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AN INVESTIGATION OF THE POSSIBLE EFFECTS OF POLLUTION
ON SOME MEMBERS OF THE PERIPHYTON IN THE LITTORAL
ZONE OF THE NORTH-EAST COAST OF ENGLAND

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Dissertation presented as part of the degree of
M.Sc. Ecology, Durham University,
September, 1967.

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An Investigation of the possible Effects of Pollution on Some Members of the Periphyton in the Littoral Zone of the North-East Coast of England.

There is a marked difference between the unpolluted water off the North Northumberland coast and the very polluted waters of the Durham coast, subject to the influence of sewage, household and industrial effluent, coal washings etc., that are present in enormous quantities in the outflow of the rivers Tyne, Wear and Tees. Current research has shown that the macrophyte-flora of the Durham coast is much restricted, both in diversity and performance, and all evidence points to this restriction being caused by the reduction of light passing through the water by suspended matter. (Bellamy et.al, 1967). The littoral macrophytes are affected directly by pollution in the sea-water and indirectly via the deep water stocks that provide replenishments for the littoral community, (Burroughs 1958). The investigation discussed here is concerned with the littoral periphyton, which is not directly dependent on deep water stocks for replenishment. Diatoms were studied particularly owing to the difficulties encountered when trying to isolate other members of the periphyton.

Pollution may affect members of the periphyton, either specifically or generally, by stimulating growth or by being toxic. Where pollution increases the amount of suspended matter, the periphyton may be subject to reduction of incident light while it is immersed, and also to changes in the substratum by deposition of suspended particles on top of the original surface.

In the time available it was decided to restrict the study to a comparison of the quantitative and qualitative distribution of periphytic diatoms at a series of sites that differed mainly in the degree of pollution of the water.

Literature.

The diversity of the habitats in the marine littoral zone is exceedingly wide, few previous studies on the periphyton in the littoral zone have been carried out in sites comparable to those sampled in the present study. A considerable number of studies of both littoral and sub-littoral benthic communities, living in sand or mud, have some relevance to this study in the problems encountered, and in factors determining distribution; important papers in this category are those by Groentved, 1960b and 1962 on shallow Danish waters, Hendey, 1951 on diatoms in Chichester Harbour, Hustedt, 1939 (Germany), Hustedt and Aleem 1951 (Plymouth area) and Mare 1942 (Plymouth area). Much work on this part of the flora has been concerned with phenomena such as diurnal migration rhythms, Palmer and Round, 1966, 1967, and on photosynthesis, for example Rowland-Taylor and Palmer, 1963, and some of these works suggest possible hypotheses to account for distribution patterns. Work on phytoplankton sometimes can be extrapolated to include periphyton communities; in 1948 Patrick reviewed the literature on factors that affect the distribution of diatoms, but referring to plankton; more detailed literature on these factors is mentioned in the results discussion.

Although the studies on rocky littoral zones by Aleem, 1950a, and Castenholz, 1963, were both remote from the North-East coast of England, the suggestion was made by the latter that "most littoral diatom species appear to be world-wide in distribution on Northern Temperate shores ..." but that it was "unknown ... whether distinct genotypes exist". The communities described by both authors show many similarities with each other and with those found in this investigation although the influence of the North Sea in many ways might be considered unique and show considerable differences from Atlantic or Pacific influence; Aleem's study was carried out at two localities on the

south coast of England and that by Castenholz in Oregon, U.S.A.

Methods of studying the periphyton have been reviewed by Cooke, 1956, Sladeckova 1962 and Wetzel, 1964, who all discuss the use of artificial substrata. Lund and Talling, 1957 reviewed the literature on general limnological methods for the algae; more specialised papers are discussed after the description of methods used. Notes on Taxonomic works are included in Appendix 2.

Terminology.

The littoral zone referred to includes the shore between mean high water spring tides (M.H.W.S.) and mean low water spring tides (M.L.W.S.); the upper littoral zone is referring to the region above mean tide level, and the lower littoral the reverse. These levels were all estimated - for example M.L.W.S. from the observed lowest level of the tide and knowledge of the predicted range - M.H.W.S. by the upper limit of some algae on exposed rocks and by the drift line. The term community is used generally, as in Aleem, 1950a, 'a biotic unit including one or more dominant species', where anything further than this is meant it is stated.

Periphyton is used throughout to describe the community discussed in this study, and includes both loosely attached cells and loosely associated organisms, but does not imply the specific type of community that forms an obvious, slimy covering on submerged surfaces, that has been specifically called periphyton by some authors. The original use of the term periphyton was restricted to the community that develops on artificial substrata. Although some American authors have used the term 'Aufwuchs' in the way that periphyton is used here, there is sometimes the implication that 'Aufwuchs' does not include organisms loosely associated with the surface, only those definitely attached (but not penetrating). Since these free cells must obviously be important in the metabolism of the community, the word periphyton is preferred. The terms benthos and benthic are in general not used in the sense that is meant by many English authors, i.e. more or less equivalent to periphyton, but in their more specific sense, of organisms living on the bottom of a body of water which therefore includes both epipelagic and epilithic organisms. Round, 1956, has suggested that the true epilithic community (i.e. on clean, bare rock) is closely related to the epiphytic community, and therefore it might be expedient

to reserve the use of these terms for the epipelagic community.

The epiphytes are considered to be organisms growing on plants, both macrophytes and microphytes. The only two components of the periphyton that are separated by the use of general terms are tychopelagic species (normally benthic species, found in the plankton when torn from the bottom) and meroplanktonic species (benthic species with planktonic resting stages) the definitions are from Hendey, 1964, and benthic is being used here in the widest sense. A discussion of some of these terms is included in Sladeczkova, 1962.

Site Selection

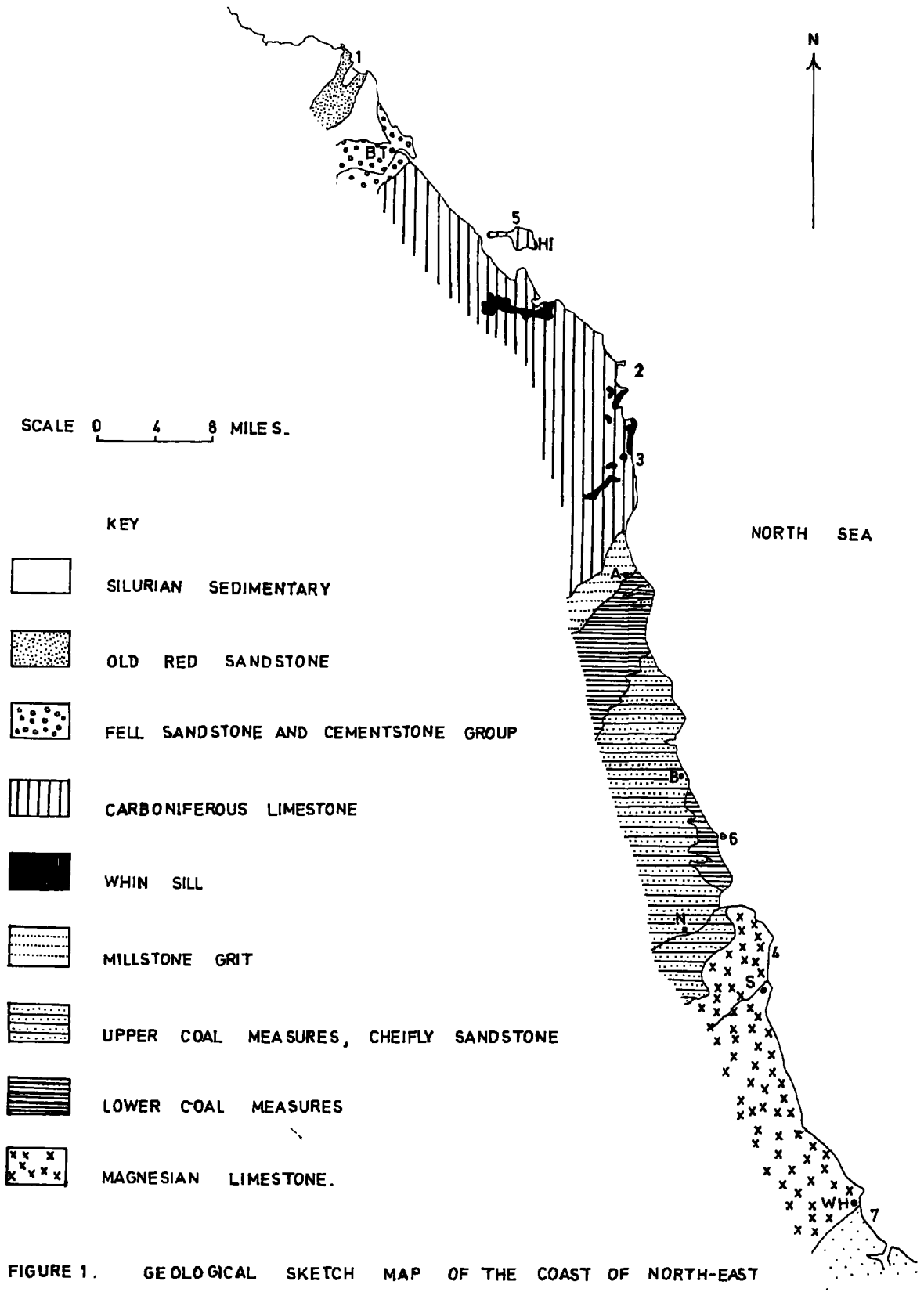
The sites sampled are shown in figure 1, description of the sites in tables 1 and 2.

The range of tides is similar at all the sites; the tidal wave moves from North to South, thus exposure and inundation by the tide does not coincide with the same amount of insolation at different sites, which introduces a source of chance variation in climatic factors between sites. There is also a residual current from North to South (Admiralty Tide Tables, 1966) this is probably important in minimising any inherent temperature differences between the most distant sites, separated by 10 degrees of longitude. It is probably also important to the dispersal of diatoms, since the sites are all sequentially affected by the same water-mass. However, the movement of the water mass is not determined by the residual current alone (this is a tidal current of geographical origin), but also by variable currents that depend on climatic factors.

(The Tidal range quoted is M.H.W.S. - M.L.W.S. to M.H.W.N. - M.L.W.N.)

Description of the geology of the area, briefly covered in figure 1 and table 1, was drawn from the following sources: Hickling, 1931, Northern Industrial Group, 1949, and Pringle, 1935. The sampling procedure was designed so that reflections of the evident differences in geology would be minimised. The exact positions of the sampling areas were chosen subjectively so that, at each site, the topography and aspect were as similar as possible. Samples were taken in these areas from exposed pools, on rocks, at all levels in the littoral zone.

The parameters shown in Table 2 are chosen as indicators of the extent of pollution. The number of coliform bacteria, estimated by a Standard Method, A.P.H.A., 1965, indicates the extent of pollution by sewage. Phosphate and nitrate, two biologically important compounds, are nutrients that



are in short supply in the open sea.

TABLE 1.

Major sites	<u>Lat.</u> <u>Long.</u>	<u>Long.</u> <u>Lat.</u>	Geology	Tidal range in feet.
1. St. Abb's Harbour Berwickshire	55° 55'N	2° 09'W	Silurian, arenaceous St. Abb's Head, Old Red Sandstone	ca. 14-7
2. Beadnell Bay Northumberland	55° 33'N	1° 37'W	Limestone	13.3-6.9
3. Craster Harbour Northumberland	55° 29'N	1° 30'W	Limestone	13.8-6.0
4. Souter Point Co. Durham	54° 58'N	1° 20'W	Magnesian Limestone	14.2-6.0
Additional Sites				
5. Holy Island Northumberland	55° 40'N	1° 47'W	Limestone	13.9-7.5
6. St. Mary's Isle Northumberland	55° 05'N	1° 26'W	Lower Carboniferous sandstone	14.6-7.1
7. West Hartlepool Co. Durham	54° 41'N	1° 11'W	Triassic Sandstone	14.1-7.4

TABLE 2

Mean Analysis Results (according to Bellamy et al, 1967).

Site.No.	Suspended matter Gms./litre	Coliform bacteria per 100 mls.	Total phosphate	Total Nitrate
1	0.02	25	30	60
2	0.04	90	42	90
3	0.03	80	48	74
4	0.20	1000	158	210
5	-	22	29	94
6	0.51	> 2000	66	96
7	0.29	> 2000	214	320

Key to place names in figure 1.

BT	Berwick-on-Tweed
HI	Holy Island
A	Amble
B	Blyth
N	Newcastle
S	Sunderland
WH	West Hartlepool

METHODS

A. Sampling

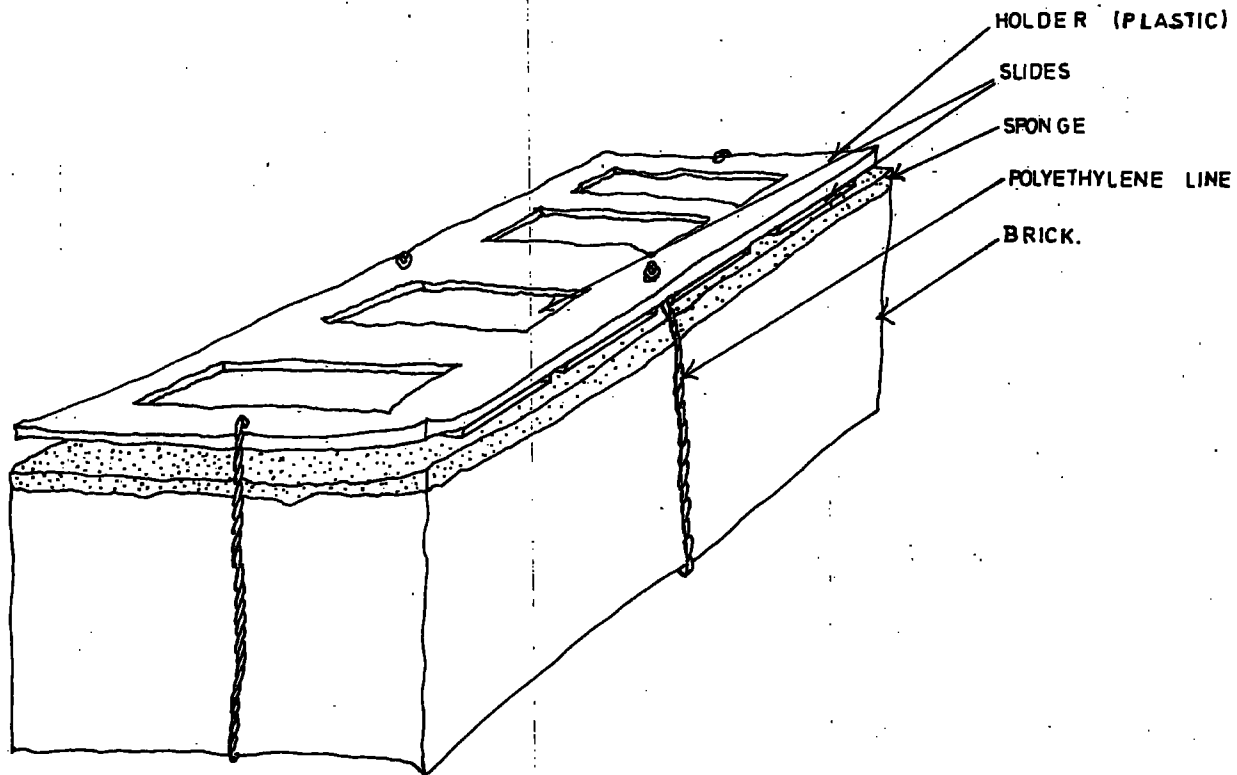
1. General

At each site rock-scrappings, silt samples and pieces of attached macrophytes were taken for examination. These samples were examined to predict the range of species that might be expected from each site, and to allow the direct observation of the growth-form of colonial species. Previous studies of littoral diatoms has indicated that the substratum type has a major effect on the community that develops on it (see Brockmann 1935, and Groentved 1960b , both studies of sediment bottoms). In order to reduce this substratum effect to a minimum, it was decided to make the main comparative study using both natural and artificial substrata common to all sites. Studies of diatom distribution indicate that the diatoms in this study are likely to be distributed in a manner described by Hendey, 1964, as a mosaic of micropopulations; thus all the samples taken are replicated both horizontally and vertically in the littoral zone of the sample area.

2. Artificial substrata

Glass microscope slides were laid down in the areas studied, and examined after various periods of exposure. Examination of these samples is simplified as the slides are viewed directly under the microscope, which would also permit quantitative comparison to be made between samples. The main disadvantage of using artificial substrata is that the community that develops may not be sufficiently like that found on natural substrata to extrapolate from one to the other. This may be due to different texture, or because the artificial substratum is inert chemically, or because

FIGURE 2. DRAWING OF BRICK USED IN ARTIFICIAL SUBSTRATUM EXPERIMENTS.



the building up of a specific community depends on a succession ending with a recurring seasonal sequence of discrete, identifiable communities, as those described by Hendey, 1964. The slides used were attached to house-bricks as shown in figure 2. When the bricks were secured by jamming in crevices in rocks, the slides were carefully cleaned with alcohol before exposure to avoid any possible non-uniformity in species-composition of the primary film of bacteria, which is important in determining the subsequent communities, Hendey, 1951. After exposure the slides were transported, either wet or dry, in plastic, screw-top staining-pots.

3. Natural Substrata.

These substrata are under the influence of the previous seasons flora, rather than the unpredictable microbial flora that must first colonise a slide. Of the natural surfaces that could have been sampled, living organisms were considered because they present a uniform surface wherever they occur, and because they could be removed from their surroundings with little disturbance of their surface. The organism chosen would be most useful if it had a widespread distribution both geographically and within the littoral zone; some algae fulfil this condition, for example Enteromorpha spp., however, the epiphytes on such a plant are likely to be far more specialised than the flora of a non-living surface. Of the commonly occurring shelled animals, the larger molluscs, such as Mytilus and Patella were considered particularly, partly because a fairly large sample area was necessary, and also these larger animals could be easily removed from their shells; by removing the animal contamination of the surface diatoms, by those in the animal's gut, is avoided. The shells can then be treated with various chemicals to remove the diatoms; if the

shell's surface area can be measured, the removal can be quantitative and used to estimate the number of cells per unit area of surface. Limpets were chosen because they were easy to identify, and because they are motile. Their movement away from their home site is important, as their epiflora must consist of more adaptable organisms than those that are sessile in the very specialised habitat of rock pools. Much of the rock that does not come under the limitations of physical factors imposed in permanent pools, is too exposed to support diatom growth to the extent that makes sampling a relatively easy procedure. An organism that has all these advantages and can be aged, would enable quantitative estimations to be used to calculate the productivity of the community. The only possibility for aging limpets is to separate a population into size classes, it is unlikely that limpets from different sites could be strictly compared if aged in this manner. It is possible that the use of this organism as a common substratum is not completely justified, if the nature of the rock on which the animal lives influences the nature of the shell laid down.

B. Removal of Diatoms from Substrata.

1. Physical removal

The general samples were not taken quantitatively; Groentved, 1960a, 1960b and 1962, describes quantitative sediment sampling in some detail. Cooke, 1956, reviews methods for scraping known areas of smooth rock surfaces. In addition to examining the suspensions of diatoms obtained from these samples, a third method was attempted. Originally devised to study fungi growing on leaves, this method was used by Margalef, 1948, to study epilithic diatoms from fresh-water streams. To examine both stones and limpet shells, the surface was prepared by washing with sea-water, alcohols, and acetone, while still wet with the acetone a viscous solution of cellodion in acetone poured on the surface, and when dry this was peeled off,

in strips. (The method as used originally incorporated staining, and used absolute alcohol and ether, instead of acetone, collodion in place of cellodion). According to Margalef this method removes the entire complement of epiliths, and its other advantage is the ease of examination of the organisms, showing both the growth form and relationships between species in the community. In theory this method could be used to give quantitative results; in practice the organisms were frequently obscured, both by indentations in the collodion and because the communities in this habitat were not spread out in a monolayer.

2. Direct observation.

Direct observation of the organisms in situ has some of the advantages of the peel method; the number of cells per unit surface area can be estimated directly, growth-forms and species relationships can be studied. The microscope slides used as artificial substrata were examined while still wet with sea-water and as this did not allow sufficient resolution of diatom frustules, they were examined after washing with various solvents and being mounted using a mountant of high refractive index. In the time taken for a diatom community to develop a great deal of silt and organic debris accumulates on the slides; vertical slides might not be so affected by this problem. In addition to transparent artificial substrata, direct observation of the epiphytes on plants which have been treated to make them transparent has been carried out in the past, and might be particularly useful on some of the thin littoral macrophytes.

3. Chemical Methods

The chemical methods that were used to remove the diatoms from the limpet shell fall into two categories: those that dissolve the shell completely, and those that dissolve mucous stipes and coverings to bring the diatoms into suspension. The latter methods

allow the surface area of the shell to be measured carefully after the removal of the diatoms, when the shell is destroyed this must be estimated from the height and a line drawn around the periphery of the base; both measurements can be made before removal without disturbing the surface. With the acid-destruction of the shell there is no loss of diatoms except for weakly siliceous species that may be destroyed. Other than acids the reagents, that dissolve mucus, that were used were sodium hydroxide (Lewin, R.A. 1962) and enzyme solution; the latter is preferred, as there was less organic debris. As time did not permit the use of more than one method for all the samples, that generally employed was acid removal. All the chemical methods were found to be an improvement on scraping, both in their simplicity and efficiency.

C. Preparation methods

a. Concentration of suspensions.

The following methods are necessary as the suspensions, obtained by chemical removal or with the washings used with mechanical removal from the substratum, are too diffuse to be examined directly. Filtration and centrifugation are also used between the stages of cleaning the diatom suspensions; cleaning is the removal of organic matter from the diatom suspensions, by washing with various chemicals, in order that the sculpturing of the frustules can be resolved: this is the means by which they are identified.

1. Centrifugation.

The centrifugation throughout the investigation has been carried out at 3,000 r.p.m. for at least 15 minutes, although this was not tested to see if it was the minimum amount necessary, its effectiveness was investigated. Ferguson Wood, 1965, has

suggested that non-continuous centrifugation is not entirely satisfactory for treating other micro-organisms of the same order of magnitude as the diatoms found here, because the smaller organisms tend to be resuspended as the centrifuge brakes, and consequently may be lost when the supernatant fluid is poured off. The difference in the number of diatoms in a sample before and after centrifuging was found to be negligible, The number was estimated in each case by counting a suspension in a haemocytometer. Thus this method can be considered to be suitable for quantitative preparations, and that there is unlikely to be differential loss of the smaller species before qualitatively examining a sample.

2. Sedimentation, see Braarud, 1958, Lovegrove, 1961, Lund and Talling, 1957, Lund et al 1958, and Lund 1959. This technique was used to investigate the efficiency of centrifugation as there is no possible loss of material by removing fluid from the sample. Although it has been widely used for the examination of plankton, in general the debris, inevitably included in bottom samples, means that it is less suitable than the other methods for concentration for this study. In addition, the inverted microscope cannot be used successfully with high-power objectives.

3. Filtration

A millipore filter of suitable dimensions could be used for the collection of diatoms from a suspension, in place of centrifugation; the main disadvantage in this method is the difficulty encountered in removing the cells from the filter. Ferguson Wood, 1956 and 1962, discusses the use of filters, and describes a method, involving fluorescence microscopy, for estimating both the number of live cells, and the number of autotrophs in a suspension, by observing the filter directly under the microscope.

Cleaning

Cleaning was carried out by one of the following methods; all involve centrifuging the diatom suspension between each stage of the washing procedure.

1. Alcohol/acetone washing, Hendey 1964.

This method does not destroy even the most weakly siliceous species. It is far less efficient than any of the subsequently described methods for the removal of organic matter. Diatoms were :

1. Suspended in distilled water.
2. Resuspended in a mixture of 50% absolute alcohol and 50% acetone.
3. A drop of suspension was placed on a coverslip, and allowed to evaporate slowly; the coverslip was lowered onto a drop of mountant placed on a slide.

2. Acid washing, Hendey, 1964.

This method involves boiling the suspension for prolonged periods with a succession of inorganic acids, which effectively removes the organic material but destroys weakly siliceous species. As it is laborious and must be used in conjunction with other methods that preserve all the species, other methods are preferred in the present study.

3. Acid and Oxidising agents, Peabody 1967.

After centrifuging, 5 grams of Potassium chromate added. With a few mls. concentrated sulphuric acid the diatoms were transferred to a boiling tube. 100 volume hydrogen peroxide was added drop by drop until there was no further reaction. Centrifuged and washed twice with distilled water. Transferred to a boiling tube with a mixture of concentrated nitric acid and concentrated hydrochloric acid in the ratio 5 : 1. After boiling for five hours in a fume cupboard, the suspension was washed with distilled water. Aleem, 1950a, suggests that species such as Amphora are lost with

acid cleaning, such as methods 2 and 3, also Round, 1960 states that species such as Cylindrotheca, Amphiprora and Nitzschia gracilis are likely to be underestimated after acid cleaning.

4. Ultra-violet irradiation and peroxide Swift, 1967

This is the most efficient method; it is relatively fast, extremely effective, has few stages and preserves weakly siliceous frustules, because the treatment is specifically destructive of organic matter. The only drawback is the need for a large, high-energy Ultra-violet source. The type of source described by Armstrong et.al., 1966, (a 1200 watt mercury arc) was not available, however Swift suggests that a smaller source would suffice for this purpose. A low-power lamp was used to irradiate the samples for a period considerably longer than that recommended for the larger source (on the assumption that damage is proportional to the product of the dose and the time of exposure). However the effect was not much greater than that of peroxide alone. It is likely that the irradiation must be above a certain threshold to penetrate the solution sufficiently to cause any damage, irrespective of the time of exposure.

Procedure.

After the addition of a few drops of 100 volume Hydrogen peroxide solution, a suspension in distilled water was irradiated for two hours. (100 volume hydrogen peroxide is extremely effective alone, for cleaning the diatoms).

The use of more than one method for cleaning all the samples collected was prohibited by the lack of time available. In general it was considered preferable to examine the entire complement of species, i.e. those preserved by methods 1 and 4; because method 4 required equipment not available, method 1, being very simple, was usually found sufficient. Frequently some acid was involved in the removal of the diatoms from the substratum, but where this had not been the case, a modified form of the acid washing technique was employed if

there was a great deal of organic matter that obscured diatoms in the final preparations. The necessity for the most rigorous type of cleaning is discussed with the consideration of the other limiting factors to the resolution possible.

C. Examination

1. Mounting

For maximum resolution of the sculpturing on diatom frustules it is necessary to use a mounting medium of high refractive index. Hendey, 1964, suggests that hyrax is the best medium, and styrax, naphrax etc. are also suitable; the best medium is probably that described by Ghazzawi, 1933, - a mixture of antimony tribromide and piperine. Hymount was used for all the slides in this investigation, although it was satisfactory, it did not compare favourably with the resolution possible with hyrax.

2. Microscopy

A microscope with a built-in light source was used with a blue filter. With a times-10 eyepiece replicate slides were covered under low-power (X10) using the mechanical stage, for the largest species (above 30 microns length). Species below 30 microns are not readily seen under low-power, but identifications were carried out with a times-100 objective, this includes species down to 5 microns. In addition phase-contrast microscopy, plane polarised light and fluorescence microscopy would have been extremely useful, not only for identification, but also for examination of live cells.

Sample Series

As it was rarely possible to sample from more than two sites on any one day it is necessary to view a time series of samples from each site, and compare these before comparison is made between different sites at the same time. Seasonal fluctuations in numbers of different species are considered with reference to the tables of frequency of littoral species in Hendeby, 1964. Dates and types of samples are shown in Appendix 1.

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13 January 1976

W B Woodward Esq
Keeper of Science Books
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South Road Durham

Dear Mr Woodward

M Sc Dissertation 'An investigation of the possible effects of Pollution on some members of the periphyton in the littoral zone of the North-east coast of England' by C Goss

I apologise for the delay in replying to your letter of 18 December 1975 about this dissertation. The missing page that you referred to was never produced. I had hoped that before submitting the dissertation I would be able to complete Table 9 and prepare Table 10, and this is the reason for the missing page number. I should therefore have deleted the reference to Table 10* and inserted a note to explain the page numbers, and I am sorry for the inconvenience caused by my failure to do this. I do not feel that any other alterations are necessary because of the absence of this table, or that it significantly affects the discussion of results.

Yours sincerely

Catherine Mitchell

Catherine Mitchell (Mrs) nee Goss..

* on page 20

RESULTS

Time did not allow examination of all samples; samples were selected to show various comparisons:

Table	Site(s)	Date(s)	Sample(s)
4	5	29/1/67	<u>Cladophora</u> , <u>Patella vulgata</u>
5	3	24/5/67	<u>P.vulgata</u> , 1" and $\frac{1}{4}$ "
6	2	8/6/67	" from different pools at the same height on shore.
7	2	May/June	<u>P.vulgata</u> , 1"
8	2/4	31/5/67, 5/6/67	"
9	samples as in Table 8, size ranges of frequent species.		
10	1/4	25/7/67	<u>P.vulgata</u> , 1"

Key to site numbers in table 1.

Key to symbols in result-tables.

-	=	not recorded
/	=	present
+	=	frequent
++	=	extremely abundant

These estimations of frequency were made subjectively.

Horizontal lines are between sub-orders, as in Hendey, 1964.

For purposes of examining their epiflora, no distinction has been made between Patella vulgata and P. aspera. Where a dimension is stated after a limpet sample, this refers to the greatest diameter of the base of the shell. It should be noted that this length does not relate directly to the surface area, although the choice of sites meant that the ratio of height of shell to the area of the base was relatively unchanging. This dimension probably only gives a very vague indication of the age of an individual, and this is almost certainly not constant for different sites.

RESULTS

Artificial substrata

All the bricks laid down at site 4 were lost, as this meant that there was no comparison, with the unpolluted site, where slides were examined, this experiment was not continued, but species lists from site 2 can be found in Appendix 4.

Sources of identification and authorities etc. are in Appendix 2

Taxonomic notes in Appendix 3.

Time prevented the inclusion of any comparative quantitative results.

TABLE 4

	Samples: <u>Patella</u>	<u>Cladophora</u>
	Holy Island - 5, 29/1/67	
<i>Paralia sulcata</i>	-	/
<i>Biddulphia aurita</i>	-	+
<i>Rhaphoneis surirella</i>	+	-
<i>Opephora pacifica*</i>	-	+
<i>Synedra affinis</i>	-	/
<i>S. investiens</i>	/	-
<i>S. tabulata</i>	-	/
<i>Thalassiothrix frauenfeldii</i>	-	+
<i>Thalassionema nitzschiodes</i>	-	/
<i>Licmophora juergensii</i>	/	/
<i>L. flabellata</i>	-	+
<i>Grammatophora angulosa</i>	/	/
<i>G. marina</i>	-	/
<i>G. oceanica</i> var. <i>oceanica</i>	+	++
<i>Rhabdonema arctuatatum</i>	/	+
<i>R. minutum/crassum</i>	/	+
<i>Achnanthes parvula</i>	-	/
<i>A. subsessilis</i>	+	-
<i>A. brevipes</i>	+	+
<i>A. longipes</i>	-	/
<i>A. sp.</i>	+	-
<i>Cocconeis britannica</i>	-	+
<i>C. dirupta</i>	-	++
<i>C. scutellum</i> var. <i>scutellum</i>	+	/
<i>C. scutellum</i> var. <i>parva</i>	-	/
<i>C. speciosa</i>	+	+
<i>C. stauroneiformis</i>	-	/
<i>Rhoicosphenia curvata</i>	/	/
<i>Amphiprora hyalina</i>	/	/
<i>Gomphonema sp.</i>	+	/
<i>Amphora coffeaformis</i> var. <i>perpusilla</i>	-	/
<i>Amphora ventricosa</i>	-	/
<i>A. sp.</i>	-	/
<i>Nitzschia acuminata</i>	-	/
<i>N. apiculata</i>	-	+
<i>N. sp.</i>	-	+
<i>Auricula dubia</i>	-	+
<i>Navicula grevilliana</i>	-	/
SO. <i>Naviculinae</i> **	3	5
Total species	18	39
In common		14
Total frequent species	11	19
In common		6

** This sub-order was not studied in detail; numbers refer to frequent species in this group.

TABLE 5

Species	Samples: <u>Patella</u> , $\frac{1}{4}$ " Craster - 3, 24/5/67.	<u>Patella</u> 1"
<i>Paralia sulcata</i>	-	+
<i>Fragilaria</i> sp	-	+
<i>Grammatophora marina</i>	-	/
<i>G. oceanica</i> var. <i>oceanica</i>	-	/
<i>Rha-bdonema arctuatatum</i>	-	/
<i>R. minutum/crassum</i>	-	/
<i>R. crassum</i>	/	-
<i>Achnanthes parvula</i>	/	-
<i>A. brevipes</i>	/	-
<i>Cocconeis scutellum</i> var. <i>scutellum</i>	-	/
<i>C. scutellum</i> var. <i>parva</i>	-	+
<i>C. speciosa</i>	-	+
<i>C. stauroneiformis</i>	-	/
<i>Anorthoneis eccentrica</i>	-	/
<i>Surirella gemma</i>	-	/
<i>Pinnularia ambigua</i>	-	+
<i>Gomphonema</i> sp.	/	/
<i>Amphora</i> sp.	-	+
Species A	-	/
Sp. B	-	/
Sp. C	-	+
Sp. D	-	/
Sp. E	-	/
Naviculinae 2 spp.	/	/
Naviculinae 1 sp.	/	-
Naviculinae 1 sp.	-	+
Total species	7	23
In common	3	

RESULTS TABLE 6

SPECIES	SAMPLES 2 - Beadnell, 8/6/67	
	P. vulgata	P. vulgata
<i>Paralia sulcata</i>	/	+
<i>Actinoptychus senarius</i>	-	/
<i>Raphoneis surirella</i>	-	/
<i>Dimerogramma (furcigerum ?)</i>	/	-
<i>Synedra affinis</i>	+	-
<i>Thalassionema nitzschiodes</i>	+	+
<i>Thalassiothrix frauenfeldii</i>	+	+
<i>Licmophora juergensii</i>	+	++
L.sp.	-	/
<i>Grammatophora angulosa</i>	/	-
<i>G. marina</i>	/	+
<i>G. oceanica</i> var. <i>oceanica</i>	+	++
<i>Auriculopsis sparsipunctata ?</i>	/	-
<i>Achnanthes brevipes</i>	-	+
<i>A. hauckiana</i>	/	-
<i>A. longipes</i>	-	/
<i>A. parvula</i>	+	-
<i>A. subsessilis</i>	/	-
<i>A. sp. indet.</i>	+	+
<i>Cocconeis britannica</i>	+	+
<i>C. clandestina</i>	-	/
<i>C. costata</i>	/	-
<i>C. dirupta/scutellum</i>	-	/
<i>C. distans</i>	/	-
<i>C. pseudomarginata</i>	/	/
<i>C. scutellum</i>	/	++
<i>C. scutellum</i> var. <i>parva</i>	-	/
<i>C. speciosa</i>	++	++
<i>C. stauroneiformis</i>	/	-
<i>C. sublittoralis</i>	/	/
<i>Rhoicosphenia curvata</i>	/	-
<i>Stauroneis sp.</i>	-	+
<i>Amphiprora hyalina</i>	/	-
<i>Tropidoneis sp.</i>	-	/
<i>Gomphonema sp.</i>	++	+
<i>Phaeodactylum tricornutum</i>	-	/
<i>Amphora coffeaformis</i> var. <i>perpusilla</i>	+	+
<i>A. ventricosa</i>	-	/
<i>A. laevis</i>	/	-
<i>A. sp.</i>	+	/
<i>Bacillaria paxillifer</i>	/	-
<i>Nitzschia acuminata</i>	+	+
<i>N. apiculata</i>	+	/
<i>N. sp. indet</i>	+	/

TABLE 6 - continued :

Surirella sp.	-	/	
Pinnularia ambigua	+	+	
Navicula grevilliana	+	+	
N. ramosissima var. minor	/	-	
N. salinicola	/	-	
N. spectabilis	-	/	
N. spp 1-6	-	/	
sp. C	++	+	
spp. D,I,G	+	-	In common
Total spp.	41	38	21
Total frequent spp.	19	18	13

TABLE 7

Species	Samples: <u>Patella</u>	
	Beadnell - 2, May	June
<i>Paralia sulcata</i>	+	++
<i>Actinoptychus senarius</i>	-	/
<i>Fragilaria</i> sp.	+	-
<i>Rhaphoneis surirella</i>	-	/
<i>Dimereogramma (furcigerum ?)</i>	-	/
<i>Opephora pacifica*</i>	/	-
<i>Synedra affinis</i>	-	+
<i>Thalassiothrix frauenfeldii</i>	-	++
<i>Thalassionema nitzschiodes</i>	-	+
<i>Licmophora</i> sp.	-	/
<i>Grammatophora marina</i>	/	/
<i>G. oceanica</i> var. <i>oceanica</i>	/	++
<i>Rhabdonema arctuatatum</i>	/	-
<i>R. minutum/crassum</i>	/	-
<i>Achnanthes parvula</i>	-	+
<i>A. hauckiana</i>	?	/
<i>A. subsessilis</i>	-	/
<i>A. brevipes</i>	+	+
<i>A. longipes</i>	-	/
<i>A. sp.</i>	-	/
<i>Cocconeis distans</i>	-	/
<i>C. britannica</i>	-	+
<i>C. clandestina</i>	-	/
<i>C. scutellum</i> var. <i>scutellum</i>	+	++
<i>C. speciosa</i>	+	++
<i>C. stauroneiformis</i>	/	/
<i>Rhoicosphenia curvata</i>	-	/
<i>Anorthoneis eccentrica</i>	/	-
<i>Surirella gemma</i>	/	-
<i>S. sp.</i>	-	/
<i>Pinnularia ambigua</i>	+	/
<i>Amphiprora hyalina</i>	-	/
<i>Tropidoneis</i> sp.	-	/
<i>Amphora laevis</i>	-	+
<i>A. sp.</i>	+	+
<i>Bacillaria paxillifer</i>	-	/
<i>Nitzschia acuminata</i>	-	+
<i>N. apiculata</i>	?	+
<i>N. sp.</i>	-	+
Naviculinae	2	-
	-	2+
	-	1
	2	2
Total species	19	38
In common		11
Total frequent species	7	17
In common		5

RESULTS TABLE 8

SPECIES	SAMPLES	
	4-Souter, 31/5/67	2-Beadnell, 8/6/67.
<i>Paralia sulcata</i>	+	++
<i>Actinoptychus senarius</i>	+	/
<i>Biddulphia aurita</i>	+	-
<i>B. obtusa</i>	++	-
<i>Corethron</i> sp.	+	-
<i>Raphoneis surirella</i>	-	/
<i>Fragilaria</i> sp.	+	-
<i>Dimerogramma (furcigerum?)</i>	-	/
<i>Synedra affinis</i>	++	/
<i>S. tabulata</i>	+	-
<i>Thalassionema nitzschioides</i>	-	+
<i>Thalassiothrix frauenfeldii</i>	-	+
<i>Licmophora</i> sp.	-	/
<i>L. juergensii</i>	+	++
<i>Grammatophora angulosa</i>	-	/
<i>G. marina</i>	/	+
<i>G. oceanica</i> var. <i>oceanica</i>	++	++
<i>Thabdonema arctuatum</i>	++	-
<i>R. (minutum/crassum)</i>	+	-
<i>Auriculopsis sparsipunctata?</i>	-	/
<i>Achnanthes brevipes</i>	-	/
<i>A. hauckiana</i>	-	/
<i>A. longipes</i>	-	/
<i>A. parvula</i>	-	+
<i>A. sessilis</i>	-	/
<i>A. sp. indet.</i>	-	+
<i>Cocconeis britannica</i>	-	+
<i>C. clandestina</i>	-	/
<i>C. costata</i>	-	/
<i>C. dirupta/scutellum</i>	-	/
<i>C. distans</i>	-	/
<i>C. pseudomarginata</i>	-	/
<i>C. scutellum</i>	+	++
<i>C. scutellum</i> var. <i>parva</i>	-	/
<i>C. speciosa</i>	++	++
<i>C. stauroneiformis</i>	+	/
<i>C. sublittoralis</i>	-	/
<i>Rhoicosphenia curvata</i>	/	/
<i>R. stauroneiformis</i>	/	-
<i>Stauroneis</i> sp.	-	/
<i>Amphiprora hyalina</i>	-	/
<i>Tropidoneis</i> sp.	-	/
<i>Gomphonema</i> sp.	-	++
<i>Phaeodactylum tricornutum</i>	-	/

Continued :

TABLE 8 - continued	4.	2.
<i>Amphora coffeaformis</i> var. <i>perpusilla</i>	-	+
<i>A. ventricosa</i>	-	/
<i>A. laevis</i>	-	/
<i>A. sp.</i>	-	/
<i>Bacillaria paxillifer</i>	-	/
<i>Nitzschia acuminata</i>	+	+
<i>N. apiculata</i>	+	+
<i>N. sp. indet.</i>	-	+
<i>Surirella sp.</i>	-	/
<i>Pinnularia ambigua</i>	-	+
<i>Navicula grevilliana</i>	+	+
<i>N. ramosissima</i> var. <i>minor</i>	-	/
<i>N. salinicola</i>	-	/
<i>N. spectabilis</i>	-	/
<i>N. spp. 1-6</i>	-	/
sp. C	+	++
spp. D, I, G	-	/
Total species	22	59
in common	14	
Total frequent species	19	19
in common	9	

TABLE 9

Frequent species occurring at more than one site.

species	site	date	site	date	site	date	site	date	site	date
	2	24.5.67	2	8.6.67	2	26.7.67	4	31.5.67	4	25.7.67
<i>Paralia sulcata</i>			22.2	+ 4.43	-		32.9	+ 8.8	-	
<i>Grammatophora</i>			25.52	+ 5.5	-		40.9	+ 10.7	-	
<i>Cocconeis scutellum</i> var. <i>scutellum</i>										
<i>Cocconeis speciosa</i>										
<i>Rhabdonema arctuatum</i>										
<i>Rhabdonema minutum</i> / <i>crassum</i>										
<i>Biddulphia aurita</i>										
<i>B. obtusa</i>										
<i>Achnanthes</i> sp.										
<i>Licmophora</i> sp.										

Mean length along apical axis or diameter, in microns \pm standard deviation, derived from at least 30 measurements, except where otherwise stated, as figures in parentheses.

Samples were from ca. 1" Limpets, and represent first 30 specimens examined from all samples at site.

Discussion of results.

In discussing results hypotheses may be proposed on the nature of the factors that are of major importance to the distributions. The possible factors can be considered as causing local fluctuations, that are observed between samples at each site, and regional variations, between the sum of the samples at one site and any other; they may be the same parameters, varying on a small and large scale, but to simplify discussion site differences and sample differences are considered separately.

Samples differences compared in Tables 4, 5 and 6.

In Aleem's 1950a paper he describes several communities and proposes horizontal zonation of these diatoms, allied to the zones described for macroscopic algae. Alternatively Hendey, 1964, proposes a mosaic of discrete recognisable communities, with some species occurring throughout. He also recognises a distinction between the associations of species found in the supra-littoral, littoral and sub-littoral. It is clear that sampling in the littoral zone should be widespread, vertically and horizontally, to include the communities making up this 'mosaic of micropopulations'. The causal factors of the mosaic envisaged are probably a combination of microclimate and interrelationships between the living organisms. The epiflora of the limpet probably has a reduced number of communities over the number occurring throughout the littoral zone, partly because its own habitat preference is limiting the number of microhabitats that are sampled, and also because its movements would exclude species that are intolerant of change. For example a limpet that spends most of its time when exposed by the tide in a rock pool, might lose epiflora species that are intolerant of high insolation, by making intermittent forays onto exposed rocks. Also the limpets were particularly selected : all these reductions in the variation of the microclimate of the habitats sampled

account for considerable similarity in the total lists compared in table 6. These are the combined species lists for replicate samples from limpets in the mid-tide region, and differences between the lists indicate the differences that are considered to be the result of the postulated heterogeneity, and show the incompleteness of the sampling on this substratum. The greater differences between the samples seen in Table 4 (comparing Cladophora and Patella vulgata) and Table 5 (comparing $\frac{1}{4}$ " limpets and mature limpets), indicate the usefulness of restricting the substrata sampled. However, it is preferable that all the communities, at least within the littoral zone, are sampled before the species found are used to indicate site differences. Also it is essential to increase the sampling area before site differences are seriously considered; the dimensions of the 'mosaic of micropopulations' being unknown. Since both tychopelagic and meroplanktonic diatoms will be introduced from the plankton to the periphyton, it is clear that a major source of settling organisms is not homogeneous. It is known that plankton species are not evenly distributed; Cassie, 1964, describes 'microswarms' of plankton organisms and analyses their dispersal in a particular water mass.

Site variations.

Although the same water-mass affects all the sites, this may not be constant because of the outflow from many major rivers in the southern half of the sample area, which will tend to make some areas brackish for much of the time and introduce many euryhaline, fresh-water species. Thus it is not known whether any uneven distribution, between sites, could be caused by differences in species-composition of the replenishment source, rather than littoral environmental factors. Brackish water would not be likely to be particularly important in the particular communities under consideration here, since only euryhaline species could survive the

extremes of salinity, caused by evaporation and precipitation at low-water, encountered on rocky shores. Comparisons of close sites such as Craster Harbour and Beadnell Bay, and separate areas within a few hundred yards, should be made before distant sites can be usefully compared. If the difference in species composition shown in Table 8, and the size differences in Table 9 were shown, by examination of more samples, to be correlated with environmental site differences, rather than chance fluctuations, speculations on causal factors might be valid.

Several of the species listed can be considered to be ubiquitous in the entire area sampled; e.g. Cocconeis scutellum, C. speciosa, Grammatophora oceanica, var. oceanica, Licmophora sp., Navicula grevilliana, Synedra spp., Nitzschia spp. and Species C. Since the frequency classes were decided upon subjectively, differences in frequency cannot be considered without quantitative sampling including these species, but it is likely from the estimates that these species differ significantly in abundance, and the differences and similarities correlate with the others discussed. Approximately the same number of individuals were examined for each sample, but as this quantity was not measured exactly, species-diversity can only be considered tentatively. Thus it was decided to investigate the size difference in the frequent species, viz. the differences in table 9, that are clearly significant. The work by Garstang, 1937, Lucas and Stubbings, 1948, and Wimpenny, C. 1933 and 1946, on size differences in diatoms dealt with plankton species. Despite the comparative homogeneity of the plankton-environment over the littoral zone, correlations between different physical parameters and size changes might extend to diatoms regardless of their habitat.

Of particular importance amongst the factors discussed is the average temperature, but Garstang criticises the simplicity of this correlation, and recommends that a great many more measurements should be made. Hendey, 1951, describes size changes in littoral diatoms, and points out that, accompanying the size differences, are shape differences, e.g. breadth/width ratio etc. This shape change implies that change from large to small forms and vice versa must be via auxospore formation, since, with normal cell division, there is no opportunity for change in the ratios of dimensions of the frustule, besides those using the depth of the girdle. It is clear that the cells used for the measurements were such different forms, e.g. Grammatophora oceanica, 40 micron cells are little wider (across the valve) than 20 micron cells. If such cells could be brought into culture it might be possible to discover whether these are different genetically or the differences result from physiology in different environments.

Discussion of species-distribution.

Hendey, 1964, considers exposure, salinity, substratum, nutrients and temperature to be the most important factors determining the distribution of littoral diatoms, in addition light and biological interactions are also discussed here. The importance of exposure is not included because samples were taken from sites selected to be exposed, also the possible effect of salinity has been mentioned already.

Substratum.

The study on periphytic diatoms at Swanage, Dorset, and Saltdean Gap, Sussex, by Aleem, 1950a, indicated that the widely different geology and slope of these two rocky shores was not important in determining diatom distribution. Both Brockmann, 1935, and

Round, 1960, found that particle size on sediment bottoms was extremely important to diatoms, but opposing generalisations were made by the two authors; Brockmann suggests that larger species are found in sand than mud, and Round's work indicates the reverse. Gilson, 1930, has shown that suspended matter sediments out of sea-water, rapidly changing the substratum with thick deposits, even on surface such as algal fronds; the suspended matter shown in table 2 indicates that if such sedimentation occurs, it will be extremely heavy at polluted sites (this effect is visible at West Hartlepool Bay, where all hard surfaces are covered with silt and only a few colonial species were found growing in the silt and on Mytilus sp., no Patella spp. were found).

A mud-loving species, such as Actinoptychus senarius, which was frequent at Souter point, and not at any less polluted site, might be indicative of the operation of this factor. Many samples taken at different distances from a pollution outfall might confirm this type of result.

Nutrients.

Smyth, 1955, working with benthic diatoms in a sea-loch, states that, for these organisms, nutrients are rarely limiting. Although diatoms on bare rock might be subject to the same shortages as plankton diatoms, (Patrick, 1948), the levels of phosphate and nitrate in Table 2 indicate that these two substances are probably in excess at all sites. Lewin and Lewin, 1960 and Lewin, 1963, have shown a great many diatoms to be both obligate and facultative auxotrophs and heterotrophs; it is clear that sewage or any other organic pollution will profoundly affect vitamins, growth substances, and organic substrates for these species. Quantitative sampling might demonstrate the result of such factors, but, in the field, effects of growth substances would be mingled with the effects of toxins from pollution; the difficulties of measuring trace

compounds, and the culture of diatoms, would also make these opposing effects difficult to separate by experiment. The effect of any dissolved substances in the littoral zone is, at times, alternatively diluted and accentuated, by rainfall and evaporation at low-water.

Seasonal fluctuations.

Table 7 was included to indicate the type of seasonal change in species that is expected over the time period covered by other sample comparisons, and because site differences could be due to the same succession of species occurring non-synchronously. The species differences over this particular month at this site are clearly no more than the sample differences in table 6, but it is not valid to extrapolate from this one example to any other month. Samples from Souter Point show that there may be a drastic reduction in numbers between the end of May and end of July.

Direct pollution effects may change the range of a particular species, for example, suspended matter reducing light at high water will probably affect the distribution of Rhabdonema spp., Grammatophora spp., Achnanthes spp., etc., which are species that 'require fairly intense, uniform illumination' Hendeby, 1964. Also with alteration of zones of macrophytes in polluted areas, distribution may be altered by inter-species relationships; Fogg, 1962, suggests that extra-cellular products from one species may be important by being toxic or beneficial to another. Changes in possible range in one parameter may alter several others and the many interacting factors produce unpredictable results, for example Rhabdonema spp. being abundant solely at a polluted site. Thus, since environmental parameters are so inter-dependent, it is most useful to view characteristics common to all species present, and to aim at measuring the complexity of the communities in relation to their environment.

CONCLUSION.

All the site differences may be due to chance sampling of different communities, and the main use to which the results may be put is to indicate the type of sampling that is probably necessary to fulfil the aims of this investigation. The following procedure might give sufficient data to estimate the effect of pollution:

1. Over at least twelve months large numbers of samples should be taken all over the littoral zone, on all types of substrates, at several sites, together with detailed measurement of environmental parameters, temperature, pH etc.
2. From the above results, particular substrata could be selected to sample at different points around a pollution outfall, together with measurement of parameters such as those in Table 2.
3. Any qualitative changes indicated from the above samples would be examined over a prolonged period, and size changes tested by a great many measurements, (see Garstang.)
4. Where any diatom species can be cultured, experiments could be designed to confirm any postulated causal relationships.
5. Quantitative sampling to examine changes in productivity, would be useful; the importance of the periphyton is stressed by Wetzel, 1963, and Groentved, who indicate that, on occasion, the periphyton is more productive, per unit surface area, than the plankton.

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SUMMARY

1. The literature and terminology of the littoral periphyton is discussed.
2. Sites sampled are described in terms of position and geology, also various parameters that reflect the degree of pollution.
3. Samples were taken from natural and artificial substrata, and their treatment before microscopical examination is described.
4. Results are principally from one natural substratum, the limpet, Patella vulgata. Samples were selected to compare the flora from some of the sites on particular occasions.
5. It was concluded that the sampling here was insufficient to postulate any correlation between the results and environmental parameters. A programme of possible sampling in the future is suggested.

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APPENDIX 1.

SAMPLES.

Site	Type of sample Limpets	Artificial substrata	Other samples
1. St. Abbs	1.11.66	-	1.11.66 rock scrapings macro-algae
	26.7.67.	-	26.7.67.
2. Beadnell	15.11.66.	-	15.11.67.
3. Craster	24.5.67.	set down 24.5.67.	24.5.67.
	8.6.67.	sampled 8.6.67.	8.6.67.
		sampled 29.6.67.	-
4. Souter	31.5.67.	set down 31.5.67.	31.5.67.
	25.7.67.	-	25.7.67.
5. Holy Is.	29.1.67.	-	29.1.67.
6. St. Mary's	15.11.66.	-	15.11.66.
7. W. Hartlepl.	-	-	11.6.67. silt, rock and <u>Mytilus</u> sp. <u>Balanus</u> sp. algae.

APPENDIX 2.

Notes on species lists.

Authorities for names are not included; all are as in Hendeby, 1964, except those marked thus *, which were identified from Hustedt, 1927-1959. Hendeby does not cover littoral diatoms from the type of site sampled here particularly thoroughly; (see his introduction, page 2, and the criticism in British Phycological Bulletin, 2 No. 6,) dwelling more particularly on coastal plankton, and benthic species from sandy or muddy sites. Hustedt describes a very large number of species, but from an extremely wide range of sites in Europe.

Those diatoms not identified to species level are either, not included in the works mentioned above, have not previously been described at all, or were too small to be seen in sufficient detail for identification with certainty. Variations of the specimens identified from the description in the above works are mentioned in the discussion of results, most notably the frequent occurrence of individuals from many different species that had frustules that were considerably smaller than the size-range given in Hendeby, while the shape and dimensions of the sculpturing remained the same.

Numbers have been used by some authors to denote the estimated frequency of a particular species of diatom (derived in the same manner as the numbers used in terrestrial phytosociology to denote cover-abundance). It should be noted that the symbols used here are indicating subjective estimations of frequency but that they bear no relation to the same symbols when they are used in tables of numerical estimations, given by these authors.

APPENDIX 4

Total species list from artificial substrata at Beadnell - 2, from slides exposed for one month (slides exposed for shorter periods had no additional species, and one month was the duration of the experiment).

Samples from slides, 29/6/67

Paralia sulcata
Stephanopyxis turris
Actinoptychus senarius
Bellerochea malleus

Thalassiothrix frauenfeldii
Licmophora sp.
Grammatophora oceanica var. *oceanica*

Achnanthes parvula

Cocconeis speciosa

C. costata

C. sp.

Campyloneis grevillii

Auricula dubia
Gomphonema sp.
Amphora coffeaformis var. *perpusilla*

A. ventricosa

A. sp.

Oekdenia inflexa

Bacillaria paxillifer

Naviculinae: 3 spp.,

