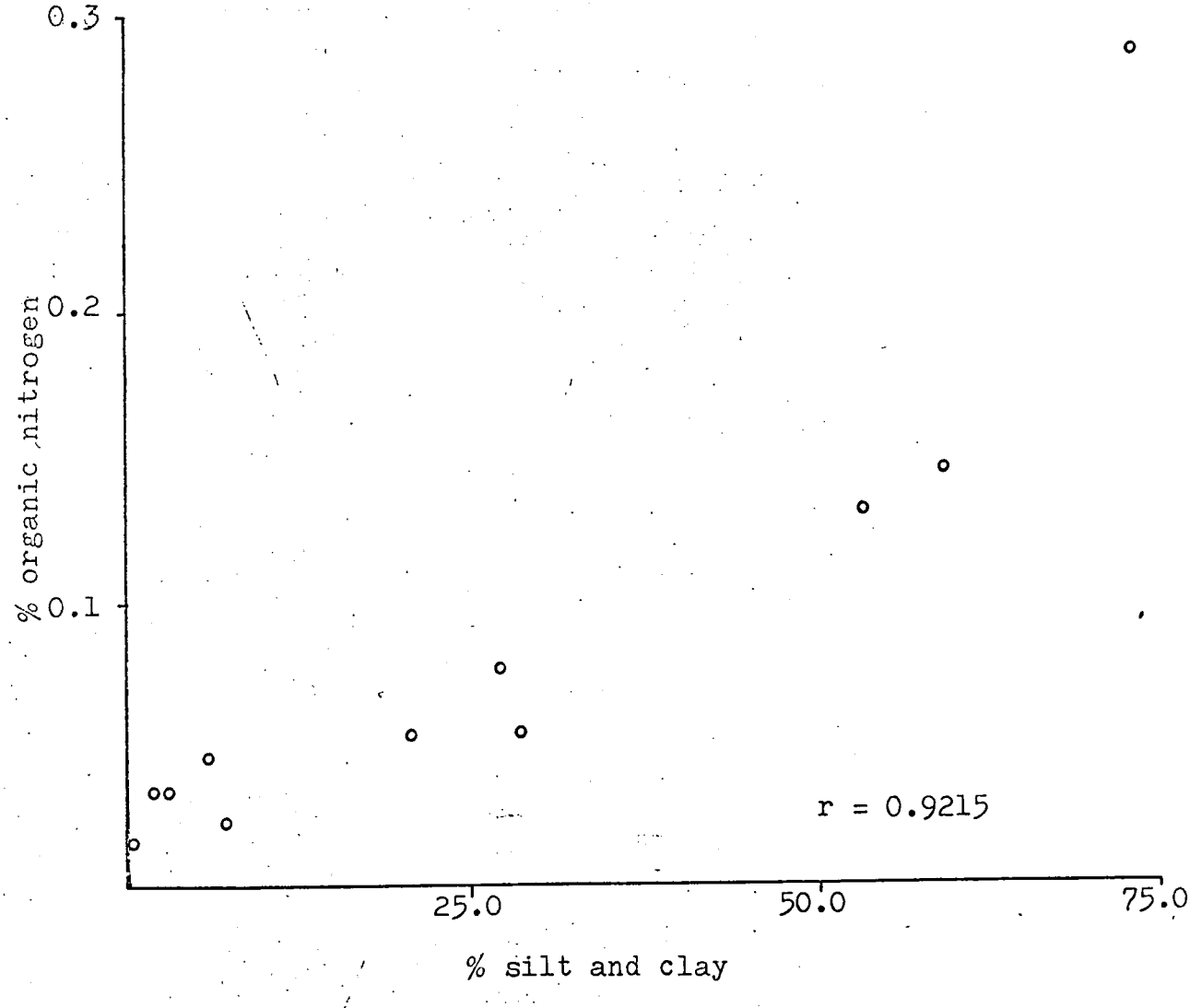
Organic nitrogen was estimated by the semi-micro Kjeldahl method involving conversion to ammonia. This method is described in Appendix 2. Three determinations were made for each sample and the mean value calculated. At the same time and sites that samples were taken for nitrogen estimation, further samples were taken at each station for bacterial counts. These were made by Mr. Adrian Meyer to find out if there was any correlation between numbers of live bacteria and amount of organic nitrogen in the substrate.

Table 1. Results of substrate analysis

Station	Percentage silt & clay	Percentage organic carbon	Percentage nitrogen	Numbers of bacteria
1	27.1	0.72	0.075	
2	73.2	2.25	0.290	4.05×10^6
3	28.3	0.44	0.052	0.70×10^6
4	7.3	0.15	0.023	
5	2.0	0.15	0.036	
6	5.8	0.15	0.046	
7	0.3	0.10	0.015	0.75×10^6
8	3.2	0.20	0.034	1.30×10^6
10	20.6	0.38	0.052	2.10×10^6
11	53.4	1.15	0.132	4.45×10^6
T	59.4	1.31	0.146	1.15×10^6

Figure 3

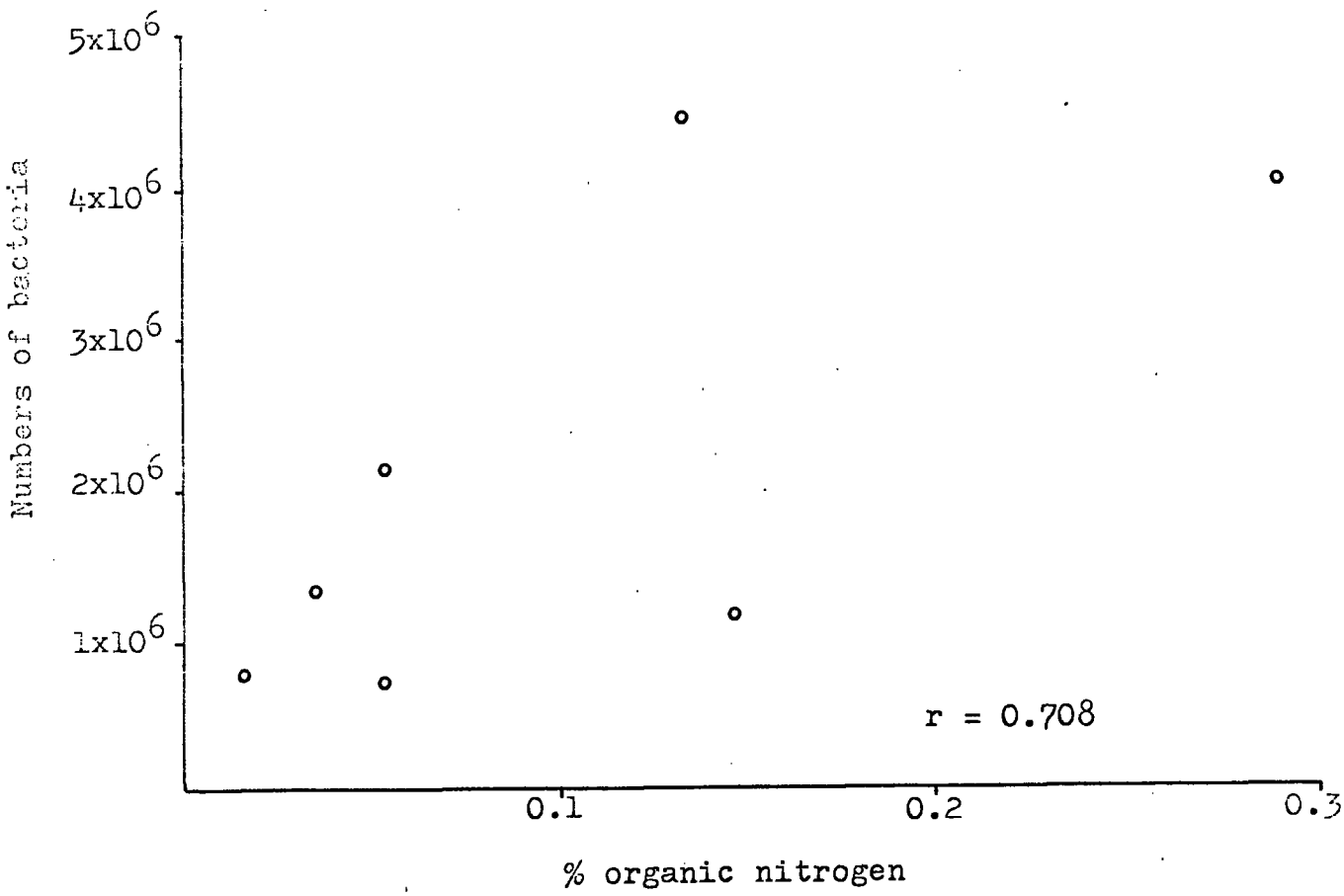

2.5
2.0
1.0
% organic carbon
r = 0.9595
25.0
50.0
75.0
% silt and clay


0.3
0.2
0.1
% organic nitrogen
r = 0.9215
25.0
50.0
75.0
% silt and clay

coefficient, r = 0.708, is significantly different from zero (0.05 P 0.02). r^2 = 50%, i.e. 50% of the variation in organic nitrogen is associated with variation in the numbers of live bacteria between samples. When dead bacteria are also considered, this indicates that the main source of organic nitrogen in the substrate is bacteria.

Figure 4. Numbers of bacteria plotted against percentage of organic nitrogen

Figure 4. Numbers of bacteria plotted against percentage of organic nitrogen



CHAPTER 3

Distribution of Macoma

(a) High tidal level sampling, stations 1 - 7 (see Fig. 1)

At each station 3 - 5 samples were taken. The surface area of each sample was 33cm x 33cm (1/9 of a square metre). The first two samples at each station were taken down to 25cm depth to find out if there were any Scrobicularia plana at the sites. If this bivalve did not occur at the site the other 3 samples were taken to only 15cm depth, but still well below the burrows of Macoma.

The substrate so obtained was then sieved through a sieve which had a mesh aperture of 1mm. The Macoma from each quadrat were kept in water from the site and brought to the laboratory where the numbers from each sample were counted. The animals were then dried in a vacuum oven at 60°C for 48 hours.

Results

The density of Macoma at each station was calculated by multiplying the mean number from the replicate samples by 9. The figures so obtained give the number per square metre.

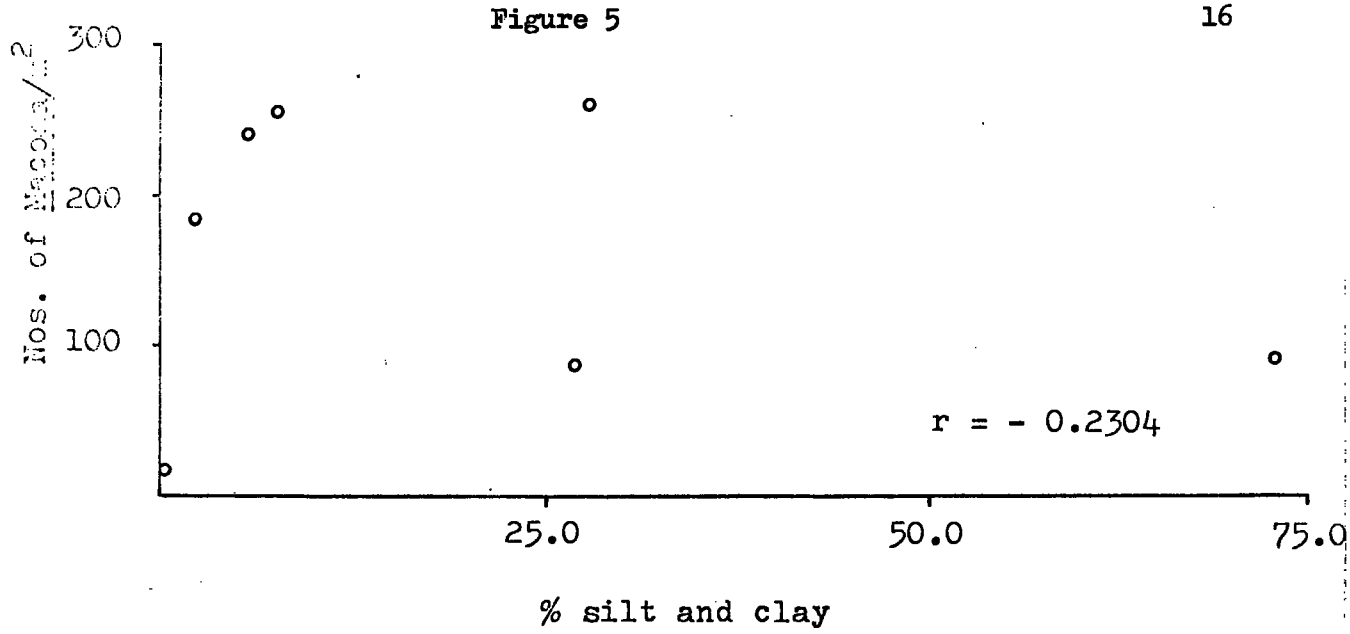
The population estimates are presented in Table 2 together with standard deviation and coefficient of variation for each set of samples. The mean numbers of Macoma at each station were then plotted against the three variables of the substrate and correlation coefficients calculated (Fig. 5).

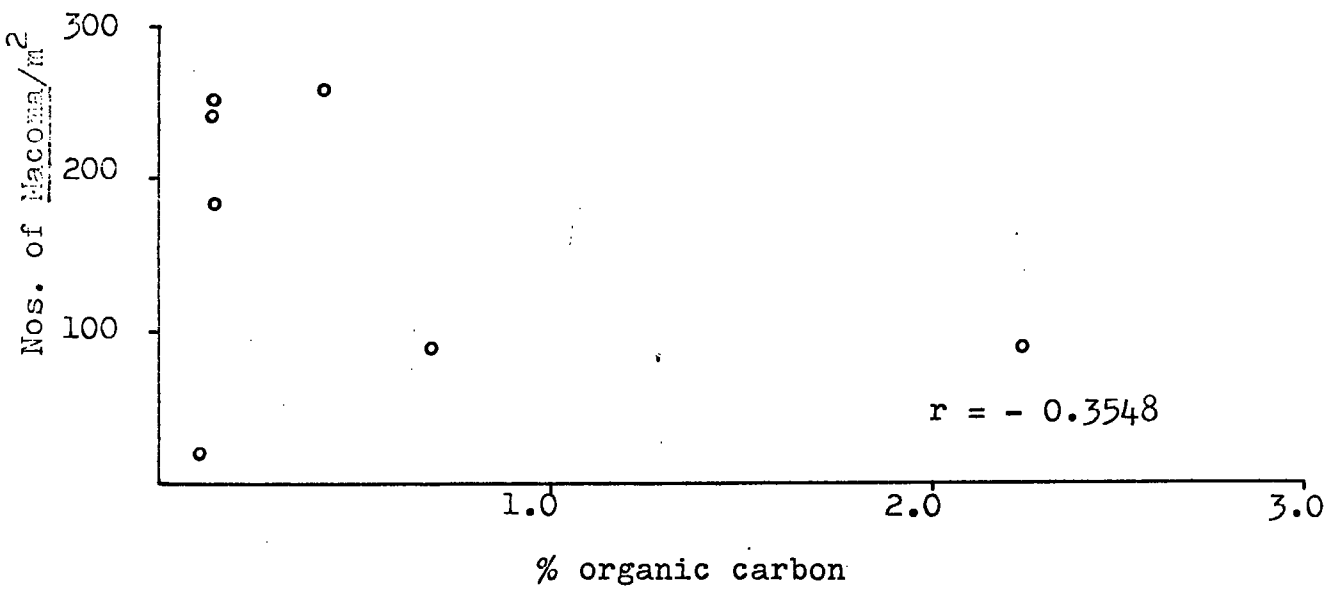
Table 2. Population estimates of Macoma obtained from replicate samples at seven high tidal level stations, together with standard deviation (S.D.) and coefficient of variation for each group of samples

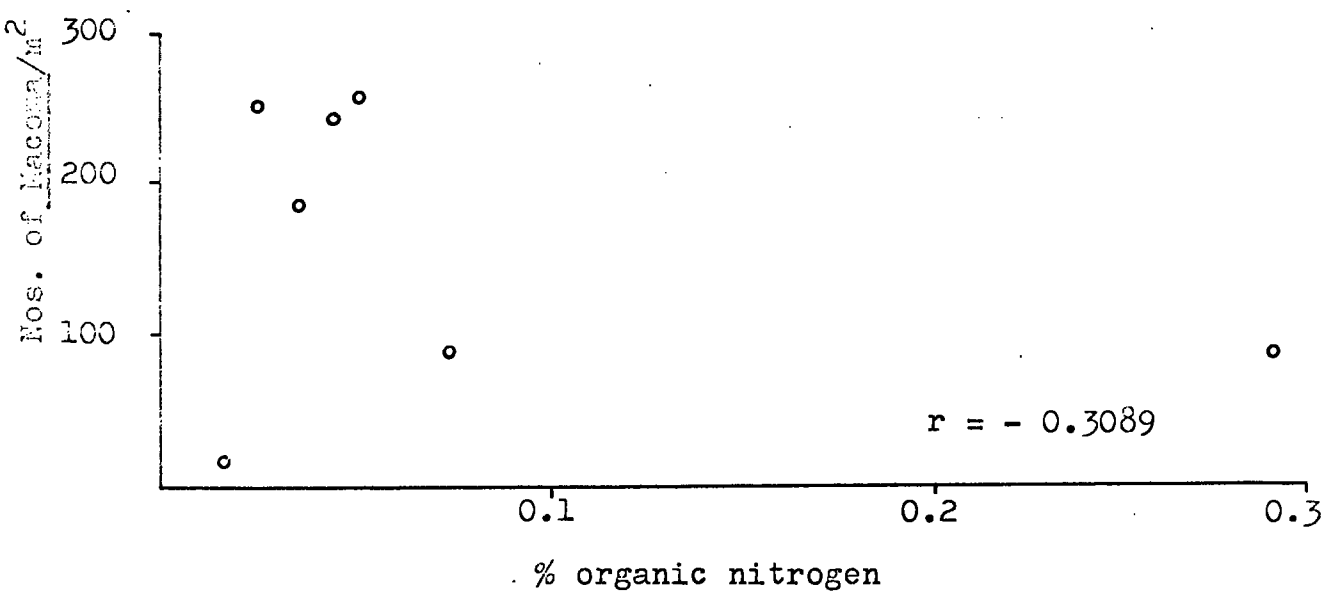
Date	Station	Number of Macoma in each replicate	Mean	S.D.	Population estimate	Coefficient of variation (100 S.D./$\bar{x}$)
27 May	1	6 - 15 - 8 - 10 - 7	9.2	3.5	82 per m^2	38.7
27 May	2	8 - 15 - 10 - 5	9.5	4.2	85 per m^2	44.2
1 June	3	26 - 25 - 30 - 23 - 37	28.2	5.5	253 per m^2	19.6
8 June	4	31 - 27 - 28 - 25	27.8	2.5	250 per m^2	9.0
8 June	5	19 - 15 - 15 - 29 - 23	20.2	5.9	181 per m^2	29.3
8 June	6	36 - 29 - 25 - 15	26.3	8.7	236 per m^2	33.3
18 June	7	1 - 2 - 2 - 0 - 3	1.6	1.1	14 per m^2	71.3

Figure 5. Numbers of *Macoma*/m^2 plotted against percentage of (a) silt and clay (b) organic carbon (c) organic nitrogen

Figure 5


Nos. of Macoma/m2
300
200
100
r = - 0.2304
25.0
50.0
75.0
% silt and clay


Nos. of Macoma/m2
300
200
100
r = - 0.3548
1.0
2.0
3.0
% organic carbon


Nos. of Macoma/m2
300
200
100
r = - 0.3089
0.1
0.2
0.3
% organic nitrogen

Discussion

The coefficients of variation for most of the groups of replicate samples are large, which implies that the confidence limits for the population estimates are very broad. Higher numbers of samples from each station would be needed to make more accurate estimates.

None of the coefficients of correlation between numbers of Macoma at the seven stations and the three measured properties of the substrate are significantly different from zero ($p > 0.1$). This means that factors other than available food in the substrate are more important in controlling the population density of Macoma at this tidal level. For example, Dunn (1967) found that settlement of spat was low in areas where time of exposure was long, i.e. at high tidal levels. Also in cold winters it is likely that mortality of Macoma in their first year of life is higher at high tidal levels than lower on the shore, due to the greater ease with which ice will form over the mud.

In the present study other animal species are not likely to have affected the density of Macoma, except possibly at station 1 where Scrobicularia plana occurs at a density of 138 per m^2. According to Green (1968) these two species do not occur together at high densities to any extent, but at low densities they can coexist at almost equal numbers/m^2, as is the case here. It is therefore possible that the density of Macoma at station 1 does not give a reliable measure of the number that could live there without the coexistence of Scrobicularia plana.

If the density of Macoma at station 1 is omitted when the correlation coefficients are calculated, these become $r = -0.2079$ (% silt and clay), $r = -0.3478$ (% organic carbon) and $r = -0.3314$ (% organic nitrogen); as before, none of them is significantly different from zero.

(b) Sampling down the shore

It has been noted by several authors (e.g. Bradfield and Newell, 1961) that Macoma usually reaches its highest density in a broad belt at or about mid-tide level on intertidal mud flats. To see if this was the case in the present study a transect was taken down the shore.

Methods and results

The transect was taken from "The Basin" (L.W.M.N.T.) to the end of the Causeway (H.W.M.N.T.), Fig. 1. Sampling sites were separated by about 20m. At each site four samples 10cm x 10cm and 10cm deep were taken and sieved. The mean number of Macoma from the four samples at each site was then calculated and multiplied by 100 to give the number of Macoma per m^2. Results from this sampling are shown in Fig. 6.

To find out if the size-distribution of Macoma at lower tidal levels differed from that at high tidal levels (see later), it was necessary to take larger samples because the number of Macoma sampled in four 10 x 10cm quadrats at each site on the transect was too low. Therefore stations 8, 9, 10 and 11 (Fig. 1)

Figure 6. Numbers of *Macoma*/m^2 at different sites on the transect

Figure 6

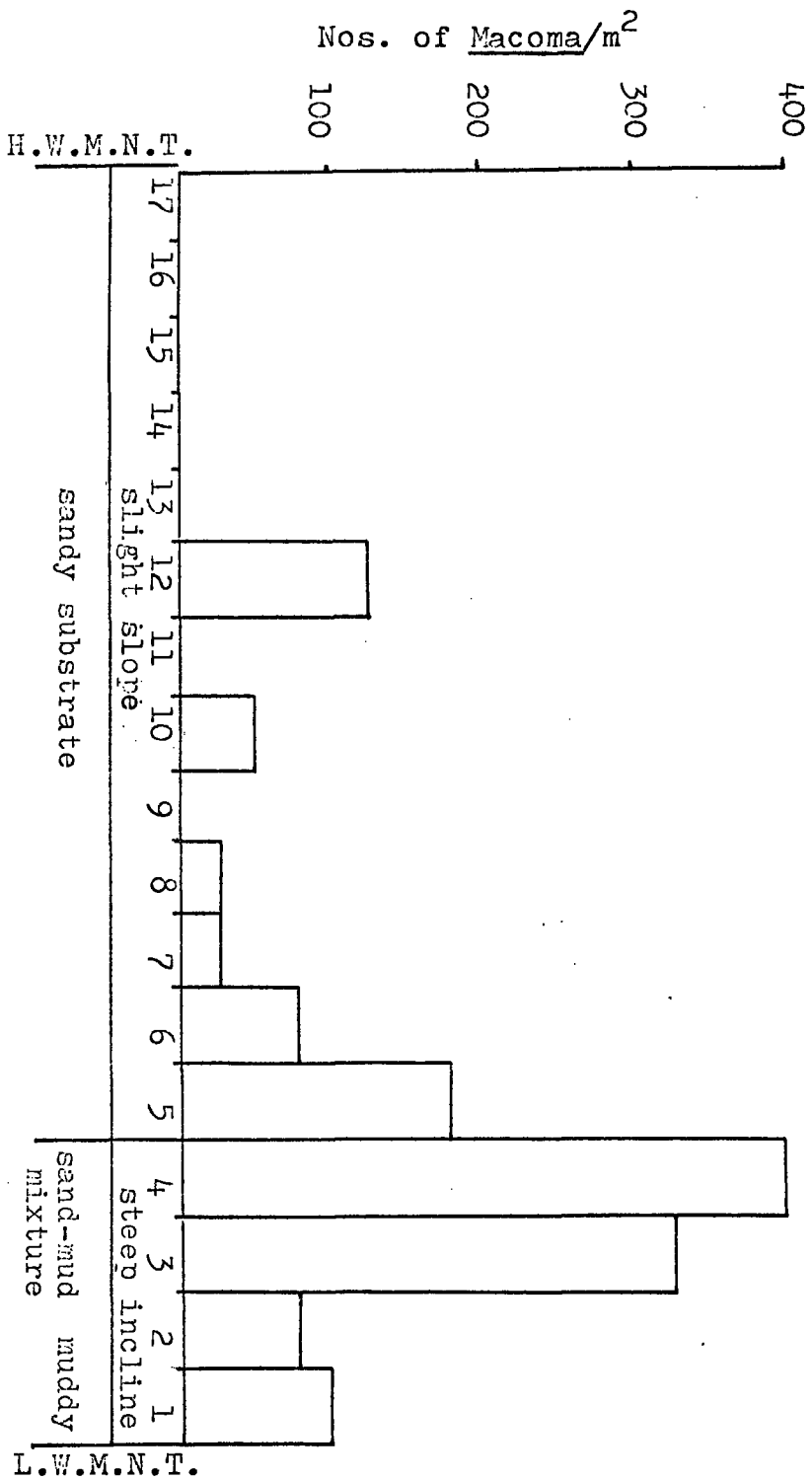

Nos. of Macoma/m²
100
200
300
400
H.W.M.N.T.
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
sandy substrate
slight slope
steep incline
sand-mud mixture
muddy
L.W.M.N.T.

Table 3. Population estimates of Macoma obtained from replicate samples at stations 8, 9, 10 and 11, together with standard deviation (S.D.) and coefficient of variation for each group of samples

Date	Station	Number of Macoma in each replicate	Mean	S.D.	Population estimate	Coefficient of variation 100 S.D. / x
18 June	8	24 - 8 - 22 - 14 - 10	15.6	7.1	140 per m^2	45.7
18 June	9	32 - 27 - 28 - 32 - 51	34.0	9.7	306 per m^2	28.7
3 July	10	70 - 67 - 68	68.3	1.6	614 per m^2	2.3
3 July	11	6 - 6 - 7	6.3	0.6	56 per m^2	9.4

were sampled in the same way as described for the high tidal level sampling. The population estimates for these stations are shown in Table 3 and substrate analyses in Table 1. Stations 11 and 8 were at the same tidal levels as transect sites 1 and 5, respectively.

Discussion

The population estimates show that the density was highest at sites 3 and 4 on the transect and at stations 9 and 10, station 10 holding the highest density of 614 Macoma per m^2. Knowing the tidal height of both ends of the transect (P. R. Evans, pers. comm.), it is clear that Macoma reaches its highest density about mid-tide level in this area.

Substrate analyses at stations 8, 10 and 11 showed that amounts of organic nitrogen and organic carbon were much higher at station 11 than at the other two stations. The scarcity of Macoma at station 11 (L.W.M.N.T.) therefore cannot be explained by limited amounts of available food in the substrate.

Stephen (1932, cited by Clay, 1962) found that young spat died quickly below low water mean tide level, and Segerstrale (1960, 1962, cited by Clay, 1962) found that the abundance of Macoma decreased as depth increased, mainly because of lack of recruitment. It is thus probable that the low density of Macoma at station 11 and sites 1 and 2 on the transect resulted chiefly from unsuccessful settlement of spat.

The dispersion of Macoma balthica in an area of high density

Holme (1950), studying the horizontal dispersion of the bivalve Tellina tenuis in the Exe estuary, found that the spacing of animals tended towards a regular distribution, indicating repulsion between individuals. As Tellina tenuis and Macoma balthica are closely related species, it was decided to investigate the dispersion of Macoma for comparison.

Methods and results

In an area of high density between stations 9 and 10 (Fig. 1) a plot 100cm x 50cm was marked on the substrate. This area was then divided into fifty adjacent 10cm x 10cm quadrats (see Fig. 7). The top 10cm of substrate from each quadrat was then dug out and sieved in the same way as used for the population estimates. The number of Macoma (irrespective of size) in each quadrat was then noted (Fig. 7). The mean number of Macoma per quadrat ($\bar{x}$) and the variance (s^2) were calculated to give a coefficient of dispersion ($s^2/\bar{x}$). In a clumped distribution this coefficient of dispersion has a value greater than one, but in even distribution less than one.

To find out if the dispersion of Macoma was significantly different from random the Chi-square test was used to compare the observed numbers of quadrats containing 0, 1, 2, etc. Macoma per quadrat with the numbers expected from a random distribution, calculated from the Poisson series.

Figure 7. Observed number of Macoma in each 10cm x 10cm quadrat

	A	B	C	D	E	F	G	H	I	J
I	3	6	2	4	4	6	6	2	5	6
II	5	3	2	3	4	1	8	5	3	4
III	5	5	4	2	3	8	5	5	5	1
IV	5	3	3	3	7	5	7	3	2	5
V	5	1	5	5	3	4	5	3	1	2

A value of chi-squared = $(O - E)^2/E$ was calculated for each class of observation (classes where E was less than 5 were grouped with adjacent classes) and the values then summed (Table 4). The value obtained, 9.1648, lies between the values expected for χ^2_4 at the 5% (9.488) and 10% levels (7.78) of significance, but closer to the former.

Discussion

The results from this study show that the distribution of Macoma in the plot of high density (404 Macoma per m^2) was significantly different from random at about the 6% level. Had more quadrats been examined, the tendency towards an even distribution might have been confirmed as a generalization.

Holme (1950) plotted the positions of individual shells in a natural population of Tellina tenuis and found that fewer individuals than expected occurred less than 1 in apart and none less than 0.6 in apart. He also found that by increasing the density of Tellina artificially on the shore shore the chances of randomly distributed individuals lying close to one another increased.

From these results Holme suggested that at moderate densities each individual occupied a "territory" determined by the range of movement of its inhalant siphon on the substrate surface. If the tendency towards an even distribution in the Macoma population studied had been confirmed, it could have been explained in the same way, as Macoma also has a long inhalant siphon which scours the surface of the substrate.

Table 4. Observed and expected numbers of quadrats containing different numbers of Macoma

Number of Macoma per quadrat	Observed frequency O	Expected frequency E*	Sign of deviation O - E	$(O - E)^2/E$
0	0	0.88	)	
			)	
1	4	3.55	) - 1.60	0.2206
			)	
2	6	7.17	)	
3	11	9.66	1.34	0.1858
4	6	9.75	- 3.75	1.4423
5	15	7.88	7.12	6.4332
6	4	5.30	- 1.30	0.3189
7	2	3.06	)	
			)	
8	2	1.54	) - 1.81	0.5640
			)	
8	0	1.21	)	

$\bar{x} = 4.04$ Total $\chi^2 = 9.1648$

$s^2 = 3.038$

* Derived from Poisson distribution with the same mean and variance

CHAPTER FOUR

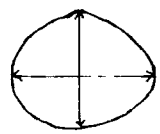
Size and age distribution

This investigation was made to find out (i) the general size and age distribution of the Macoma population, and (ii) if there were any differences in size distribution and growth rate between different stations at the same tidal level and/or between stations at different tidal levels.

In the Dee estuary Stopford (1951) found that the settlement of spat was heaviest in June - July. Thus, sampling in May and June should not detect any considerable number of individuals spatted in the spring. The smaller individuals sampled during this study probably came from late in last year's (1972) spawning season. The scarcity of animals under $10mm^2$ ($\leq$ 3.5mm on length basis) lends support to this.

Measurements of animals

When the collections of animals had been dried, each one was measured across its longest horizontal axis and longest vertical axis (see diagram).



Multiplying the figures so obtained gave an index of the area of the shell. This index was thought to be a more logical measure of growth rate than the length of the shell that has been used by other workers.

Table 5. Mean area indices of Macoma balthica at the end of each year of growth

Station	Tidal level	End of 1st year	End of 2nd year	End of 3rd year	End of 4th year	Increase in area index per year			
						1st	2nd	3rd	4th
1	High	25mm^2	66mm^2	96mm^2	121mm^2	25mm^2	41mm^2	30mm^2	25mm^2
3	High	40 "	81 "	121 "		40 "	41 "	40 "	?
4	High	32 "	74 "	110 "	151 "	32 "	42 "	36 "	41 "
9	Mid	39 "	95 "	150 "	197 "	39 "	56 "	55 "	47 "
					Mean	34.0mm^2		42.5mm^2	

Figure 8a. Histograms showing the number of Macoma in each $10mm^2$ size-class as a percentage of the total stations 1, 2, 3, 4 and 5

Figure 8a

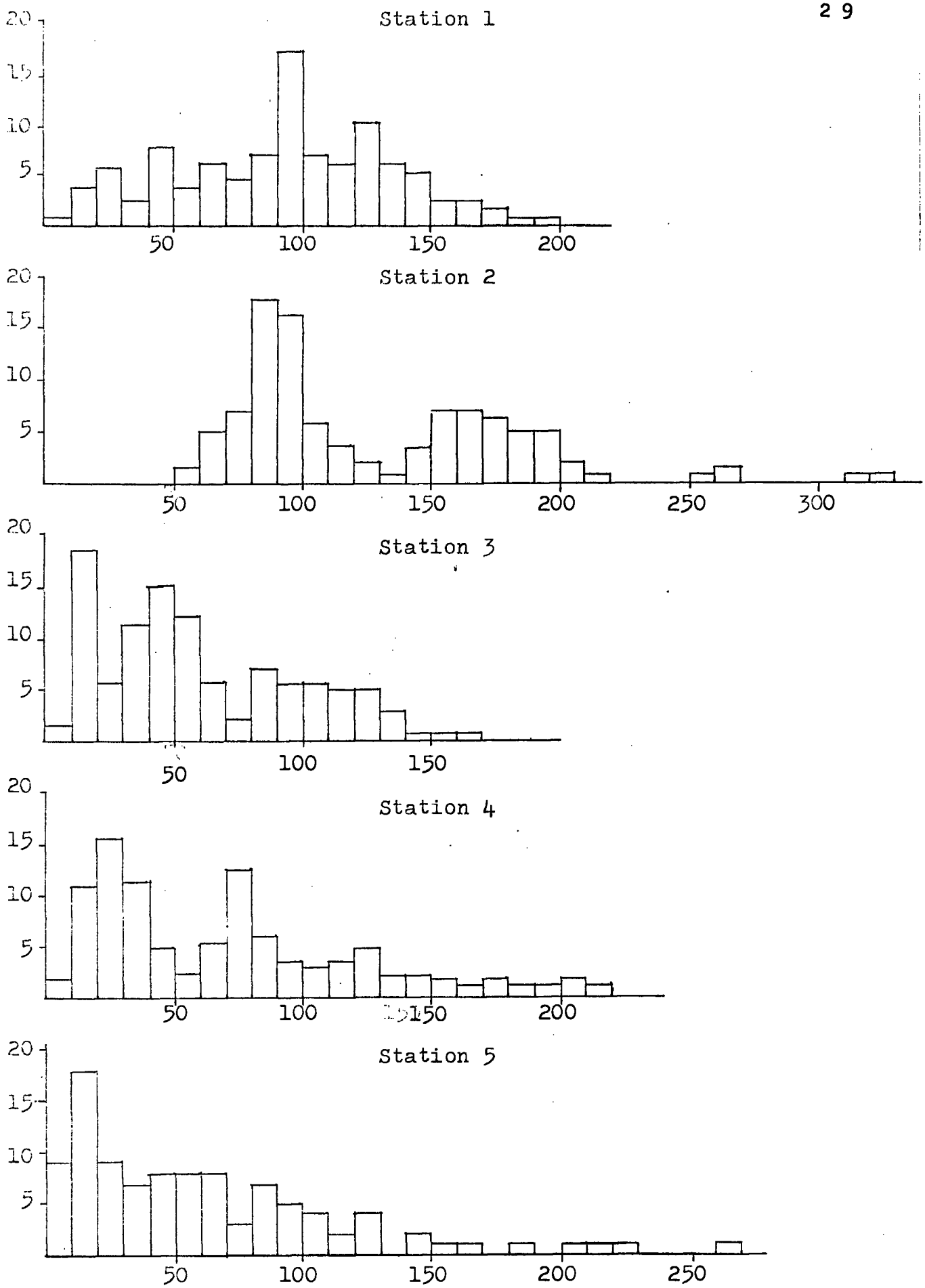
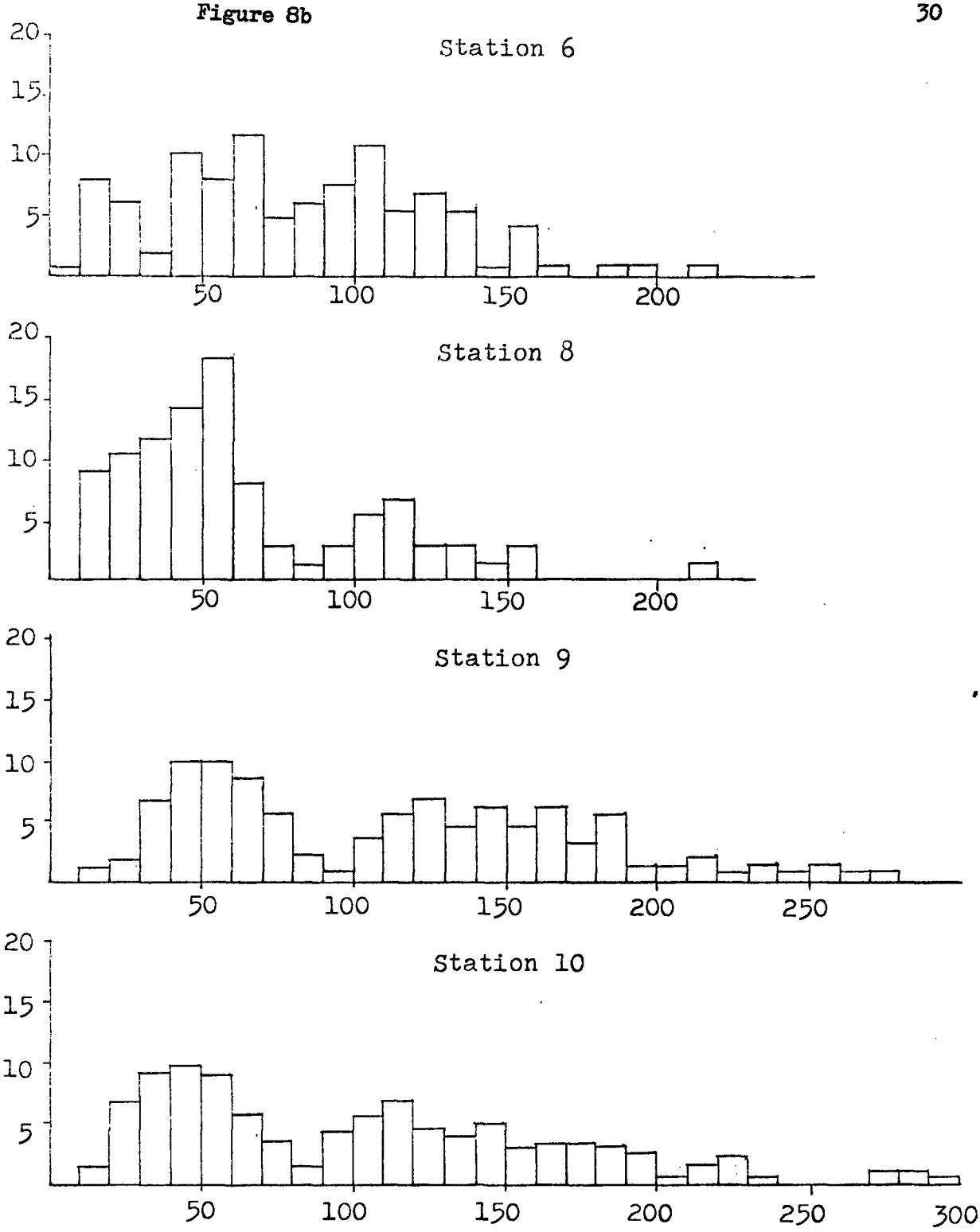

Station 1
20
15
10
5
50
100
150
200
Station 2
20
15
10
5
50
100
150
200
250
300
Station 3
20
15
10
5
50
100
150
Station 4
20
15
10
5
50
100
150
200
Station 5
20
15
10
5
50
100
150
200
250

Figure 8b. Histograms showing the number of *Macoma* in each $10mm^2$ size-class as a percentage of the total stations 6, 8, 9 and 10

Figure 8b


Station 6
20
15
10
5
50
100
150
200
Station 8
20
15
10
5
50
100
150
200
Station 9
20
15
10
5
50
100
150
200
250
Station 10
20
15
10
5
50
100
150
200
250
300

Discussion

Growth rate : The results presented in Table 5 show that after the first year the increase in area index of the shell is similar from year to year throughout the life span of the animal. As expected, the size reached by the end of the first year is lower. T-test between the means 34.0mm^2 and 42.5mm^2 (see Table 5) shows that growth rate in the first year of life is probably significantly lower than that in the second and third year ($t = 1.70$, $p \sim 0.1$).

The growth rate was lowest at station 1, most probably because of reduced salinity at this station resulting from a fresh water inflow. (Very little freshwater enters elsewhere on the mudflats). In a review of the literature, Clay (1962) records that "in the old port of Wimereux, in which the sea water only enters at the highest tides of the full moon, Macoma is only half as large as in marine positions. In the Baltic also, the animals are, on the whole, noticeably smaller than in open water". Dunn (1967) also found evidence of reduced growth rates in the upper reaches of the Ythan estuary, Scotland.

The highest growth rate occurred at station 9, sited at about mid-tide level. These results agree with those of Brady (1943) who found that the growth rate of Macoma at Budle Bay (3 miles south of Lindisfarne) was higher at lower tidal levels, most likely as a result of increased feeding time during submersion.

Size distribution from measurement of area indices

Most of the histograms (Fig. 8a and b for the different stations show distinct peaks at one or more small size-classes. As the size increases, the peaks become less distinct, probably as a result of increased overlap between age-classes. The results of the growth rate study allow the age-class represented by each peak to be decided tentatively.

The histogram for station 1 shows a major peak at 90 - 100mm^2. According to Table 5 the animals in this size-class are likely to be 3 years old; there is also a smaller peak at 120 - 130mm^2 showing the presence of 4 years old individuals. From the histogram it is obvious that settlement and/or survival of spat has been rather poor at this station in 1971 and 1972. The maximum life-span seems to be 5 or even 6 years.

At station 2 the growth rings on Macoma shells were very obscure, so ageing was almost impossible. However, because the sampling was made early in the growing season, the peaks on the histogram can be used to give an indication of growth rate. It is clear that one year old animals were completely missing from this site in 1973. The peak at 80 - 90mm^2 probably represents 2 years old animals. If this is correct, then the growth rate at this station is almost as high as at station 9, even though at a higher tidal level.

A high growth rate at station 2 could be explained by the very high levels of organic matter (debris) at this station (Table 1). The time for feeding may also be longer

at station 2 than at the other high tidal level stations, due to the softness of the surface deposits, with small mean particle size aiding retention of a surface water film.

At station 3 the growth rings on the shells were not very clear, though better than at station 2. The histogram for this station shows a high percentage in the 10 - 20mm^2 size-class with another peak at the 40 - 50mm^2 class, corresponding to the first year group as judged by growth rings. The only possible explanation for the smaller size-class is a highly successful settlement of spat late in the 1972 spawning season.

The histograms for the three other high tidal level stations (4, 5 and 6), which all represent similar substrate, do not show much variation, except that one year old animals are not as common at station 6 as at the other two.

It is clear from this study that the size-frequency distribution differs between stations at similar high tidal level. The differences are mostly caused by differences in the success of settlement and survival of spat at any one station from year to year. At these high tidal levels the maximum life span seems to be 5 to 6 years, but only low percentage of the population lives longer than 4 years.

Several workers have found that size-distribution changes with the time of exposure of the substrate. Segerstrale (1927, cited by Clay) concluded that a downshore migration took place during the development of the animal. If so, one would expect a dominance of larger size groups at lower tidal levels.

Selective predation could also affect the size-distribution at different levels on the shore. Goss-Custard (1969)

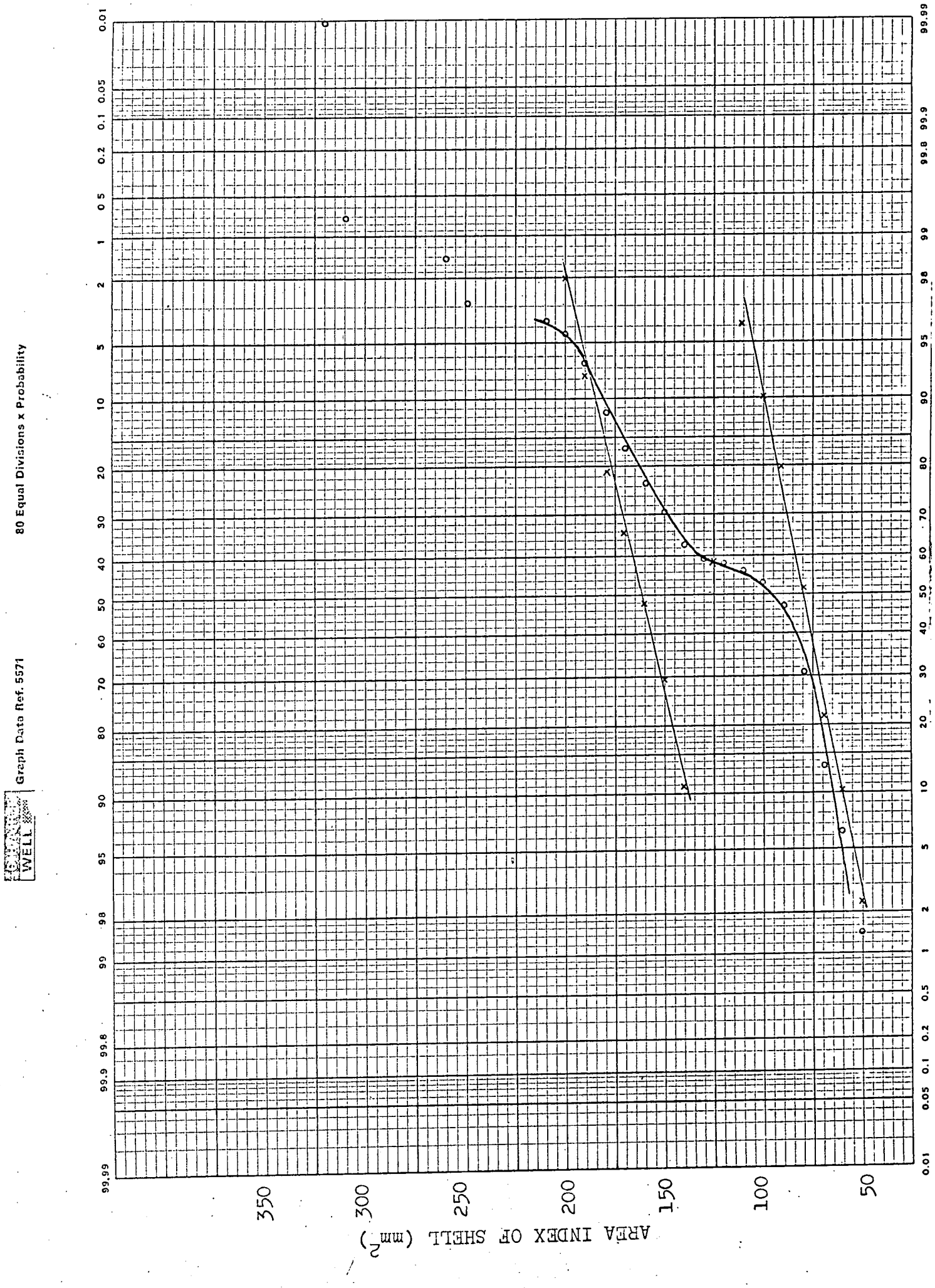
found that Redshank Tringa totanus, feeding in the Ythan estuary, had a preference for Macoma, ranging from 7 - 13mm in length (c.a. 40 - 150mm^2 on area index basis). As the time available for feeding by wading birds increases with distance from low water mark, size-preferences in their feeding could obviously affect the size-distribution of prey down the shore. However, in the present study the size-frequency distributions of Macoma at the lower stations (8, 9 and 10) do not provide evidence for migration or selective predation, perhaps because any such trends are masked by year-to-year variations in spat-fall at the different sites. The only marked difference between upper and lower levels seems to indicate higher growth rates lower down on the shore.

The most widely used graphical method for separating polymodal frequency distributions of size-classes into age-classes employs probability paper (Cassie, 1954). This method could be used only for results from station 2 in this investigation, because overlap between age-classes was too great at other sites. For station 2 the percentage cumulative frequency (PCF) distribution was plotted on arithmetic probability paper. The best fitting sigmoidal curve was then drawn through the points and the point of inflexion between the first two sub-populations located (see Fig. 9). This point occurred at the 59% PCF level which implies that the first sub-population (smallest size-class) comprised 59% of the total population.

There was no overlap between second and third sub-populations, therefore the proportion of the total population made up by the second sub-population could be calculated directly by subtracting 59% from 96.5% (the PCF level dividing the second

Figure 9. The size-class distribution of Macoma from station 2 plotted on probability paper, showing point of inflexion and calculated straight-line plots

Figure 9


AREA INDEX OF SHELL (mm²)
350
300
250
200
150
100
50
80 Equal Divisions x Probability
Graph Data Ref. 5571
WELL

and last size-classes in the population). The second size-class thus formed 37.5% of the total. To produce a straight line plot for the smallest sub-population, selected points were multiplied by $^{100}/_{59}$ and plotted on the same probability diagram. The same was done for the second sub-population except that before multiplying the selected points, 59% was subtracted from each PCF and the resultant percentages were multiplied by $^{100}/_{37.5}$. From the straight lines so produced, the means for each sub-population could be obtained by reading off the size at the 50% PCF level. Standard deviations for each mean were then calculated by subtracting the size at the 16% PCF level from the mean.

Results

Sub-population	1	59%	$\bar{x} = 80mm^2$	S.D. = 15.0
Sub-population	2	37.5	$\bar{x} = 162mm^2$	S.D. = 19.0

CHAPTER 5

Dry weight measurements

This part of the project was carried out to find out the relationships betweeen the dry weights of flesh and shells and the area index of *Macoma* size.

As the growth rate investigation showed that there was a difference in size of equal-age animals between stations it was decided to use animals from the same sites, including station 2, to find out if the dry weight of flesh and shells showed the same trend as the rate of growth.

Before drying, the animals were kept not longer than 5 - 6 hours in water from the sampling sites. It is therefore likely that they did not have enough time to clear their gut completely. Thus the dry weight of "flesh" is probably slightly too high, as it may include some sediments ingested prior to collection.

There was also a difference in date of sampling at the different sites; animals from station 9 were collected $3\frac{1}{2}$ weeks later than animals at stations 1 and 2. The animals at station 9 have thus had a slightly longer period in the 1973 growing season in which to gain weight. However, according to Ansell & Trevallion (1967), who studied *Tellina tenuis* in Scotland, this depends on when the spawning season begins. They found that the body weight was minimal from February to early May, but increased greatly during May. In June when the spawning season began, the body weight fell and fluctuated further throughout

the spawning season as a result of changing balance between growth of body and gonad and losses of material in spawning.

Hancock and Franklin (1972) found that the dry weight of *Cardium edule* was minimal at a similar time as found for *Tellina tenuis*. Therefore any relationship between dry weight of flesh and area index of shell of *Macoma* cannot be regarded as constant throughout the year.

Methods and results

Each animal from the five stations that had an area index above $50mm^2$ was weighed separately; both total weight and weight of flesh were obtained. The mean weights of shell and dry flesh for each size-class at the different stations are presented in Tables 6 and 7.

Correlation coefficients were calculated for $\log_{10}$ dry weight of flesh against $\log_{10}$ area index of shell, and $\log_{10}$ dry weight of shell against $\log_{10}$ area index of shell for each station. The correlation coefficients, r, together with the regression coefficients b (slope of regression line) and a (intercept on the ordinate) are presented in Tables 8 and 9.

To find out if the regression lines (see Fig.10) for different stations were significantly different, the slopes were compared by t-tests. For $\log_{10}$ dry weight of flesh against $\log_{10}$ area index of shell, t-tests showed that *b* for station 1 was significantly lower than b for all other stations. Also, b for station 9 was significantly higher than all the others. Values of t for these comparisons are given in Table 10.

Table 6. Mean dry weight of shell in each size-class (50mm^2) at stations 1, 2, 3, 4, and 9. Numbers in brackets give the number of measurements behind each mean

Size-class	Station 1	Station 2	Station 3	Station 4	Station 9
320		1205.7 (1)			
310		882.5 (1)			
300					
290					
280					
270					879.2 (1)
260		565.0 (2)			929.6 (1)
250		508.8 (1)			743.4 (2)
240					676.5 (1)
230					812.7 (2)
220					536.7 (1)
210		424.7 (1)		582.7 (2)	619.9 (3)
200		294.8 (3)		506.9 (2)	472.8 (2)
190	436.1 (1)	282.7 (7)		475.1 (4)	395.4 (2)
180	411.1 (1)	255.1 (7)		412.1 (1)	352.5 (9)
170	364.8 (2)	246.7 (9)		443.1 (2)	335.4 (5)
160	365.5 (3)	212.9(10)	261.5 (1)	322.2 (2)	303.6(10)
150	312.9 (3)	185.2(10)	249.0 (1)	299.3 (4)	239.7 (6)
140	261.4 (7)	172.5 (5)	267.3 (1)	249.2 (4)	233.9(10)
130	220.2 (8)		215.1 (4)	193.8 (4)	177.6 (6)
120	181.9(13)	131.4 (3)	159.1 (7)	169.2 (7)	176.2(10)
110	168.0 (8)	83.9 (5)		130.6 (7)	129.6 (9)
100	144.3 (9)	69.7 (8)	112.2 (8)	111.5 (5)	113.7 (6)
90	104.8(20)	62.8(22)	88.7 (8)	84.4 (6)	100.3 (1)
80	75.4 (8)	53.9(23)	75.5(10)	75.2(11)	65.8 (4)
70	64.9 (6)	43.6(10)	58.0 (3)	64.2(19)	49.4 (9)
60	52.4 (7)	38.4 (5)	44.6 (8)		38.6(14)
50	28.6 (5)	33.6 (2)	31.6(17)		27.0(15)

Table 7. Mean dry weight of flesh in each size class ($\geq 50mm^2$) at stations 1, 2, 3, 4 and 9. Numbers in brackets give the number of measurements behind each mean

Size-class	Station 1	Station 2	Station 3	Station 4	Station 9
320		196.3 (1)			
310		109.3 (1)			
300					
290					
280					
270					105.5 (1)
260		109.1 (2)			104.0 (1)
250		106.0 (1)			87.1 (2)
240					74.4 (1)
230					96.7 (2)
220					78.8 (1)
210		86.6 (1)		49.8 (2)	83.1 (3)
200		70.2 (3)		47.6 (2)	77.7 (2)
190	36.0 (1)	74.0 (7)		44.8 (4)	72.2 (2)
180	34.3 (1)	71.5 (7)		40.3 (1)	69.6 (9)
170	35.4 (2)	63.9 (9)		44.2 (2)	65.4 (5)
160	32.3 (3)	58.4(10)	47.1 (1)	43.6 (2)	57.1(10)
150	35.7 (3)	53.8(10)	47.4 (1)	37.3 (4)	55.0 (6)
140	27.6 (7)	51.6 (5)	55.5 (1)	36.3 (4)	45.7(10)
130	26.5 (8)		42.1 (4)	33.4 (4)	43.4 (6)
120	24.9(13)	44.1 (3)	37.7 (7)	29.0 (7)	38.8(10)
110	23.8 (8)	35.8 (5)		26.5 (6)	32.2 (9)
100	24.0 (9)	31.9 (8)	29.5 (8)	22.3 (5)	24.3 (6)
90	19.3(20)	28.4(22)	26.6 (8)	21.3 (6)	26.3 (1)
80	17.2 (8)	26.1(23)	23.1(10)	17.7(11)	17.9 (4)
70	16.9 (6)	20.8(10)	17.8 (3)	15.4(19)	13.9 (9)
60	14.5 (7)	22.8 (5)	14.8 (8)	11.1 (9)	11.2(14)
50	9.6 (5)	14.9 (2)	10.6(17)	9.9 (4)	8.0(15)
					6.4(16)

Table 8. Analysis of the relationships between $\log_{10}$ dry weight of flesh and $\log_{10}$ area index of shell

Station	Correlation coefficient r	Slope of the regression line b	Standard Deviation of b	Intercept with ordinate a	Standard deviation of a
1	0.8365	0.914	0.060	- 0.502	0.121
2	0.9560	1.227	0.032	- 0.937	0.067
3	0.9683	1.314	0.062	- 1.153	0.127
4	0.9556	1.142	0.038	- 0.920	0.079
9	0.9812	1.638	0.028	- 1.863	0.058

Table 9. Analyses of the relationships between $\log_{10}$ dry weight of shells and $\log_{10}$ area index of shell

Station	Correlation coefficient r	Slope of the regression line b	Standard Deviation of b	Intercept with ordinate a	Standard deviation of a
1	0.9734	2.027	0.047	- 1.939	0.096
2	0.9786	1.944	0.035	- 1.969	0.073
3	0.9688	1.946	0.092	- 1.827	0.185
4	0.9740	2.120	0.056	- 2.174	0.113
9	0.9548	1.983	0.055	- 1.919	0.112

Figure 10. Regression lines for $\log_{10}$ dry weight of flesh against $\log_{10}$ area index of shell

Figure 10

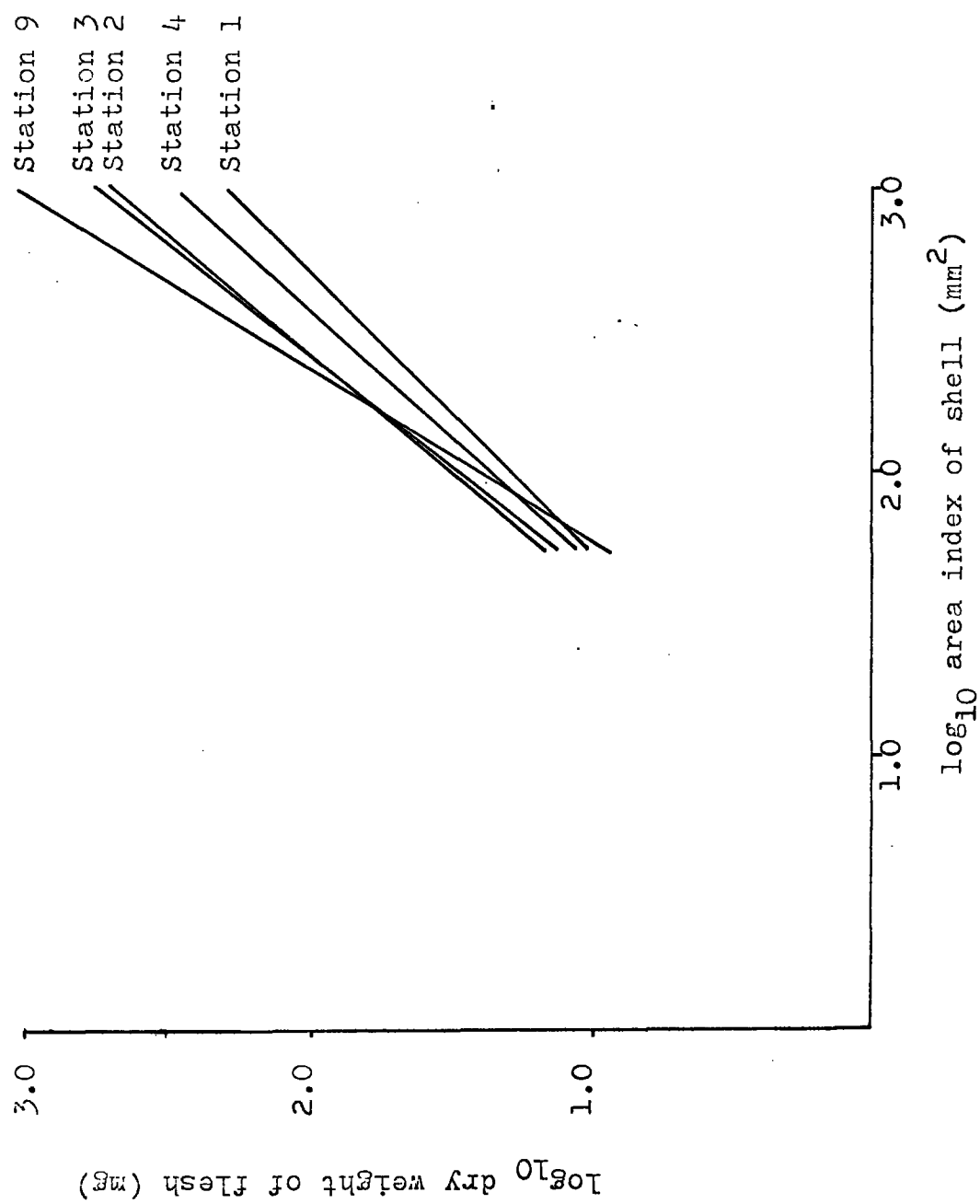

Station 9
Station 3
Station 2
Station 4
Station 1
3.0
2.0
1.0
log10 dry weight of flesh (mg)
1.0
2.0
3.0
log10 area index of shell (mm2)

Table 10. Comparisons of slopes of the regression lines $\log_{10}$ dry weight of flesh against $\log_{10}$ area index. Values of t together with significance level. t = 1.96 (p , 0.05) and higher indicate significant difference

	Station 1 b = 0.914	Station 2 b = 1.227	Station 3 b = 1.314	Station 4 b = 1.142	Station 9 b = 1.638
Station 1		4.603 (0.001)	4.609 (0.001)	3.180 (0.01)	10.900 (0.001)
2	4.603 (0.001)		1.243	1.702	9.687 (0.001)
3	4.609 (0.001)	1.243		2.339 (0.05)	4.732 (0.001)
4	3.180 (0.01)	1.702	2.339 (0.05)		10.317 (0.001)
9	10.900 (0.001)	9.687 (0.001)	4.732 (0.001)	10.317 (0.001)	

Figure 11. Regression lines for $\log_{10}$ dry weight of shells against $\log_{10}$ area index of shell

Figure 11

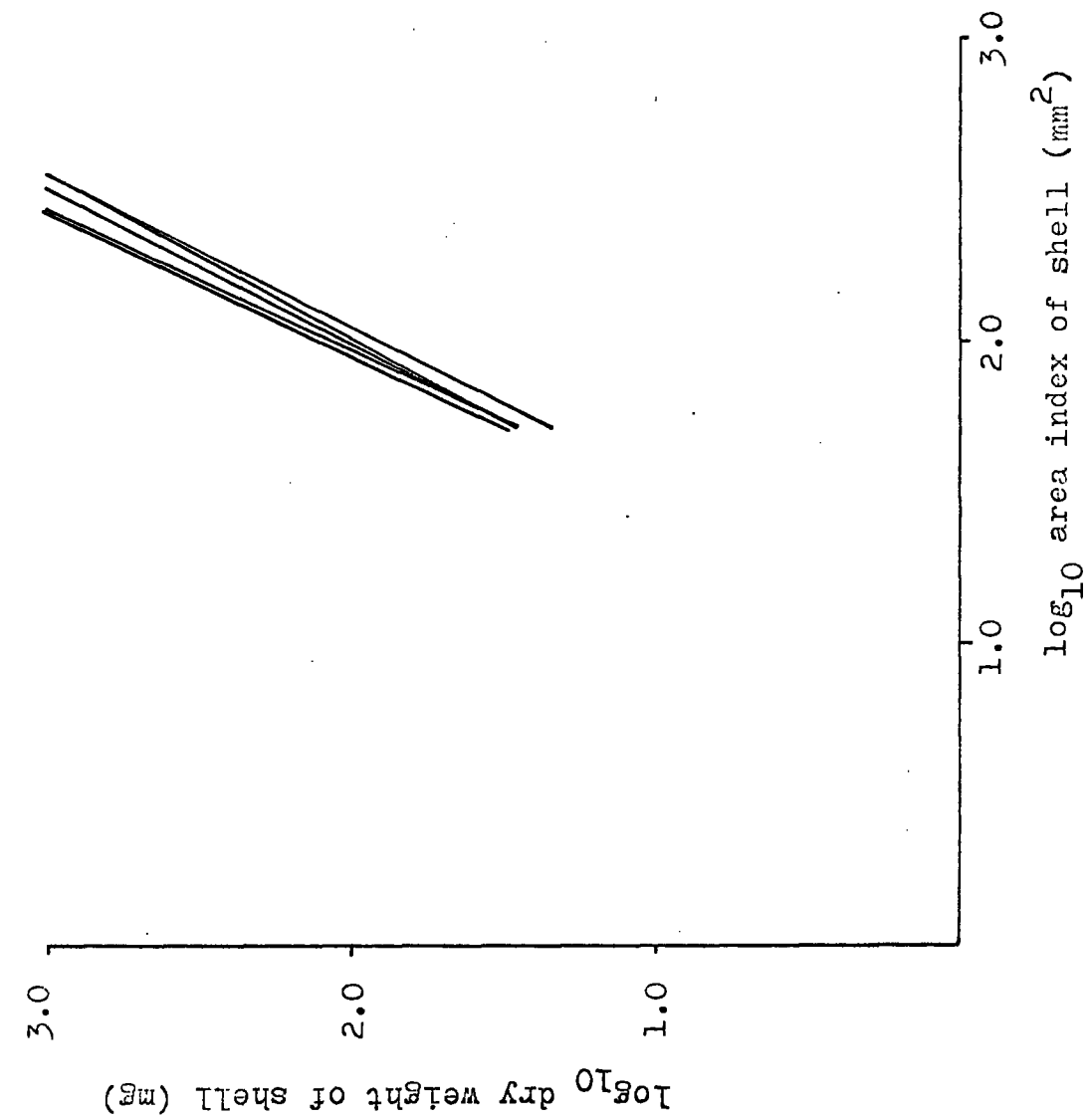

3.0
2.0
1.0
log10 dry weight of shell (mg)
1.0
2.0
3.0
log10 area index of shell (mm2)

Table 11. Comparisons of slopes of the regression lines $\log_{10}$ dry weight of shells against $\log_{10}$ area index of shell. Values of t, t = 1.96 or higher indicate significant difference

	Station 1 b = 2.027	Station 2 b = 1.944	Station 3 b = 1.946	Station 4 b = 2.120	Station 9 b = 1.983
Station 1		1.429	0.783	1.277	0.610
2	1.429		0.020	2.686 (0.01)	0.602
3	0.783	0.020		1.617	0.345
4	1.277	2.686 (0.01)	1.617		1.754
9	0.610	0.602	0.345	1.754	

Table 12. Comparisons of intercept with ordinate a for $\log_{10}$ dry weight of shells against $\log_{10}$ area index of shell

		Station 1 a = - 1.939	Station 2 a = - 1.969	Station 3 a = - 1.827	Station 4 a = - 2.174	Station 9 a = - 1.919
Station	1		0.245	0.552	1.592	0.136
	2	0.245		0.727	1.535	0.370
	3	0.552	0.727		1.619	0.439
	4	1.592	1.535	1.619		1.605
	9	0.136	0.370	0.439	1.605	

For $\log_{10}$ dry weight of shells against $\log_{10}$ area index of shell (Fig. 10), t-tests showed that values of b did not differ between station, with one exception : b for station 4 was significantly higher than b for station 2 (see Table 11).

Having found that the slopes of the regression lines did not differ between stations, the intercepts with the ordinate (a) were compared by t-tests (see Table 12). As these did not show any significant differences it can be concluded that the values represented by the different lines do not differ significantly.

Discussion

All the correlation coefficients for $\log_{10}$ dry weight of flesh against $\log_{10}$ area index of shell are significantly different from zero ($p > 0.001$) which indicates that area index of shell gives reliable information about weight of flesh inside the shells at a given station, but as the slopes of the regression lines for different stations are significantly different, the same size of shell does not hold the same amount of flesh at different stations. Increase in weight of dry flesh with increased size of shell is lowest at station 1 but highest at station 9. These results agree with the results from the growth rate study, where station 1 had the lowest increase in area index of shell per year, but station 9 the highest. However, the increase in shell weight with increased size does not differ between stations, except at

stations 4 and 2, as mentioned before. As the correlation coefficients for $\log_{10}$ dry weight of shells against $\log_{10}$ area index of shell are all significantly different from zero ($p > 0.001$) but neither slopes nor intercepts with ordinate differ significantly between stations, the area index of shell gives reliable information about the weight of shell at all sampling sites examined at Lindisfarne.

The results from this investigation indicate that environmental factors causing low or high growth rate have the same effects on rate of increase in dry weight of flesh with increased size of shell; however, the thickness of the shells formed at different stations does not seem to be affected.

Calorific content

Calorific contents of animals from different size-classes and different stations were determined to find out if there were any differences in body composition, as indicated by calorific value/gm between size-classes and/or between stations.

Methods

After the dried flesh had been removed from the shells and weighed, it was dried again in a vacuum oven at 60°C for 12 hours to remove any moisture that might have been adsorbed during weighing.

Thereafter animals from a chosen size-class and station were combined until their weight was within the range 0.25gm to 0.40gm. A pellet was then made and weighed accurately.

To determine the calorific content of each pellet a Ballistic Bomb Calorimeter was used, according to the instructions given in Appendix 3. When each pellet had been"bombed",the amount of inorganic matter was weighed and subtracted from the initial weight of the pellet to find out the amount of organic matter burnt. A calibration curve was made for the calorimeter by burning different weights of benzoic acid which has known calorific content of 6319 cal/gm. The calorific content of the organic matter in each pellet could then be read from this curve.

Table 13. Calorific content presented as Kcal/g of organic matter

Size-class	Station 1	Station 3	Station 4	Station 9	Mean
30-50	4.93	5.36	5.30	5.26	5.21
50-70		5.28	5.10	5.20	5.19
70-90	4.99	5.46	5.15	4.98	5.15
90-110	4.94	5.00	5.20	5.20	5.09
110-130	4.90	5.15	5.05	5.50	5.15
130-150	4.90		5.45	4.96	5.10
150-170			5.29	4.85	
170			4.97		
Mean	4.93	5.25	5.19	5.14	
S.D.	0.0014	0.0293	0.0239	0.0488	

t-test between 3 and 1 significant at ($t = 3.9506$)
$0.01 < p < 0.002$

t-test between 9 and 1 ($t = 2.085$)
$0.1 < p < 0.05$

t-test between 4 and 1 ($t = 3.611$)
$0.01 < p < 0.002$

Results and discussion

The results are presented in Table 13. Only single measurements have produced most of the values given in the table as there was insufficient material for many duplicate determinations. As may be seen, there is no evidence of differences in calorific value (per gram of organic matter) from different size-classes. But when the means for different stations are compared, t-tests show that the mean for station 1 is significantly lower than the means for stations 3 and 4 (see Table). It is thus not possible to regard the calorific content as being constant per gram of organic matter from Macoma in the Lindisfarne area.

The results from this investigation show the same trend as those from the growth rate study and dry weight measurements, namely the animals at station 1 have not only the lowest growth rate of shell area per year and lowest increase in dry weight of flesh with increased size, but also have significantly lower calorific content per gm of organic matter.

CHAPTER 6

Heavy metal analyses

Marine organisms are able to accumulate in their tissues and skeletons many of those heavy metals occurring in very low concentrations in their external environment. Concentration factors of hundreds or even thousands are commonly found (Bryan 1971).

Accumulation of this kind introduces heavy metals into the food web and makes them available to predators, e.g. fish and birds, in high concentrations.

In the present investigation, concentrations of cadmium and lead in the entire soft parts of Macoma balthica and Scrobicularia plana were determined. Concentrations of calcium were also determined, because high concentrations may cause interference with the analyses for cadmium and lead, and give inflated values for these metals.

Methods

Wet ashing of the dried flesh was performed with fuming nitric acid. 1.0g samples containing several animals previously dried at 60°C were transferred to glass vials and 5ml of fuming nitric acid added with 2 drops of octan-1-ol to prevent frothing. Two small glass balls were also placed in each vial. The samples were then heated in an apparatus similar to that described by Thompson and Blacnflower (1972). The samples were digested at

100°C, below the boiling point of nitric acid, for 45 minutes before the heat was increased until the mixture boiled steadily but not too vigorously. When all the acid had been evaporated from the sample, a yellow residue remained on the bottom of the vial. 1ml of fuming nitric acid was then added to the residue and the solution evaporated as before. This procedure was repeated several times to produce as clean a residue as possible. The glass vial was then removed from the heating apparatus and left to cool. Finally, the residue was dissolved in minimum quantity of 1:1 HCl. Control solutions were prepared by evaporating from glass vials the same amount of fuming nitric acid as needed for the digestion of the tissue samples. Any residues formed were then dissolved in 5ml of 1:1 HCl. The samples were now analysed for cadmium and lead, using the Unicam SP90 Atomic Absorption Spectrophotometer.

Solutions containing a range of known concentrations of these metals were made and the percentage absorption for each concentration determined. From the calibration curves so obtained the concentrations of cadmium and lead in unknown solutions could be read.

Before analysing for calcium certain precautions had to be taken since the percentage absorption is liable to be depressed if the sample solutions contain phosphates, sulphates or any oxygenated anions. The best way to overcome this interference is to add a solution of a "releasing agent" to the sample solution. The most widely used is lanthanum chloride.

In the present study the sample solutions were diluted in 5% lanthanum chloride solution and distilled water until the concentration of calcium was between 5 and 20 p.p.m. in 1% lanthanum chloride solution.

Standard solutions of calcium were made; these contained 0, 5, 10, 15 and 20 p.p.m. in 1% lanthanum chloride solution.

The results from the calcium analyses showed concentrations of 5 - 7000 p.p.m. in the dry bivalve flesh samples, equivalent to about 1000 p.p.m. in the HCl solutions analysed. To find out if concentrations of this order of magnitude interfered with cadmium and lead absorption analyses, a solution was made containing 1000 p.p.m. of calcium, but no cadmium or lead. This solution was analysed for apparent cadmium and lead. The results showed that the effects of calcium in this concentration in the sample solutions on other metal determinations were negligible.

Results and discussion

As the effects from calcium were negligible, and the control solutions did not show any detectable amounts of cadmium and lead, the results are presented as obtained in Table 14a and b. The results from each sample were multiplied by five, the dilution factor.

Analyses for heavy metals in British coastal waters (Preston *et al.* 1972) showed concentrations of 0.00041 p.p.m. for cadmium and 0.00021 p.p.m. for lead in the North Sea coastal area.

Table 14a. Concentration of cadmium and lead in the entire soft parts of *Macoma balthica* together with concentration of calcium. All concentrations as p.p.m. in the samples dried at 60°C.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Mean
Cadmium	1.65	1.75	1.35	1.60	1.70	1.60	1.80	1.90	1.50	1.67
Lead	10.00	10.50	9.75	10.00	12.75	12.25	11.50	11.75	12.25	11.17
Calcium	4800	4000	4500	5600	5400	4500	5200	5100	4800	4878

b. Concentration of the same elements in the entire soft parts of *Scrobicularia plana*

Cadmium	0.65	0.60	0.65	0.60	0.60	0.70	0.55	0.65	0.60	0.62
Lead	12.00	12.75	12.25	12.00	11.50	11.90	10.50	12.00	11.90	11.87
Calcium	6150	6600	7200	6400	6800	7100	7550	7250	6850	6878

Comparing the concentrations of these two metals in the sea-water with those in the tissues of the two bivalve species gives concentration factors of 4000 and 1500 for cadmium in Macoma and Scrobicularia respectively, but much higher values for lead, namely 53000 and 56000 respectively.

It should be remembered that sea-water is not the only available source of heavy metals. As the two molluscs feed mainly from the sediments and so could undoubtedly absorb metals from them, it would be necessary to analyse the sediments for cadmium and lead to get better knowledge of the real biological concentration factors.

The concentration of cadmium in Macoma is significantly higher than in Scrobicularia ($t = 15.533$ $p > 0.001$) yet the levels of lead are comparable ($t = 1.620$ not significant). This indicates that the two species have similar ability to accumulate lead, but Macoma accumulates cadmium more easily than Scrobicularia.

The nearest industrial sources of heavy metal contamination to Lindisfarne are probably Blyth (40 miles S.) and the Forth estuary (40 miles N.). The concentration of heavy metals in the sediments at Lindisfarne should therefore not be high.

Preston et al. (1972) analysed limpets Patella vulgaris from coastal waters in the Irish Sea for heavy metals. At the most contaminated sampling solutions they found concentrations up to 35 p.p.m. for cadmium and up to 85 p.p.m. for lead in the dried flesh of the animals. These concentrations are much higher than those found in the present

investigation, as expected if contamination in the Lindisfarne area is low. However, as the ability to accumulate heavy metals differs between species, a comparison between Macoma from different areas would be more reliable. The fact that the lead/cadmium concentration ratio was so much higher at Lindisfarne (6.7:1 for Macoma, 19:1 for Scrobicularia) than in limpets from the Irish Sea (up to 2.4:1) may reflect a difference in the ability of bivalves and gastropods to accumulate these metals, or may merely reflect differing levels of contamination in their respective foods.

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Summary

1. The distribution of Macoma balthica on Holy Island Sands and Fenham Flats, Lindisfarne was investigated.

2. It was found that the density of Macoma at high tidal levels was not limited by available food in the substrate.

3. The highest density of Macoma was found at about mid-tide level, although available food in the substrate was much higher at lower tidal levels.

4. The dispersion of Macoma in an area of high density was examined.

5. Direct evidence of growth rate was obtained by measuring an area index from growth rings on the shells.

6. Size and age distributions of Macoma populations were investigated at several sites, both at high tidal levels and lower down the shore.

7. Dry weight measurements of flesh showed that there was a marked difference between stations in the rate of increase in dry weight with increased size of shell.

8. Dry weight measurements of shells showed that in general there was no marked difference in the rate of increase in shell weight with shell size at different stations.

9. Analyses for calorific content of animals from different sites showed that calorific content per gram of organic matter was significantly lower for animals from the low growth rate site.

10. Analyses for cadmium and lead in the entire soft parts of Macoma balthica and Scrobicularia plana were carried out. Both concentrated lead to a similar degree, but Macoma contained cadmium levels twice as high as Scrobicularia.

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Appendix 1

The Walkley Black method for estimation of organic carbon in deposits.

1. The oven dried substrate samples were allowed to cool in a desiccator.
2. Accurately weighed finely ground sample was transferred to a 500ml Erlenmeyer Flask. Samples of 1.2g were suitable for substrates containing low percentages of organic carbon, and 0.9g for those rich in organic carbon.
3. 10ml of 1 N potassium dichromate were added, followed by 20ml of conc. sulphuric acid (containing 12.5g/l silver sulphate to overcome interference by chlorides). (The addition of sulphuric acid was made cautiously). The flask was then left to stand for 3 min on an asbestos sheet.
4. 200ml of water were added, followed by 10ml of 85% phosphoric acid and 1ml of diphenylamine indicator solution.
5. Titration was by addition of 1 N ferrous sulphate in small lots until the colour of the solution flashes from purple to green, then the excess of dichromate was restored by adding 0.5ml of potassium dichromate and the titration was completed by adding ferrous sulphate, drop by drop, until the last trace of the blue carbon disappeared.

Appendix 2

The semi-micro Kjeldahl method

1. From the oven dried substrate samples cooled in a desiccator accurately weighed, finely ground aliquot 0.5 - 1.0g was transferred to a digestion flask.
2. 2g of catalyst (made up of 32g potassium sulphate, 5g of mercury sulphate, and 1g of selenium powder, well mixed) were added to the digestion flask followed by 3ml of A.R. conc. sulphuric acid.
3. The mixture was then heated gently over micro-burner for 5 minutes. The heat was then increased so that the mixture boiled steadily for a further 45 minutes.
4. When fully digested, the mixture was allowed to cool.
5. The digestion flask was then fitted to a "Quickfit" semi-micro distillation apparatus which had already been steamed through with distilled water.
6. 20ml of freshly mixed 40% sodium hydroxide and 40% sodium sulphide (9 volumes of alkali to 1 volume of sulphide) were poured into the funnel of the distillation apparatus.
7. Before the alkali was run into the digested solution, the end of the condenser was dipped into a flask containing 10ml of saturated boric acid + 2 drops of mixed indicator (methyl red and methylene blue).
8. The alkali solution was then run into the digestion flask and the ammonia liberated was collected in the saturated boric acid.
9. The mixture in the digestion flask was then steam distilled until about 25ml of the boric acid mixture had been collected.

10. The solution of ammonia absorbed in saturated boric acid was then titrated with 0.025 N HCl until the end point deep blue to lilac was reached.

Note : To determine whether there was any nitrogen in the reagents used, this procedure was repeated for a mixture where A. R. glucose had been digested instead of substrate.

The amount of 0.025 N HCl to reach the end point for this mixture (0.03ml in this case) was subtracted from the other titres before calculating the nitrogen content of the samples.

Appendix 3

Bomb Calorimeter instructions

1. Make pellet (about 0.3 - 0.4gm) and weigh accurately.
2. Put pellet in crucible on end of standard 2" 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 atmospheres).
9. Close oxygen control valve on bomb unit and check that pressure in bomb holds steady at 25 - 26 atm.
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.