DOCTOR OF PHILOSOPHY

The population biology of Littorina obtusata (L) (Gastropoda: Prosobranchiata).

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THE POPULATION BIOLOGY OF LITTORINA OBTUSATA (L.) (GASTROPODA: PROSOBRANCHIATA).

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ABSTRACT.

Wave action was responsible for movement of L. obtusata and caused high mortality on some shores by washing the animals on to the strand line. L. obtusata from wave exposed shores were adapted to wave action by crevice seeking behaviour, laying eggs on rock rather than weed, the young taking refuge within bladders of F. vesiculosus, and the adults being able to maintain their position in strong water currents, partly by possessing a more streamlined shell. The stronger the wave action, the smaller the adult, due partly to lowered winter growth on exposed shores.

Conditions of extreme shelter were better tolerated by animals inhabiting extremely sheltered shores than by those inhabiting the more exposed shores. Animals from extremely sheltered shores were less active in summer than in winter, and less active than animals from the more exposed shores. They were also better able to withstand high temperatures and lowered oxygen tensions.

Carcinus maenas caused considerable mortality at Ynys Faelog, and attacked an introduced population till the density fell to approximately that of the surrounding indigenous population. Low down on certain shores were found animals with crab resistant shells, though the reason for the resistance was obscure.

L. obtusata exhibited a variable geotaxis when out of water in the field, but a constantly negative geotaxis out of water in the laboratory, the rate of movement being slower when Fucus was the substrate provided. Geotaxis was always positive under water. The behaviour is explained as an adaptation to regaining their position on Fucus if dislodged. A marked positive rheotaxis was observed in a current of deep water, but not demonstrated in a film of moving water. L. obtusata maintained their position under Fucus as they were negatively phototactic.

Animals on more exposed and on densely populated shores were smaller and laid fewer eggs, though the individual eggs were of the same size.

Natural populations were seen to exhibit a bimodal frequency distribution. Young were mainly hatched in the summer, mature the next summer and died the spring and following summer, breeding continuously after sexual maturity.

No active migration of different size groups was in evidence.

Primary production of Fucus and its rate of consumption by L. obtusata on a densely populated shore were estimated. Food may have limited the size attained at Trearddur Bay.

The distribution of the following digenean Trematode parasites on Anglesey were noted; Cryptocotyle lingua (Creplin), Microphallus similis (Jagerskiol), and Cercaria lebouri (Stunkard). There was a seasonality in the infestation rate. Females and males were equally parasitised. The sex ratio was biased slightly in

favour of females.

The parasites caused an increase in size attained by the host, and mixed infections were rarer than expected.

CONTENTS.

Introduction

Littorina obtusata (L.) is a prosobranch gastropod living between mean high water neaps and mean low water neaps, on rocky shores with a good cover of fucoids. (Barkman 1955, Colman 1940, Dexter 1947 and Parr 1966)

It is found over large areas of the temperate zone, on the American Atlantic seaboard from Cape Cod to Labrador, and in Europe from the Straits of Gibralter to the southern shores of Greenland (Dautzenberg and Fischer 1914). It is absent from the Baltic Sea, its absence being explained by its susceptibility to the influence of freshwater. Hertling (1928) found the eggs to be incapable of surviving a salt concentration of 25% or less.

On the shore the distribution of L. obtusata is closely associated with Fucus spp. and Ascophyllum nodosum (Le Jol.) upon which it feeds (Barkman 1955 and Baaker 1959). Though in parts of the North of Scotland (Nicol 1956) Laminaria is preferred. The association is not an absolute one for L. obtusata is reported as absent from shores with a good cover of fucoids, but which are either of vertical rock rising from a sandy shore, or else one subject to moderate wave action (Barkman 1955).

L. obtusata is a highly polymorphic species. Dautzenberg and Fischer (1914) first described the colour varieties, since when a considerable body of work has been directed at the distribution and significance of colour variation, (Barkman 1955 and Parr 1966) and an intensive study by Sacchi (1961, 1963, 1964, 1964b, 1966 and 1967) who discovered the dwarf form to be a separate species:- Littorina mariae (Sacchi 1966b).

The sex ratio is slightly biassed in favour of females, there being a slight but probably insignificant difference between the colour varieties (Sacchi 1966b).

The eggs are laid on Fucus in gelatinous masses of from ninety to a hundred eggs from one to two hours after copulation during high water at night (Thorsen 1946) there being no means by which the female can store spermatozoa (Fretter and Graham 1962). According to Elmhirst (1923) breeding in the Clyde area of Scotland is continuous from February to September. Hatching takes place three to four weeks after laying at a temperature of 13-14°C. (Thorsen 1946), and is recorded in the Plymouth Marine Fauna as occurring between February and March in the Plymouth area.

The young hatch in the crawling stage as miniature replicas of the adult half a millimeter long, there being no planktonic stage (Barkman 1955). Though Tattershall (1920) reported L. obtusata eggs from South Ireland hatching as a late veliger stage, this has never been observed subsequently.

Little is known of the subsequent life history of L. obtusata, though it is suspected by Barkman of maturing in about one year after hatching.

Predators have been found to include the common shore crab Carcinus maenas (L.) (Crothers unpublished), the oyster catcher Haematopus ostralagus and the herring gull Larus argentatus (Moore 1959b) whilst the young ones are taken by the purple sandpiper Calidris maritima (Freare 1966).

Aims

The object of the present work was to examine natural, and artificial populations of Littorina obtusata (L.) in the field, and by measuring recruitment, growth and mortality, try to ascertain how the population structures observed were dynamically maintained. Further experiments were performed both in the laboratory and in the field to elucidate the effects of certain factors such as wave action and predation on the natural populations of L. obtusata.

In recent years increasing attention has been paid to field ecology of many marine species. The work of Smith and Newell (1955) and Newell (1958) investigated the dynamics of zonation of L. littorea at Whitstable, whilst Williams carried out an intensive study on the breeding cycle and population structure of the same animal on a shore in Mid Wales,(Williams (1963).

The effects of predation of the common shore crab Carcinus maenas were shown by Kitching et al (1966) to have a profound effect on Nucella lapillus (L) at Loch Ine. It would probably attack many other gastropods including L. obtusata.

Few workers appear to have studied populations of L. obtusata in the field, some poineer work was done by Colman (1933) and several papers of Sacchi 1961; 1963; 1964 and 1965 considered L. obtusata populations, but were mainly concerned with colour and morphological forms. Neither worker observed populations throughout all the seasons of the year.

Littorina obtusata is a good animal for such an ecological study. It is a common littoral animal on the shores of Anglesey and has no sublittoral component to its population. The adults are sufficiently large to enable them to be easily collected and present no difficulties in differentiating between individuals as do many colonial animals. Finally the young hatch as a non pelagic, miniature replica of the adult from eggs which are laid on the shore. There is thus limited interchange of genetic material from one shore to another unlike the situation in L. littorea.

Description Of The Shores Used For This Study

It was thought better to give a description of all the shores at the beginning, rather than describe them in the text as the need arose. Classification of the shores according to Ballantine (1961) are given as an approximate indication only of the conditions prevailing on each shore. The word "exposed" is taken throughout to mean "exposed to wave action" unless otherwise stated.

The first three shores described are all composed of isolated rocks embedded in a matrix of beach material and in the mid littoral zone covered with Ascophyllum nodosum (Le Jol.) and Fucus vesiculosus (L) or a mixture of both. The population of Littorina obtusata (L) on each weed covered rock could be studied as an entity in itself separate from the populations on other rocks. This was very useful in transplant experiments, when an experimental population could be set up on any isolated rock; and the population observed and measured as necessary.

FOUR MILE BRIDGE

Of all the shores studied, that of Four Mile Bridge (see fig. 1 and plate1) is the most protected from wave action. Wave fetch at the shore varies between 10 and 100 meters depending upon the height of the tide. The shore is very unusual. Whereas most shores sheltered to this degree are estuaries and subject to considerable influx of freshwater. The influence of freshwater at Four Mile Bridge is negligible. The shore, which is gently sloping, faces South West.

The beach material consists of mud, embedded in which are isolated, weed covered rocks varying in weight from 2 kgms. to 50 kgms. All the rocks even the smallest, are sufficiently stable in these conditions to be covered with weed. On the Ballantine exposure scale this shore may be considered as extremely sheltered.

BLACK ROCK

(See fig. 1 and plate 2.)

Black Rock lies to the North East Entrance to the Menai Straits and is subject to wave action to a much greater extent than is Four Mile Bridge, the wave fetch being in the order of 10 miles in a North Easterly direction, though it is considerably less in all other directions. The gently shelving beach material is of sand in the winter, but becomes muddy in the summer months owing to the deposition of fine material on top of the sand. Rocks on this shore are not stable unless they are greater than approximately fifteen kgms. in weight; and even above this weight they are sometimes moved by exceptionally violent North East Gales. On the Ballantine scale the shore would be described as sheltered.

RED WHARFE BAY

This north facing shore (see fig. 1 and plate 3.) is more exposed to wave action, than either Black Rock or Four Mile Bridge. The wave fetch being 100 miles over an arc of 30 degrees to the north.

The beach material is of limestone cobbles, and is more steeply shelving than the beach of the previous two shores.

The force of waves is sufficient to render unstable all but the heaviest rocks. Any rock less than one tonne is usually bare of weed, either because it is unstable or because its height is insufficient for its upper surfaces to be clear of the churning action of the cobbles which make up the beach material.

Ascophyllum is practically absent from this shore and the evesiculate form of Fucus vesiculosus forms the mid littoral algal cover.

The Littorina obtusata population on this shore is extremely sparse. This shore according to the Ballantine exposure scale is fairly sheltered.

RHOSNEIGR

(See fig. 1 and plate 4.)

This shore differs from the first three in being composed not of isolated rocks, but continuous reefs separated by sandy areas. The reefs are formed of vertically bedded hornfels, which have eroded to form a very dissected surface.

A few isolated weed covered rocks exist, one of which was used in a transplant experiment.

The shore faces south west and is a typical fairly sheltered shore on the Ballantine scale.

The shore is interesting as those parts which are gently shelving and highly dissected fall biologically in the category of fairly sheltered, but those other parts which are more steeply shelving and of a smoother nature would be classed as semi-exposed.

The population of L. obtusata is more dense than at Red Wharfe Bay, and so provided most of the L. obtusata for experiments requiring animals from fairly sheltered conditions.

PENMON

A shore similar to that of Rhosneigr in being composed of a continuous mass of rock which is largely covered with Fucus vesiculosus (see fig. 1 and plate 5). Penmon differs from Rhosneigr in facing eastwards, being more steeply shelving and composed of limestone, which, except for potholes, has eroded into a more or less smooth surface devoid of the crevices which are so characteristic of Rhosneigr.

The population of L. obtusata was a very sparse one, probably as there were few crevices in which to shelter from heavy wave action.

The shore is separated by only a narrow strait three hundred meters wide, from an off-shore island, the home of a large colony of seabirds. It was A good shore, therefore, to observe the effects of birds on introduced populations of L. obtusata.

RHOSCOLYN AND TREARDDUR BAY

These two shores resemble each other in facing south and being composed of continuous rock with a complete cover of Fucus vesiculosus and Ascophyllum nodosum (see fig. 1 and plate 6 and 7).

Both are sheltered shores, protected from wave action by being part of a bay, and by lying behind masses of rock to seaward.

Both these shores were chosen for study, for although they are similar in many physical respects, the population of L. obtusata at Rhoscolyn was more heavily parasitised by dignetic trematodes.

Both shores would be described as sheltered according to the Ballantine scale.

At Trearddur Bay two shores were used. They were designated shore one and shore two. Most of the work was carried out on shore one and reference to Trearddur Bay is taken to indicate shore one unless otherwise stated. Shore two was without the isolated sand patches of shore one, and possessed a population of L. obtusata twice as dense as that on shore one. When large numbers of L. obtusata were collected they were taken from shore two as large numbers could be collected in a short time.

The shores were in decreasing order of exposure.

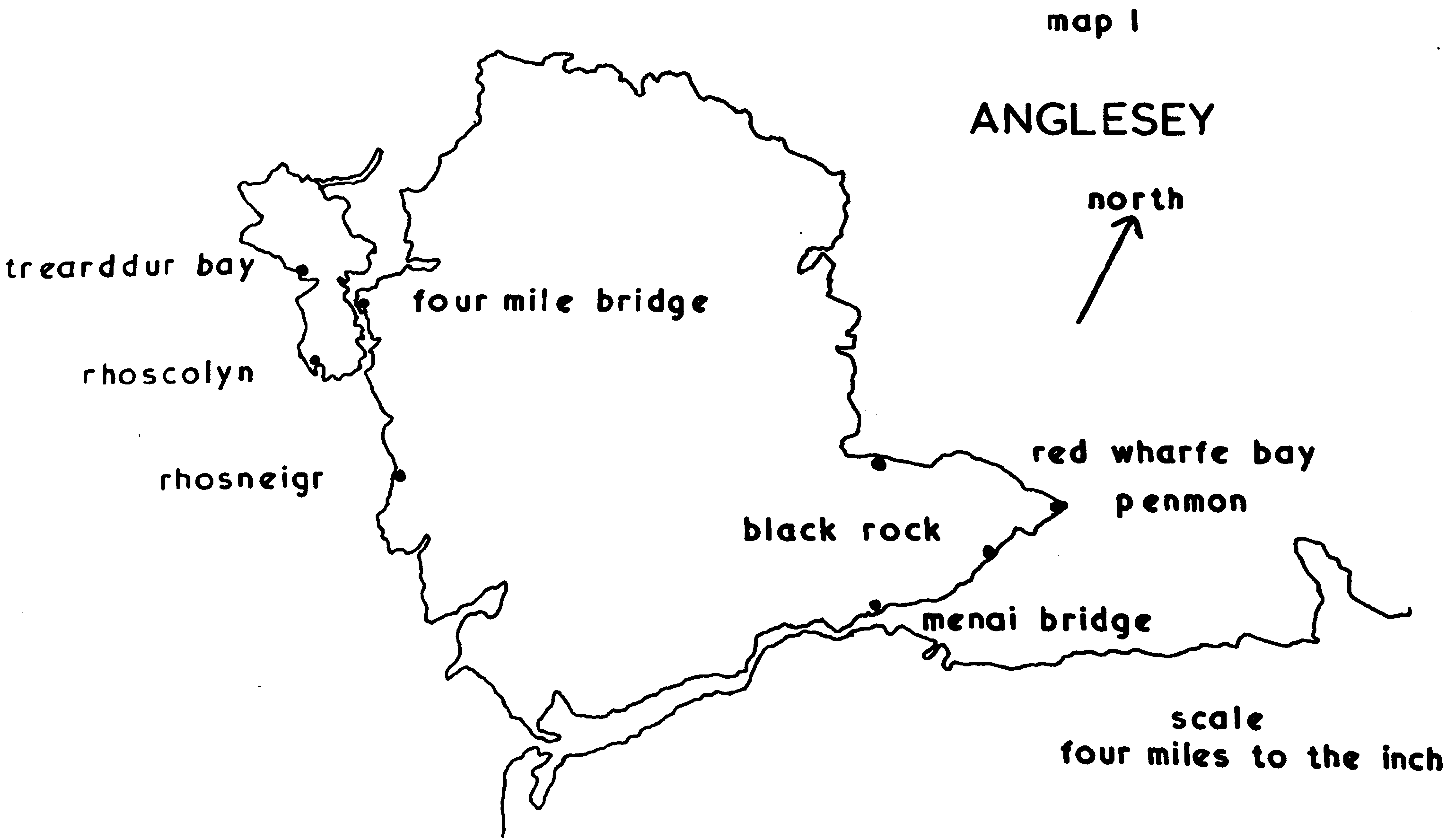
Rhosneigr, Red Wharf Bay and Penmon	(fairly sheltered)
Rhoscolyn and Trearddur Bay	(sheltered)
Black Rock	(sheltered)
Four Mile Bridge	(extremely sheltered)

As L. obtusata were never found on shores of greater exposure than fairly sheltered, no such shores were used in this study.

Though the method of describing the exposure of shores used by Ballantine (1961) is based on an essentially circular argument and may be criticised because of this, it does provide a quick method of describing the effect of wave action on any particular shore in the British Isles.

Figure 1.

Map of Anglesey to show the position of the shores mentioned in the text.


map I
ANGLESEY
north
trearddur bay
four mile bridge
rhoscolyn
rhosneigr
red wharfe bay
penmon
black rock
menai bridge
scale
four miles to the inch

Plates 1-7.

Photographs of the shores on Anglesey mentioned in the text.

Plate 1	Four Mile Bridge
2	Black Rock
3	Red Wharfe Bay
4	Rhosneigr
5	Penmon
6	Rhoscolyn
7	Trearddur Bay

FOUR MILE BRIDGE

BLACK ROCK

RED WHARF BAY

RHOSNEIGR

PENMON

RHOSCOLYN

TREARDDUR BAY

SECTION 1.

THE EFFECT OF WAVE ACTION ON L. OBTUSATA.

SECTION I.

THE EFFECT OF WAVE ACTION ON L. OBTUSATA

PART I THE STABILITY OF THE POPULATION AT BLACK ROCK

Introduction

Newell (1958a) discovered that individuals of Littorina littorea at Whitstable tended to remain in one position on the shore. Although they made small feeding excursions they usually returned to the same place. Barkman (1955) states that experiments by Ebbinge Wubben at Den Helder, showed L. obtusata to make extensive migrations only if the shore was either bare of weed, or was covered with a continuous layer of it. On shores with isolated tufts of weed the winkles remained on one tuft. The laboratory experiments of Van Dongen (1956) tended to corroborate this finding.

The work of Van Dongen showed L. obtusata to be attracted by Fucus chemically and it seemed unlikely they would leave one tuft of Fucus and migrate to a more distant one. As Van Dongen never attempted any field experiments and Barkman gives the impression that the observations of Ebbinge Wubben were not very extensive, more work needed to be done on the movement of individuals in the field.

The first experiment was conducted as a preliminary investigation to discover whether individuals of L. obtusata remained in one position on the shore as did L. littorea at Whitstable according to Newell (1958a).

Methods

At Black Rock one isolated boulder measuring one tenth of a square meter in plan view was chosen in the upper mid littoral zone.

On December 26th, 1965 all the L. obtusata on this rock retained by a one quarter inch sieve were marked with a file mark across the spire of the shell.

Only animals retained by the quarter inch standard Greening sieve were used in this experiment, smaller animals being difficult to find and easily overlooked. The rock was searched starting from one side and working towards the other. Subsequent similar searches being made till a search failed to reveal any animals. In practice the first search usually recovered all the animals and the second search served as a check.

On December 27th the population was re-examined and the number of marked L. obtusata remaining on the rock counted. The number of unmarked animals was also counted, these representing ones which had moved onto the rock from elsewhere in the period of twenty-four hours. The unmarked animals were marked with file marks in the same manner. The population was re-examined on January 12th, 1965.

Results and Conclusions

Of the forty-one L. obtusata marked on December 26th, thirty-two remained on December 27th. In addition to these sixteen animals had moved onto the rock from elsewhere. Of the forty-eight animals present on December 27th only three remained on January 12th. These had been joined by fourteen unmarked animals making a total of seventeen L. obtusata on the rock on January 12th.

Unlike L. littorea at Whitstable, individuals of L. obtusata at Black Rock were not only highly mobile, but the number of individuals on the rock varied widely. None of the marked winkles which were lost could be found by searching the rocks nearby.

L. obtusata is only able to make slow progress across sand (Barkman 1959), and in the first period of twenty-four hours would not have been able to crawl actively out of the area which was searched.

Observation both in the laboratory and in the field using an aqualung showed that L. obtusata does not leave the Fucus on which it feeds, unless it is dislodged.

The inference that wave action was responsible for the loss of animals from the rock was investigated by comparing , in Part II, the rate of loss from the same rock at Black Rock with that from a similar rock on the much more sheltered shore at Four Mile Bridge.

PART 2 .THE EFFECT OF WAVE EXPOSURE ON THE STABILITY OF POPULATION OF L.OBTUSATA.

Introduction

No work has been done on the effect of wave exposure on the stability of L. obtusata populations, though Barkman (1959) mentions that on wave exposed shores L. obtusata gives way to the dwarf form now known as L. mariae (Sacchi). On shores of even greater wave exposure no L. obtusata or L. mariae are to be found.

Experiment I Comparison of the rate of loss of animals at Black Rock and Four Mile Bridge.

The two shores used for this investigation were the sheltered shore at Black Rock and the extremely sheltered shore at Four Mile Bridge. The rock at Black Rock which had been used in Part I was again used. A similar sized rock at Four Mile Bridge in the upper mid littoral zone was used for the comparison.

All the animals found on the experimental rocks on each shore were graded for size using a quarter inch Greening standard sieve. The smallest L. obtusata which this sieve retained weighed 0.2 grams. Only those animals retained by the quarter inch sieve were used in this experiment, as many of the animals smaller than this would often be overlooked even in the most efficient search and would therefore tend to invalidate the results. The animals which were retained by the quarter inch sieve were marked with one file mark across the top of the spire.

Every time the rocks on each shore were visited, the total numbers of marked and unmarked animals retained by the sieve were noted; and every unmarked animal retained by the sieve was marked.

The results are shown in fig. 2. They showed that the rate of loss of L. obtusata from Black Rock was very much greater than from Four Mile Bridge.

It appeared likely that the difference in wave action experienced by the two shores was responsible for the observed difference in the loss rates.

The high loss rate at Four Mile Bridge in early April might be attributible to the activities of fishermen digging for bait rather than to natural causes, on some occasion the rock was found to have been moved and even turned over.

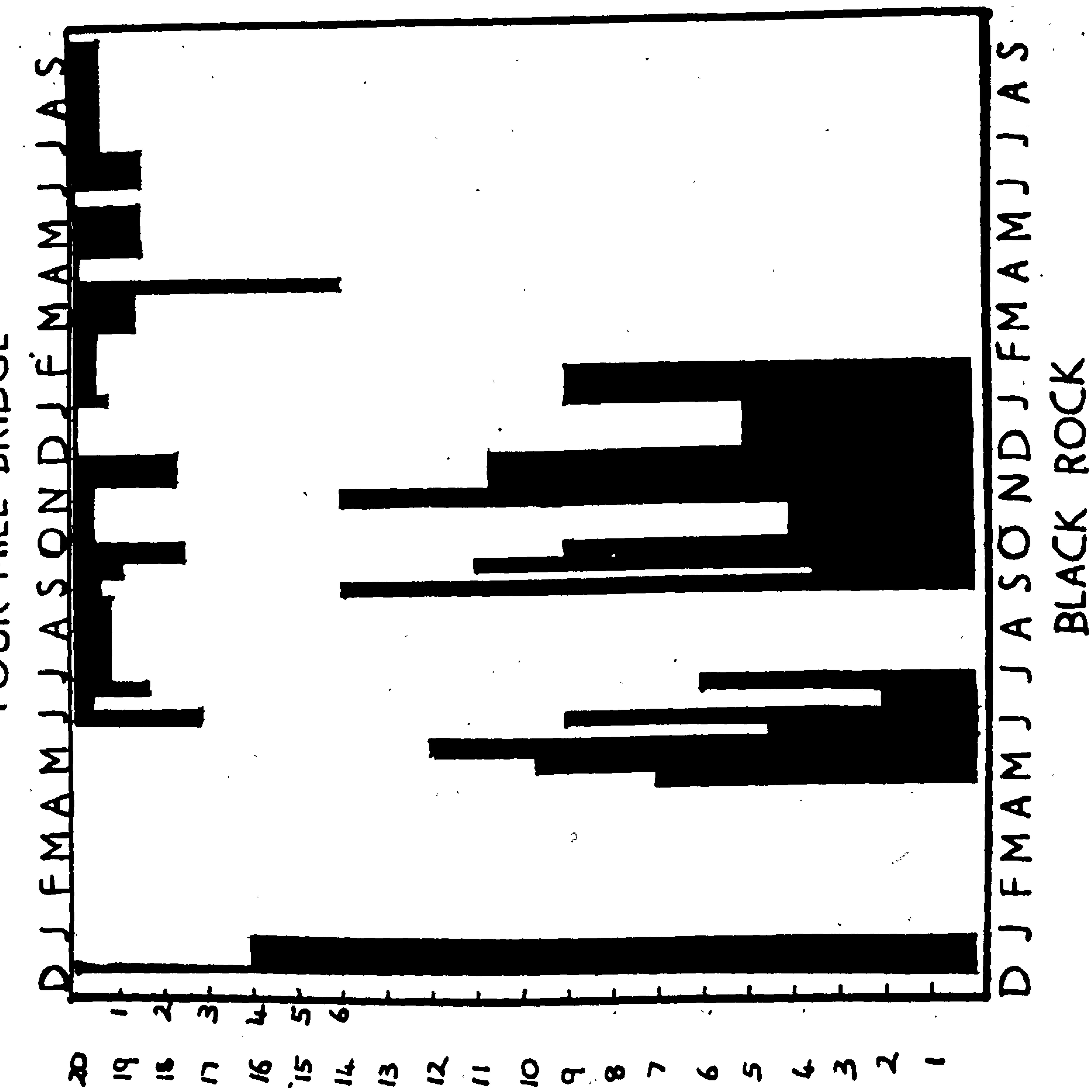
Despite the strong evidence in favour of wave action as the cause of the difference in the observed loss rate, there still existed the possibility that the animals may have been actively leaving the experimental rocks. The difference in loss rate reflecting a greater tendency amongst Black Rock animals to move actively from one rock to another.

This possibility is the subject of experiment 2.

Figure 2.

Daily loss rate (m) in percent of *L. obtusata* living at Four Mile Bridge and those living at Black Rock between December 1966 and September 1967.

FIG.2



DAILY LOSS OF L.OBTUSATA IN PERCENT
RIGHT HAND SCALE FOUR MILE BRIDGE

LEFT HAND SCALE BLACK ROCK

Experiment II <u>To determine the rate of loss on the fairly sheltered shore at Red Wharfe Bay of animals collected from the extremely sheltered shore at Four Mile Bridge.</u>

On March 2nd and March 11th, 1966, 107 and 262 L. obtusata respectively were collected from Four Mile Bridge. They were marked by means of a file mark and placed on a boulder covered with Fucus vesiculosus in the upper mid littoral zone at Red Wharfe Bay. The rock was revisited on March 4th and March 14th respectively, and all the remaining marked animals were counted.

The numbers remaining (see table 2a and 2b, p.14) indicated that when transplanted to the more exposed shore at Red Wharfe Bay, the L. obtusata from Four Mile Bridge were lost more quickly than they had been on their natural shore. They were lost more rapidly even than were the Black Rock animals at Black Rock, (see fig. 2, p.11).

To discount the possibility of the effects of the road journey being responsible for the high rate of loss at Red Wharfe Bay of L. obtusata collected at Four Mile Bridge, the following transplant was performed.

A hundred animals were collected from the shore at Four Mile Bridge on March 19th, 1966 and taken on a twenty mile road journey, marked with a file mark and returned to Four Mile Bridge.

The rate of loss of these animals (see table 2c, p.14) was comparable with the rate of loss of animals which had not been removed from the shore. (See fig. 2, p.11)

Discussion

L. obtusata living at Four Mile Bridge showed a smaller loss rate than the animals living at the more exposed Black Rock.

When transplanted to more exposed shores, the L. obtusata from Four Mile Bridge showed a marked increase in loss rate. As it was shown not to be the road journey which was affecting the loss rate, the most likely cause was wave action, the greater the wave action, the greater the rate of loss.

Many of the animals washed from a rock may never be able to find another supporting a growth of Fucus, especially when the rocks are sparsely scattered over the shore. Animals not reaching the cover of Fucus will be washed up on the strand line and die. Many such animals, many of them still alive have been found on the strand line after storms.

Table 2.

If the rate of loss of *L. obtusata* per day between two consecutive observations is regarded as proportional to the number present at the beginning of each day and if :-

N_o = number of *L. obtusata* present at an observation.

n = number of animals remaining at the next observation.

x = number of days between the two observations.

y = the ratio of the number present at the beginning to the number present at the end of the day.

then

$$y^x = \frac{N_o}{n}$$

$$x \log y = \log \frac{N_o}{n}$$

$$\log y = \frac{1}{x} \log \frac{N_o}{n}$$

If m = the number of *L. obtusata* lost each day expressed as a percent of the number present at the beginning of the day.

then

$$m = 100 - \frac{100}{y}$$

TABLE 2

Comparison of loss rates from sheltered and less sheltered shores

Table 2a and 2b

Rate of loss on the fairly sheltered shore at Red Wharfe Bay, of *L. obtusata* collected from the extremely sheltered shore at Four Mile Bridge.

2a First Transplant

Date	Number Present	Daily Loss (m) in %
March 2. 1966	107	
March 4.	61	34.5

2b Second Transplant

Date	Number Present	Daily Loss (m) in %
March 11. 1966	262	
March 14.	25	55

Table 2c - CONTROL

Rate of loss on the extremely sheltered shore at Four Mile Bridge of *L. obtusata* collected from Four Mile Bridge.

Date	Number Present	Daily Loss (m) in %
March 19. 1966	154	
March 21.	154	0.0
March 30.	131	1.7

PART 3 COMPARISON OF THE RATE OF LOSS ON MORE EXPOSED SHORES, OF ANIMALS COLLECTED FROM BOTH MORE EXPOSED AND MORE SHELTERED SHORES.

Introduction

The adverse effects of wave action in removing L. obtusata from its habitat of Fucus covered rock, presents an opportunity on the more exposed shores, for selection of animals more resistant to the dislodging effect of wave action.

The ensuing experiment compared by means of transplants, the rate of loss on more exposed shores of L. obtusata collected from both more sheltered and more exposed shores.

Procedure and Results

The following transplants were performed. In all cases the shore of origin was indicated by marking the shell with a trianglar file.

On March 19th, 1966, animals were collected from the extremely sheltered shore at Four Mile Bridge, and from the sheltered shore at Black Rock and placed on an isolated rock covered with Fucus vesiculosus on the fairly sheltered shore at Red Wharfe Bay.

On April 25th, 1966, a second set of animals collected from Four Mile Bridge and Red Wharfe Bay were placed on the same isolated rock at Red Wharfe Bay.

A similar transplant was made on April 6th, 1967 with animals collected from Four Mile Bridge together with ones from the fairly sheltered shore at Rhosneigr and placed on an isolated rock covered with Fucus vesiculosus at Rhosneigr.

The rates of loss (see Table 3a, 3b, and 3c, p.16 & 17) showed that L. obtusata originating from the more exposed shores were lost more slowly than those collected from the extremely sheltered shore at Four Mile Bridge.

When on February 17th, 1966 L. obtusata collected from the shores at Black Rock and Rhosneigr were placed on a rock at Black Rock, they were seen to exhibit rates of loss which did not significantly differ. (Table 3d p17)

Discussion

The results of the transplants indicated an addption on the part of L. obtusata living on shores subject to appreciable wave action, an adaptation which allowed them to maintain better their position on F. vesiculosus covered rock subjected to wave action.

The similar rates of loss at Black Rock of animals collected from Black Rock and Rhosneigr (see Table 3d, p.17) may be accounted for by the similar effect on L. obtusata of wave action on the two shores.

Rhosneigr is exposed to the greater wave fetch, but the shore is of continuous highly dissected rock. L. obtusata dislodged by wave action have a good chance of becoming washed into a crevice and not onto the strand-line.

Table 3.

Comparison of the rates of loss of L. obtusata collected from both more exposed and more sheltered shores, and placed on more exposed shores.

Table 3a.

Rate of loss on the fairly sheltered shore at Red Wharfe Bay of L. obtusata collected from the sheltered shore at Black Rock and the extremely sheltered shore at Four Mile Bridge.

Collected from Four Mile Bridge			Collected from Black Rock	
Date	Number Present	Daily Loss (m) in %	Number Present	Daily Loss (m) in %
March 19.1966	185		191	
March 21	71	38	118	21

Table 3b.

Rate of loss on the fairly sheltered shore at Red Wharfe Bay of L. obtusata collected from Red Wharfe Bay and from the extremely sheltered shore at Four Mile Bridge.

Collected from Four Mile Bridge			Collected from Red Wharfe Bay	
Date	Number Present	Daily Loss (m) in %	Number Present	Daily Loss (m) in %
April 25.1966	102		87	
April 26.	30	70.6	67	23.0
May 2.	1	84.0	40	7.4

Table 3c.

Rate of loss on the fairly sheltered shore at Rhosneigr of *L. obtusata* collected from Rhosneigr and the extremely sheltered shore at Four Mile Bridge.

	Collected from Four Mile Bridge		Collected from Rhsoneigr	
Date	Number Present	Daily Loss (m) in %	Number Present	Daily Loss (m) in %
April 6. 1967	150		150	
April 8.	49	43.0	78	28.0
April 10.	16	43.0	48	22.0

Table 3d.

Rate of loss on the sheltered shore at Black Rock of *L. obtusata* collected from Black Rock and the fairly sheltered shore at Rhosneigr.

	Collected from Rhosneigr		Collected from Black Rock	
Date	Number Present	Daily Loss (m) in %	Number Present	Daily Loss (m) in %
Feb. 17.1966	130		128	
Feb. 23.	70	10.0	70	10.0
March 3.	49	5.0	46	6.0
March 14.	20	8.0	18	8.0
April 26.	2	5.0	3	4.0

Though Black Rock is less exposed to wave action, the shore, being of isolated boulders in a matrix of sand, presents little opportunity for a dislodged winkle to escape the strand-line.

The difference between the effect on L. obtusata of wave action on the two shores is counterbalanced by the effect of the different topography of the two shores. The more continuous rock substrate and hence fucoid cover at Rhosneigr allowing the animals to regain their position more readily than at Black Rock.

It is uncertain whether the resistance to wave action exhibited by L. obtusata inhabiting the more exposed shores is genetically fixed in the population.

The young of L. obtusata hatch without the interpolation of a planktonic phase, from eggs laid on members of the Fucaceae. Though some dispersal occurs from shore to shore, populations on different shores are to a large extent genetically isolated from one another, so allowing them to develop their peculiar characteristics in response to the environment.

PART 4 THE NATURE OF THE ADAPTATION TO WAVE EXPOSURE

Introduction

It was established from the foregoing transplant experiments that L. obtusata living on wave exposed shores were adapted to resist being washed away by wave action. This section is devoted to the nature of the adaptation which enabled L. obtusata to resist being dislodged by wave action.

Theoretical considerations suggest three possible lines along which this adaptation may have taken place.

Firstly a more depressed shell would be less affected by the fast water currents associated with heavy wave action. Secondly the behaviour pattern of animals living on exposed shores may have been modified to avoid areas of the microhabitat subjected to the stronger water currents. Finally the foot of animals on exposed shores may have undergone modifications to allow it to grip the substrate more firmly.

Part 4 is divided into three:-

Part 4a. Adaptation of shell shape
Part 4b. Resistance to experimentally produced currents
Part 4c. Adaptations in behaviour

Part 4a Adaptation of shell shape.

Using diving techniques it was possible to observe winkles in their natural habitat under water. Of necessity the dives had to be made in calm weather, though it was possible by fanning currents of water over the winkles by hand to simulate to some extent the water currents the animals were likely to encounter in nature.

Observation showed that a sudden current of water dislodged the winkles from their position very easily. If, however, they were given warning by being tapped or by being subjected to small water currents first, they reacted by clamping their shells down hard onto the substrate. They were also usually found to turn so that they faced the direction of the current.

When clamped down it was impossible to dislodge them, even by the strongest currents which could be produced by hand.

It must be realised that the currents produced by hand were in no way comparable with the strength of the currents likely to be encountered during a storm. They showed, however, that L. obtusata responded to currents by clamping down and facing the direction of the current.

The orientation of L. obtusata to experimentally produced currents in the laboratory is discussed in the section on behaviour (section 4 p.67).

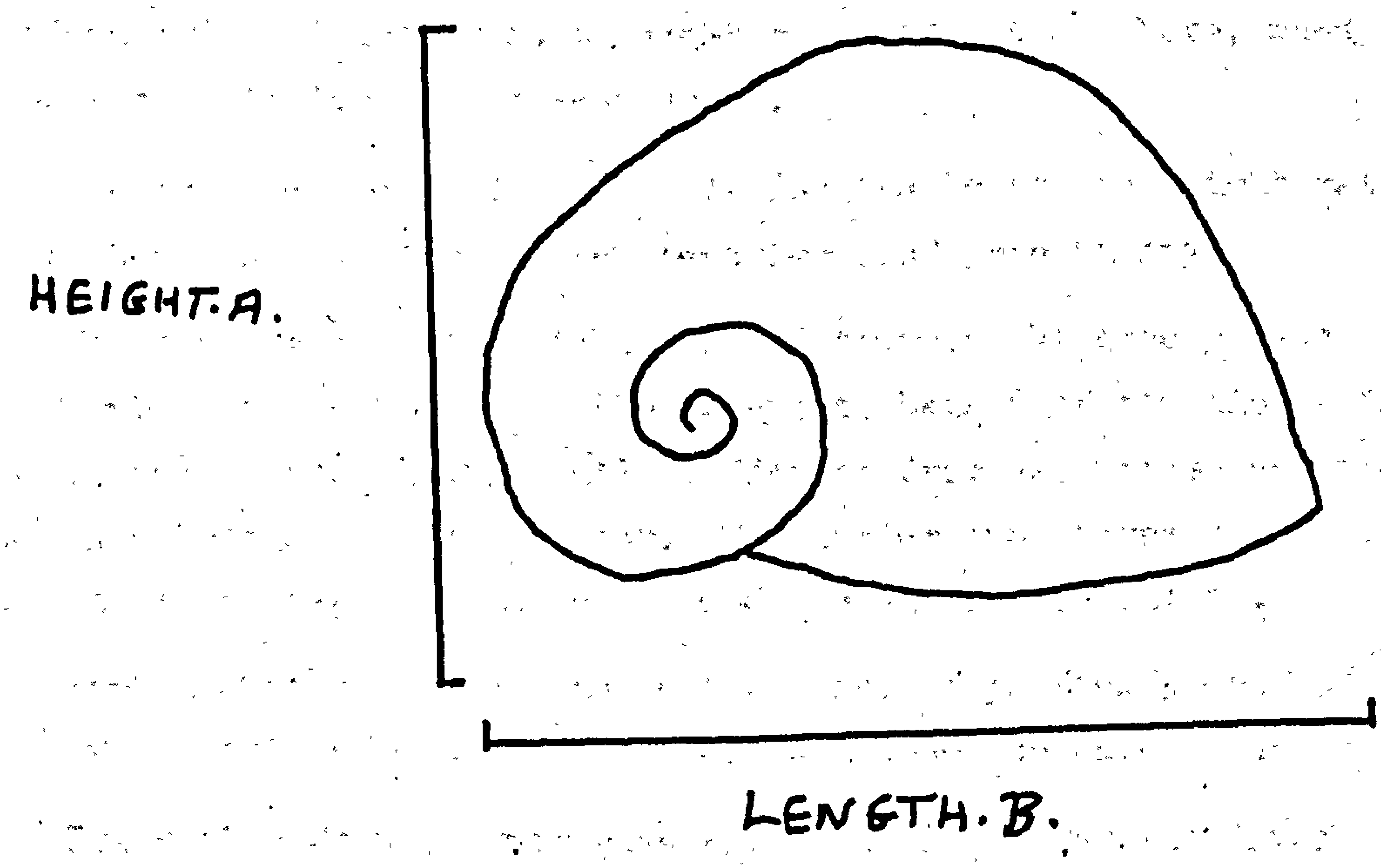
As the animals clamped down onto the substrate, only the shell required consideration in relation to stream lining.

A classical streamlined shape is usually thought of as possessing an aerofoil cross section. This shape relies for its efficiency upon a constant direction of water flow. On a rocky shore the water currents resulting from wave action are likely to flow in many directions.

A lower shell would be affected less by multi-directional water currents, and would benefit more from the presence of depressions and cracks in the rock.

The aerofoil shape may be of some advantage to animals feeding on <u>Fucus</u>. <u>Fucus</u> being a lax plant streams out in the direction of the water currents. This results in the flow of water flowing mainly from the base to the apex of the plant providing the winkle with a constant relative direction of flow.

As <u>L. obtusata</u> move onto the rock when subject to heavy seas on exposed shores, the factor considered important to the streamlining of the shell was the ratio of the height (A) of the shell to its length (B). (See fig. 3)



<u>Fig. 3.</u> <u>The shell of L. obtusata</u>

<u>Methods</u>

The length and height of the shells from Four Mile Bridge, Ynys Faelog, Rhoscolyn and Rhosneigr were measured.

The four shores represent a gradation from extreme shelter to fair shelter in that order.

The shells measured were always those of adult animals as the inclusion of young shells which were still growing would have confused the issue. An adult shell was recognised by the rounded, smooth nature of the shell lip. The lips of the shells of young animals which were still growing were sharp, friable and uncalcified. The measurements were performed using a measuring frame.

The measuring frame consisted of two wooden blocks mounted on a common wooden base and joined by a brass rod. On the brass rod was mounted a slider, the lower half of which abutted against the animal to be measured, holding it against the left hand wooden block. The upper half of the slider was a pointer which moved over a scale of millimeters.

The use of the frame precluded errors which would have arisen from measuring the curved surface of a shell, by eye, against a ruler.

The results (see table 4, p.22) showed difference in the means of the ratio of length to height. To confirm that the difference was significant the results were subjected to an analysis of variance and a variance ratio table (see table 5a, p.22) was constructed.

With a value for the variance of a hundred and four, a significant difference existed in the length: height ratio between at least some of the shores.

As the number of degrees of freedom of the residual value was high, it was considered permissible to use the expression $\frac{\sqrt{0.0047}}{\sqrt{n}}$ as an assessment the standard error of the length: height ratio from each shore, where n is the number of measurements taken from each shore.

The means and confidence limits for the length to height ratio are shown in Table 5b at the ninety nine per cent level of probability.

The results of the analysis showed that at the ninety nine per cent level of probability the ratios of the length to height of the shells from Rhosneigr and Rhoscolyn were significantly different from each other and from those of Four Mile Bridge and Ynys Faelog. The latter two shores showed difference from each other at the ninety nine per cent level of probability.

The difference between the animals from Ynys Faelog and Four Mile Bridge was difficult to explain in terms of resistance to wave exposure.

The shells from Rhosneigr were relatively longer and lower than those of any of the other three shores and were therefore considered to be better adapted to resisting the high speed water currents associated with the heavy wave action experienced at Rhosneigr.

The Rhoscolyn shells were intermediate in nature, while those of Four Mile Bridge and Ynys Faelog were relatively shorter and taller.

Part 4b Resistance to experimentally produced currents

Introduction

In the following experiment L. obtusata from the fairly sheltered shore at Rhosneigr and the extremely sheltered shore at Four Mile Bridge were subjected simultaneously to strong water currents in the laboratory.

Table 4.

Measurements of the ratio of length to height of shells of L. obtusata from four shores on Anglesey.

Shore	Ballantine Exposure	Number of Shells Measured	Means of Length to Height Ratio
Rhosneigr	Fairly sheltered	60	1.661
Rhoscolyn	Sheltered	61	1.582
Ynys Faelog	Very sheltered	60	1.463
Four Mile Bridge	Extremely sheltered	56	1.498

Table 5. Analysis of length to height ratios

Table 5a.

Variance ratio table of length to height ratios of shells of L. obtusata from four shores on Anglesey.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Between shores	1.466	3	0.488	104
Residual	1.0969	233	0.00470	-
TOTAL	2.563	236	-	-

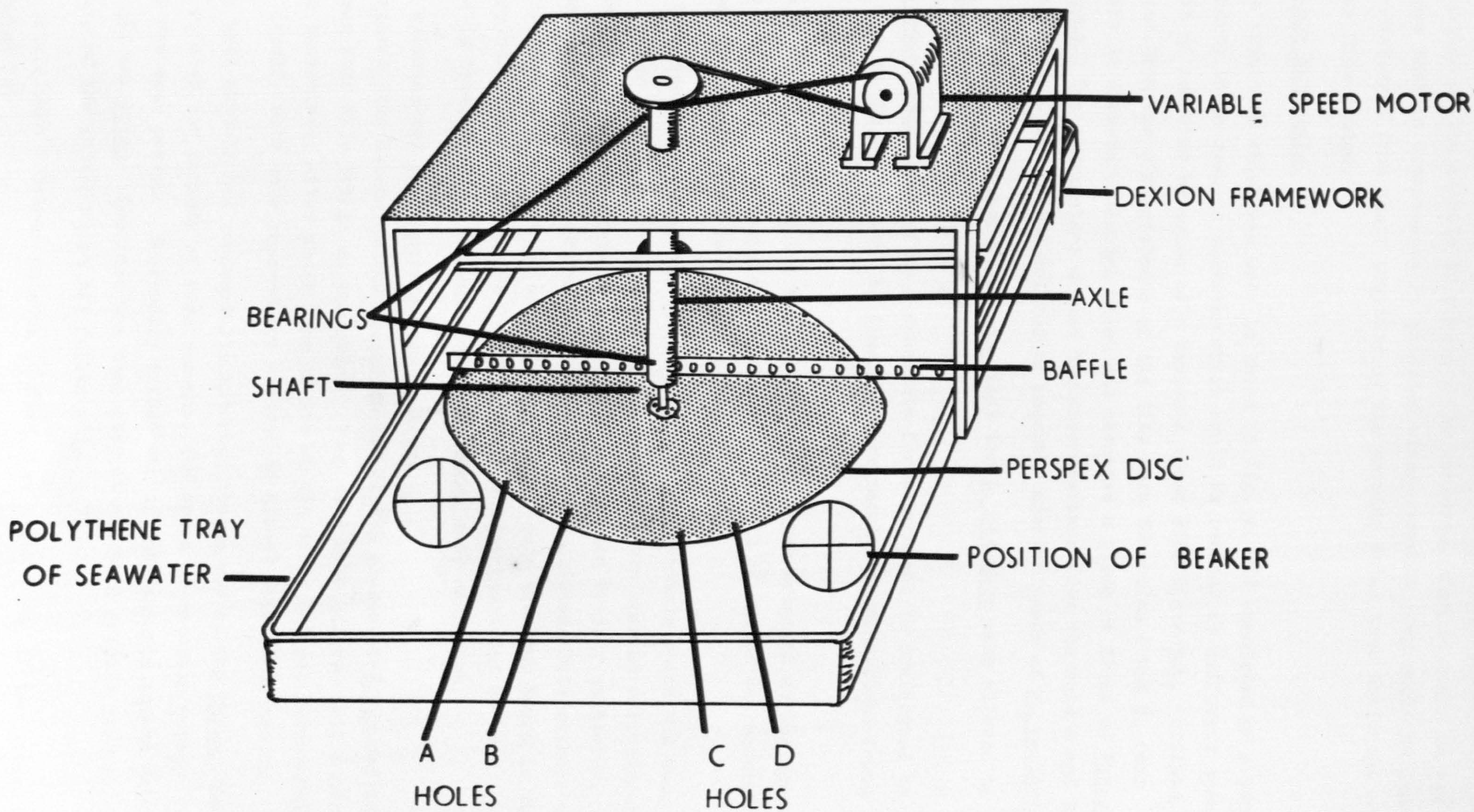
Table 5b.

Confidence limits at the ninety nine per cent level of probability of the means of the length to height ratio of shells of L. obtusata on four shores on Anglesey.

Shore	Means of the Length: Height Ratio	Ninety-nine per cent Confidence Limits
Rhosneigr	1.661	± .022
Rhoscolyn	1.582	± .022
Ynys Faelog	1.463	± .022
Four Mile Bridge	1.498	± .023

Figure 4.

The apparatus used to subject L. obtusata to water currents in the laboratory.


VARIABLE SPEED MOTOR
DEXION FRAMEWORK
AXLE
BEARINGS
BAFFLE
SHAFT
PERSPEX DISC
POLYTHENE TRAY OF SEAWATER
POSITION OF BEAKER
A B
HOLES
C D
HOLES

If the relatively long, low shape of the shells from Rhosneigr conferred any advantage on the animals in strong water currents, then it would be reasonable to suppose that in experimentally produced water currents they would be less readily dislodged from their position by the passing water than would the animals from Four Mile Bridge.

Methods and Materials

The apparatus which was used is shown in fig. 4. It consisted of a perspex disc, thirty three cms. in diameter which could be rotated at different speeds in a bath of seawater by means of a variable speed electric motor. Drilled one centimeter from the circumference of the disc were two holes A and B, four milimeters in diameter, through which was threaded a piece of frond of *Fucus vesiculosus*. Ten centimeters around the circumference from the hole A and B, two more holes, C and D were drilled to accept a similar piece of *F. vesiculosus*.

It was upon these pieces of *Fucus* that the *L. obtusata* were allowed to attach themselves during the experiment.

F. vesiculosus was used as a substrate for attachment in preference to the perspex disc as it was thought to simulate more nearly the conditions found in nature.

The swirling action of the water in the bath which occurred during the rotation of the disc was dampened by means of a length of dexion and several liter beakers placed so as to arrest the water flow.

As the experiment proceeded it became clear that animals which had been kept for longer than two or three hours in the laboratory reacted very differently to the experimental conditions from those which had been freshly collected. The experiment was therefore restarted and only freshly collected adult animals were used during the experiment. No animal was kept for more than two hours in the laboratory before being used. For this reason the experiment had to be conducted in three stages, a fresh collection being made each time.

The experimental procedure was as follows.

An animal from Rhosneigr was placed on one of the pieces of *Fucus* and an animal from Four Mile Bridge on the other. They were both allowed half a minute to attach themselves, after which time if one or the other had not successfully attached itself, both were removed and allowed to attach themselves again.

When both animals had successfully attached themselves to the *Fucus*, the disc was rotated for periods of five seconds, the speed of rotation being increased for each period. Eventually either one or both animals became detached and fell off the *Fucus*. The disc was then stopped and both animals removed, and a note made of the animal which had fallen off.

The trials were arranged in sets of four, the positioning of the animals being altered for each trial to nullify the effect of any difference in the two pieces of Fucus.

The set of four trials was arranged as follows:-

If R1 and R2 were two animals from Rhosneigr, and F1 and F2 two from Four Mile Bridge. The two pieces of Fucus were A-B and C-D.

Trial	Fucus A-B	Fucus C-D
1	R1	F1
2	F1	R1
3	F2	R2
4	R2	F2

Results

The Results are shown as three contingency tables, (Tables 6a, 6b and 6c). They were analysed by a chi squared test, using Yates correction for continuity.

Table 6.

The comparative resistance of *L. obtusata* collected at Four Mile Bridge and Rhosneigr to experimentally induced currents in the laboratory.

Table 6a October 27th, 1967

	Dislodged	Not dislodged	Total	
Rhosneigr	6	22	28	
Four Mile Bridge	25	3	28	$\chi^2 = 23.5$
TOTAL	31	25	56	

Table 6b April 4th, 1968

	Dislodged	Not dislodged	Total	
Rhosneigr	13	15	28	
Four Mile Bridge	22	6	28	$\chi^2 = 4.9$
TOTAL	35	21	56	

Table 6c April 5th, 1968

	Dislodged	Not dislodged	Total	
Rhosneigr	11	17	28	
Four Mile Bridge	24	4	28	$\chi^2 = 11.0$
TOTAL	35	21	56	

TOTAL χ^2 for the three tables is 39.4 for 3 degrees of freedom.

The contingency table indicated a significant difference for each section of the experiment, the total chi squared indicating a significant difference at the 99.9% level of probability.

In all three sections of the experiment the animals originating from the fairly sheltered shore at Rhosneigr maintained their position better than those originating from the extremely sheltered shore at Four Mile Bridge.

A similar experiment using L. obtusata from the fairly sheltered shore at Red Wharfe Bay in place of those from Rhosneigr showed them to be also better able to resist being dislodged than those from Four Mile Bridge (Table 7).

Table 7.

The comparative resistance of L. obtusata collected from Four Mile Bridge and Red Wharfe Bay to experimentally induced currents in the laboratory.

	Dislodged	Not dislodged	Total	
Red Wharfe Bay	0	14	14	
Four Mile Bridge	14	0	14	$\chi^2 = 24$
TOTAL	14	14	28	

Discussion

Under the experimental conditions, the animals from Rhosneigr and Red Wharfe Bay, both shores of fair shelter, were better able to maintain their position in high speed water currents than were the animals collected from Four Mile Bridge. This ability was probably partly at least due to the difference in length to height ratio, but may also to some extent have reflected the greater ability of the foot to grip the substrate.

The greater ability of the L. obtusata from Rhosneigr and Red Wharfe Bay to maintain their position in strong water currents no doubt contributed to their superior performance on fairly sheltered shores when compared with those transplanted there from the extremely sheltered shores. (see tables 2 and 3, p. 14, 16, 17)

Part 4c Adaptations in behaviour

Introduction

While some work has been done on the behaviour of L. obtusata in the laboratory (Barkman 1955 and Van Dongen 1956), no field work appears to have been done on the behaviour of the animal in relation to wave exposure.

Methods

It was not possible to observe the reaction of L. obtusata to heavy wave action directly by diving. Populations of L. obtusata were therefore observed throughout the year on five shores to discover whether any changes occurred which could be observed at low tide and which could be interpreted as a reaction to conditions of heavy wave action.

The five shores chosen were those at Black Rock, Four Mile Bridge, Rhoscolyn, Rhosneigr and Trearddur Bay. These shores were chosen as they represented a wide range of exposure to wave action.

Results and Discussion

No difference was observed in the behaviour of the populations on any of the shores except at Rhosneigr.

L. obtusata normally lived on the Fucus upon which it fed and laid its eggs. At Rhosneigr from October to April, a high proportion of the population moved off the Fucus and onto the rock where it remained attached in crevices. A high proportion of the egg masses, often over half the total counted in any area (see Table 8), were found attached to the rock surface and not to the fronds of the Fucus. In the rock crevices, L. obtusata would be protected to a great degree from the effect of the heavy winter wave action.

In view of the above observation it was surprising to find no mention of such behaviour in the literature. It is further more stated by some that L. obtusata relies entirely upon Fucus as a substrate on which to live and lay its eggs. (Barkman 1955 and Sacchi 1964 and 1966). There can however be little doubt as to the significance of this behaviour, especially as it was not observed on any of the other four shores at any time of year.

As this behaviour seemed to be peculiar to the Rhosneigr animals it was thought to be a behaviour pattern developed in response to wave exposure and which would be absent in animals from other shores. It was decided therefore to investigate this hypothesis more closely by transplanting animals from a more sheltered shore to a shore of exposure comparable with Rhosneigr.

The second shore at Trearddur Bay presented itself as a good choice for a source of winkles as the winkle population at Trearddur Bay was particularly dense and therefore lent itself to the collection of large numbers of animals without difficulty. Chosing the shore which was to receive these animals presented some problems. It was impracticable to transplant them to Rhosneigr partly because of the difficulty of marking 4,000 animals but mainly because the egg masses which they laid would be indistinguishable from the ones laid by the indigineous animals. It was therefore decided to take them to Porth Leven in Cornwall as that shore had been denuded of its original population by the influx of Torrey Canyon oil at Porth Leven in April, 1967. The Fucus had to a large extent recovered so the habitat was well suited to receiving the Trearddur Bay winkles.

4,000 winkles, collected from Trearddur Bay on 10th March, 1968, were released at Porth Leven two days later. Regular observations revealed that these animals lived and layed their eggs on the rock in winter. During the summer months, on the other hand, they left the rock, and lived and laid their eggs on the weed. The experiment suggested that this behaviour, exhibited only when the winkles were exposed to heavy waves, was certainly latent in the animals from Trearddur Bay, and probably in those from other shores as well. If all winkles adapted themselves quickly to wave exposure by hiding in crevices, then this behaviour cannot be cited to explain why animals transplanted from the extremely sheltered shore at Four Mile Bridge failed to survive on fairly sheltered shores. It must be remembered that Trearddur Bay is a shore affording only moderate shelter, whereas at Four Mile Bridge, where shelter is extreme, the animals may possibly have lacked an inherent ability to adapt in this way.

It would have been interesting to conduct the experiment with winkles from Four Mile Bridge. However, the low density of animals on the shore, and the restricted area of the shore itself, precluded large scale collection. Similar natural limitations to collection existed on other extremely sheltered shores in Anglesey.

Table 8.

The site of egg masses of Littorina obtusata on the upper mid littoral zone at Rhosneigr, between January 24th, 1967 and May 14th, 1968.

Date	Number of Egg Masses on Rock	Number of Egg Masses on Fucus	Proportion of Masses on the Rock in Per cent
January 24.1967	4	0	100
February 3	30	32	48.5
February 8	11	0	100
May 5	0	37	0.0
June 16	3	53	5.4
July 8	0	43	0.0
August 15	0	57	0.0
October 2	3	18	14.0
November 23	4	13	24.0
January 11.1968	18	65	22.0
February 27	24	25	41.0
April 4	10	7	59.0
May 14	13	22	37.0

PART 5 THE EFFECT OF WAVE EXPOSURE ON GROWTH AND SIZE OF L. OBTUSATA.

Introduction

Little is known of the effect of wave exposure on population characteristics of L. obtusata. Barkman (1955) mentioned the adverse effect of surf showing certain varieties to be more surf resistant. Barkman also noted the small size of adult L. obtusata living on wave exposed shores.

Sacchi (1961) corroborated the observation of Barkman in finding the var. citrina and reticulata to be more surf resistant than the var. olivacea, and in a later publication Sacchi and Rastelli (1966) described the surf loving dwarf form as a new species, L. mariae (Sacchi and Rastelli). The observations of Barkman are in some doubt as he almost certainly included L. mariae in his results for L. obtusata. The effect of wave action on growth and size of L. obtusata was therefore uncertain.

Part 5 is divided into two.

Part 5a considers the effect of wave exposure on the adult size of L. obtusata whilst Part 5b considers the effect of wave exposure on the growth of the young.

Part 5a. The effect of wave exposure upon adult size of L. obtusata in the field.

Methods

Adult L. obtusata were collected from fourteen shores, representing a wide range of conditions of wave exposure. Collections were made only of adult animals whose shell lip had become rounded off, they were weighed individually, and the population mean weight calculated for each shore. The shores from which collections were made are shown in Table 9a (p.32) in order of decreasing wave exposure.

The shores were graded according to the Ballantine exposure scale (Ballantine 1961). The order of the shores within each Ballantine class was based on wave fetch. Shores exposed to waves with a fetch of more than 100 miles were graded according to the size of arc over which such waves could reach the shores without being refracted by more than 10°.

The remaining shores were graded according to the distance of land at ninety degrees to the shore.

Results and Discussion

The weights of the adult populations were plotted against degrees of wave exposure of the shore of origin. (Fig. 5, p.31) The points of the graph show the mean weight of the adult population decreases with increasing wave exposure, and provided the three points enclosed in the triangles are ignored, the points lie approximately on a straight line. It was justifiable to ignore the points

Figure 5.

The mean weight of the adult population of L. obtusata plotted against the degree of wave exposure on the shores listed in Table 9a.

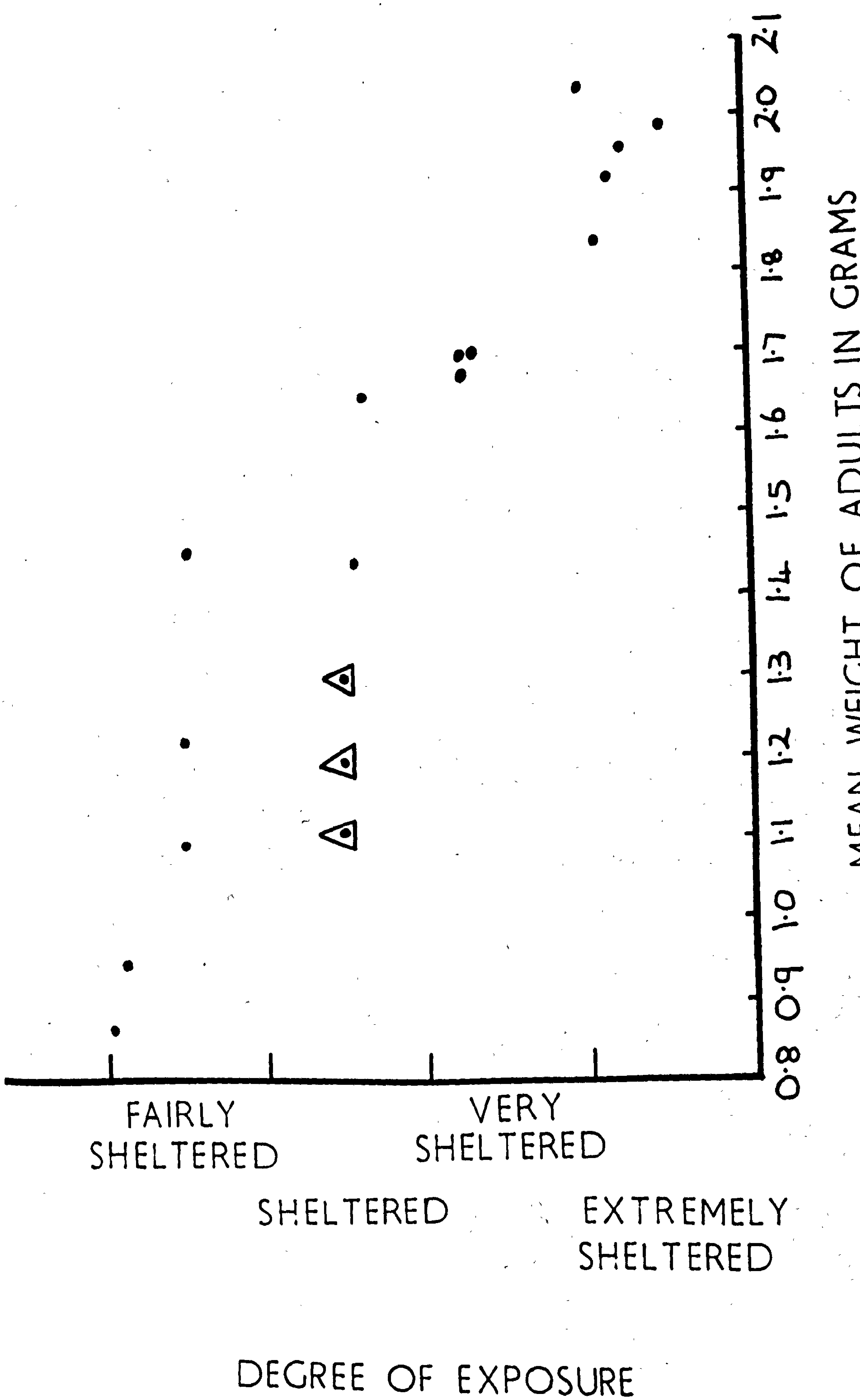

MEAN WEIGHT OF ADULTS IN GRAMS
0·8
0·9
1·0
1·1
1·2
1·3
1·4
1·5
1·6
1·7
1·8
1·9
2·0
2·1
FAIRLY SHELTERED
SHELTERED
VERY SHELTERED
EXTREMELY SHELTERED
DEGREE OF EXPOSURE

Table 9a.

Shores used to compile Figure 5 in order of decreasing wave exposure.

Shore	County	Ballantine Exposure Type
Chapel Porth	Cornwall	Fairly sheltered
Red Wharfe Bay	Anglesey	Fairly sheltered
Daymer Bay	Cornwall	Fairly sheltered
Polridmouth	Cornwall	Fairly sheltered
Rhosneigr	Anglesey	Fairly sheltered
Rhoscolyn (upper and lower shore)	Anglesey	Sheltered
Trearddur Bay (upper and lower shore)	Anglesey	Sheltered
Trearddur Bay (second shore)	Anglesey	Sheltered
Black Rock (upper and lower shore)	Anglesey	Sheltered
Rock (seaward shore)	Cornwall	Sheltered
Rock (landward shore)	Cornwall	Very sheltered
Glyn Garth	Anglesey	Very sheltered
Ynys Faelog	Anglesey	Very sheltered
Golant	Cornwall	Very sheltered
Four Mile Bridge	Anglesey	Extremely sheltered

Table 9b.

Winter and Spring growth rates of L. obtusata on five shores on Anglesey. Growth rate is expressed in percent increase in weight per week.

Shore	Winter Growth Rate October - February	Spring Growth Rate March and April
Four Mile Bridge	4.5	4.0
Black Rock	3.6	2.8
Rhoscolyn	0.3	3.5
Trearddur Bay	0.9	5.4
Rhosneigr	0.8	5.5

enclosed in the triangles as there was evidence to suggest that the animals on the three shores concerned (two shores at Trearddur Bay and one at Rhoscolyn) were suffering from overcrowding.

There were two possible reasons for the effect of wave exposure in decreasing the size of adult L. obtusata. Firstly, during heavy wave action the animals may not be able to feed, secondly small animals may be favoured by conditions of wave exposure as they would be better able to shelter in small crevices than would the larger animals.

Part 5b. The effect of wave action on the growth of L. obtusata in the field.

Methods

Populations of L. obtusata were sampled at approximately six week intervals throughout the year on the following shores. Rhosneigr (fairly sheltered), Trearddur Bay and Rhoscolyn (sheltered), Black Rock (very sheltered), and Four Mile Bridge (extremely sheltered).

To sample the population, Fucus vesiculosus was cleared completely from several patches in the upper F. vesiculosus zone on each of the shores. Collecting from several small patches rather than one large one helped to reduce any error due to clumping of the population.

The Fucus was placed in containers together with all the L. obtusata living on the weed which had been removed, and also any which were on the rock underneath the weed.

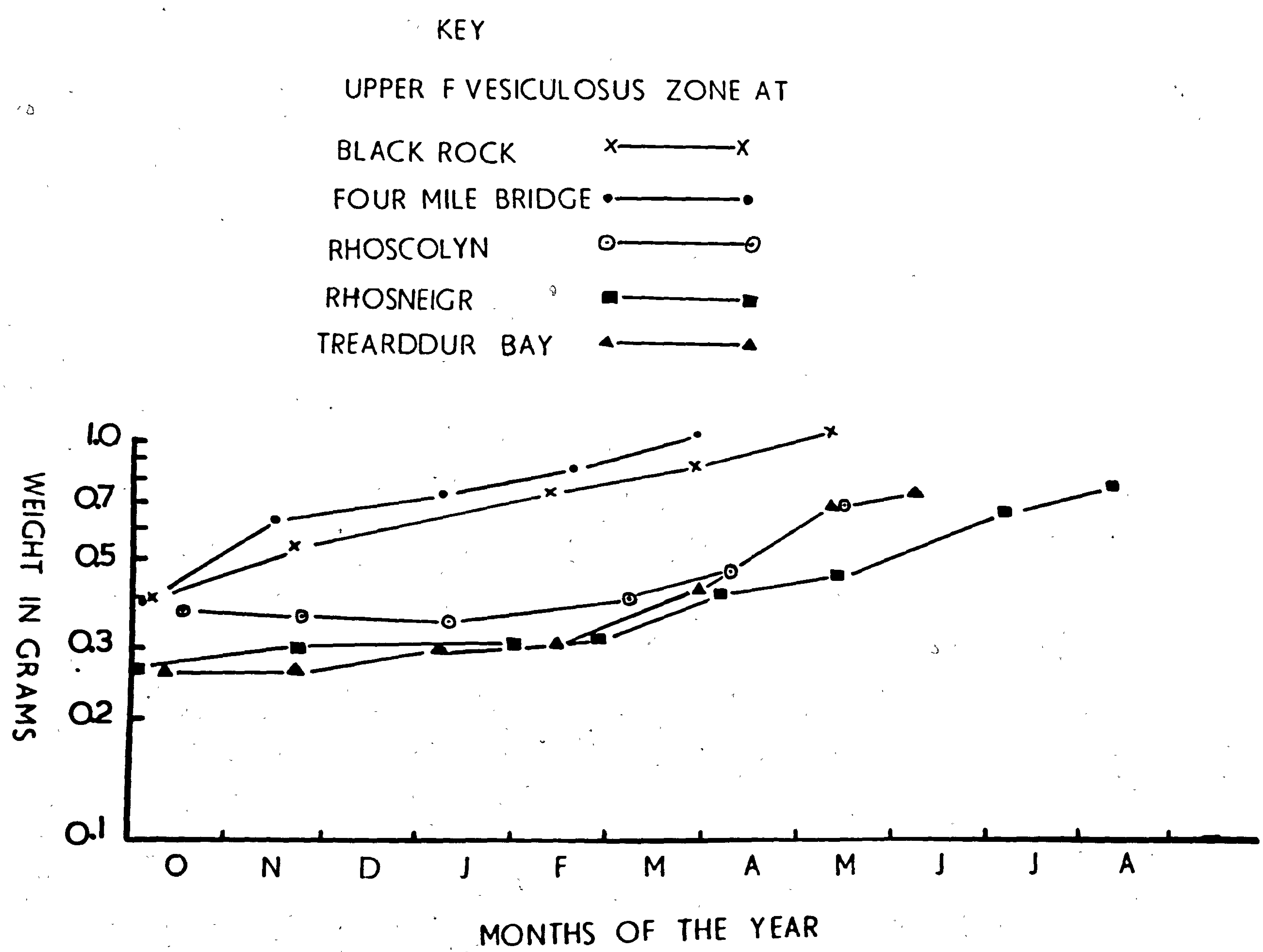
The Fucus was hand sorted in the laboratory under bright illumination, and then allowed to stand in a bath of fresh water for four hours when any animals which had been overlooked were washed off. Most of the large animals were found by hand sorting, but many of the smaller ones were missed, being recovered by the freshwater washing. The very small ones washed out by freshwater were separated from the pieces of seaweed and other debris by washing them through a set of graded sieves.

The animals (except those below 0.015 gms.) were individually weighed. Those heavier than 0.1 grams. were weighed to the nearest 0.1 gms, and those below 0.1 gms. to the nearest 0.02 gms. Those animals lighter than 0.015 gms. were not weighed, but were counted. When the number of those small animals exceeded two hundred, their number was estimated by shaking them in the smallest (1/32") sieve. The sieve was divided into eight equal segments, and the number estimated by counting those contained in one segment and multiplying by eight. The accuracy of the extraction technique for the very small ones was not great enough to justify a more exact method of counting.

The inaccuracy was particularly manifest with the collection from Rhosneigr where numerous small ones were found inside the bladders of F. vesiculosus. This behaviour was considered to be an adaptation to avoiding the action of the surf.

Figure 6.

The growth of the young of L. obtusata on five shores in Anglesey between October 1967 and May 1968.


KEY
UPPER F VESICULOSUS ZONE AT
BLACK ROCK
FOUR MILE BRIDGE
RHOSCOLYN
RHOSNEIGR
TREARDDUR BAY
1.0
0.7
0.5
0.3
0.2
0.1
WEIGHT IN GRAMS
O
N
D
J
F
M
A
M
J
J
A
MONTHS OF THE YEAR

Treatment of Samples

The weights of the individuals in each sample were arranged as a cumulative frequency, and plotted on arithmetical probability graph paper.

The paper (Harding 1949) converts the typical sigmoid cumulative frequency curve of a normal distribution into a straight line, or in the case of a polymodal population, a line which is the resultant of two or more straight lines.

Results

L. obtusata breeds continuously throughout the year, though a peak production of young takes place between May and August. Unlike L. littorea which has a well defined breeding season (Williams 1964), the plot of the cumulative frequency of L. obtusata (see fig. 15 - 25, pp.132-141) produced a line which was only very obscurely polymodal, thus making analysis difficult.

It was usually possible to distinguish a separate adult population, except in June, July and August, when the previous years young were reaching adult size. The very small individuals usually formed a more or less distinct population, possibly owing to a rapid recruitment and mortality. Between these two populations a third indistinct population could be distinguished between October and May, representing animals which had survived the critical very small size.

The middle and lower, and the middle and upper population often blended into one another. With only one sample, delimitation of the middle population would not have been justified. Large numbers of samples were however available, and as the delimitations of the middle populations were all mutually consistent, the analysis was considered justified.

The mean weight of the middle populations were read from the graphs (fig. 15-25, pp.132-141) and plotted against the time of year for all the shores under consideration (see fig. 6, p.34).

Figure 6 shows winter growth from October to February of the middle population at Rhosneigr, Trearddur Bay and Rhoscolyn to average 0.66% per week over the three shores, whilst growth over the same period of the middle population at Black Rock and Four Mile Bridge averaged 4.05% per week over the two shores (see Table 9b, p.32). The difference in winter growth rate between the animals on the shores with large adults (Black Rock and Four Mile Bridge) and the animals on shores with small adults (Rhoscolyn, Rhosneigr and Trearddur Bay) was analysed by the Student's 't' test and found to be significant to the 99% level of probability.

The subsequent spring growth calculated as percent increase in weight per week during March and April showed no significant difference between the two groups of shores (Table 9b, p.32).

The spring growth rate of animals at Rhoscolyn, Rhosneigr and Trearddur Bay taken as a group was significantly greater than the winter growth on the same shores. The spring growth rate of the animals at Black Rock and Four Mile Bridge showed no significant increase on the winter rate.

There was therefore a strong suggestion that the animals from Rhoscolyn, Rhosneigr and Trearddur Bay were not as large as those from Black Rock and Four Mile Bridge because they do not grow so fast in the winter. Since the former shores are more exposed to wave action than the latter, it is highly suggestive that winter wave action severely limits winter growth rate.

SECTION 2.

THE EFFECT ON L. OBTUSATA OF CONDITIONS OF EXTREME SHELTER.

SECTION 2.

THE EFFECT ON L. OBTUSATA OF CONDITIONS OF EXTREME SHELTER

Introduction

The absence of wave action, though not detremental to a grazing animal like L. obtusata, provides conditions for the settlement of fine sediment, both as a muddy substrate, and as a layer of sediment covering the fucoids upon which L. obtusata depends for food.

The presence of sediment is said to be detremental to L. obtusata by clogging its respiratory surfaces (Barkman 1955).

The temperature of the sea is higher in the summer round sheltered shores than it is round more exposed shores (Sundene 1962). It is doubtful if the temperature rises sufficiently to cause heat death in L. obtusata though the combination of high temperature and organic sediment has been found by Wieser and Kanwischer (1957) to seriously deplete the oxygen tension under Fucus on hot cloudy days.

From this consideration, the adverse conditions experienced on the extremely sheltered shore at Four Mile Bridge were possibly more pronounced in the summer. If these conditions were sufficiently unfavourable a detectable genetic line of shelter resistant winkles may have been selected for at Four Mile Bridge.

Section 2 is devoted to the ways in which specimens of L. obtusata from Four Mile Bridge were adapted to conditions of extreme shelter.

PART 1 THE EFFECT OF EXTREME SHELTER IN THE FIELD - COMPARISON OF THE LOSS RATE AT FOUR MILE BRIDGE OF ANIMALS COLLECTED FROM BOTH FOUR MILE BRIDGE AND MORE EXPOSED SHORES

As a preliminary investigation, L. obtusata collected from both the fairly sheltered shore at Red Wharfe Bay and the extremely sheltered shore at Four Mile Bridge were placed together on one Fucus vesiculosus covered rock at Four Mile Bridge. The shore of origin was indicated by file marks on the upper part of the shell.

During subsequent visits to Four Mile Bridge, the numbers of animals on the rock originating from both Red Wharfe Bay and Four Mile Bridge were counted.

Reference to Table 10 (p.39) shows the rate of loss at Four Mile Bridge of animals collected from Red Wharfe Bay was greater than the loss rate of the indigenous animals.

Most of the animals lost from the rock were found as empty shells on the mud within a short distance of the experimental rock. They had not been washed up on to the strand line as there was insufficient wave action to move winkle shells. It was uncertain whether these animals had died by becoming dislodged from the weed and falling onto the mud from whence they were unable to regain the weed, or whether after death from another cause they subsequently fell onto the mud.

The difference in loss rate between animals collected from Four Mile Bridge and those from Red Wharfe Bay, suggested an adaptation to the adverse effects of extreme shelter on the part of L. obtusata living at Four Mile Bridge.

As there is an increase in the severity during the summer months of some of the adverse effects of extreme shelter, the difference in loss rate at Four Mile Bridge of animals originating from Four Mile Bridge and more exposed shores might be expected to increase in the summer. The following set of transplants compared the loss rates of animals collected from Four Mile Bridge and from Rhosneigr when both were placed on a rock covered with Fucus vesiculosus at Four Mile Bridge.

It was not possible to use Red Wharfe Bay as a source of winkles as there were not enough of them. Instead collections were made at Rhosneigr where winkles were abundant and large numbers could be removed without fear of overcollecting.

The same rock covered with F. vesiculosus on the upper part of the littoral zone at Four Mile Bridge was used for duration of the experiment. Throughout the year winkles were collected from Rhosneigr and an equal number from Four Mile Bridge. The winkles were marked by means of a file mark as before to distinguish them, and then placed on the selected rock at Four Mile Bridge. Subsequent counts of the animals remaining on the rock from each shore were continued until the number of animals remaining from one or other of the shores had become too depleated to be of any value. The remaining animals were then removed and the experiment repeated with a different set of animals.

Table 10.

Rate of loss on the extremely sheltered shore at Four Mile Bridge of L. obtusata collected from both Four Mile Bridge and the fairly sheltered shore at Red Wharfe Bay.

	Collected from Four Mile Bridge		Collected from Red Wharfe Bay	
Date	Number Present	Daily Loss (m) in %	Number Present	Daily Loss (m) in %
February 22.1966	100		100	
February 24	88	8.3	74	14
March 3	70	4.0	29	15
March 15	44	3.7	7	11
May 28	12	1.6	0	--

Figure 7.

The apparatus used to maintain aquarium cultures of L. obtusata.

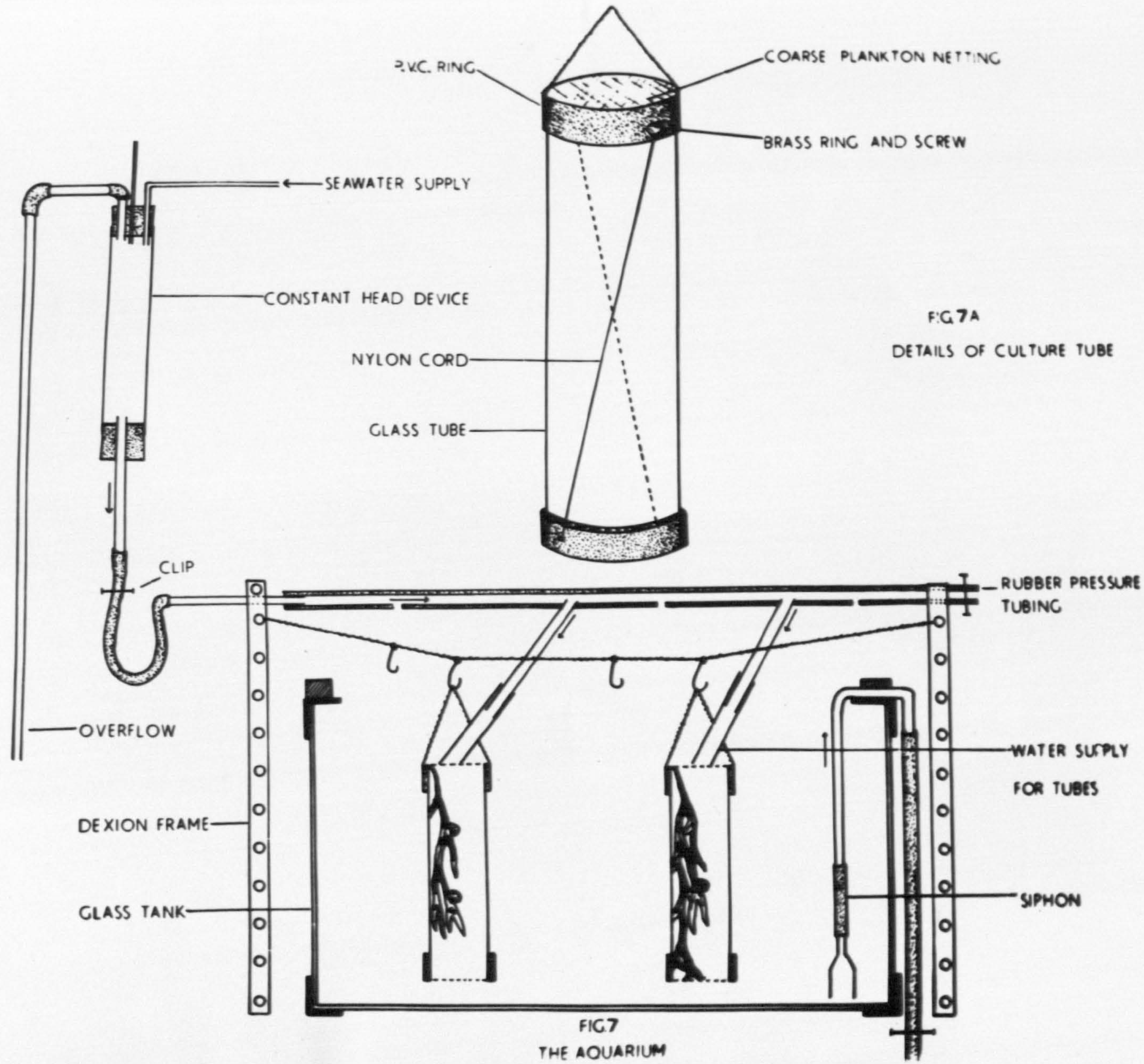


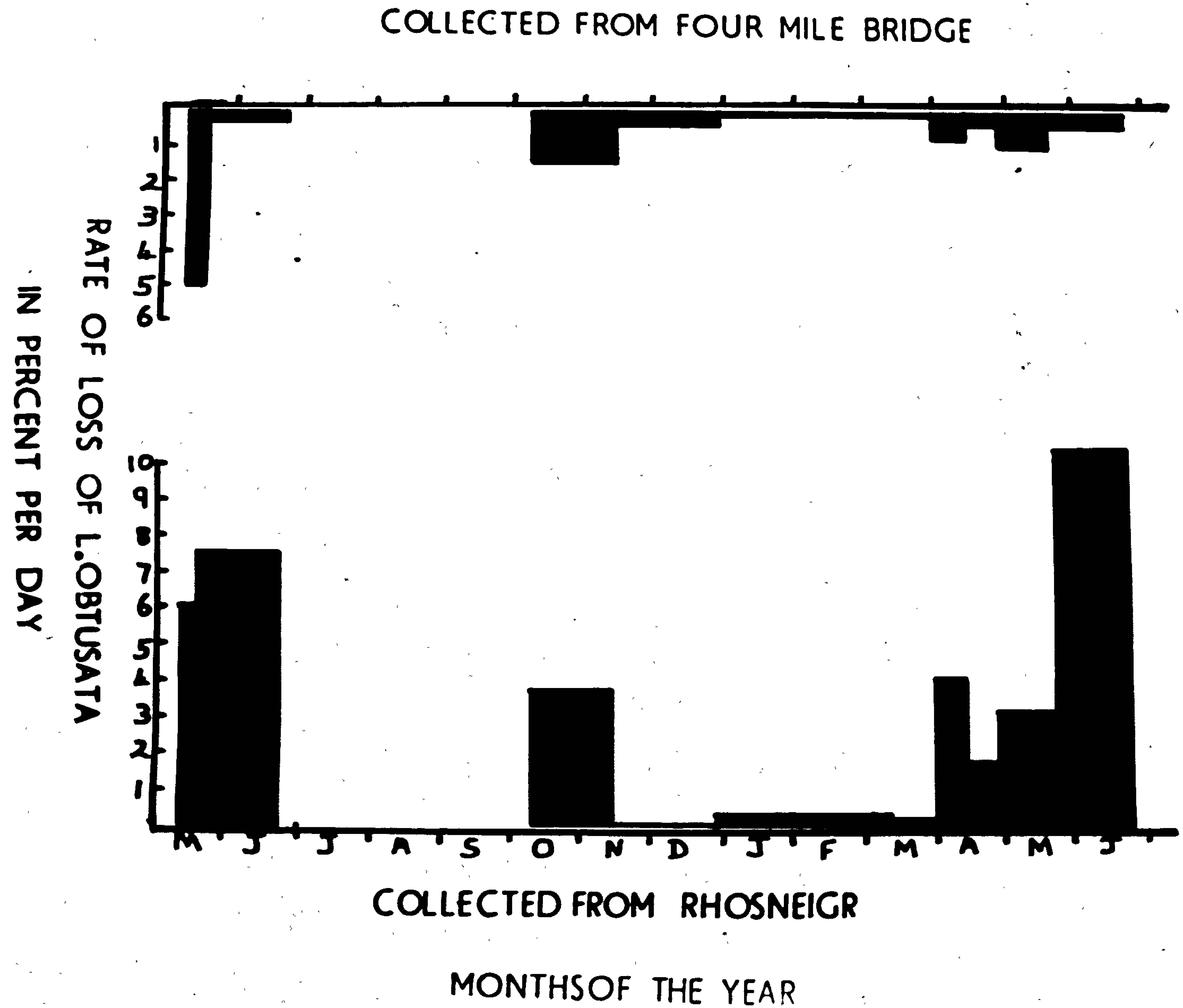
FIG.7A
DETAILS OF CULTURE TUBE

FIG.7
THE AQUARIUM

Figure 8.

The daily loss rate (m) in percent at Four Mile Bridge, of animals collected from Rhosneigr and Four Mile Bridge.

FIG.8



COLLECTED FROM FOUR MILE BRIDGE
RATE OF LOSS OF L.OBTUSATA
IN PERCENT PER DAY
1
2
3
4
5
6
10
9
8
7
6
5
4
3
2
1
M J J A S O N D J F M A M J
COLLECTED FROM RHOSNEIGR
MONTHSOF THE YEAR

The daily rates of loss (m) in percent from Table 54 p.167 (for definition see p.13) were plotted in the form of a graph (fig. 8 p.41).

The results show, with one exception, the daily loss factor of the animals from Rhosneigr to be greater than that of the animals from Four Mile Bridge.

The results were then grouped together according to the time of year. The results from March 28th to June 16th being grouped as summer losses and those from September 27th to March 28th as winter losses.

As there were good reasons for supposing the rates of loss were not normally distributed, the original figures of the numbers of animals lost in each experimental period were drawn up in the form of a series of contingency tables. The tables for the summer losses (table 11b, p.44) using the additive properties of χ^2 gave a total χ^2 for four degrees of freedom of 252.7, which was significant at the 99.9% level of probability. The winter losses (table 11a, p.43) posed a problem in analysis. The results between October 30th and December 12th showed a greater loss amongst the Rhosneigr animals. As two of the expectations in this period were less than 5 a value for significance was not calculated, but it was considered to be sufficiently small to ignore. The winter losses then showed a significant difference, though less so than the summer ones. The lowest χ^2's were observed in the middle of the winter, suggesting the difference in the loss rates between the Four Mile Bridge and Rhosneigr animals to be markedly greater in the summer.

The results indicated an adaptation on the part of L. obtusata inhabiting the shore of Four Mile Bridge to withstand conditions which were inimical to the animals transplanted from Rhosneigr. The Rhosneigr animals were not successfully migrating from the experimental rock to others in the vicinity as many empty shells of animals from Rhosneigr could be found near the experimental rock. They were seldom found on other rocks nearby.

The increase in the rate of loss of Rhosneigr animals in the summer months suggested that the effective agents were more pronounced in the summer.

Table 11. Contingency Tables

Analysis of the rate of loss at Four Mile Bridge of *L. obtusata* collected from Rhosneigr (RN) and Four Mile Bridge (FMB).

Table 11a Winter Losses

Sept. 27 - Oct. 30

	RN.	FMB.	
Lost	60	38	98
Left	40	62	102
	100	100	200

$\chi^2 = 8.8$

Oct. 30 - Dec. 12

	RN.	FMB.	
Lost	0	2	2
Left	30	60	90
	30	62	92

$\chi^2 = 0.054$

Dec. 12 - Feb. 10

	RN.	FMB.	
Lost	8	6	14
Left	22	54	76
	30	60	90

$\chi^2 = 0.17$

Feb. 27 - March 17

	RN.	FMB.	
Lost	12	2	14
Left	88	98	186
	100	100	200

$\chi^2 = 6.25$

March 17 - March 28

	RN.	FMB.	
Lost	32	10	42
Left	56	88	144
	88	98	186

$\chi^2 = 16.75$

TOTAL $\chi^2 = 31.97$

Table 11b Summer Losses

March 28 - April 10

	RN.	FMB.	
Lost	11	3	14
Left	45	85	130
	56	88	144

$\chi^2 = 8.5$

April 10 - May 23

	RN.	FMB.	
Lost	34	30	64
Left	11	55	66
	45	85	130

$\chi^2 = 17.5$

May 10 - May 17

	RN.	FMB.	
Lost	36	32	68
Left	64	68.	132
	100	100	200

$\chi^2 = 0.2$

May 23 - June 7

	RN.	FMB.	
Lost	91	7	98
Left	9	93	102
	100	100	200

$\chi^2 = 139$

May 17 - June 16

	RN.	FMB.	
Lost	58	7	65
Left	6	61	67
	64	68	132

$\chi^2 = 87.5$

TOTAL χ^2 = ~~1042.7~~ 252.7

PART 2 THE EFFECT OF HIGH WATER TEMPERATURE.

Introduction

High summer water temperature experienced on shores sheltered from wave action has been shown in some cases to preclude the survival of certain species. Alaria esculenta has been shown to be unable to survive if the water temperature rises above 18°C. (Sundene 1962); and Barkman (1955) considers high temperature to be important in limiting the southern distributions of L. obtusata.

An investigation into the relative abilities of L. obtusata from Rhosneigr and Four Mile Bridge to withstand high temperatures was considered desirable in the light of the results of the transplant experiment (see fig. 8, p.41).

If the animals from Four Mile Bridge were better able to withstand higher temperatures than the Rhosneigr animals, the phenomenon was more likely to be manifest in the middle and at the end of the summer. The experiments were therefore conducted in July and November.

Materials and Methods

L. obtusata collected from Rhosneigr and Four Mile Bridge were marked with file scratches on the shells to identify them. The animals were then placed in two glass liter beakers, fifty animals from each shore being placed in each beaker.

The water was aerated continuously and the animals were left for twenty-four hours to acclimatise to room temperature (approximately 19°C.) The water in the beakers was then replaced with seawater from a bucket which was at room temperature, and the beakers placed in a constant temperature bath which was also at room temperature. The bath was allowed to heat up at the rate of one centigrade degree every seven minutes until the desired temperature was reached. For the duration of the experiment air was vigorously bubbled through the water in the beakers by means of aeration blocks.

The experiment was controlled with fifty animals from each shore in a liter beaker at room temperature. The water in the control beaker was aerated in the same manner as the experimental water.

The animals in the water bath exuded large quantities of mucus for a short time after the commencement of heating. The water was therefore replaced after two hours with fresh seawater which had been allowed to stand in beakers in the temperature bath and reach the appropriate temperature.

After certain periods of time, samples of L. obtusata were removed from the experiment and placed on a tray to cool gradually to room temperature. The tray was then flooded with seawater at room temperature. The animals were allowed twenty four hours to recover. Death was considered to have occurred if the animals after twenty four hours had not regained their sensitivity.

Usually the assessment of sensitivity was simple as most of the animals, both dead and alive, were not fully withdrawn into their shell. The shells of the animals which were completely withdrawn were cracked open. If no movement of the body resulted, they were said to be dead. In the case of death the decision was usually reinforced by the disorganised appearance of the digestive gland and gonad area.

A preliminary experiment showed a possibility that the L. obtusata from Four Mile Bridge were dying more quickly than those from Rhosneigr. It was noticed that most of the animals from Rhosneigr had crawled higher in the beaker than those from Four Mile Bridge. The Rhosneigr animals were therefore probably experiencing different experimental conditions than those from Four Mile Bridge.

In all subsequent experiments, discs of nylon netting pushed into the beakers were used to prevent any of the animals from crawling upwards.

Results

In each of the experiments (see Table 12, p.48) survival rate of the Four Mile Bridge animals was found to have exceeded that of the Rhosneigr animals.

As the number of hours required for the fifty percent mortality point to be reached appeared to vary from one experiment to the other, the results from each experiment were treated as independent sets of data, and were accordingly analysed by the Chi Squared Test using the Yates correction for continuity.

Since the survival rate of the animals from Four Mile Bridge was always the greater of the two, the individual chi squared values were able to be added to give a total chi squared of 46.75 for nine degrees of freedom. The difference in the survival rate of the two groups of animals (those from Rhosneigr and those from Four Mile Bridge) was therefore significant at the 99.9% level of probability.

Discussion

It is difficult to interpret the ecological significance of the difference in temperature tolerance between the Four Mile Bridge and Rhosneigr animals. The temperatures on the two shores are unlikely to differ significantly when uncovered by the tide, though the surrounding sea water at high tide is probably at a higher temperature at Four Mile Bridge than at Rhosneigr owing to the greater mixing on the more exposed shore. The higher water temperature at Four Mile Bridge was considered unlikely to rise sufficiently to cause heat death to the animals transplanted from Rhosneigr.

According to Theadle et al (1969) conditions of low oxygen tension and the presence of hydrogen sulphide are better tolerated at lower temperatures.

It was thought possible for greater temperature tolerance of the animals native to Four Mile Bridge to be part of an adaptation for surviving under conditions of lowered oxygen tension, or increased hydrogen sulphide content of the organically rich sediment at Four Mile Bridge.

It is reasonable to postulate that at a high temperature, the resistance to lowered oxygen tension or presence of hydrogen sulphide is likely to be greater amongst the animals which are better adapted to high temperatures.

Table 12.

Temperature tolerance of L. obtusata from a fairly sheltered shore (Rhosneigr (RN) compared with L. obtusata from an extremely sheltered shore (Four Mile Bridge (FMB.)

Date	Temp. in ^{o}C	Time at Temp. in Hours	Total Number		Percent Dead		Contribution to χ^2
			FMB.	RN.	FMB.	RN.	
Nov. 10.1967	32	17	50	50	22	48	5.83
"	32	19	50	50	48	92	21.0
"	32	21	50	50	72	94	7.1
Nov. 14.1967	34	15	50	50	84	96	2.8
Nov. 16.1967	34	15	100	100	66	78	3.06
July 29.1969	34	16	150	150	85	93	5.35
July 31.1969	34	13.5	50	50	44	48	0.04
"	34	14	50	50	66	76	0.77
"	34	14	50	50	43	58	0.80

TOTAL $\chi^2 = 46.75$

PART 3 THE EFFECT OF LOW OXYGEN TENSION UPON L. OBTUSATA FROM FOUR MILE BRIDGE AND RHOSNEIGR.

It has been shown by Wieser and Kanwischer (1957) that on hot days when the sun was obscured by cloud, the oxygen content of the environment under tufts of Fucus became extremely depleted, and sometimes was even reduced to zero. This is known to be partly due to oxygen absorption by the mud (Allee 1923). As L. obtusata lives permanently under Fucus, the lowering of oxygen tension must be an important factor in the life of the animal.

Wieser and Kanwischer went on to demonstrate that animals which were not capable of leaving the weed habitat were adapted to survive under conditions of very low oxygen tension. More recently Theadle et al (1969) showed that animals of muddy substrata were better able to withstand low oxygen tension than animals of clean substrata.

It was decided to compare the relative abilities of L. obtusata from Rhosneigr and Four Mile Bridge to withstand lowered oxygen tension in the laboratory.

Materials and Methods

If L. obtusata was kept in a jar of seawater through which nitrogen was bubbled to keep it deoxygenated, the water quickly became foul and the animals died.

A group of L. obtusata kept in a similar jar as a control through which air was bubbled at the same rate as the nitrogen in the experimental jar also died. It was therefore decided to pass a continuous stream of freshly deoxygenated water through a column of L. obtusata.

The apparatus is shown in fig. 9, p.50.

The animals were kept in a glass tube with an internal diameter of two and a half cms. and one meter long. They were placed in the tube in groups, equal numbers of animals from Rhosneigr and Four Mile Bridge. Each group of animals was separated from the group above and below it by a wad of cotton cloth and a loosely fitting rubber bung with a hole bored in its centre.

Deoxygenated water was passed in at the bottom of the tube and out at the top, the rate of flow being controlled by a clip on the rubber tubing at the in-flow end. Animals could be removed from the tube during the experiment in groups without disturbing the rest, by means of a long stiff piece of wire hooked at the end. This was passed down between the rubber bungs and the tube wall, till the appropriate point was reached. The hook was then turned to hook under the chosen bung. The bung was in this way withdrawn together with all the animals above it.

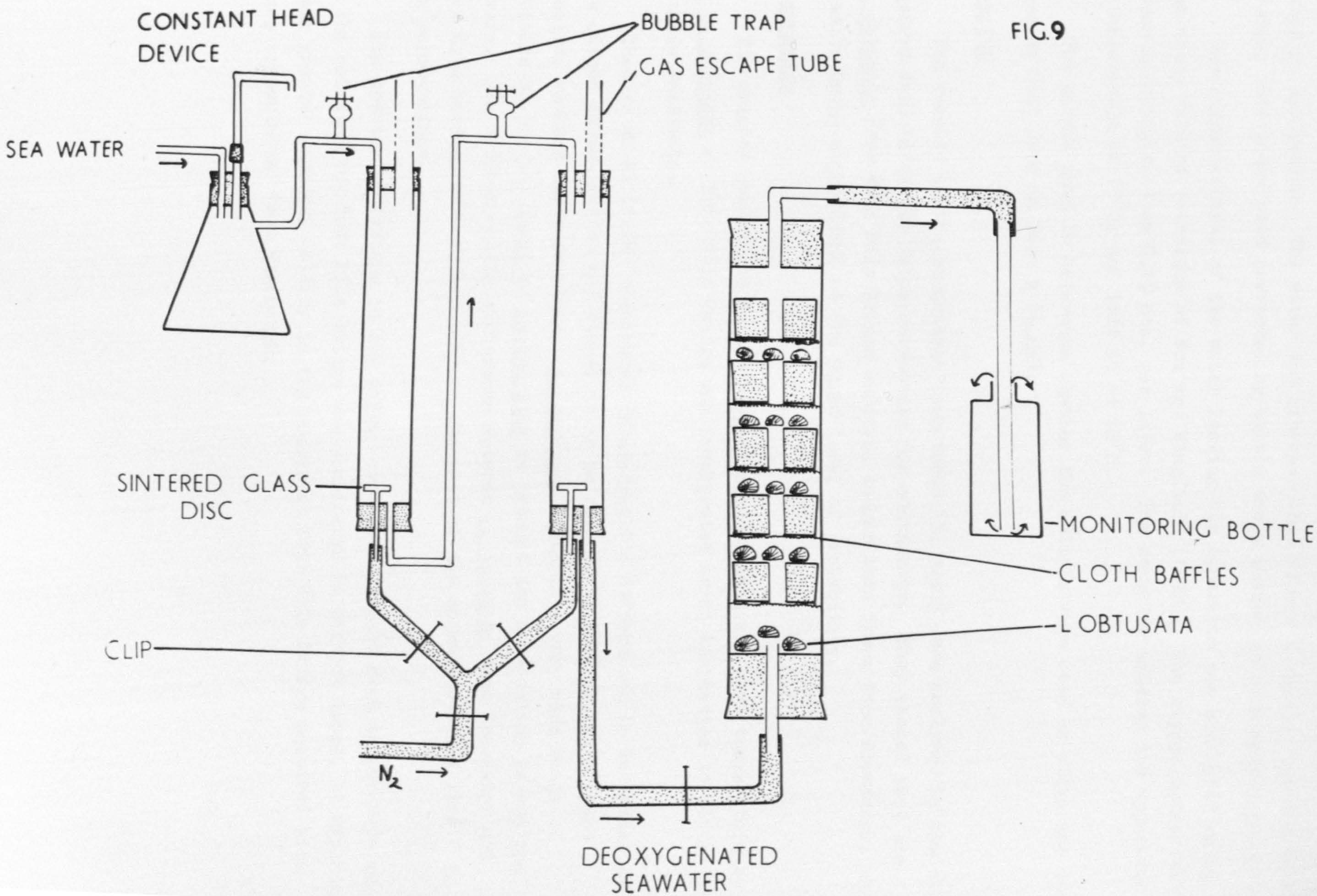
The water was de-oxygenated as follows:-

Nitrogen from a cylinder was passed through a pressure reduction valve, then via polythene tubing to two sintered glass discs situated one in each of two glass tubes two cms. from the bottom. The tubes were of internal diameter three cms. and twenty cms. long.

Figure 9.

The apparatus used to pass a stream of deoxygenated seawater simultaneously over L. obtusata from Rhosneigr and Four Mile Bridge.

FIG.9


CONSTANT HEAD
DEVICE
BUBBLE TRAP
GAS ESCAPE TUBE
SEA WATER
SINTERED GLASS
DISC
CLIP
N2
DEOXYGENATED
SEAWATER
MONITORING BOTTLE
CLOTH BAFFLES
L OBTUSATA

The scintered glass discs produced a stream of very small bubbles of nitrogen.

Seawater was passed from a constant head device down through each of the two tubes in succession. The water and nitrogen thus formed a counter current flow system. Air locks were prevented by bubble traps placed at strategical points.

The oxygen content of the water leaving the apparatus was monitored using the micro winkler technique of Fox and Wingfield (1938). The oxygen concentration never rose higher than 0.16 ccs. per liter. The seawater entered the apparatus at a temperature of 17°C. and left it at 18°C.

The method used to determine whether the animals were dead or alive was the same as that used in Part 2 (p.45).

Results

The results of the experiment (see table 13, p.52) were analysed by the chi squared test using the Yates correction for continuity. They showed that the L. obtusata from Four Mile Bridge survived longer than those from Rhosneigr; the results being significant at the 99.5% level of probability.

Discussion

The greater resistance to low oxygen concentration and high temperature shown by L. obtusata at Four Mile Bridge was interpreted as an adaptation to living on a muddy substrata.

Theadle et al.(1969) discovered inter-specific differences in tolerance of low oxygen concentration and showed it to be related to the habitat of each species, whether muddy or clean. L. obtusata lives in a very wide range of habitats so long as Fucus or Ascophyllum is present and wave action is not too severe. The intra-specific difference between L. obtusata from Rhosneigr and Four Mile Bridge shows versatility on the part of the animal to adapt itself to its surroundings.

The greater resistance to low oxygen concentration and high temperature shown by the animals from Four Mile Bridge was considered in part at least, to explain their greater survival ability in the summer at Four Mile Bridge compared with those transplanted from Rhsoneigr.

Table 13.

Survival rate of L. obtusata from Four Mile Bridge (FMB.) and Rhosneigr (RN.) under conditions of low oxygen concentration.

Date August 24.1969.

Number of Hours Without Oxygen.	Total		Percent Dead		Contribution to χ^2
	FMB.	RN.	FMB.	RN.	
49.0	15	15	0	20	3.03
54.25	15	15	13.3	66.6	5.2
54.75	33	33	18.2	42.5	3.2
62.75	37	37	16.2	46	5.2

TOTAL χ^2 16.6 For 4 degrees of freedom

PART 4 COMPARISON OF THE ACTIVITY OF L. OBTUSATA FROM RHOSNEIGR AND FOUR MILE BRIDGE.

Introduction

It was discovered (see Table 13, p. 52) that of the *L. obtusata* from Four Mile Bridge and those from Rhosneigr, the former were better able to withstand lowered oxygen concentration. A less active animal is better able to withstand lowered oxygen concentration than a more active animal (Wieser and Kanwischer 1957). An investigation was conducted into the possibility that the *L. obtusata* from Four Mile Bridge were less active than those from Rhosneigr, at least in the summer.

The activities were measured on five occasions throughout the year. October 3rd 1967, January 20th 1968, March 28th 1968 and July 5th 1968. As a control, the activity of *L. obtusata* from Rhosneigr which were kept for forty eight hours on the upper mid littoral zone at Four Mile Bridge were compared on June 30th 1968, with those originating from Four Mile Bridge.

Materials and Methods

The activity of each animal was measured by timing the period it took to crawl to the circumference of a circle of twelve cms. diameter, from the moment that it was positioned in the centre. Fifteen circles were used simultaneously to shorten the duration of the experiment and to ensure that the animals were kept in the laboratory for as short a time as possible before being used.

The circles were drawn in blue chalk on a light grey plastic tray which sloped at an angle of fifteen degrees.

The room used for this experiment was maintained in permanent blackout, and constant artificial illumination was provided by means of two fluorescent tubes, so eliminating the effect of change in light intensity between one experiment and the next.

Animals were always used within four hours of being collected, and were kept in the laboratory on clean damp *Fucus vesiculosus* to await treatment.

A set of fifteen animals was selected from one shore, placed in a sieve and vigoriously shaken under water for half a minute to act as a stimulus. They were then placed one by one in the centres of the chalk circles facing up the slope. The times they took to reach the circumference of the circles were noted. They were allowed a period of fifteen minutes from the start of the experiment to do this. Any animals not reaching the circumference in the time allowed were counted. The time was taken from the positioning of the first of the fifteen animals.

An animal was placed every four seconds, it taking a minute to place all of them. In practice therefore, the last animal to be placed was allowed only fourteen and not fifteen minutes to reach the circumference.

At the end of the first fifteen minutes all the animals were removed and the circles thoroughly washed with seawater to remove any mucus which might affect the activity of the succeeding set of animals. The second set was taken from the Rhosneigr animals. This alternation was continued for the remainder of the experiment.

Results and Discussion

The results were plotted in the form of a graph (Fig. 10 p.55). If there were no differences in the activity of the animals within each shore sample, after a short time lag, the numbers leaving the circles per unit time would be proportional to the number remaining. The number remaining when plotted against time would then form an exponetial curve. Any differences in activity between animals within a shore sample would be indicated by departure from a straight line of the function of the log of the number remaining plotted against time.

The animals from Rhosneigr (see Fig. 10 p.55) on every occasion showed the greater activity. The activity of the Rhosneigr animals was greater in the July sample than in any of the other samples.

The converse applied to the animals from Four Mile Bridge which showed the least activity in the two summer samples.

The animals collected from Rhosneigr and left for forty eight hours at Four Mile Bridge showed greater activity than the indigenious animals, but less activity than the animals collected directly from Rhosneigr on July 5th.

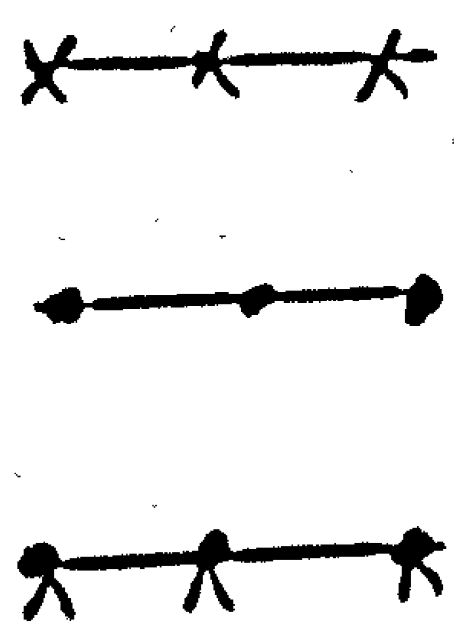
As the animals from Four Mile Bridge had been shown to be more resistant to low oxygen concentration, their lesser activity may be interpreted as part of an adaptation to an environment which sometimes became depleated of oxygen in the hot weather. The results of the control experiment (Fig. 10E, p.58) suggested a degree of acclimatisation on the part of the animals transplanted from Rhosneigr, results which may also have been brought about by death of the more active animals from Rhosneigr during the period of forty eight hours for which they were at Four Mile Bridge.

As the activity of the transplanted Rhosneigr animals was not as low as those from Four Mile Bridge, there appeared to be a limit to the degree to which the Rhosneigr animals could become acclimatised to conditions at Four Mile Bridge.

Figure 10.

The activity of L. obtusata from Four Mile Bridge and Rhosneigr at different times of year.
The activity was plotted as the log 10 of the number of animals remaining in the circles at a given time for a further explaination see text.

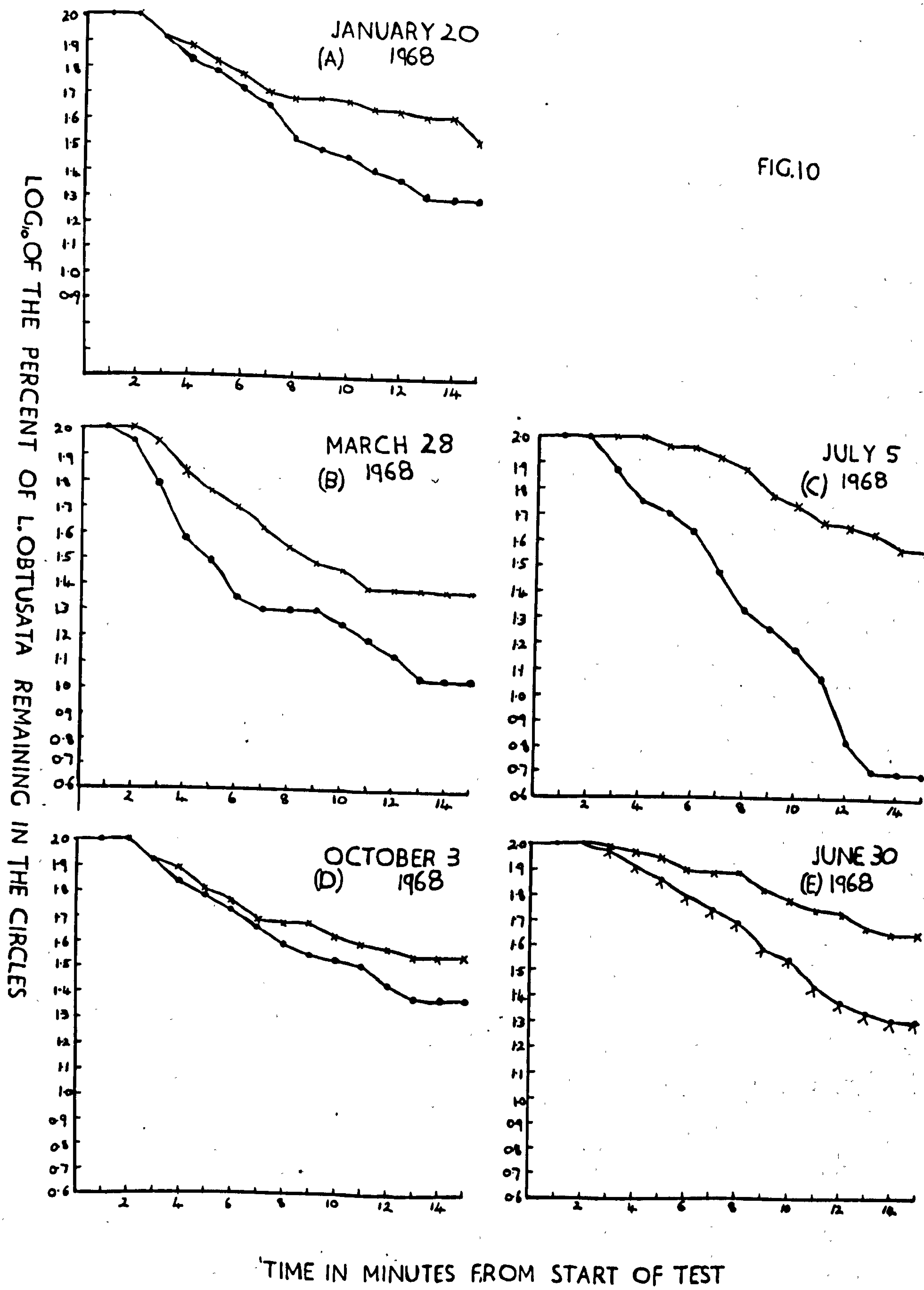
KEY.



FROM FOUR MILE BRIDGE

FROM RHOSNEIGR

FROM RHOSNEIGR AND PUT OUT AT FOUR MILE BRIDGE



LOG10 OF THE PERCENT OF L. OBTUSATA REMAINING IN THE CIRCLES
JANUARY 20 (A) 1968
MARCH 28 (B) 1968
JULY 5 (C) 1968
OCTOBER 3 (D) 1968
JUNE 30 (E) 1968
TIME IN MINUTES FROM START OF TEST

FIG. 10

SECTION 3.

THE EFFECT OF PREDATION OF THE COMMON SHORE CRAB

CARCINUS MAENAS

SECTION 3.

THE EFFECT OF PREDATION OF THE COMMON SHORE CRAB, CARCINUS MAENAS (L).

PART 1 CRAB PREDATION IN THE FIELD

The shell of Littorina obtusata is a very thick structure and must be able to resist the attacks of many would be predators. Moore (1939b) has observed oyster catchers pecking out the soft parts, and herring gulls dropping them onto rocks to break open the shells, and even swallowing them whole. Sacchi (1961) watched gulls at Roscoff selectively taking orange and red colour varieties.

Observation on the Anglesey shores failed to reveal any bird predation, At Penmon, winkles were placed on top of the Fucus and watched from a distance through binoculars. Though in the vicinity of a herring gull colony, no attacks were noted. Predation by the herring gull can not however be ruled out.

The shells of the young L. obtusata are known to be susceptible to attack by the purple sandpiper (Freare 1966) and probably contribute to the diet of a number of shore inhabiting birds. The effect of this predation on the populations of L. obtusata is difficult to assess, but probably amounts to a small fraction of the total mortality. Crabs in many parts of the world are known to break open the shells of gastropods to obtain the food within. Indeed some species of crabs rely on this source of nutrient and their chelae are modified for the purpose (Shoup 1968). In Britain the work of Kitching et al (1966) and Walker (1967) showed the common shore crab Carcinus maenas to be responsible for considerable mortality amongst Nucella lapillus; whilst Barnes (1968) has demonstrated that C. maenas readily attacks the common mussel Mytilus edulis. It was of considerable interest therefore to make the following observations while aqualung diving at Black Rock.

A number of L. obtusata were placed on the sand underwater at Black Rock to investigate their ability to crawl over the flooded substrate. Several specimens of the common shore crab, Carcinus maenas, were seen to gather around to investigate the winkles. The way in which the crabs manipulated the winkle shells with their chelae seemed to indicate experience in such manoeuvres. The crabs were observed for a period of approximately twenty minutes but none were seen to do more than investigate the shells. Observing by means of diving is not an ideal method as a considerable disturbance is created by the exhaled air. Although no winkle was ever attacked successfully it was thought the crabs might not have been behaving normally under such disturbed conditions; and the possibility that they did prey on winkles when undisturbed remained a strong possibility. It was decided to investigate the matter further by keeping crabs together with winkles in an aquarium in the laboratory at Menai Bridge.

The floor of a glass tank 100 x 50 cms. was covered with sand so that the winkles placed on it would be unable to crawl towards and up the sides and so escape the attacks of the crabs.

The water was aerated by means of an aeration block and seawater constantly ran through the tank to maintain clean conditions for the duration of the experiment.

On May 5th 1967, twenty winkles were collected from Ynys Faelog and placed on sand in the bottom of the tank. It was decided to use only adult animals whose shell mouths had become rounded and thickened.

Three large specimens of shore crab were then collected by trapping from the pontoons of Menai Bridge pier. The three crabs were placed with the winkles and left there for forty eight hours.

Upon re-examination all the winkle shells were seen to have been opened and the contents eaten by the crabs. The shells were in various stages of disintegration, and it was difficult to determine the manner of attack from the pieces which remained. Subsequent observations showed that the crab held the shell with one chelae the other being used to break the shell lip, the breaking process continuing around the body whorl until the whole of the body whorl of the shell had been cracked from the columella. The columella was thus usually left intact with the upper shell whorls.

In the course of the subsequent five days the same three crabs attacked and consumed 80 more specimens of L. obtusata making a total of 100 animals consumed in one week.

C. maenas is extremely catholic in its taste indeed C. M. Yonge (1949) said of it:-

"Nothing that is edible comes amiss to this most active and voracious of scavengers."

On most shores therefore the distructive potential of a crab on winkle populations is probably never fully realised as other food is available. The ability of C. maenas to attack L. obtusata with such expediency probably indicates a high proportion of the gastropod in the diet of the crab.

It appeared reasonable to suppose that if crab attacks were frequent in nature then the characteristic pieces of shell would be found on the strandline of the shores where winkle populations were established. A search of strandlines on several shores on Anglesey failed to reveal such evidence of crab attack. Pieces of shell were found on many of the shores examined but the number found was never very great and did not seem to be important when compared with the large numbers of empty but intact shells discovered on the strandlines.

It was probable that the small pieces left after crab attack were quickly lost by being broken up further and mixed with the sand and pebbles of the strandline. On the shore of the Menai Straits where C. maenas was particulary large and common, the population of L. obtusata was surprisingly sparse. The shores of the Straits at Ynys Faelog were considered the most promising for observing crab attack under natural conditions.

As the L. obtusata population at Ynys Faelog was sparse it was decided to introduce more animals.

The shore was first searched to confirm that pieces of shell resulting from crab attack were not common in the vicinity chosen for the experiment. Three thousand L. obtusata were collected from Trearddur Bay on June 13th 1968 and placed in the Ascophyllum zone at Ynys Faelog. The animals were scattered in an area four yards square.

The use of so small an area insured that if any crab attack ensued, the pieces of shell remaining after such an attack would be concentrated in one place and easily detected.

The search for pieces of shell was made a week after the winkles had been released. This search revealed considerable evidence of crab attack. Fresh pieces of shell being found in quantity on the mud and gravel between stones and cracks in the rock. The number of released animals which could be found at the end of the week was considerably depleted. A thirty minute count in the area revealed 234 living animals remaining out of the original three thousand.

The possibility of the animals having died as a result of the unfamiliar muddy conditions encountered at Ynys Faelog was discounted since only a few empty but intact shells were discovered during the search. It also seemed unlikely that the reduction in numbers of animals could be attributed to their dispersal from the area in which they were released as this has been shown to be a slow process on very sheltered shores.

If C. maenas is predatory on L. obtusata to the extent suggested by the foregoing observations, crab predation offers itself as an explaination of the sparsity of the Ynys Faelog population of L. obtusata. Bearing in mind the statement of Barkman (1955) that in his experience L. obtusata does not thrive well on shores with a large amount of fine sediment, it could be argued that excessive sediment was the factor keeping down the density of the Ynys Faelog population.

Were it not for the relatively dense population of L. obtusata on the muddy shore at Four Mile Bridge this argument would have carried considerable weight. The fact that the specimens of C. maenas at Ynys Faelog were both larger and more numerous than at Four Mile Bridge lent support to the theory that it was crab predation which was responsible for the difference in the two populations of L. obtusata.

PART 2 THE PHENOMENON OF CRAB RESISTANCE.

During the course of the two years 1965 - 1967 it became apparent that on certain shores in Anglesey notably at Trearddur Bay and Rhoscolyn the winkles varied with the level on the shore which they inhabited. Those living towards the lower end of the L. obtusata zone were considerably larger than ones in the middle and upper parts of the zone. It was later realized there was not only a difference in size but the form of the shell mouth differed in the two groups of animals.

The difference was observable only in the adult shells where no more addition of new material was taking place at the shell lip.

The investigation was continued by selecting a shell considered typical of the upper shore type and one typical of the lower shore type.

The animals were removed from the two shells by dipping them in boiling water and winkling them out with a pin. Each shell was then sawn along a line shown in fig. 11a (p.60).

An "eclipse" junior hack saw was used for this purpose. The plane of the saw blade was kept normal to the external surface of the shell whorl at the point where the cut was being made. The resulting sections were then examined. The shell from the lower part of the L. obtusata zone (fig. 11c, p.60) appeared to be thicker than that from the upper part of the zone (fig. 11b, p.60). The shape of the section of the shell lip also differed. The lip of the lower shore shells were thickened and tapered. The measurements were made by means of a pair of callipers.

The animals with the thicker shell would be expected to have a larger shell to animal body weight ratio. To verify this winkles were collected from both the upper and lower parts of the L. obtusata zone at Trearddur Bay on three separate occasions. On June 7th and June 14th and October 5th, 1968, twenty of each type were collected. Each group of twenty winkles were blotted dry and weighed. The animals were then plunged into boiling water and the bodies removed from the shells by means of a needle knife.

Most of the animals were removed from their shells complete, some however broke during the operation. The shells of these animals were cracked open and the remains of their bodies taken out with foreceps. Great care was taken not to lose any of the shells thus cracked. The empty shells were then weighed and the difference between the two weights used to calculate the total body weight of the twnty specimens.

The results are shown in Table 14, p.62. They were analysed by means of the Students "t" Test. The value obtained for 't' was 8.7. As the value of 't' for three degrees of freedom of the ninety nine percent level of significance is 8.6 it may be concluded that animals from the upper and lower parts of the L. obtusata zones differed significantly from one another. There was a clear indication that the

Figure 11.

Drawings of the shells of L. obtusata.

11a. The whole shell as the animals rests on the substrate showing the line along which it was cut.

11b. The sectioned shell of an upper shore (crab susceptible) type to show the thin lip. Maximum thickness of shell at lip 1.5 mm. Thickness of shell of body whorl 1.4 mm.

11c. The sectioned shell of a lower shore (crab resistant) type to show the thick lip. Maximum thickness of shell at lip 2.1 mm. Thickness of shell of body whorl 1.7 mm.

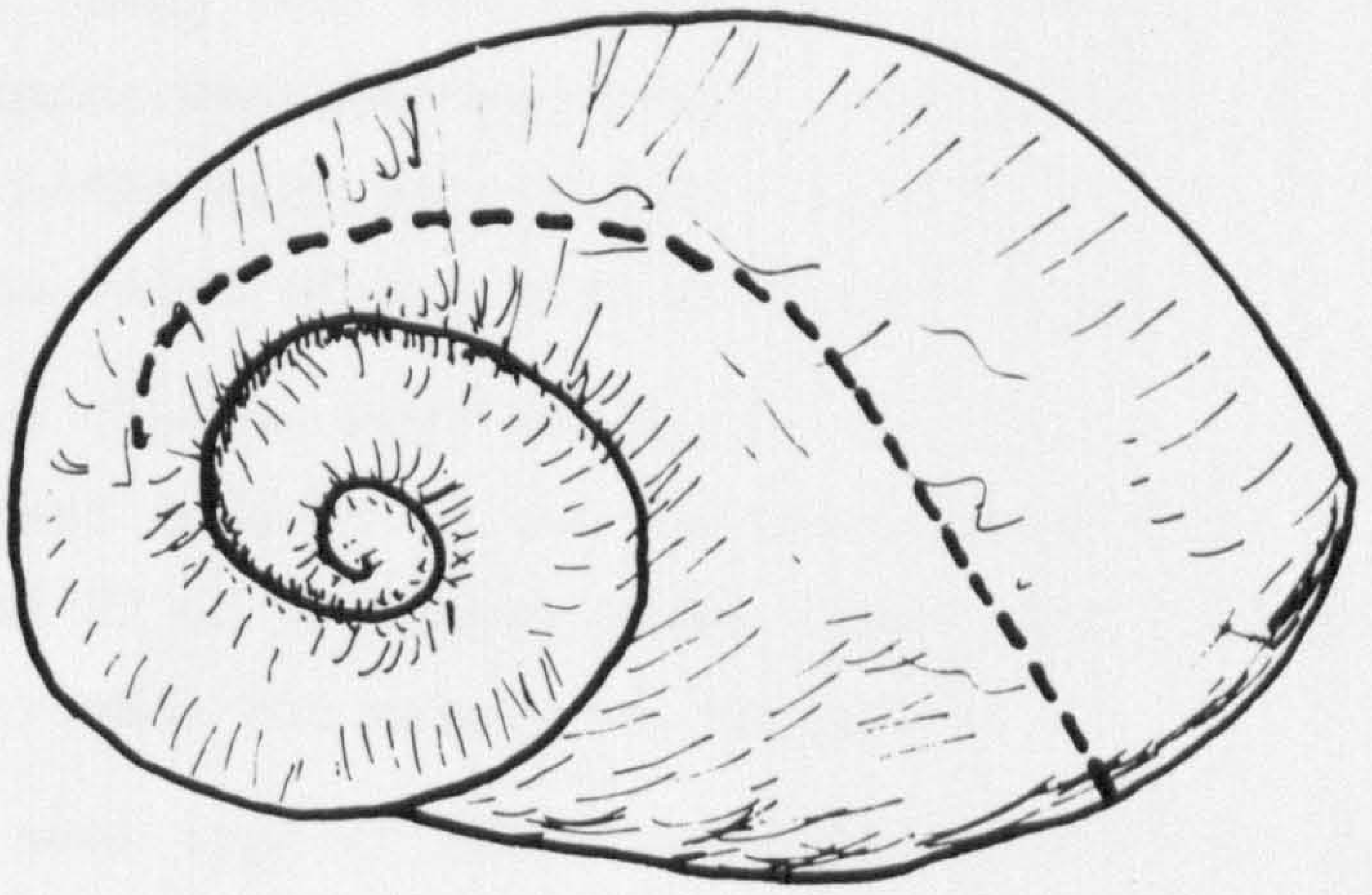


FIG.11A

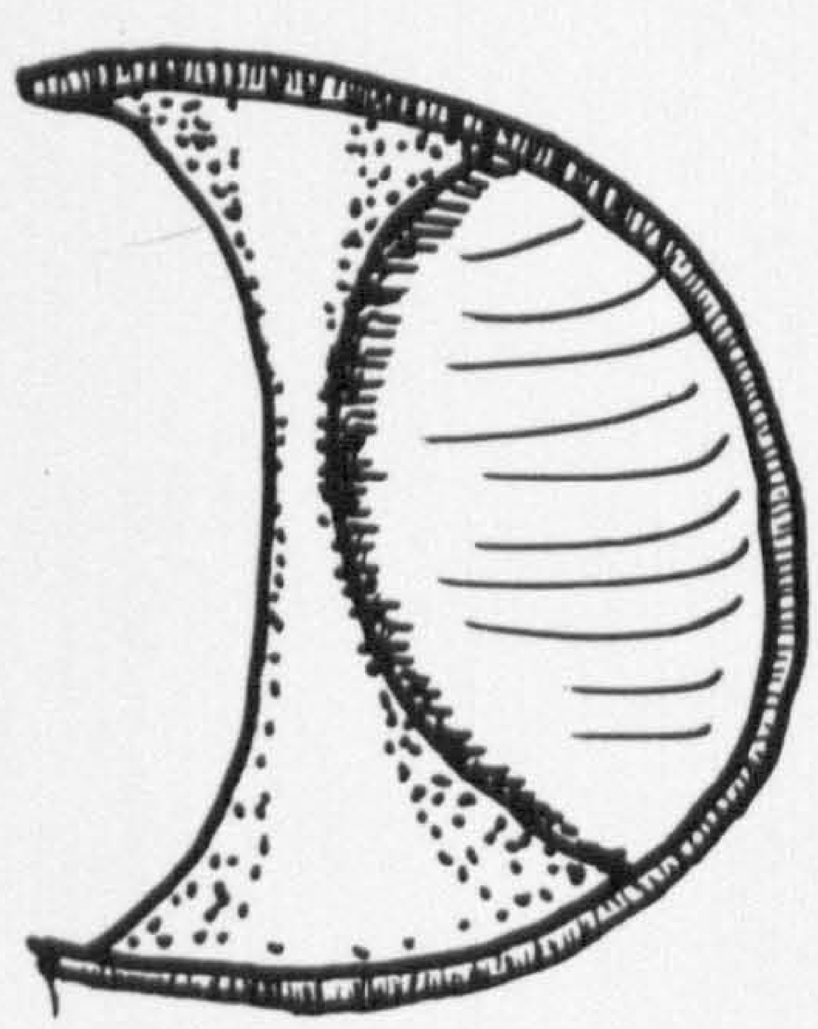


FIG.11 B

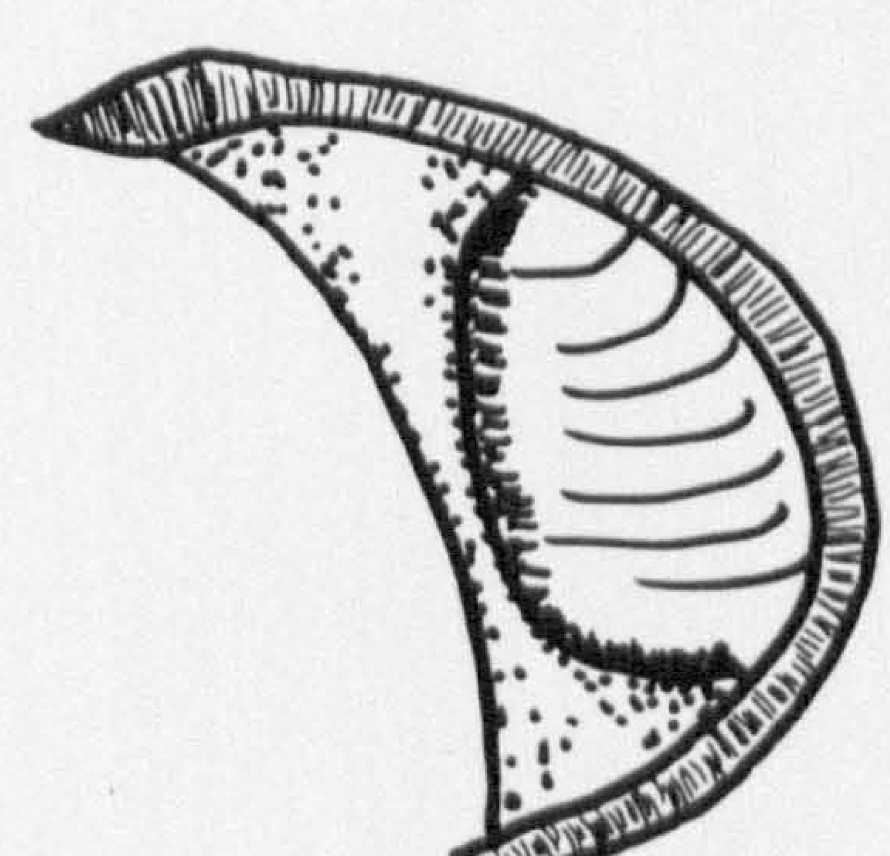


FIG.11C

FIG.11

ratio of shell weight to the weight of animal was greater for the lower shore animal than for those from the upper shore.

Fotrty four winkles were collected from the upper part of the L. obtusata zone and fourty four from the lower Trearddur Bay. All the specimens were adults.

Two common shore crabs were trapped at Menai Bridge pier. The crabs weighed 85 gms. and 74 gms. Each crab was placed in a plastic container half a meter deep containing running seawater and aerated continuously with aeration blocks. The bottoms of the baths were covered with sand to prevent the winkles from crawling towards the sides of the bath and hence escaping.

The lower shore winkles were identified by means of a file mark across the top of the shell, then to each of the two baths were added 22 winkles of the lower shore type and 22 of the upper shore type. The containers were covered over with polythene to prevent the escape of the crabs.

The number of each type of shell remaining intact was noted on four subsequent occasions and the experiment terminated on March 15th, 1968. It can be seen from Table 15, p.62 that the shells of the animals from the lower shore at Trearddur Bay were attacked less readily than those from the upper shore. Indeed the crabs seemed unable to break open the shells of the lower shore animals except in two cases.

Table 14.

Shell to Body Weight at Trearddur Bay

U = Upper Zone   L = Lower Zone

		Weight of 20 Shells in gms.	Weight of 20 Bodies	Shell to Body Weight Ratio
June 7th	U	19.2	7.9	2.43
	L	25.2	7.4	3.41
June 14th	U	20.4	6.5	3.14
	L	29.3	7.9	3.71
October 5th	U	16.0	6.0	2.67
	L	25.6	7.1	3.60
	U	15.0	6.1	2.46
	L	25.2	7.5	3.36

T for 3 degrees of freedom 8.7 using the method of differences.

Table 15.

Effect of Thick Shells on Crab Predation

Days Since Commencement	Numbers of Animals Intact: Container 1. (85 gm. Crab)		Container 2. (74 gm. Crab)	
	Upper Shore	Lower Shore	Upper Shore	Lower Shore
0	22	22	22	22
4	15	22	21	22
10	4	20	14	22
14	0	20	7	22
21	0	20	0	22

PART 3 CRAB RESISTANCE ON OTHER SHORES.

The phenomenon of crab resistance in gastropods has been demonstrated in Thais lapillus by Kitching et al (1966) and it is also discussed by Flatterly and Walton (1922). In the case of L. obtusata there is considerable temptation to conclude that crab activity is greater lower on the shore, resulting in the selection of thicker shelled crab resistant winkles on the lower part of the littoral zone.

This conclusion however tempting is not justifiable since the work of Moore (1936) indicates that in Nucella lapillus it was the proportion of mussel in the diet and not crab activity which was responsible for the thickness of the shell.

Crothers. J. (1968 and in a personal communication) considers crab activity to be comparable over the whole of the shore at least up to mean high water neaps. Nevertheless the lower shore winkles are likely to be exposed to crab attack for longer periods.

The following sets of samples of L. obtusata were taken to detect the presence of crab resistant animals on other shores. The samples were treated as previously to determine the shell to body weight ratio.

Samples were taken from the upper and lower L. obtusata zone at Four Mile Bridge and the upper shore at Ynys Faelog, the lower part of the Ynys Faelog shore possessed no weed and hence no winkle population.

Samples from the upper and lower L. obtusata zone were taken from Glyn Garth which lies on the Menai Straits one mile north east of Ynys Faelog.

The Student's "t" Test was used to analyse the results. Table 17 (p.64) showed that at Ynys Faelog the ratio of shell to body weight was not as great as on the low shore at Trearddur Bay. Inspection of the shells from Ynys Faelog showed the shell mouth was not in any degree thickened, and the shells certainly were not crab resistant as a hundred of them had been eaten in a week by three crabs, (see Section 3, Part 1, p.57).

If the shell type found on the lower shore at Trearddur Bay had been produced solely in response to crab predation, its effect on the type of shell at Ynys Faelog should be at least as pronounced if not more so, because of the large number of crabs.

Table 16 (p.64) showed the shells from the lower L. obtusata zone at Glyn Garth did not differ significantly from those from the upper zone, as the value for 't' was only 1.36.

Table 18 (p.64) similarly failed to disclose any difference in the upper and lower zone animals at Four Mile Bridge.

The shells from the upper and lower L. obtusata zones at Black Rock and Rhosneigr also failed to show the presence of thickened shells with tapered mouths.

On the shores used for this study, well defined crab resistance was confined to the lower shores at Trearddur Bay and Rhoscolyn.

DISCUSSION OF SECTION 3.

It has been shown from laboratory work that crabs were able to successfully attack *L. obtusata* by cracking open their shells to obtain the food within. In the field pieces of freshly broken shell were to be found by careful searching and it was supposed that these were produced as a result of crab activity.

When the winkle population at Ynys Faelog, where crabs abound, was artificially increased, the pieces of freshly broken shell became more abundant. The parallel fall in the introduced population indicated they were being killed, probably by crabs.

The natural population of *L. obtusata* at Ynys Faelog was a sparse one (seven grams of *L. obtusata* per K.gram of weed) but that at Four Mile Bridge was considerably more dense (twenty seven grams of *L. obtusata* per K.gram of weed) despite the similar muddy conditions which prevail on the two shores.

The crab population at Ynys Faelog was unusually large both in the number of crabs and in the size which they attained. The crabs at Four Mile Bridge were relatively few and smaller in size.

Carcinus moves up and down the shore with the tides, moving into deeper water at low tide (Crothers 1968), they thus spend most of their life submerged. Some shores however provide plenty of inter-tidal shelter under rocks which is used by the crabs when the tide is out. Where there is abundant shelter, many crabs tend to remain in the littoral zone when the tide is out.

Ynys Faelog is a shore with plenty of shelter, plenty of food and a good sublittoral refuge for crabs to move into if they do fail to find satisfactory littoral shelter. The shore at Four Mile Bridge is in this respect totally different. There is very little shelter for the crabs above low water mark and at low tide the sublittoral refuge is reduced to a strip only a few feet wide.

This may explain the difference in the crab populations on the two shores.

After the work on the effect of wave exposure had shown that populations of *L. obtusata* were very sensitive to wave action, this factor was evoked as a partial explaination of the differences in the winkle populations of the two shores. The very narrow strip of water at Four Mile Bridge provides an extremely small wave fetch. The Menai Straits at Ynys Faelog is considerably wider being in the order of half a mile. This argument was never considered to be particularly convincing as the more continuous weed cover at Ynys Faelog would tend to counteract the effect of the greater wave exposure.

The effect of crab predation seemed to provide a more credible explaination for the population difference. The absence of a deep water refuge together with a lack of shelter for crabs at Four Mile Bridge prevented the crab population from

Table 16.

Shell to Body Weight at Glyn Garth.

Sample		Weight of 20 Shells in gms.	Weight of 20 Bodies in gms.	Shell to Body Weight Ratio
Upper Shore	1	29.2	10.2	2.86
	2	26.5	8.3	3.19
	3	28.0	8.1	3.46
Lower Shore	1	27.3	8.5	3.22
	2	29.8	9.1	3.28
	3	31.8	9.2	3.46

't' for 3 degrees of freedom 1.36

Table 17.

Shell to Body Weight at Ynys Faelog.

Sample	Weight of 20 Shells in gms.	Weight of 20 Bodies in gms.	Shell to Body Weight Ratio
1	28.5	8.5	3.35
2	31.3	9.6	3.26
3	28.7	8.6	3.34
4	29.7	8.8	3.38

Table 18.

Shell to Body Weight at Four Mile Bridge.

Sample		Weight of 20 Shells in gms.	Weight of 20 Bodies in gms.	Shell to Body Weight Ratio
Upper Shore	1	26.9	7.78	3.5
	2	29.35	8.65	3.4
Lower Shore	1	32.2	9.8	3.3
	2	33.5	9.85	3.4

reaching the density at which it began to seriously affect the winkle population.

Ynys Faelog in providing shelter littorally and a good deep water refuge allowed the crab population to become unusually large to such a degree that its effect on the density of the winkle population became pronounced. Kitching et.al. (1966) similarly believed crab predation seriously affected the population density of <u>Thais lapillus</u>, and on on some shores was responsible for its absence.

SECTION 4.

THE BEHAVIOUR OF L. OBTUSATA.

Part 1a. The effect of Fucus vesiculosus on negative geotaxis out of water in the laboratory.

Specimens of L. obtusata were collected from Ynys Faelog and placed on damp Fucus vesiculosus in the laboratory to await trial.

A flat wooden board 20 x 12 cm. was covered with pieces of F. vesiculosus frond. The fronds were fixed to the board by drawing pins to form a continuous cover. A second smoothe piece of wood the same size but without weed was used as a control.

Both pieces of wood were dampened and layed horizontally. One L. obtusata was placed on each piece and allowed to attach itself.

When both animals were attached, the pieces of wood were placed vertically. After a period of ten minutes the animals were removed and the distance they had crawled from the starting point noted. The experiments were performed ten times.

From Table 19 (p.69) it can be seen that whilst the winkles on the wood climbed rapidly, those on the Fucus fronds moved very little.

As was to be expected from field observations the active negative geotaxis of L. obtusata was much reduced on Fucus vesiculosus.

Part 1b. Geotaxis in the field and observation of the modifying effect of Fucus vesiculosus.

Laboratory observations of Barkman (1955) indicated that L. obtusata rarely move downwards. If the upward movement of L. obtusata on substrata bare of weed was as constant in the field as it was found to be by Barkman in the laboratory, quantities of winkles would be found in the field marooned on top of weed bare rocks. As this was not the case, the animals must behave differently under natural conditions. Observation showed that the only times they are found off the weed on most shores, is after they have been displaced by wave action.

To simulate this situation as naturally as possible, a hundred animals were collected from the F. vesiculosus at Black Rock just as it was being covered by the tide.

The animals were immediately scattered on the sand inside a circle of stones which had just been covered by the tide.

The circle of stones was formed of rocks picked from the beach, some with F. vesiculosus growing on them and some without. The circle was approximately two meters in diameter.

The animals were then left until the tide again receeded and left them uncovered. As the sea was completely calm, the winkles were not displaced by wave action.

Once uncovered the winkles began to crawl. Some encountered other winkles and they attached themselves to each other, but most continued crawling until they reached the stones of the circle.

Table 19.

Rates of crawling on wood and Fucus vesiculosus.

Each trial lasted for ten minutes.

Distance Crawled in cms. (All the animals crawled upwards).

Trial	On Wood	On Fucus
1	10	1.25
2	5.0	2.5
3	12.5	1.25
4	17.5	0.0
5	17.5	1.25
6	13.5	2.5
7	15.0	1.00
8	22.0	0.0
9	19.0	1.25
10	16.5	3.5

Those encountering weed covered stones crawled up them and onto the weed. Once on the weed they crawled under the edge of one of the fronds and stayed there. Those which arrived at stones bare of weed, crawled up them, and upon reaching the top, continued crawling usually in the same direction down the other side. No animal remained on bare rock.

This observation showed that the negative geotaxis of L. obtusata was readily reversed in the field, thus enabling the animal to leave weed bare rocks.

On many shores detached pieces of F. vesiculosus were seen lying on the sand and the rock. In view of the present observations, and those of Barkman 1955 who states that L. obtusata will not readily leave Fucus, it was decided to test the behaviour of L. obtusata encountering detached pieces of F. vesiculosus.

Several pieces of F. vesiculosus fronds of approximately 10 cms. long were placed in the path of the animals which were crawling away from the circle of stones

Animals encountering the pieces of F. vesiculosus crawled onto them and carried out exploring movements along the edge of the pieces. The movement continued from three to seven minutes after which time the winkles left the pieces of weed and continued crawling, not necessarily in the direction they had been crawling when they first encountered the weed.

Further tests were carried out in the same manner using large clumps of detached weed. An animal encountering such a clump behaved in a manner similar to those encountering a weed covered rock. They appeared to be unable to differentiate between weed growing on rock, and clumps of weed loose on the sand.

The difference in behaviour of winkles on detached clumps and isolated pieces of frond was probably determined, by the ability to find a raised edge of weed under which to crawl. The behaviour of the animals on the edge of a piece of weed was possibly light controlled. The attraction of F. vesiculosus was not always an over-riding factor. It has been shown in Section 3, Part 3, that on exposed shores from the middle of Autumn to the middle of Spring a large proportion of the L. obtusata population leave the Fucus for rock crevices.

It may be concluded that the negative geotaxis, and the affinity for Fucus were both behaviour patterns which may be modified to meet the conditions encountered in the natural environment.

Part 1c. Geotaxis above and below water in the field.

It has been shown that the negative geotaxis which appeared so constant in the laboratory, was less so in the field, as all the experiments were performed above water, the behaviour of L. obtusata under water required investigation. The following experiment compared the direction of movement of L. obtusata above and below water.

The work was conducted at Polridmouth, Cornwall on April 3rd and October 5th, 1969.

Two flat pieces of rock were selected and placed horizontally on the edge of a rock pool above the water level. Twenty four L. obtusata were then collected from F. vesiculosus just as it was being uncovered by the tide. The two flat rocks were dampened and twelve L. obtusata put onto each one, six facing one way and six in the opposite direction. After all the animals on the first rock had become attached, it was raised to a vertical position, and propped in that position with the direction of the sun in the plane of the rock and to the right of it. The second rock was immersed in the rock pool and turned to the vertical position and propped under water with the direction of the sun once again in the plane of the rock. Both rocks were placed such that with reference to the direction which the animals were placed, six faced upwards, and six faced downwards.

When each rock had been left for one minute, the number of animals crawling upwards, and the number crawling downwards on each rock were counted. Those not moving and those moving horizontally were discounted. Often several animals fell off when the rock was being moved to the vertical position, those too were discounted. The trial was repeated twenty one times.

Results

The results see table 20 (p.72) show that both above and below water more animals crawled upwards than crawled downwards, the greater percentage of animals crawling upwards on the rocks under water. Under water only SIX out of a hundred and SIXTY one animals crawled downwards.

Discussion

Negative geotaxis under water was in the field as well as in the laboratory remarkably constant.

The value of a variable reaction to gravity above water has already been discussed, the value of a constantly negative geotaxis under water is probably a behaviour which enables L. obtusata to crawl out of rock pools.

L. obtusata relies for food and shelter on Fucus vesiculosus or Ascophyllum nodosum both of which are seldom found in rock pools. The ability to leave a rock pool is ensured by the geotaxis which is constantly negative whilst the animal is under water.

If under water L. obtusata always crawled upwards it might be expected that if crawling on an isolated, submerged rock, they would reach the top and stay there unable to move down owing to the constancey of the geotaxis under water.

The negative geotaxis has been shown to be subject to modification above water allowing the L. obtusata to leave isolated weed bare rocks. The following experiment was performed to see whether a similar modification allowed L. obtusata to leave isolated weed bare rocks under water in rock pools.

Table 20.

The direction of movement of L. obtusata on rock on the shore at Polridmouth Cornwall, October 5th, 1969.

	Under Water		Above Water	
	Crawling Upwards	Crawling Downwards	Crawling Upwards	Crawling Downwards
	4	1	3	2
	8	0	2	1
	4	0	5	2
	7	0	1	1
	1	1	3	3
	7	0	4	6
	4	0	6	3
	5	0	6	1
	6	0	0	4
	7	0	6	2
	10	0	3	1
	11	0	6	3
	8	0	8	1
	9	0	8	0
	6	0	3	2
	6	0	12	0
	5	1	5	2
	9	1	6	2
	7	1	10	1
	10	0	4	2
	11	1	2	9
	9	0	3	6
TOTALS	154	6	106	54

BEHAVIOUR OF L. OBTUSATA ON ISOLATED ROCKS UNDER WATER

Materials and Methods

Eight slabs of schist were selected from the beach at Polridmouth. They were layed horizontally, dampened and a number of *L. obtusata* placed on each one (the number depended on the size of the rock) and allowed to attach themselves. Four of the rocks were elevated to the vertical and wedged in position. The other four were immersed in a rock pool, then they too were wedged in a vertical position. The eight rocks were left for half an hour after which time the numbers of animals within two centimeters of the top of the rocks and those further than two centimeters including those that had left the rock were counted separately.

L. obtusata on the rocks above water (see Table 21, p.74) crawled downwards many of them escaping from the rock, and others attaching themselves in the shade at the base. Those on the rocks in the water became marooned at the top of the rocks.

Discussion

The negative geotaxis exhibited by *L. obtusata* below water was so constant that for the duration of the experiment, none of the animals left the submerged rocks and most of them crawled to the top of the rock and stayed there. *L. obtusata* crawling on isolated weed bare rocks in rock pools appear to have little chance of escaping from the rock pool and finding a patch of *Fucus*. It can only be supposed that occasions when *L. obtusata* require to leave isolated rocks in rock pools are too rare to allow selection for the necessary modifications in the negative geotaxis.

Table 21.

The position adopted by L. obtusata on isolated rocks both submerged and above water. Animals within 2 cms. of the top were classed as "on top".

	Above Water		Below Water	
	On Top	Not on Top	On Top	Not on Top
	0	7	5	1
	0	5	5	0
	0	6	8	2
	0	15	31	2
TOTALS	0	33	49	5

PART 2. RHEOTACTIC BEHAVIOUR

Compared with the number of works containing references to geotaxis and phototaxis, there is little mention of rheotaxis in L. obtusata.

Barkman (1955) showed that a thin film of water draining down a vertical glass plate, opposed the negative geotaxis, preventing L. obtusata from crawling upwards. Barkman's experiment was corroborated in the field by Ebbinge Wubben (Barkman 1955) who is reported to have seen L. obtusata crawling downwards and sideways under the action of a thin film of draining water. Sacchi and Rastelli (1966) however considered L. obtusata to be positively rheotactic under all conditions.

The following two experiments were therefore carried out to determine the reaction of L. obtusata to both a thin film of moving water and to a current of water which completely immersed the animals hereafter referred to as deep water.

Part 2a. Reaction to a thin film of moving water.

Introduction

This experiment was performed to test the findings of Barkman (1955) that a thin film of moving water causes L. obtusata to move downwards.

Materials and Methods

A light grey plastic tray one meter square was placed at an angle of 15^{o} on a wet bench in a blacked out room. Constant illumination was provided by means of a fluorescent tube, thus eliminating the effect of changing light intensity. Against the upper edge of the tray was placed a tank with a lipped edge. The lip of the tank overhung the upper edge of the plastic tray. The tank was tilted so that water overflowing from it ran evenly down the plastic tray. The rate of water flow into the tank was adjusted to two liters per minute, thus producing a film of water draining over the tray.

On the tray twelve circles, twelve cms. diameter were drawn in blue blackboard chalk. Taking the direction directly up the slope as zero degrees two marks were made on the circumference of the circles at seventy five degrees and two hundred and eighty five degrees.

L. obtusata were collected from Rhosneigr and Ynys Faelog to test the reaction of animals from both exposed and sheltered shores respectively.

No animals were kept in the laboratory for more than three hours before being used.

The animals were treated as follows.

The tray was first wetted and one L. obtusata was placed in the centre of each of the twelve circles facing directly upwards. Twenty minutes were allowed for the animals to reach the circumference, any animals not reaching the circumference within that time were removed. An animal was considered to have crawled upwards if the mid point of the shell lip passed above the marks on the circumference, and was considered not to have crawled up if the mid point of the shell lip passed below the two marks. The animals from Rhosneigr were used first.

At the end of each trial of twelve, the circles were washed thoroughly to remove any mucus which might have affected the succeeding trials. The next trial was performed in the same way with the animals from the same shore but with the addition of a film of flowing water.

The trials were continued until one hundred and fifty two shore had been tested, seventy one with a film of draining water, and eighty one without.

Results

The results (see Table 22, p.77) were arranged in two contingency tables and analysed by means of the Chi Squared Test using the Yates correction for continuity.

Neither the L. obtusata from Rhosneigr nor those from Ynys Faelog showed any tendency to move down the tray under the influence of a film of draining water. There was instead a suggestion that the animals from Rhosneigr were crawling upwards in response to the draining water, though the results do not show significance even at the ninety five percent level of probability. The animals from Ynys Faelog showed a greater tendency to crawl upwards under the influence of the water film which was significant to the ninety nine percent level of probability.

Discussion

The results of this experiment do not corroborate the findings of Barkman (1955) who maintained that the influence of draining water is sufficiently strong to counteract negative geotaxis of L. obtusata on a vertical glass plate.

Barkman quotes Kanda (1916) as reporting the negative geotaxis of L. littorea to be greater on smoother surfaces, and for greater angles of slope.

Barkman maintained that those reactions could probably be extended to include L. obtusata, thus explaining the immobility of L. obtusata in his moving film experiment which was performed on a vertical glass plate. The L. obtusata he thought were held by the opposing responses of negative geotaxis and their reaction to crawl downward in the presence of a film of moving water. In the present experiment the use of plastic instead of glass, and a fifteen degree slope instead of a vertical one would have led to a very definite downward movement if Barkman's theory were correct. As neither the L. obtusata from Rhosneigr nor those from Ynys Faelog showed any tendency to move downwards, Barkman's theory can not be universally applicable. Barkman does not reveal how long his animals were kept in the laboratory before being used. The length of time the animals are kept is of great importance as their activity declines after being kept for more than a few hours.

Table 22.

The effect of a thin film of moving water on the movement of L. obtusata from Rhosneigr and from Ynys Faelog. May 9th, 1968.

Table a L. obtusata from Rhosneigr.

	With Water	Without Water	Total
Crawling upwards	31	26	57
Not crawling upwards	10	22	32
Total	41	48	89

$\chi^2 = 3.55$

Table b L. obtusata from Ynys Faelog.

	With Water	Without Water	Total
Crawling upwards	26	12	38
Not crawling upwards	4	21	25
Total	30	33	63

$\chi^2 = 14.6$

Figure 12.

The apparatus used to pass a current of deep water over *L. obtusata* in the laboratory.

SECTION 4.

THE BEHAVIOUR OF L. OBTUSATA

There is a considerable volume of work on the behaviour of the Littorinidae in the laboratory. The bulk of this work refers to L. obtusata (LITTOREA). (Haseman 1911, Hayes 1927, Kanda 1916, Minnich 1942, and Newell 1958b). In the field too, L. littorea has received some attention (Dexter 1943, Gowonloch 1926, and Newell 1958a). Some work has been concerned with the behaviour of L. obtusata in the laboratory but very little has been extended to field observations.

PART 1 GEOTACTIC BEHAVIOUR

It is well established that when L. obtusata are kept in an aquarium tank without any of their food plant (Fucus or Ascophyllum) the animals crawl up the sides of the tank and even leave the water. They appear to be negatively geotactic both in and out of the water. This phenomenon has been observed by several workers (Baaker 1959, Barkman 1955, Van Dongen 1956, and Janssen 1960).

Barkman investigated the negative geotaxis in some detail, showing it to become weaker on gentler slopes and rougher surfaces; and even becoming positive if the winkles were desiccated. The influence of temperatures on the geotaxis was studied by Janssen (1960) who discovered that below $0^{o}C$. it became positive.

Barkman (1955) found that, even in an aquarium in which the water rose and fell in an immitation of tidal rhythm, the winkles climbed the tank sides, coming to rest just above high water level.

The relavance of negative geotaxis to L. obtusata in the field was tentatively ascribed by Barkman to the maintainance of its positions on the shore. When the animals are covered by the tide they move up the shore but as they are uncovered they move downwards under the influence of draining water being negatively rheotactic. They thus, claims Barkman, remain in their correct zone on the shore.

From personal observation, except in restricted drainage channels, water ceased to drain off a shore very shortly after it was uncovered. Barkman's theory also assumes that winkles spend a large proportion of their time off the weed and on rock. Diving experience on Anglesey has shown that unless dislodged, L. obtusata remain on Fucus or Ascophyllum. The fronds of these two fucoids float vertically owing to the presence of air bladders, one frond thus being more or less isolated from the others. As L. obtusata were never seen to move from one frond to another, it was doubtful if extensive tidal migrations ever took place on Anglesey.

As the fronds of F. vesiculosus and Ascophyllum float almost vertically when under water, a constant negative geotaxis would result in the animals crawling to the apices of the fronds. Diving experience showed that the winkles were distributed over the entire length of the frond, and not all concentrated at the tips. The Fucus must therefore have a modifying affect on the negative geotaxes. This effect was investigated further in the laboratory.


OUTLET FLAP
L.OBTUSATA
F. VESICULOSUS
WIER
COARSE NETTING
BAFFLE
SEAWATER SUPPLY

FIG.12

REACTION TO A CURRENT OF DEEP WATER.

Introduction

Observation whilst diving showed that when currents of water were fanned over L. obtusata which were feeding on Fucus vesiculosus, they orientated to face the direction of the current. The following experiment was performed to investigate the orientation of L. obtusata to experimentally induced currents in the laboratory.

Materials and Methods

The apparatus used for the experiment is shown in fig. 12 (p.78). It consisted of a wooden trough 60 by 10 cms. Seawater entered at the right hand end and then flowed through a set of six, two centimeter diameter holes drilled in a plywood baffle. The water then flowed through a piece of course plankton netting (2mm. mesh) and over a weir three centimeters high. This system effectively dampened the turbulence of the inflowing water. The exit of the trough at the left hand end was controlled by a wooden flap which could be opened and closed to allow exit for the water.

The trough was arranged to slope at an angle of two degrees. With the rate of flow of seawater at a maximum, the mean current velocity was approximately 18 centimeters per second over the floor of the trough. The floor of the trough was carpeted with fronds of F. vesiculosus pinned to the wood with drawing pins.

The experiment was conducted as follows.

With the trough full of seawater, but with the flap shut and the water turned off, ten L. obtusata were placed on the Fucus, five facing left (downstream) and five facing right (upstream). The rate of flow of water was gradually increased by simultaneously opening the flap and increasing the volume of seawater entering, the animals thus had time to attach themselves and were not washed away by any sudden current of water.

The water was run at maximum speed for half a minute and then turned off. The number of animals facing upstream (right) and the number facing downstream (left) were counted. Those facing at right angles to the direction of the current and those which had climbed the sides of the trough were discounted.

A control experiment was performed in which the animals were put onto the floor of the trough as above, but were allowed to remain for half a minute in the stagnant seawater, after which time they were counted in the same manner.

Results

The results of the experiment and its control are shown in Table 23 (p.80) from them the following contingency table was compiled.

The table was analysed for significance using the Chi Squared Test and applying the Yates correction.

Table 23.

The reaction of L. obtusata to a current of deep water. When the water was flowing animals facing right faced upstream.

	With Current of Seawater.	Without Current of Seawater.	Total
Facing right	91	26	117
Facing left	17	23	40
Total	108	49	157

Total $X^2 = 15.6$

The Chi Squared value of 15.6 showed the difference between the experiment and the control was significant at the 99.9% level of probability. L. obtusata orientated in a current of water to face upstream.

Discussion

The orientation to face upstream in water currents can be interpreted as a reaction to offer the least resistance to the passing water. This reaction would be of great value in helping to prevent being washed away by strong water currents on the shore.

Some workers have discovered positive rheotaxis (crawling upstream) in L. obtusata (Sacchi and Rastelli 1966).

It is possible to interpret this as a secondary effect of its orientation behaviour in water currents. If L. obtusata in a current of water faces upstream and it crawls in the direction it is facing, it will appear to possess a true rheotaxis.

Table 23.

The effect of a current of deep water on the orientation of L. obtusata from Rhosneigr. August 20th, 1969.

Table 23a.

	Facing Upstream (right)	Facing Downstream (left)
	5	1
	3	0
	4	2
	1	4
	7	1
	5	1
	6	2
	8	1
	8	0
	7	0
	7	1
	6	1
	7	0
	7	2
	5	1
	5	0
Total	91	17

Table 23b.

The effect of deep static water on the orientation of L. obtusata from Rhosneigr.

	Facing Right	Facing Left
	3	1
	2	2
	2	2
	3	0
	3	2
	1	4
	5	3
	3	5
	4	4
Total	26	23

PART 3 PHOTOTACTIC BEHAVIOUR.

Littorina obtusata is reported by some authors to be negatively phototactic. Experiments by Barkman (1955) indicated that L. obtusata moves away from well lit ted areas. Charles (1960) discovered that L. obtusata illuminated by polarised light, moved parallel to the plane of polarisation, further investigation showed that the winkles orientated themselves so that minimum light fell upon their eyes. Janssen (1960) however considered L. obtusata to be positively phototactic, although the sign reverses below 3°C.

The apparent discrepancy between workers in this field may be due to the great varability of L. obtusata, since the above work was carried out, the taxon "Littorina obtusata (L)" has been divided into two. The larger, Littorina obtusata (L), and the smaller Littorina mariae, spec. nov. (Sacchi and Rastelli 1966).

Although the two species are difficult to separate in the field, they are found to exhibit different behaviour responses and physiological properties, (Sacchi 1961, 1964, 1966, Sacchi and Rastelli 1966), L. mariae being found less negatively phototactic than L. obtusata.

It is well established that L. obtusata are found under fronds of Fucus and Ascophyllum (Barkman 1955), observations on Anglesey confirmed this finding. One observation made shortly after dawn at Rhosneigr revealed a large number of L. obtusata on the upper surface of Fucus vesiculosus. It was thought that during night time L. obtusata may have been living on the upper surface of the Fucus, crawling underneath the fronds as the light intensity increased at dawn.

Rhosneigr was revisited at dawn and a count made of all the L. obtusata which could be seen on the upper surface of fucoids in a period of half an hour. A similar count was made at noon on a subsequent day.

At dawn on May 13th, 1968, 784 animals were counted on top of the Fucus, but at noon on May 21st, 1968, only 18 were counted.

This observation strongly suggested that it was the influence of light which caused L. obtusata to remain under the Fucus during the day time. A laboratory experiment was performed to investigate the effect of light on the position L. obtusata maintained on Fucus vesiculosus.

Phototaxis in the Laboratory.

Materials and Method.

The room was maintained in black out for the duration of the experiment.

A wooden frame was erected upon which were placed, next to each other, two perspex discs 20 cms. in diameter. One disc was black and the other transparent. The black disc was arranged to be illuminated from above by an anglespoise lamp with a cryselco 40 watt opalite bulb, 60 cms. above the disc. The transparent disc was illuminated from below using the same bulb. The lamp was situated to one side of the disc and the light brought to bear by means of a mirror, so avoiding the effect of convected heat. The total light path was arranged to be 60 cms.

A carpet of F. vesiculosus one frond deep was placed on each disc. Animals freshly collected from Rhosneigr were used for the experiment, no animal being kept in the laboratory for more than three hours. The two discs were used alternately, twenty animals being placed on top of the Fucus of the appropriate disc. Each experiment was allowed to run for half an hour. At the end of that period the numbers of animals on the top and underneath the weed, on each of the discs was counted. There was some difficulty in interpreting the position of the animals, as many had adopted positions which could be interpreted in either way. Since it is the eyes which are important in sensing light, an animal was considered to be "on top" if it was facing upwards and "underneath" if it was facing downwards. In the cases when it was impossible to intrepret the position of the animal, they were considered to be on top of the disc illuminated from above, and underneath on the disc illuminated from below. The experiment can therefore not be critised as being biased in favour of the expected result.

Results and Discussion

Although the results of the experiment (see Table 24, p.84) showed a definite difference between animals lighted from below and those lighted from above, they were not as convincing as may have been expected from the field observation. It should however be remembered that the condition in the laboratory differed markedly from those in the field.

It may be inferred from the results of this experiment, and from the field observations, that light was probably a factor responsible for L. obtusata being found underneath Fucus in the field during the day time.

Table 24.

The reaction of L. obtusata to light.

20 animals were used on each disc.

Numbers on top of Fucus fronds

Disc Lighted from Above	8	6	4	10	10	6	6	8	5	7
Disc Lighted from Below	12	14	14	14	16	15	8	12	11	13

SECTION 5.

INVESTIGATION OF NATURAL POPULATIONS.

SECTION 5.

INVESTIGATION OF NATURAL POPULATIONS.

Introduction

Littoral population ecology is still in an underdeveloped state when compared with many terrestrial fields. Smith (1953) pointed out that our knowledge of even the commonest of intertidal animals is far from complete.

Moore (1937) had worked on Littorina littorea but it was not until the work of Smith and Newell (1955) that the Littorinidae received a detailed ecological study. The work of Smith and Newell was expanded to include the shores of Cardigan Bay by Williams (1964a) who discovered that this population differed in several important respects from that of Smith and Newell at Whitstable.

At the same time Williams (1946b and 1964c) also studied populations of Gibbula umbiliculis (da Costa) and Monodonta lineata (da Costa), thus forming a useful basis for comparison between populations of three different species.

Littorina obtusata has received mainly physiological study (Bakker 1959, Barkman 1955, Van Dongen 1956, Janssen 1960, Sacchi 1961, 1963, 1964a, 1964b, 1966 and 1967) and its many colour forms have received quantative attention from Sacchi (1961 and 1963), Moyse (1966) and Parr (1966).

Populations of L. obtusata have never been studied as a dynamic entity though it is difficult to imagine an animal more ideal for such a study. It is restricted to the intertidal zone and easily removed from the shore without damage. The limit of each individual is perfectly clear and its eggs which are layed intertidally are easily collected and counted, and hatch as miniature adults without a planktonic stage.

Two lines of study were open in the present investigation. Either following the methods of Newell, Smith and Newell, and Williams, study could be concentrated on single population of L. obtusata, or without precedent, the study could include several populations on shores of different types studied in less detail.

The latter approach was considered more fruitful for although of necessity each population received less attention, it was possible to observe the ways in which the several populations reacted to different environmental conditions.

The absence of a planktonic larva was helpful as each population, being isolated from its neighbours except for non larval migration was able to develop its own characteristics in response to environment.

PART 1 METHODS.

The methods for investigating the natural populations were many, and the results often interpreted in the light of the combined data from several distinct operations. It was therefore beneficial to consider firstly the methods used and secondly the results.

Methods

1a. Choice of shores
1b. Sampling techniques
1c. Treatment of samples
1d. Microscopic examination
1e. Treatment of the eggs

Part 1a. Choice of shores.

In selecting the shores to be studied, the following factors were taken into account:-

(1) Wave Action.

It was shown earlier (Section 1) that L. obtusata was sensitive to wave action as many became dislodged from their position on the shore. If they were unable to regain the weed they were very likely to become washed up onto the strandline.

(2) Nature of Substrate.

L. obtusata would be more likely to regain the weed if the shore consisted of large, continuous reefs of rock with isolated patches of mobile substrate, (sand or mud) in between, than if it consisted of isolated boulders embedded in a mobile substrate.

The presence of crevices in the rock has also been shown to be important in resistance to wave action in the winter, the presence of crevices being advantageous to survival of L. obtusata on shores exposed to wave action.

The presence of large quantities of mud and a thin layer of sediment on the fronds of the weed has been shown to be a likely detrimental factor to the survival of L. obtusata in the hot summer months.

(3) Presence of Parasites.

It was found by James (1963) that the incidence of parasitism by larval Trematodes in L. saxatilis varied on different shores. A brief survey on Anglesey revealed that different populations of L. obtusata varied very widely in the degree to which they were parasitised.

Since parasitised animals are unable to reproduce and on some shores the incidence of parasitism rose to 50% of the potential breeding population, the effect parasite level might be detectable when comparing heavily and lightly parasitised populations.

(4) Population Density.

Finally the populations chosen had to be sufficiently large to withstand destructive sampling over a long period without showing any detectable effect.

The following shores were used for the study. (For a full description of shores see pages 3, 4 and 5).

	Sheltered from Wave Action	Not Sheltered from Wave Action
Substrate more or less continuous rock	Rhoscolyn (heavily parasitised)	Rhosneigr (with crevices)
	Trearddur Bay (lightly parasitised)	Penmon (without crevices)
Substrate of isolated rocks	Four Mile Bridge (muddy substrate extreme shelter)	Red Wharf Bay
	Black Rock (sandy substrate)	

Shores with some combination of factors were never discovered. For instance a combination of high wave exposure and high parasite incidence was never found even though populations in the vicinity of gull colonies were examined.

Part 1b. Sampling Techniques.

The use of a quadrat for sampling was considered to be impracticable. As *L. obtusata* lives mainly on *Fucus vesiculosus*, the size of the habitat was considered to be proportional to the weight of *F. vesiculosus* frond available for crawling and feeding. Samples were taken every month throughout the course of a year and on some shores over a longer period. Where the shore extended from the upper *F. vesiculosus* zone to the *F. serratus* zone, separate samples were collected from three levels on the shore, the upper and lower parts of the *F. vesiculosus* zone and the upper part of the *F. serratus* zone.

At Four Mile Bridge there was no *F. serratus* zone; at Black Rock and Rhosneigr the *L. obtusata* population on *F. serratus* was too sparse to be sampled adequately. The same applied to the lower part of the *F. vesiculosus* zone at Rhsoneigr. In order to sample these parts of the shores adequately large quantities of weed would have had to be removed to the laboratory. Such amounts were not only impractible to work with, but would have unduely denuded the shore of its weed cover.

The once well developed lower *F. vesiculosus* zone at Four Mile Bridge would have provided valuable information on migration throughout the year under extremely sheltered conditions. The whole of the lower part of the shore was however destroyed by bait digging fishermen who lived in a housing estate completed after the present work was started. The shore at Four Mile Bridge is almost unique in England and Wales, it is both sheltered as a moderate sized river, and has practically no fresh water influence. The destruction of such a scientifically valuable shore under lies the immediate need for effective conservation on Anglesey.

Part 1c. Treatment of Samples.

The samples of weed were hand sorted under illumination from a 60 watt lamp situated as close as possible to the weed compatable with convienience. Too feeble a source of illumination was found to induce large sampling errors.

All the L. obtusata encountered were removed and all the eggs were counted. (see Treatment of eggs.)

The sample of weed was then washed with seawater and allowed to drain for an hour, to eliminate the effect of dehydration on the weight of the sample and the effect of sediment on the weight of the samples from Four Mile Bridge.

The weed was then soaked in fresh water for four hours and at the end of that time vigourously washed, where most of the remaining winkles fell off.

The winkles were separated from pieces of debris by means of a set of graded sieves ranging from $\frac{1}{2}$ inch mesh to $\frac{1}{32}$ inch mesh. The animals smaller than 0.01 gms. were retained by the latter sieve, and were counted directly if there were less than two hundred. If there were more, the sieve was divided into eight equal segments, and the number estimated by counting those contained in one segment and multiplying by eight.

The method of extraction by washing provided a useful check on the efficiency of the hand sorting method. Nearly all of the very small ones recovered were removed in this way. The hand sorting method for the animals larger than 0.1 gm. was shown to be very efficient, niney five percent or more being recovered.

The animals heavier than 0.1 gm. were then weighed individually. Those below 0.1 gms. were often too numerous to weigh separately. A set of standard animals was therefore kept whose weights were known. The sample winkles were compared by eye with the standard animals and alotted to 0.02 gram weight classes.

The shells were divided into two groups, adults and juveniles depending upon the condition of the shell lip. If the lip was sufficiently rounded and thickened that it was not possible to break it with the finger nail of the third digit, it was considered as adult. This distinction though somewhat arbitary had the merit of being both rapid and easily applied in the field with the minimum of equipment.

The use of weight as an indication of size rather than shell height was preferred, as it has been shown that the shape of the shells differed on different shores.

Part 1d. Microscopic Examination.

Those animals removed from the weed by the fresh water extraction method were discarded after weighing as they were mostly dead. Of the other animals, the ones sufficiently large to possibly be mature were graded for size using a set of standard sieves. The holes in the sieves were found not to be identical in size. The largest hole in each sieve was therefore found and marked with paint. Only animals not able to pass through the painted hole were said to be retained by that particular sieve. The reassortment into size groups by means of the sieves was largely a matter of convenience. Often several collections were made on a single day. They were all sorted and weighed and then placed in the culture tubes to await dissection. It was impractible to keep separate all the weight groups from all the samples.

The shells of the animals were then cracked open using two pieces of slate in a hammer and anvil arrangement, twenty animals at one time being placed on the anvil and cracked in quick succession.

The animals were then dissected to determine their sex and their breeding condition. Mature males were recognised by their enlarged glandular penis, and the presence of spermatozoa in the vas deferens. Females were deemed mature if the capsule and accessory glands were fully enlarged and the oviduct contained reproductive material which showed as a cream coloured mass.

At the same time the digestive gland and gonad area was searched for the presence of larval Trematodes. The presence of these parasites was soon found to be reflected in a greatly reduced penis size and empty vas deferens in the male and a reduced capsule and accessory gland together with an empty oviduct in the female. Even parasite infections so light as to be missed by all except the most careful search were seen to produce these effects. The animals were therefore assumed to be free of Trematode parasites if the reproductive structures were fully developed. If these structures were under developed a search was made for Trematode parasites. In all cases animals which had an adult shell type but reduced reproductive structures, were found to be parasitised.

The condition of the reproductive structures was in the large animals and adult animals considered a reliable indication of the presence or absence of the parasites.

Part 1e. Treatment of the eggs.

The eggs of L. obtusata were easily recognised. Some workers have found difficulty in distinguishing between the eggs of L. obtusata and those of Lacuna spp. The eggs of the latter however were distributed in concentric rings within their jelly, whilst those of the former were not. Confusion did however exist between the eggs of L. obtusata and L. mariae. It would not be possible to tell them apart unless the eggs were hatched and the young animals reared to maturity.

The egg masses were usually attached to Fucus spp. or Ascophyllum but were often also attached to the rock substrate on the FAIRLY sheltered shore at Rhosneigr. They were also observed on Ulva lactuca and Porphyra umbilicalis when populations were particularly dense.

During the hand sorting of the weed the numbers of egg masses were counted and the number of developing embryos in each mass estimated.

To count directly the number of embryos is each mass would have been impractible. The following method of estimation was therefore adopted.

A number of egg masses were collected from the shore and arranged in size groups, such that each size group contained masses with the same number of embryos, as estimated by eye. One mass from each size group was then dissected and the number of embryos counted accurately. It was assumed that the other masses in the same size groups contained the same number of embryos. This was checked by counting accurately the number of embryos in two other masses in each size group. The estimated and true numbers were found to be within twenty percent of each other.

Once the numbers of embryos in each mass were quantified, twenty one masses were selected as follows.

3 masses containing 50 embryos
" " " 100 "
" " " 150 "
" " " 200 "
" " " 250 "
" " " 300 "
" " " 400 "

These masses were placed in twenty percent alcohol and were used as standards with which to compare the sample masses. The sample masses were thus grouped into size classes from fifty to four hundred embryos increasing by intervals of fifty.

In this way the number of egg masses and eggs per kilogram of Fucus was estimated for each sample taken.

PART 2 RESULTS - THE EGGS.

Part 2a. The difference in egg numbers between seasons and between shores.

Part 2b. Time taken for eggs to hatch.

Part 2c. The fecundity of females.

(1) Method of judging the maturity of females.

(2) Estimation of the number of eggs layed in a life time.

Part 2a The difference in egg numbers between seasons and between shores.

The numbers of eggs found on the shores throughout the sampling period are shown in Fig. 13 (p.93). The figures refer to the numbers of eggs from the upper parts of the shore only. The eggs of L. mariae were layed on the middle and lower parts of the shore, and would thus confuse the results.

Eggs were found throughout the year on all the shores studied. Though there was considerable fluctuation in the numbers present there appeared to be little seasonality; although all shores showed a depression in the numbers of eggs between August and October. The depression was probably in part due to a shorter hatching time in the warmer summer conditions but difference in hatching time could not explain the large depression found at Rhoscolyn and Trearddur Bay. At that time of year the previous summers young were maturing and the adults dying off, there might therefore have been fewer laying females.

The constancy in the number of eggs was a striking feature of the results from Four Mile Bridge, the counts of the density of newly hatched young and of adults were also more constant for this shore than for any other.

Also estimated from each collection was the average number of eggs per egg mass and the number of masses found for each mature, unparasitised female collected from the same area as the eggs. From this data were calculated the number of eggs per mature, unparasitised female.

The results are shown in Tables 25, 26, 27, 28 and 29 (p.94 and 95), and from them the variance ratio tables shown in Table 30 (p.97) were constructed. Before any analysis of variance was initiated, an 'F' test was performed to detect any significant difference between the variances of each shore sample. As no significant difference was detected the method of analysis of variance was considered valid.

All the variance ratio analysis showed significance between shores at the 99% level of probability. The data were therefore examined in more detail to determine between which shores the significant differences lay. Table 31 (p.98) shows the means, standard errors and confidence limits of the number of eggs per mass (Table 31a, p.98), the number of masses per unparasitised mature female (Table 31b, p.98) and the number of eggs per unparasitised mature female (Table 31c, p. 98).

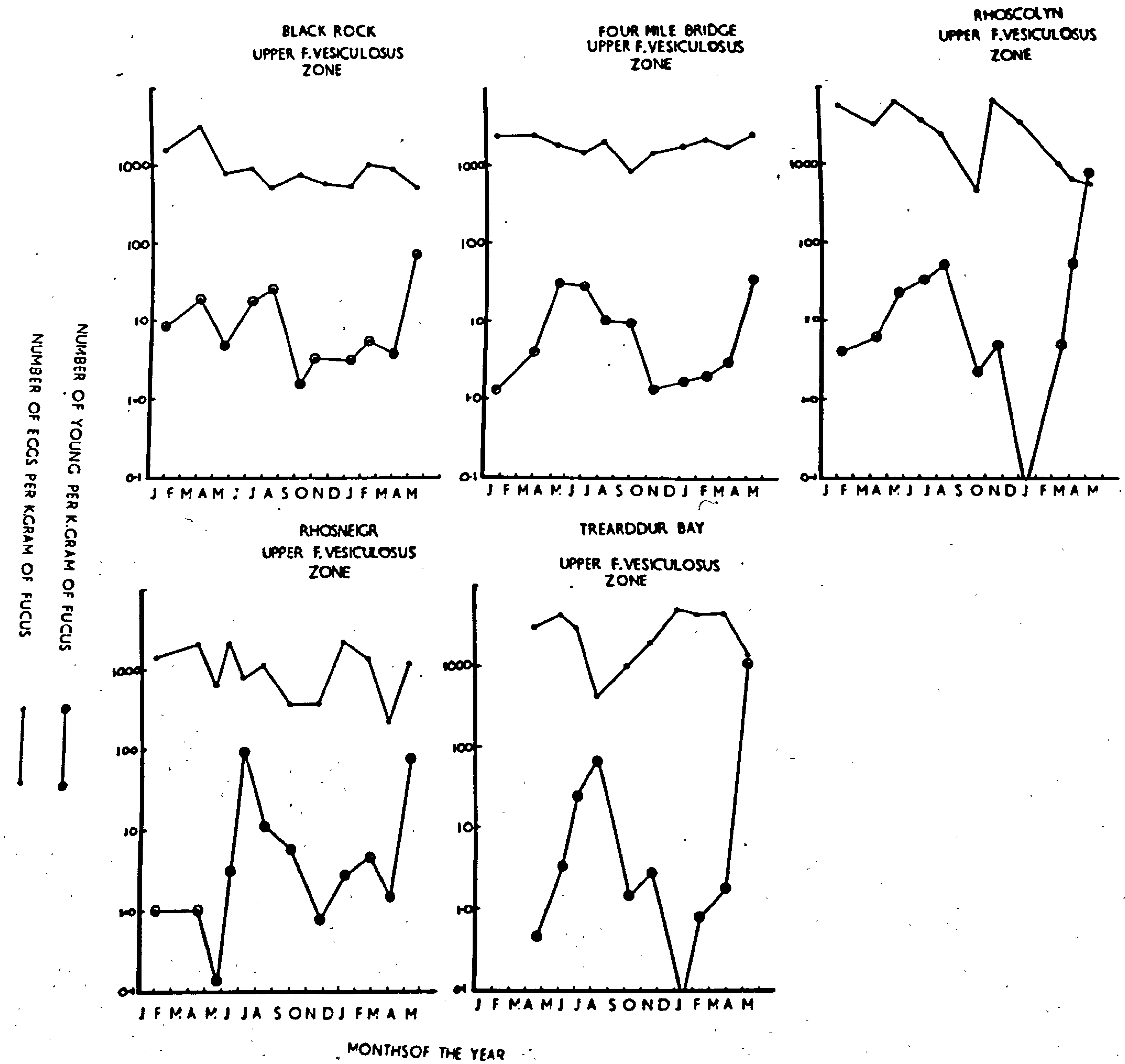
At the 99% level of probability the mature unparasitised females at Black Rock and Four Mile Bridge layed significantly more eggs than those from Rhoscolyn, Trearddur Bay and Rhosneigr. They did it by laying significantly larger masses than those from Rhoscolyn and Trearddur Bay, and significantly more masses than those from Rhosneigr.

It was logical to expect the egg masses from Rhoscolyn and Trearddur Bay to be smaller than those from Black Rock and Four Mile Bridge as the adult females were considerably smaller (see fig. 5, p.31).

The large masses layed by the small sized females at Rhosneigr was unexpected. A possible explaination does suggest itself. <u>L. obtusata</u> normally lays eggs on <u>Fucus</u>. There is probably therefore an inhibitory mechanism preventing the eggs from being layed elsewhere. At Rhosneigr the animals often have re-course to shelter in crevices in the rock where many of their eggs are also found. The inhibiting mechanism may prevent the females from laying on the rock until the number of mature eggs in storage is sufficient to over come the inhibition to laying on rock. Such a theory would explain the large egg masses, and also the small number of them per female. It would not in itself explain the smaller number of eggs layed by each female. This might be explained either by egg loss due to wave action, or by the inability of the females to obtain sufficient food whilst they are in crevices.

Figure 13.

The numbers of eggs and young smaller than 0.015 gms. per kilogram of F. vesiculosus on the upper F. vesiculosus zone on five shores in Anglesey from May 1967 to May 1968.


BLACK ROCK
UPPER F. VESICULOSUS
ZONE
FOUR MILE BRIDGE
UPPER F. VESICULOSUS
ZONE
RHOSCOLYN
UPPER F. VESICULOSUS
ZONE
RHOSNEIGR
UPPER F. VESICULOSUS
ZONE
TREARDDUR BAY
UPPER F. VESICULOSUS
ZONE
1000
100
10
1·0
0·1
J F M A M J J A S O N D J F M A M
MONTHS OF THE YEAR
NUMBER OF YOUNG PER K.GRAM OF FUCUS
NUMBER OF EGGS PER K.GRAM OF FUCUS

Tables 25, 26, 27, 28 and 29.

Average number of eggs per mass, and masses and eggs per mature unparasitised female, throughout the sampling period of one year.

Table 25.

Black Rock

Date	Ave. No. of Eggs Per Mass.	Ave. No. of Masses Per Mature Unparasitised ♀	Ave. No. of Eggs Per Mature Unparasitised ♀
May 5.1967	193	3.0	580
July 7.	216	3.8	820
Aug. 14.	230	1.27	294
Oct. 10.	190	1.75	330
Nov. 21.	204	1.0	206
Jan. 8.1968	230	1.25	288
Feb. 13.	220	1.52	338
March 30	250	1.64	410
May 13.	220	1.6	350

Table 26.

Rhoscolyn

Date	Ave. No. of Eggs Per Mass.	Ave. No. Of Masses Per Mature Unparasitised ♀	Ave. No. of Eggs Per Mature Unparasitised ♀
May 22.1967	112	3.46	390
July 9.	134	1.78	237
Aug. 15.	197	1.09	214
Oct. 16.	125	0.24	30
Nov. 23.	172	1.77	305
Jan. 11.1968	172	2.44	420
March 18.	150	1.35	204
April 4.	167	1.64	275
May 16.	162	1.12	183

Table 27.

Trearddur Bay

Date	Ave. No. of Eggs Per Mass.	Ave. No. of Masses Per Mature Unparasitised ♀	Ave. No. of Eggs Per Mature Unparasitised ♀
June 6.1967	147	1.25	185
July 7.	138	1.14	155
Aug. 8.	151	0.283	43
Oct. 10.	158	0.28	42
Nov. 22.	240	0.51	122
Jan. 11.1968	210	1.7	355
Feb. 15.	217	2.4	510
April 1.	228	2.02	460
May 14.	220	1.2	220

Table 28.

Four Mile Bridge

Date	Ave. No. of Eggs Per Mass	Ave. No. of Masses Per Mature Unparasitised ♀	Ave. No. of Eggs Per Mature Unparasitised ♀
May 17.1967	200	1.80	360
July 5.	240	1.51	360
Aug. 15.	250	1.80	455
Oct. 3.	226	0.70	158
Nov. 15	200	1.17	234
Jan. 9.1968	245	1.66	405
Feb. 21.	280	1.44	400
April 1.	238	1.8	430
May 14.	240	2.67	640

Table 29.

Rhosneigr

Date	Ave. No. of Eggs Per Mass	Ave. No. of Masses Per Mature Unparasitised ♀	Ave. No. of Eggs Per Mature Unparasitised ♀
May 22.1967	197	1.00	197
July 8.	230	0.85	198
Aug. 15.	243	0.9	220
Oct. 2.	236	0.21	52
Nov. 23.	250	0.184	46
Jan. 11.1968	270	0.72	193
Feb. 27.	236	0.585	138
April 4.	230	0.31	73
May 14.	200	0.7	133

Table 30.

Variance Ratio Tables

Table 30a.

Variance ratio table of number of eggs per mass.

Source of Varation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Between Shores	40229	4	100.57	23.8
Residual	16874	40	421.8	
Total	67103	41		

Table 30b.

Variance ratio table of the number of masses per mature unparasitised female.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Between Shores	9.732	4	2.433	7.06
Between Seasons	11.967	8		
Residual	11.008	32	0.344	
Total	32.707	44		

Table 30c.

Variance ratio table of the number of eggs per mature unparasitised female.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Between Shores	425152	4	106288	7.4
Between Seasons	260210	8		
Residual	460887	32	14403	
Total	1246249	44		

Table 31.

Means, standard errors and confidence limits associated with the data in Tables 25 to 30.

Table 31a.

Number of eggs per mass.

Shore	Means	Standard Error	95% Limits	99% Limits
Black Rock	217	6.84	± 13.4	± 17.5
Four Mile Bridge	235.4	6.84	± 13.4	± 17.5
Rhoscolyn	154.5	6.84	± 13.4	± 17.5
Trearddur Bay	190.8	6.84	± 13.4	± 17.5
Rhosneigr	232.4	6.84	± 13.4	± 17.5

Table 31b.

Number of masses per mature unparasitised female.

Shore	Means	Standard Error	95% Limits
Black Rock	1.87	0.195	± 0.38
Four Mile Bridge	1.617	0.195	± 0.38
Rhoscolyn	1.54	0.195	± 0.38
Trearddur Bay	1.198	0.195	± 0.38
Rhosneigr	0.613	0.195	± 0.38

Table 31c.

Number of eggs per mature unparasitised female.

Shore	Means	Standard Error	95% Limits	99% Limits
Black Rock	402	40	± 78	± 103
Four Mile Bridge	382	40	± 78	± 103
Rhoscolyn	251	40	± 78	± 103
Trearddur Bay	232	40	± 78	± 103
Rhosneigr	139	40	± 78	± 103

Part 2b. Time taken for the eggs to hatch.

The only reference to hatching time for the eggs of L. obtusata is that of Thorsen (1946) who quotes hatching time as three to four weeks at 13-14°C.

To determine the hatching period at different times of the year the following procedure was adopted.

At Four Mile Bridge, chosen for its isolated boulders and lack of wave action, all the eggs were removed from the weed on one rock. The rock was revisited five days later to give time for more eggs to be layed. All the newly layed egg masses were marked by punching in the Fucus lamina next to the mass with a small round hole by means of a pair of leather punches. The rock was revisited at subsequent times to monitor the development of the eggs. More than one set of masses could be followed simultaneously by using different sized holes as markers. When one or more of the embryos had left the egg mass, the hatching was considered to have taken place but if all the embryos had left, the mass was considered to have provided only a maximum estimate of hatching periods. The results in Table 32 showed the development time to vary from 80 days in the winter to 18 days in the summer.

Table 32.

Number of days taken for L. obtusata eggs to hatch from the time of laying.

Approximate Date Laid	Days Taken to Hatch.
Dec. 13	79
Jan. 18	80
March 8	55
April 9	50
June 23	18

Part 2c. The fecundity of females.

(1) Method of judging the maturity of a female.

Sexual maturity of the females was judged by the condition of the oviduct, the capsule and accessory gland. If the oviduct was full of reproductive material and the glands swollen the females were jidged mature.

This method of judgement was tested by setting up a laboratory culture of animals in different stages of shell growth. The animals were grouped into four shell type classes as follows.

Class 1 Shell lip rounded off.

Class 2 Shell lip with no horney zone, but not rounded off.

Class 3 With a narrow horney zone at the shell lip.

Class 4 With a wide horney zone at the shell lip.

Each class was placed in a hanging culture tube with F. vesiculosus in the laboratory. The animals used were collected from Black Rock, Four Mile Bridge and Rhosneigr, a separate set of cultures made for the animals from each shore.

After sufficient time had been allowed fr the females to lay, the cultures tubes were opened and the number of egg masses in each were counted. The masses were always to be found on the Fucus and never on the glass sides of the tubes. The animals were then dissected and examined, and the number of females which had swollen oviduct and glands were counted.

Only those tubes (see Table 33, p.101) with at least some females whose oviducts and glands were swollen, contained egg masses. It was thus established that animals without swollen oviducts and glands were not mature. Whether all females with swollen ducts and glands were capable of laying eggs was not absolutely established, but it seemed a reasonable assumption that they were.

Throughout the year all females which were not parasitised, and which were fully grown, were found to possess swollen oviducts and glands.

As at all times of the year, egg masses were found on the shore, females, once mature probably continued to lay eggs until they died or became infected with parasites.

(2) Estimation of the number of eggs layed in a life time.

From the dissections of females of L. obtusata collected from five shores on Anglesey was found the weight at which sexual naturity occurred. The weights are shown in Table 34 (p.101)

Extrapolation of the graph plottings of Fig. 6 suggests the sizes shown in Table 34 were reached in July of the second summer on the shore by those animals hatching the previous summer. This is corroborrated by Fig. 14 (p.131) which shows an increase in the number of adults in the middle of the summer on most of the shores. The number of adults then decreases throughout the spring and early summer. The reason for these losses is uncertain, but wave action must be responsible for a large proportion of the deaths.

It was difficult to estimate the average life of a mature female as the breeding cycle is so prolonged, but probably a mature female is able to live for ten months, and under good conditons the time is almost certainly longer.

The choice of ten months as the life of a mature female was based on the variation of the number of adults per kilogram of Fucus (see fig. 14 p.131) which showed on most shores a decrease in adults in the early summer, with a subsequent increase in late summer.

Using the figures of the number of eggs per mature unparasitised female from Four Mile Bridge (Table 28, p.95), together with the times taken for the eggs to hatch at different times of the year (Table 32, p.99) it was possible to gain a rough estimate of the number of eggs laid per mature, unparasitised female per month (see Table 35, p.102) for the ten months from August to May.

Table 33.

Egg laying by L. obtusata in the laboratory.

Table 33a.

From Rhosneigr. Laying April 17 - May 6.1967.

Shell Class	Number of Animals	Number of Egg Masses	Number of Females with Swollen Oviduct and Associated Glands
1	60	16	28
3	60	10	25
4	60	0	0

Table 33b.

From Rhosneigr. Laying February 10 - 22.1967.

1	25	12	13
3	25	7	9
4	25	0	0

Table 33c.

From Black Rock. Laying April 8 - 18.1967.

1	57	15	18
2	63	8	18
3	30	5	10
4	45	3	6

Table 33d.

From Four Mile Bridge. Laying February 10 - 22.1967.

1	40	12	17
3	40	2	4
4	40	0	0

Table 34.

The weight at which females reach sexual maturity on five shores on Anglesey. The shores are arranged in order of decreasing exposure.

Shore	Weight when Maturing in Gms.
Rhosneigr	1.0 - 1.3
Rhoscolyn	0.9 - 1.2
Trearddur Bay	0.9 - 1.2
Black Rock	1.3 - 1.6
Four Mile Bridge	1.5 - 1.8

Table 35.

Approximate number of eggs laid per mature, unparasitised female per month at Four Mile Bridge.

Month	Number of Eggs laid Per Mature Unparasitised Female Per Month
August	1620
September	1620
October	210
November	120
December	150
January	150
February	150
March	180
April	240
May	3600
Total Eggs Per Life	8040

The Four Mile Bridge figures were used in deference to the smaller variation of the number of females present per Kilogram of Fucus (see fig. 14, p.131). On the other shores heavy wave action was liable to wash animals off the weed so injecting a certain inaccuracy into the estimate of the number of eggs per mature, unparasitised female.

Many mature females may live for longer or shorter periods than ten months. Mature females living through the whole summer when egg production was high may well have produced more than the estimated eight thousand eggs per life time. Conversely, females living for a shorter time, and the smaller females on some of the other shores probably laid less.

The figure for the fecundity of L. obtusata has been converted to calories by Graham (personal communication) the approximate value being 1,200 calories per female per life time. By comparison L. littorea produces 200 calories of eggs per annum at a shell height of 2.0 cms. and 400 calories at 2.3 cms. rising to 1.270 calories at 3.0 cms. (Graham unpublished)

As L. littorea continues to grow after sexual maturity, some of its calorific value is directed towards somatic growth, whilst L. obtusata which stops growing at sexual maturity presumably uses most of its calorific intake for the production of reproductive material.

In an average life time Graham calculated that L. littorea produces in the order of 600 calories. The figure is based on the observation that the dominent size groups is 2.3 cms. (5-6 years old) at Porth Cwyfan (Anglesey). Older animals and the larger animals on some of the other shores almost certainly produce more than this.

PART 3 HATCHING AND SUBSEQUENT GROWTH

Part 3a. Size of animals at hatching.

The size of animals hatching from the egg masses on a very sheltered shore where the adults were large, was compared with the size of animals hatching from masses on a fairly sheltered shore where the adults were significantly smaller.

The following proceedure was adopted. Egg masses were collected from the very sheltered shore at Rock (Cornwall) and the fairly sheltered shore at Polridmouth (Cornwall). Only egg masses from which at least some young had already hatched were used, to ensure all the masses chosen were at the same stage of development.

In the laboratory the young were dissected from their gelatinous investment, five animals being taken from each of eleven egg masses collected from each shore. In all, 110 animals were measured. The young animals were placed on a slide and and the longest axis of their shells measured with a micrometer eye piece to the nearest division (one division equals 16.6μ).

The young animals from Rock were found to have a mean longest shell axis of 523μ with a standard error of 22.7μ, whilst those from Polridmouth had a mean longest shell axis of 517μ with a standard error of 21.6μ.

The size of the young hatching from the egg on the two shores therefore showed no significent difference. The significant difference in size of the adults must be attributed to subsequent difference in growth rates.

If animals hatching at the same size can be assumed to grow from the same size eggs, then the larger females on the more sheltered shores, it may be concluded, lay the same size eggs, but lay them in larger masses. They also lay more eggs per unit time than the smaller females from the more exposed shores.

Part 3b. Growth of the young from exposed and sheltered shores using culture methods.

Introduction

The small mean sizes of the adults on fairly sheltered shores and on densely populated shores when compared with those inhabiting the more sheltered and less densely populated shores might be due to either genotypic or phenotypic effect or a mixture of both. The problem was investigated by culturing the young of both the large and the small adults under identical conditions.

Three approaches were made.

(1) Laboratory culture.

(2) Field culture methods:-

- (I) Culture in the Menai Straits hanging from a raft.
- (II) Culture in the Fowey estuary (a drowned river valley in Cornwall) ten feet down the anchor chain of a mooring buoy.

(1) Labortory culture methods.

Some difficulty was experienced in maintaining L. obtusata alive in the laboratory over long periods. This was due partly to the animals tendancy to climb the sides of the aquarium under the influence of their strong negative geotaxis. Even if escape was made impossible, the animals remained at the highest level in the aquarium.

Further difficulty was experienced with the food plant, Fucus vesiculosus, which rapidly decayed if it was left in contact with the bottom of the tank for more than two days.

The problems were largely overcome using a hanging culture technique (see fig. 7, p.40). The animals were kept in glass tubes (fig. 7a, p.40) a hundred mms. in diameter and three hundred mms. long. Rings were cut from P.V.C. tubing the internal diameter of which was approximatley a hundred MMS. Across the end of each ring was stretched course plankton netting and stuck in place with P.V.C. cement, supplied by Yorkshire Imperial Plastics. These rings formed lids with which to close both ends of the glass tubing.

Into the upper and lower lid were screwed two brass screws diametrically opposite each other. Nylon cord was attatched to the screws in the lower lid and fitted over the screws in the upper lid by means of a brass ring tied to its end. As the cord was just sufficiently long to fit over the brass screws, a slight twisting of the upper lid relative to the lower enabled the lids to form a firm seal on the glass tubing (see Fig. 7a, p.40).

When F. vesiculosus was required, pieces were wedged by means of their stipe, between the netting of the upper lid and the edge of the glass tube. As the weed was hanging downwardsin the tubes, it remained in a healthy condition for a long time.

The tubes containing weed and animals were hung with nylon cord from hooks suspended by means of nylon cord stretched between two members of a dexion frame-work. (Fig. 7b, p.40)

The seawater supply was maintained at a constant rate by means of a constant head device. The rate of flow was regulated by means of a clip attached to the rubber tubing. The water was fed to the culture tubes through rubber pressure tubing, in whose walls, eight holes were bored with a cork borer.

Into each hole was placed a short piece of glass tubing and over the glass tubing, was located a larger diameter glass tube which channeled the water onto the top of the culture tube, where it trickled through the plankton netting and into the culture tube. The system of concentric tubes allowed flexibility in the distance between culture tubes and the holes in the pressure tubing.

The tank was drained by means of a siphon. A clip attached to the outlet tube adjusted the rate of flow of water. It was possible by regulating the rate of inflow and out-flow of the water to produce a rise and fall in the water level of any desired frequency; although it was not possible to simulate the harmonic motion of the tides. In practice a fast flow of water minimised the tendency for the system to become blocked by debris.

The rise and fall of the water level was a great help in providing a flushing effect which maintained a fresh environment in the culture tubes.

Using this method, L. obtusata could be kept indefinitely in the laboratory. The only limiting factor was the reliability of the seawater system. The laboratory system contained sufficient suspended debris to periodically block the seawater taps. Such blockages were sometimes fatal to the cultures.

Eight cultures could be maintained simultaneously, and any tube removed for examination without disturbing the rest of the tubes. It was thus possible to make several collections on one field trip and maintain the animals in a healthy state until they were required.

Using this culture technique, L. obtusata showed no tendency to climb the sides of the culture tube, most of them remained on the weed, and were seen to crawl both upwards and downwards, even when crawling on the glass walls of the tubes.

Young of the small adults from Rhoscolyn were kept in one tube, and young of the large adults from Four Mile Bridge were kept in another. As the dimensions of the glass tube, the water supply and the amount of food available in both tubes were identical, it was thought advantageous to keep the animals separately as it would then be possible to tell when each set of animals began to lay eggs.

In each tube were a set of smaller young, and a set of larger ones, so providing a duplicate sample from each shore. The larger ones were marked to distinguish them from the smaller ones by means of a file scratch on the shell.

The cultures worked well for a while, but the intermittent and frequent blocking of the seawater system probably had an adverse effect on growth, and eventually was responsible for the death of the cultures.

The results are shown in Table 36 (p.107).

Animals from both shores maintained similar growth rates even through the winter; though in the field (see Fig. 6, p.34) those at Rhoscolyn grew less quickly in the winter than those at Four Mile Bridge.

There was a suggestion of faster growth by the larger group from Four Mile Bridge when compared with the similar group from Rhoscolyn, but the reverse was true for the smaller size groups. There was therefore no overall evidence to suggest that under similar conditions, the animals from Four Mile Bridge grew any more rapidly than those at Rhoscolyn.

Egg laying by the animals from both shores occurred between September 25th and October 25th, at which time the largest animals from Four Mile Bridge weighed

1.35 gms. and those from Rhoscolyn 1.0 gms. The animals from Rhoscolyn were therefore maturing at the normal size, but those from Four Mile Bridge were maturing at a smaller size than when in the field.

The laboratory conditons were not ideal, and were probably responsible for stunting the growth, the effect being no more pronounced than the field conditions at Rhoscolyn, but worse than the field conditions at Four Mile Bridge.

The time at which maturity was reached seems to depend on some factor other than size.

Methods 2 and 3 Field culture methods.

For culturing L. obtusata both on the raft in the Menai Straits andon the anchor chain of the buoy at Fowey; the young winkles were placed in plastic netting bags of 2mm. mesh, whilst closed by being stitched with nylon string.

The single bag used on the raft in the Menai Straits contained young winkles from Trearddur Bay (small adults) and Four Mile Bridge (large adults). Three size groups were used from each shore, and were marked with file marks to distinguish them.

The experiment lasted only three weeks. The results are shown in Table 37 (p.107).

Two bags were used in the culture at Fowey. The young L. obtusata used were from Polridmouth (small adults) and Golant (large adults).

Three size groups were used from each shore and two bage were used so that all the samples were duplicated. The animals were not sampled as frequently as was desirable, because each weighing involved two diving outings which had to be arranged when shipping was not occupying the buoys.

The results are shown in Table 38. (p.108).

The results from the two growth experiments show a slight tendency for the young of large adults to grow more rapidly than those of small adults though the results were not conclusive. In the Fowey bag experiment many of the smaller ones died, whether this was due to the effect of small crabs or to other causes was uncertain.

The very small difference in the growth rates between young of large and small adults was insufficient to account for the adult size difference.

Comparative growth rates in the field during the winter months were however lacking.

An interesting decrease in the growth rates in the Fowey occurred between July 16th and August 25th. The F. vesiculosus being used as food had become encrusted with various Polyzoa and colonial Ascidians which covered as much as 90% of the frond surface. The effect on the winkles ability to obtain their food must have been deleterious. This observation leads to the suspicion that L. obtusata was excluded from low levels of shore by competition for weed by encrusting forms.

Table 36.

Rate of growth in the laboratory in percent increase in weight per week of L. obtusata from Rhoscolyn and Four Mile Bridge. Numbers in brackets refer to average weights in gms.

Date	Larger Group of Young		Smaller Group of Young	
	Four Mile Bridge	Rhoscolyn	Four Mile Bridge	Rhoscolyn
5/5/67	(.37)	(.32)	(.16)	(.11)
19/5	9.5 (.45)	9.1 (.38)	14 (.21)	12.3 (.15)
7/6	4.3 (.49)	7.6 (.46)	6.9 (.25)	10.0 (.19)
23/6	9.4 (.61)	7.9 (.55)	13.6 (.34)	13.8 (.26)
5/7	12.3 (.74)	7.4 (.62)	15.4 (.43)	10.4 (.31)
14/8	6.0 (1.03)	5.0 (.82)	6.8 (.63)	8.1 (.48)
25/10	1.4 (1.19)	1.0 (.91)	2.4 (.82)	3.1 (.66)
11/11	2.1 (1.26)	3.2 (.98)	2.8 (.88)	3.0 (.71)

Egg laying commenced between September 25th and October 25th with the animals from both shores.

Table 37.

Rate of growth on the raft in the Menai Straits, in percent per week of L. obtusata from Tearddur Bay and Four Mile Bridge.

From Trearddur Bay

	July 30	Aug. 22	Percent Increase per Week
Average weight in Gms. small group	0.30	0.37	6
Average weight in Gms. large group	0.40	0.48	5

From Four Mile Bridge

	July 30	Aug. 22	Percent Increase per Week
Average weight in Gms. small group	0.30	0.37	6
Average weight in Gms. large group	0.40	0.52	7

Table 38.

Rate of growth in the two bags in the Fowey estuary, in percent per week of L. obtusata from Polridmouth and Golant. Numbers in brackets refer to average weight of each size group. in gms

First Sample.

	June 17	July 16	Aug. 25
	(0.088)	9.5 (0.128)	0.7 (0.133)
From Golant	(0.20)	8.4 (0.28)	6.3 (0.41)
	(0.30)	11.2 (0.465)	2.8 (0.546)
	(0.105)	7.6 (0.143)	- -
From Polridmouth	(0.27)	7.9 (0.37)	0.6 (0.383)
	(0.39)	6.3 (0.51)	2.4 (0.586)

Second Sample.

	June 17	July 16	Aug. 25
	(0.079)	11.2 (0.123)	- -
From Golant	(0.20)	11.3 (0.312)	2.0 (0.35)
	(0.42)	10.2 (0.627)	1.4 (0.68)
	(0.125)	- -	- -
From Polridmouth	(0.23)	13.0 (0.38)	- -
	(0.43)	8.4 (0.605)	3.6 (0.74)

Table 38b.

Rate of growth three feet above mean sea level on the pier piles at Menai Bridge in percent per week of L. obtusata from Trearddur Bay. Figures in brackets refer to the average weight of each size group. in gms

	Feb. 21	April 8	May 5	June 14
Group 1 (6 animals)	(0.84)	8.5 (0.144)	14.2 (0.24)	11.7 (0.45)
Group 2 (10 animals)	(0.151)	8.5 (0.255)	13.0 (0.41)	10 (0.70)
Group 3 (10 animals)	(0.35)	4.2 (0.46)	7.6 (0.61)	9.4 (1.01)
Group 4 (8 animals)	(0.55)	3.8 (0.70)	6.8 (0.90)	11.7 (1.17)

TABLE 38 C

As TABLE 38, but animals collected from Polridmouth (small adults) and ROCK (LARGE ADULTS) TO COMPARE WINTER and SPRING growth

	Feb 24	March 25	JUNE 21
FROM ROCK	(0.197)	2.3 (0.216)	5.7 (0.460)
	(0.370)	3.9 (0.43)	4.0 (0.750)
FROM POLRIDMOUTH	(0.192)	3.7 (0.222)	2.3 (0.370)
	(0.375)	7.6 (0.505)	3.5 (0.820)

A further growth experiment was conducted whose purpose was threefold. Firstly, to compare the winter and summer growth rates of animals from the same shore, secondly, to compare the growth rates of different size groups and thirdly to act as an check in the graphical analysis of populations growth in the field.

On February 21st, 1968, L. obtusata of various sizes were collected from the upper F. vesiculosus zone at Trearddur Bay. They were sorted into four size groups and marked with file scratches to distinguish them. Each size group was weighed and placed with F. vesiculosus in a plastic netting bag of 2 mm. mesh and tied and to the pier piles of Menai Bridge town pier approximately three feet above mean sea level. The animals were removed periodically from the bags and weighed, at the same time fresh F. vesiculosus was introduced. The experiment continued till just after June 14th when the bags were found to have been slit open.

Table 38b (p.108) shows the growth rate during the summer to be greater than during the winter and early spring, and the growth of the smaller size groups to be proportionately about twice that of the larger groups. The growth rate of the larger groups during the period from February 21st to April 8th corresponded very well with the growth rate during the winter of the animals in the field at Black Rock and Four Mile Bridge. The growth rate was considerably greater than that of the animals in the field at Trearddur Bay, lending support to the theory that wave action may have been responsible for the slow winter growth rate at Rhoscolyn, Rhosneigr and Trearrdur Bay.

Part 4. The population structure

The numbers of animals per Kilogram of weed were plotted in the form of a cumulative frequency on arithmetical probability graph paper. This paper, devised by Hazen (1916) and described for biological work by Harding (1949) has rulings designed to convert the sigmoid cumulative frequency curve of a normal distribution to a straight line whose position is determined by the mean and slope by the standard deviation of the normal distribution. Polymodal distributions, provided each mode is basically normal, result in a cumulative frequency plot which is the resultant of two or more straight lines. The straight lines are usually easy to plot in if the population modes are sufficiently distinct. By first dividing the curve with line running vertically through the points of inflection, the plots within each pair of vertical lines may then be treated as a separate population and plotted as such on the graph paper. Each of the separate populations, being normally distributed will form a straight line plot.

The method may be criticised owing to the arbitrary method of determining the points of inflection. For polymodal populations, however, the graphical method provides a useful analysis if complete accuracy in assessment of mean and standard deviation is not required.

The cumulative frequency curves for populations of L. obtusata are shown in Fig. 15-24 (p.132 to 141). The curves were polymodal, though the modes were ill defined, there being only well defined points of inflection representing the change between the growing and the adult populations. The poor definition of the modes within the growing population reflected the ill defined breeding season of L. obtusata.

The typical curve between October and April for the total population of animals still growing showed a more or less straight line component representing the animals hatched since the summer. This was followed by a change in slope of the line, which was interpreted as representing the population of young animals hatched in the summer. The summer hatched individuals formed a distinct population as represented on the graphs (fig. 15 - 25, p.324w) because of the high proportion of the animals hatching in the summer months (see fig. 13, p.93).

The shape of the curves from the lower F. vesiculosus and upper F. serratus zones was unreliable owing to the almost certain presence in the samples of young L. mariae. The following discussion therefore applies only to the upper F. vesiculosus zones, where the young L. mariae were assumed to be absent as the adults were seldom found.

The summer curves showed an influx of young forming up to 90% of the total numbers of L. obtusata. The estimate of young was almost certainly too low, many of the smaller specimens being missed as they were living in bladders or were lost in the sand of the shore during collection.

Throughout the autumn and winter the typical curve represented a tri-modal population. A population of young animals which was continuously being augmented by recruitment from late hatching eggs. A population of older animals, the survivors of the great summer production of young, and finally a population of adults and near adults which were now at least a year old.

There was considerable blending of the three populations, the middle often being almost obscured by the late hatching young. During the winter little change occurred at Rhoscolyn, Rhosneigr and Trearddur Bay, possibly owing to wave action. Considerable growth however took place in both the lower and middle populations at Black Rock and Four Mile Bridge.

During the spring, all populations on all the shores continued to grow till the middle population merged with the upper one in the early summer, leaving a bimodal population throughout the summer months.

The largest number of L. obtusata hatched in the summer. These animals matured in the following summer and died probably after continuously breeding, in the following spring and early summer.

Because of the great blending of the different populations, it was not possible to measure the growth rate throughout the life time of the animals, though the growth of the middle population was able to be followed between October and May (see Table 9b, p.32 and Fig. 6, p.34).

Migration of particular size groups probably did not occur. None of the cumulative frequency curves from any level on the shore showed any loss or gain of one particular size group contemporary with a complementary change at any other level.

There was however undoubtedly a mass movement of animals upwards, probably under the influence of wave action, this could not be shown by the cumulative frequency curves, but was demonstrated at Trearddur Bay in the following manner.

The upper part of the intertidal zone at Trearddur Bay is composed of shingle, the lower part of the shingle being well within the F. vesiculosus zone but bare of weed.

A large boulder (weight 40 kgms. approximately) bearing a good growth of F. vesiculosus was deposited on the shingle on March 20th, 1968 after being cleared of winkles. The boulder was revisited on April 4th when 112 adult L. obtusata were removed, and again on May 14th when 76 were removed. These animals were all alive, and could only have been washed upwards from lower levels. Though Barkman (1955) suggests L. obtusata will only leave the clump of Fucus if it can scent another, all the water bearing scent from the weed covered boulder would have percolated down through the shingle and not have reached the main population of L. obtusata. Those animals moving upshore onto the boulder could not therefore have been moving under the guidance of scent.

Had the boulder not been in place the animals would have been washed onto the strand line. The total of 188 therefore represented potentially dead animals; strengthening the view that wave action is the greatest mortality factor on some shores.

PART 5. VARIATION IN THE POPULATIONS.

The data for the number of adults (fig. 14, p.131) and the number of eggs and young (fig. 13, p.93) per kilogram of Fucus suggested there was less variation from month to month at Four Mile Bridge than on any of the other shores. Each of the three groups of data (numbers of adults, eggs and young) were normalised by dividing each monthly value by the yearly mean (from May 1967 - May 1968). The variance was calculated from each shore for each group of data. The variances are shown in Table 39 (p.113). No significance test was performed as there were good reasons for supposing the original data might not be strictly normal, nevertheless inspection established the variance of the Four Mile Bridge data to be low for each of the three groups.

The reasons for the small variance of the Four Mile Bridge data were likely to have been twofold. Firstly, the mortality of the young was probably high in the summer owing to the anoxicus conditions, secondly, the mortality of the adults in winter was not affected by wave action, and thus was less markedly seasonal.

In this respect the low variance of the number of young and the number of adults at Rhosneigr was incongruous. It must be remembered however that many of the young took refuge in bladdars and were missed, whilst the numbers of adults lost in the winter would have been minimised by the very dissected nature of the rock surface.

Table 39.

Variances of the number of eggs, young and adults per kilogram of *Fucus* on five shores on Anglesey, between May 1967 and May 1968.

As all the data have been normalised, the variances within each column are comparable.

	Variance of Number of Eggs.	Variance of Number of Young.	Variance of Number of Adults.
Four Mile Bridge	0.07	2.14	0.12
Black Rock	0.06	2.22	0.24
Rhoscolyn	0.75	5.97	0.62
Trearddur Bay	0.38	7.25	0.24
Rhosneigr	0.42	2.77	0.20

PART 6. AVAILABILITY OF FOOD AS A LIMITING FACTOR.

The adults of L. obtusata on the second (continuous) reef at Trearddur Bay were particularly small (see fig. 5, p.31) the mean weight being 1.1 gms. The population was also more dense than on any other observed shore on Anglesey. (see Table 42, p.118) There seemed a possibility that the small size was partly at least due to lack of food. The food plant (F. vesiculosus) looked in very poor condition, many of the fronds having been completely stripped of lamina except at the very apex.

The rate of consumption of the food plant by L. obtusata, and the rate of production of new fronds was estimated.

Growth of F. vesiculosus.

Ynys Faelog was chosen for the measurement because the population of L. obtusata was sparse. As the fronds were marked by means of small round holes, it was advantageous to have as few extraneous holes as possible produced by the feeding winkles.

The rate of growth of fronds of F. vesiculosus was measured as follows. Several fronds were marked with a small round hole three mms. in diameter and ten mms. from the apex with a pair of leather punches. The distance of each hole from the apex was measured at intervals of time to determine the rate of growth at different seasons of the year. The measurements were performed on plants both in the upper and lower F. vesiculosus zones for comparison.

Growth of the plants in the lower zone (fig 26, p.115) was considerably greater than growth in the upper part. Growth was also greater in the summer than in the winter.

Consumption of F. vesiculosus by L. obtusata.

All the L. obtusata found in 2.8 kgms. of F. vesiculosus from Trearddur Bay were placed together with one kilogram of F. vesiculosus in a plastic netting bag of two mm. mesh. The total weight of the winkles was 390 gms. The top of the bag was closed by stitching with nylon string. The bag could therefore be opened easily when required. The F. vesiculosus chosen for the bag always consisted of clean, non fruiting fronds, as the presence of fruiting fronds produced deposits of rotting conceptacles in the bottom of the bag. A similar control bag was filled with one kilogram of F. vesiculosus but with no winkles and closed in a like fashion.

Both bags were tied to the fixed piles of Menai Bridge pier, approximately three feet above mean sea level. From time to time the bags were removed, the Fucus weighed and replaced with fresh weed. The total weight of the animals was adjusted to 390 gms. by removing some of the larger ones.

The weight of F. vesiculosus consumed by 100 gms. of L. obtusata per day is shown in Table 40 (p.117) and was calculated from the difference in weight between the experimental and control weed.

Figure 26.

The length of a piece of F. vesiculosus frond typical of the upper and the lower F. vesiculosus zone to show the seasonal growth.

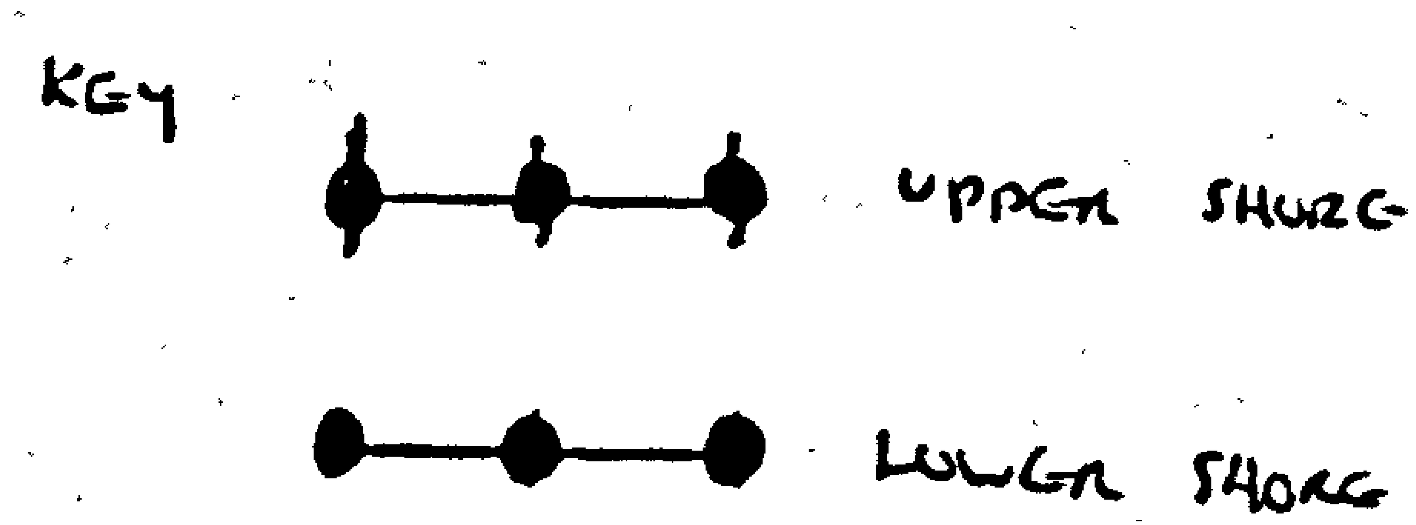
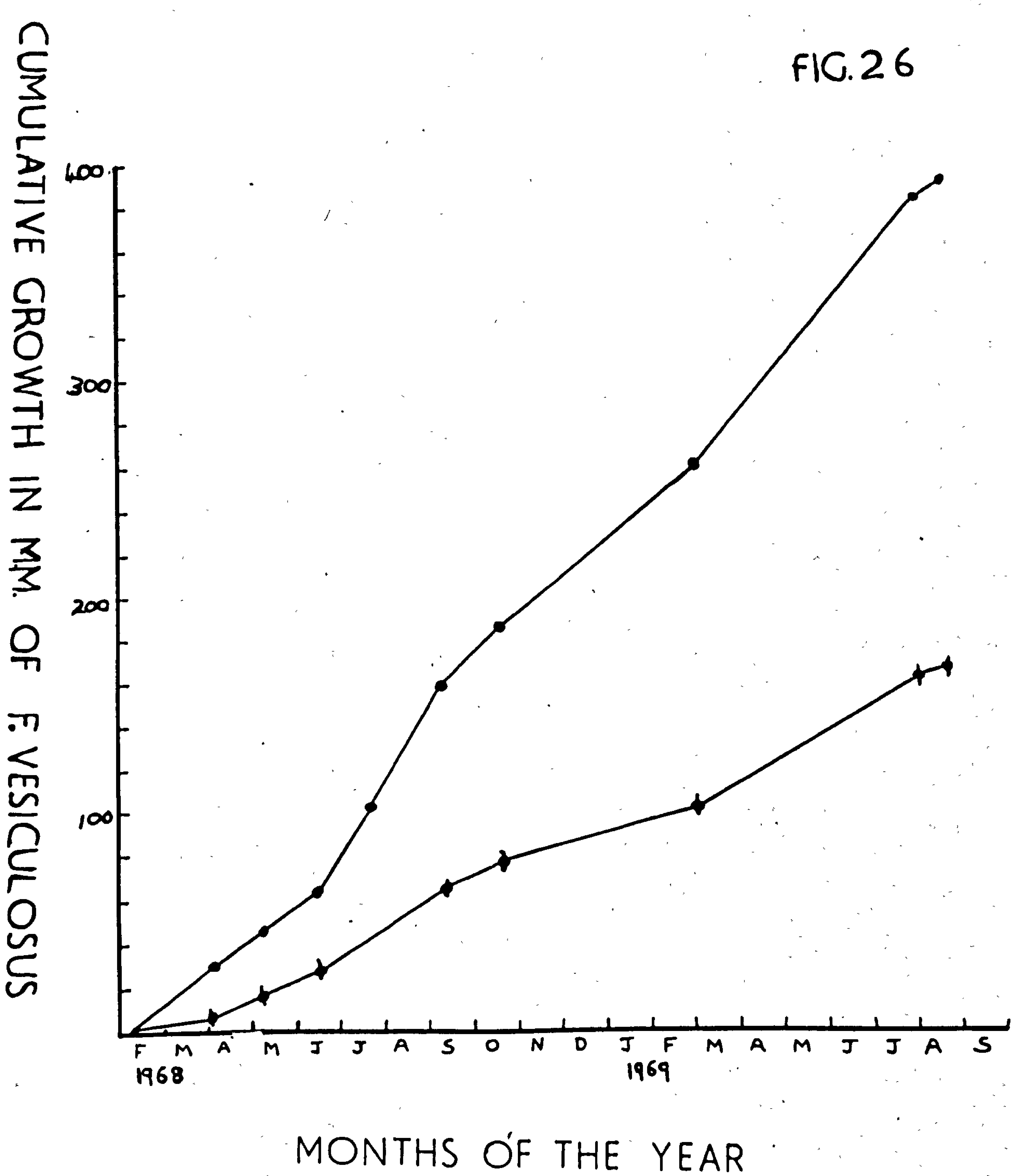


FIG. 26


CUMULATIVE GROWTH IN MM. OF F. VESICULOSUS
400
300
200
100
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
1968
1969
MONTHS OF THE YEAR

To estimate the total weight of lamina consumed by the L. obtusata on 1 Kgm. of Fucus at Trearddur Bay, the following method was adopted.

Observation showed L. obtusata to eat only the lamina of the frond, and to leave the midrib intact. As only the lamina was used for food it was the weight of lamina consumed which was considered pertinent.

Throughout the year samples of F. vesiculosus were taken from the upper F. vesiculosus zone on the second shore at Trearddur Bay. The weed was weighed and the number of actively growing apices counted. Fruiting apices and the very minute apices of the regeneration squamules were not included. The number of apices per K.gm of F. vesiculosus at Trearddur Bay is shown in Table 41 (p.117). The weight of lamina per unit length of frond was estimated by selecting several intact fronds, measuring the total length of fronds, cutting the lamina from the middle and weighing it separately. It was thus possible to use the figures of frond growth from the upper zone at Ynys Faelog to estimate the total weight of lamina produced by one K.gm of Trearddur Bay weed per day, see Table 43 (p.118).

Using the figures of density of animals per K.gm of weed at Trearddur Bay (see Table 42, p.118) and the weight of weed consumed in the bags under Menai Bridge pier, it was possible to calculate the probable weight of weed eaten by the winkles living in one K.gm of weed at Trearddur Bay.

The results of the comparison of consumption and production are shown in Table 43 (p.118).

During the winter months production was seen to exceed consumption, but during the summer months the reverse was true. Whilst it should be remembered that the figures are approximate, they do show food production was probably not far in excess of consumption, and may in fact have been less.

When all the additional loss of the food by wave action and other factors is taken into consideration, together with the probable inefficiency on the part of the winkle in finding the remaining edible parts of the plant, it appeared highly probable that on the second reef at Trearddur Bay, food was a limiting factor in the L. obtusata population.

If food was a limiting factor, it might explain the very small size of adults on that shore, their mean size was smaller even than that of the adults living on the first shore at Trearddur Bay only twenty meters away, (the adult mean weights being 1.1 gms and 1.3 gms. respectively) and exposed to the same degree of wave action.

Table 40.

Daily rate of consumption of <u>F. vesiculosus</u> in grams, by 100 gms. of <u>L. obtusata</u>.

Period	Gms. of Fucus/day/100 gms. of L. obtusata.
Feb. 21 - March 6	0.810
March 6 - April 8	1.9
April 8 - May 5	3.5
May 5 - June 14	2.4
July 30 - Aug. 23	3.6

Table 41.

Number of growing apices per K.gram of <u>F. vesidulosus</u> from the second shore at Trearddur Bay.

Date	
Sept. 13.	4,300
Dec. 8.	4,270
March 4.	4.100
July 30.	3,250

Table 42.

Density of L. obtusata, in gms, per K.gram of F. vesiculosus on the second shore at Trearddur Bay.

Date	Density in gms/K.gm of F. vesiculosus.
Aug. 14	202
Nov. 22	130
Feb. 15	82
April 1	90
May 14	150

Table 43.

Comparison of the rate of production (in gms/day) of one K.gm of F. vesiculosus on the second shore at Trearddur Bay with the rate of consumption by L. obtusata (in gms/day) found in one K.gm of F. vesiculosus on the same shore.

Date	Production	Consumption
Feb. 21 - March 6	2.38	0.65
March 6 - April 8	2.55	1.7
April 8 - May 5	4.1	4.2
May 5 - June 14	3.15	3.7
July 30 - Aug. 23	4.05	8.2

PART 7. THE DIGENEAN PARASITES.

Introduction

Since the work of Pelseneer (1906) our knowledge of the taxonomy and occurrence of the digenean parasites of the Littorinidae has been increased by several workers (Lebour 1911, Rees 1932 and 1935 and Stunkard 1932 and 1957) whilst James in a great volume of work (James 1964a, 1968a, 1968b, 1968c and 1968d) concentrated on elucidating the life cycles of the parasites and hence the true identity of many of the cercariae, finally producing (James 1968e) a key to the digenean parasites of the Littorinidae at Dale, Pembs.

The relation between molluscan host and digenean parasite has been described by many workers. The host tissue reactions have been described by Pan (1963) and James (1965), whilst the effect on the molluscan biochemistry and cell physiology was considered by Rees (1936) Cheng and Snyder (1962 and 1962a), Pan (1965) and James (1965). The cells of the digestive gland were found by all workers to suffer, an effect attributed by James (1965) to the starvation of the cells due to digestive tubule collapse under pressure from the mass of parasite tissue. The loss of glycogen and increase in neutral fats have been noted by both Cheng and Snyder (1962) and James (1965). The former considers the effect, the direct enzymatic effect of the parasite to be responsible whilst James favours lack of oxygen in the affected areas as the better explaination.

The present work considers the effects on the size of L. obtusata of parasitism by members of the digenea, the relative susceptibility of the sexes to infection, and the distribution and seasonal fluctuations of five digenean species on Anglesey.

The final size attained by parasitised Peringia ulvae (Pennant) has been found to be abnormally large (Rothschild 1936) whilst Pan (1965) discovered the effect of Schistosoma mansoni was to increase the growth rate of Australorbis glabratus whilst the parasite was immature, but stunted the growth after cercariae had began to emerge.

Most workers agree that the effect of the digenea is in many cases fatal to the molluscan host, though Mayerhof and Rothschild (1940) kept in captivity a specimen of Littorina littorea for five years whilst infected with Cryptocotyle lingua (Creplin).

Females of Peringia ulvae were found by Rothschild (1938b) to be infected more frequently than the males, the infection ratio often being as high as 16 to 1, though as the author conceds, sex reversal may have confused the issue; apparantly one gravid female was found to possess a penis.

A regular seasonal rhythm in the infestation rate by the digenea has been reported by many (Kemp and Gravely 1919; Manson-Bahr and Fairley 1920; Soparker 1921; Rees 1932; and Probert 1966) the latter considering the peaks and depressions in the infestation rate of Bithynia tentaculata and Lymnaea stagnalis to be the result of the interaction of the life cycles of host and parasite. Seasonal occurrence of parasites in marine mollusca has not been investigated, and as all the work suggesting seasonality was performed with freshwater molluscan hosts, the present work was considered timely.

Mixed infections of parasites have been found by Probert 1966 to be exceedingly rare, the reason he believes is possibly due to the host tissue reaction preventing the development of a second miracidium, but conceeds that the host may also lose its attractiveness.

Mixed infections are claimed to represent the result of simultaneous penetration by miracidia of two species.

Methods

For the methods adopted see p.88. The parasite species were identified with the help of the key of James (1968e) and Dr. B. L. James himself.

Part 3a. Susceptibility of the sexes.

Out of 10,893 animals dissected 5,605 were females and 5,288 males. (X^2 significant to 99% probability level) showed females to be more common than males (see also Sacchi and Rastelli (1966) who reached the same conclusion). The infestation rate of the adult females was 18.6% and of the males 18.0%. There was therefore little evidence to suggest a higher incidence of parasitism in the females. There was also no evidence to suggest parasites caused a sex reversal in either direction. Females always possessed at least traces of the capsule and accessory gland and no penis, whilst the males possessed a penis, even if only a small one, and no trace of a capsule or accessory gland.

The size of the penis in infected males and also the size of the capsule and accessory gland in the infected females varied widely, seeming to bear little relation to the severity of the infestation. It can only be suggested that the maturity of the winkle at the initial entry of the parasite was responsible for the difference. If infected before maturity the glands and penis never develope beyond a rudiment, but if infected after maturity, whilst considerable regression takes places, they never become as small as before maturity.

Part 3b. The effect on growth and size.

The method of dividing the animals into size groups with a set of graded sieves, resulted in obtaining a set of adults of large size and a set of adults of small size. The infestation rate (Table 44a, p.123) on all five shores was higher amongst the large adults than amongst the small ones. Table 44b (produced as a result of the work of John Cotter for an honours project) confirmed these findings for Church Island in the Menai Straits.

If the animals were growing large as a result of parasitism, it should have been possible to demonstate in the size range occupied by the medium and small sized adults, a greater infestation rate amongst those still growing (near adults) compared with those which had ceased growth (adults) as judged by the nature of the shell lip.

Unfortunately the group of near adults included some animals which were immature hence young and only lightly parasitised. These acted to damp down the effect being sought. Nevertheless Table 45 (p.124) showed on all the shores the higher rate of parasitism amongst the near adults than amongst the adults.

There was thus strong evidence to suggest a growth stimulating effect of the parasites. Whether growth was stimulated as suggested by Pan (1965) or whether the absence of the onset of sexual maturity resulted in the absence of the growth checking stimulus was uncertain.

Part 3c. Seasonality.

The percent infestation of the adult animals throughout the year in Anglesey is shown in Fig. 27 (p.122). It must be remembered that fluctuation in infestation rate is almost certainly compounded of the parasite life cycle, the host life cycle and the movements of the definitive host (usually a gull or wader). Good correlation between the shores of all the variations throughout the year was therefore not to be expected.

Fig. 27 (p.122) should be compared with Fig. 14 (p.131) which shows the density of adult L. obtusata on the five shores throughout the same period, and sampled on the same dates.

Except at Rhosneigr, the upper F. vesiculosus zones of all the shores studied showed a decline in the infection rate in April and May, followed by a summer rise and an October depression. This pattern followed closely that described for the infestation of Bithynia tentaculata in a South Wales lake by Probert (1966). Reference to Fig. 14 (p.131) shows the April depression coincided with the depression of adults, suggesting the parasitised adults were dying before the healthy ones. The subsequent peak may be explained partly by the death of the healthy adults and perhaps partly by an increase in miracidial activity, and the October depression by recruitment from the ranks of the lightly parasitised young.

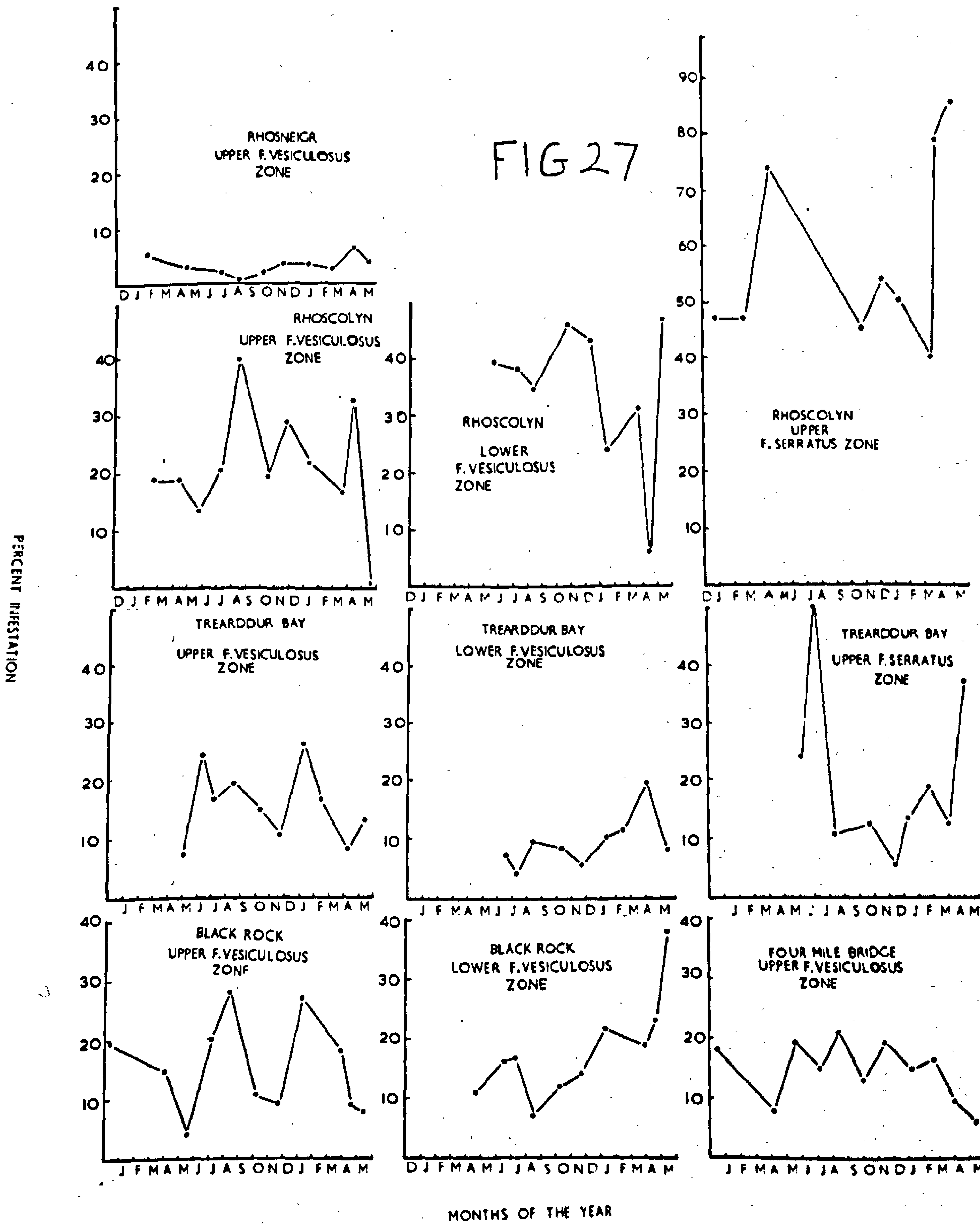
The lower F. vesiculosus and upper F. serratus zones at Trearddur Bay followed this pattern, but less clearly. The upper F. serratus zone at Rhoscolyn showed the October depression in 1967 but the rest of the pattern was not consistent, likewise the whole of the pattern from the lower F. vesiculosus zone at Rhoscolyn.

It may be concluded that either the coincidence of the pattern in the upper F. vesiculosus zones and over the whole shore at Trearddur Bay was spurious, or that the patterns showed a basic seasonality, but other factors were obscuring the pattern on the mid and lower shore at Rhoscolyn.

Figure 27.

The rate of infestation of L. obtusata by digenean parasites on five shores in Anglesey. The rate of infestation is expressed in percent of the total adult population.

FIG 27



PERCENT INFESTATION
RHOSNEIGR UPPER F. VESICULOSUS ZONE
RHOSCOLYN UPPER F. VESICULOSUS ZONE
RHOSCOLYN LOWER F. VESICULOSUS ZONE
RHOSCOLYN UPPER F. SERRATUS ZONE
TREARDDUR BAY UPPER F. VESICULOSUS ZONE
TREARDDUR BAY LOWER F. VESICULOSUS ZONE
TREARDDUR BAY UPPER F. SERRATUS ZONE
BLACK ROCK UPPER F. VESICULOSUS ZONE
BLACK ROCK LOWER F. VESICULOSUS ZONE
FOUR MILE BRIDGE UPPER F. VESICULOSUS ZONE
MONTHS OF THE YEAR

Table 44a.

Percent infection with digenean Trematode larvae of both small and large adults of L. obtusata on five shores in Anglesey.

F.v.z. = Fucus vesiculosus zone   F.s.z. = Fucus serratus zone.

Shore	Percent Infection Large Adults	Small Adults
Black Rock (upper F.v.z.)	15.5	11.5
Black Rock (lower F.v.z.)	14	5
Four Mile Bridge (upper F.v.z.)	56	10
Rhoscolyn (upper F.v.z.)	48	25
Rhoscolyn (lower F.v.z.)	41	23
Rhoscolyn (upper F.s.z.)	55	40
Rhosneigr (upper F.v.z.)	5.6	0.8
Trearddur Bay (upper F.v.z.)	32	12
Trearddur Bay (lower F.v.z.)	21	64
Trearddur Bay (upper F.s.z.)	25	10

Table 44b.

The infestation rate of adult L. obtusata of different shell lengths by digenean parasites on the upper F. vesiculosus zone at Church Island, Menai Straits, Anglesey, a very sheltered shore. Data due to Cotter (1969).

Shell length in mm.	Percent Infected.
7-9	2
9-11	10
11-13	14
13-15	12
15-17	21
17-19	81

Table 45.

Percent infection with digenean Trematode larvae of adults and near adults in the size range of the smaller adults on five shores on Anglesey.

F.v.z. = *Fucus vesiculosus* zone F.s.z. = *Fucus serratus* zone.

Shore	Percent Infection: Adults	Percent Infection: Near Adults
Black Rock (upper F.v.z.)	15	26
Black Rock (lower F.v.z.)	16	29
Four Mile Bridge (upper F.v.z.)	10	21
Rhoscolyn (upper F.v.z.)	26	28
Rhoscolyn (lower F.v.z.)	41	60
Rhoscolyn (upper F.s.z.)	55	79
Trearddur Bay (upper F.v.z.)	11	12.5
Trearddur Bay (lower F.v.z.)	6.1	11
Trearddur Bay (upper F.s.z.)	24	56
Church Island (upper F.v.z.)	8	17.2

The Church Island data due to Cotter (1969).

Little can be said with certainty, but in the light of the occurrence of seasonality in fresh water digenean parasites, the pattern seen on the upper F. vesiculosus zones at Black Rock, Four Mile Bridge, Rhoscolyn and the whole shore at Trearddur Bay may well have represented genuine seasonality.

Since the completion of this work my attention was drawn to a paper by James (1969) who discusses the seasonality of the digenean M. similis in L. rudis. It is interesting to note the similarity in the seasonal pattern of infestation rate between L. rudis and L. obtusata on those shores which showed similarity in the seasonal pattern. James ascribes the seasonal pattern entirely to the breeding cycle of the host. Why a similar pattern should occur in a host with a different breeding cycle is uncertain.

Part 3d. The distribution and relative abundance of digenean species.

The percent infestation by three parasite species was recorded throughout the year on five shores in Anglesey. The species involved were Microphallus similis (Jagerskiol), Cryptocotyle lingua (Creplin), Cercaria lebouri (Stunkard).

Other species of digenea were also encountered, some frequently others rarely, they were however not identified with certainty. The more common of these fell into two groups which may be named groups A and B. Group A included all the cercariae of the opecoelid cotylomicrocercous type whilst group B contained echinostome cercariae of the group typified by the genus Himasthla.

The distribution and relative abundance of the parasites, see Table 46 (p.126) shows Microphallus similis to be the most common digenean parasite on all the shores, ranging from 77.7 % to 98.7% of the total parasite infestation. At Black Rock on both the upper and lower F. vesiculosus zones it was present almost to the exclusion of all other species. Group A (cotylomicrocercous cercariae) were found to comprise between zero and 15.2% of total infestation, and there was a suggestion that they were more important lower on the shore. The other three species together represented 4.98% of the total number of infestations examined. Whilst the numbers of those three species were too low to allow concrete conclusions to be drawn as to their distribution, there was a suggestion that Cryptocotyle lingua favoured Four Mile Bridge whilst Cercaria lebouri was found more commonly at Trearddur Bay. The reason for the different distribution of the species was not clear, but probably depended upon the movements of the definitive hosts.

Part 3e. Mixed Infections.

Mixed infection were found to be rare. During the study, a total of fourteen mixed infections were found. The only mixtures counted were of Microphallus similis and any one of the other species. The sporocysts of M. similis were small and oval whilst the sporocysts or rediae of all the other species were larger and elongate. Thus under the binocular dissecting microscope mixed infections were easily detected and were not likely to be overlooked.

Table 46.

The relative abundence of five species of digenetic Trematode larvae on five shores in Anglesey.

F.v.z. = *F. vesiculosus* zone   F.s.z. = *F. serratus* zone.

Shore	Percent of Total Parasite Infections					Total Number of Infections
	Microphallus Similis	Group A	Group B	Cryptocotyle Lingua	Cercaria Lebouri	
Black Rock (upper F.v.z.)	95	3.3	0.85	0.85	0	118
Black Rock (lower F.v.z.)	98.7	0	0	0	1.3	77
Four Mile Bridge (upper F.v.z.)	77.7	13.7	3.45	5.15	0	233
Four Mile Bridge (lower F.v.z.)	80	15	2.5	2.5	0	40
Rhoscolyn (upper F.v.z.)	90	4.2	4.9	0.55	0.25	365
Rhoscolyn (lower F.v.z.)	88.6	7.5	2.25	1.13	1.52	267
Rhoscolyn (upper F.s.z.)	89.5	8.34	1.54	0.31	0.31	325
Rhosneigr (upper F.v.z.)	83.7	14	0	2.3	0	43
Trearddur Bay (upper F.v.z.)	86.2	7.64	1.9	0.46	3.8	210
Trearddur Bay (lower F.v.z.)	81.4	15.2	1.7	0	1.7	118
Trearddur Bay (upper F.s.z.)	79	12.2	4.05	0	4.75	147
Total Percent	86.75	8.27	2.62	1.13	1.23	1943

whereas looking for a second species of elongate larva amongst an infestation of another would have been a difficult process susceptible to inaccuracy.

A chi squared test was conducted to determine whether the number of mixed infections was significantly lower than would have been expected on the basis of random assortment of the parasite species amongst their hosts. The calculations were based on the combined data from all the shores except that at Rhosneigr.

Number of <u>L. obtusata</u> infected with <u>M. similis</u>	1689
Number of <u>L. obtusata</u> infected with any other Trematode	251
Number of <u>L. obtusata</u> examined	9333
Chance of observing an infection with <u>M. similis</u>	$\frac{1689}{9333} = \frac{1}{5.64}$
Chance of observing an infection with any other Trematode	$\frac{251}{9333} = \frac{1}{37.2}$
Chance of observing a mixed infection	$\frac{1}{5.64 \times 37.2} = \frac{1}{210}$
Number of mixed infections expected	$\frac{9333}{210} = 44$
Number of mixed infections observed	$= 14$

$$= \frac{(44-14)^2}{44} + \frac{(9289 - 9319)^2}{9289} = 20.5$$

For one degree of freedom this is significant to 99.9% level of probability. Whilst it is conceded that the grouping of heterogeneous data constituted a logical inaccuracy, it may be said in this case to have produced results which were significant despite the lumping rather than because of it.

Strict logic would require each sample to be analysed separately and the total number of expected mixed infections to be calculated by addition of the individual samples. Such an accumulation of small totals would have introduced an error which incurred the possibility of not only being large, but also unpredictable in its effect.

There were thus significantly fewer mixed infections than expected, implying that either secondary infections did not develop owing to the presence of the primary infection, or there existed a mechanism which prevented penetration by a miracidium of an already infected host. Such a mechanism would have obvious selective advantages for the parasite.

GENERAL DISCUSSION.

L. obtusata depends upon the large Fucoids for both food and living space. The food is provided by the lamina of the fronds and requires little searching for the animal to find it. On most shores therefore food is readily available and little appetitive energy is expended to obtain it. There is however considerable disadvantage in using *Fucus* for a substrate to live on, as the animals are highly susceptible to wave action, especially towards the limit of their exposure tolerance. As food is abundant, it is advantageous to grow to reproductive size in a short time. The ten months required for *L. obtusata* to reach maturity is probably a reflection of the super abundant food coupled with a precarious habitat. The precarious nature of the habitat is probably also responsible for the cessation of growth at maturity. Unlike *L. littorea*, *L. obtusata* ceases to grow after maturity, thus all the available energy is channeled into reproduction. A long life and continuous growth would be of little advantage to an animal so easily lost by wave action.

The use of an egg phase attached either to rock or Fucoid substrate is able to act as a very important resevoir of young animals should a large proportion of the adult popultion be killed by an exceptionally heavy surf. The large numbers of eggs present on the shore during the winter is possibly a surf adaptation. The importance of the eggs as a resistant resevoir of young was emphasised in a somewhat artificial manner during the Torrey Canyon incident of 1966. All the adult and young *L. obtusata* at Chapel Porth on the north coast of Cornwall were destroyed, but the eggs survived. The subsequent population of *L. obtusata* at Chapel Porth may have been the result of hatching of the surviving egg masses, though this is not completely certain as fresh material may have arrived by dispersal from other shores.

As the young of *L. obtusata* never enter the plankton, the efficiency of dispersal was in some doubt. Hunter et al (1964) found one living young *L. obtusata* in fresh water some distance from the sea, otherwise there is no other evidence in the literature of the dispersal of *L. obtusata*. Evidence again came from the results of the oil pollution. The oil from the Torrey Canyon completely destroyed *L. obtusata* and the eggs at Porth Leven, and even one year later no *L. obtusata* or *L. mariae* were to be found. Two years following the oil influx *L. mariae* were common at Porth Leven. These animals must have arrived by dispersal from other shores. The egg stage attached to fronds of *Fucus* or young in bladders of *F. vesiculosus* seemed to be likely methods of dispersal.

The possession by an animal of a planktonic larva has three main advantages. Firstly as a means of effective dispersal and genetic exchange, secondly the abundant food in the plankton allows rapid development of the young and lastly the delicate larval forms are hydrostatically protected.

As L. obtusata already lives on an abundent food supply there is less need for a planktonic larva, and as dispersal is effected to a limited degree by the weed born eggs and young, colonisation of new and denuded habitats is ensured. The absence of a planktonic larva also allows the populations on each shore to develop their own characters in response to the particular conditions on each shore and hence increases the survival rate.

The question of population regulation is one to which increasing attention is being paid by ecologists both in the laboratory and in the field. In the artificial conditions of the laboratory, the presence of population regulative mechanisms which rely upon feed back from population density (density dependent mechanisms) have been demonstrated more than once. The situation in the field presents many problems owing to the diversity of the natural environment. The advocates of the view that populations are regulated in a non density dependent fashion (Andrewartha 1957, Birch 1957 and Klomp 1964) belive that as food never becomes limiting, regulation must be mediated by such density independent factors as weather.

The proponents of density dependent factors in population regulation accept, or should accept the importance of physical factors such as weather in determining the average population density, but propose in addition the operation of a density dependent factor. This factor may not be active except at unusually high or low densities (Milne 1957) or may be operative continuously (Brinkhurst, 1966, Eisenberg 1966, and Reynoldson 1957). The factor may however only operate at only one part of the life history of the animals and may represent only a very small proportion of the total mortality. The term "Key Factor" being used by Morris (1957) to describe this phenomenon in the regulation of populations of the spruce budworm.

The identity of the density dependent factor may be exceedingly obscure and difficult to detect. Its presence may however be more easily demonstrated than its identity (Eisenberg 1966, Soloman 1961).

In the case of L. obtusata, no conclusive evidence of density dependent factors was obtained, though several suggestive pieces of evidence emerged. The degree of wave action, the continuity of the rocky substrate and its degree of dissection were clearly important density independent factors determining the average population level, and competition for crevices may in addition have added a density dependent factor, though this is not conclusive.

The predatory activities of the shore crab at Ynys Faelog were able to destroy large numbers of individual animals, returning the artificially increased density to approximately its original value. There are good reasons for supposing the predation to be density dependent. The crabs probably mainly subsist on waste material of human activities until the L. obtusata population reaches a sufficiently high density to provide a viable alternative source of food, whereupon it is attacked. Such a mechanism would operate in a density dependent fashion.

On the other shores density dependent factors were even more elusive. The smaller size of the adult animals on the shores with a dense L. obtusata population and the smaller number of eggs they layed were highly suggestive, especially when it was found that grown in similar conditions the young of both large and small adults grew at more or less equal rates and matured at approximately the same size. In this case food supply may have been the density dependent factor. In addition to this, destruction of the lamina for food resulted in less living space being available.

The presence of a density dependent factor operating on some of the very sparsely populated shores such as that at Red Wharfe Bay and especially Perran Porth (Cornwall) where only three animals were found in forty minutes appeared unlikely. The populations on these shores were probably maintained by passive dispersal of the eggs and young from other shores.

The mechanism of confinement of L. obtusata to the area between M.H.W.N. and M.L.W.N. has received some attention in the past. The overall upward movement of L. obtusata under the effect of wave action would certainly result in lower densities on the lower shore and higher densities on the upper shore, its absence from Pelvetia being possibly a result of the narrow fronds providing inadequate foothold to prevent the animals being washed up onto the strand line. On the extremely sheltered shore at Four Mile Bridge, L. obtusata were often found on Pelvetia.

Its absence sub-littorally is not easily explained. The animals survive and grow well when continuously immersed, and though the absence of food plant may exclude the winkles sub littorally, it must be remembered that Laminaria is the food plant of L. obtusata in North Scotland. The results of the Fowey bag experiment suggest competition from various encrusting polyzoa and colonial Ascidians may eliminate L. obtusata by covering up its food supply. The reason for the failure of the eggs was obscure, but whatever the cause, it seems to indicate on the inability of L. obtusata to survive and breed sub-littorally.

Figure 14.

Numbers of large L. obtusata per kilogram of Fucus on five shores on Anglesey.

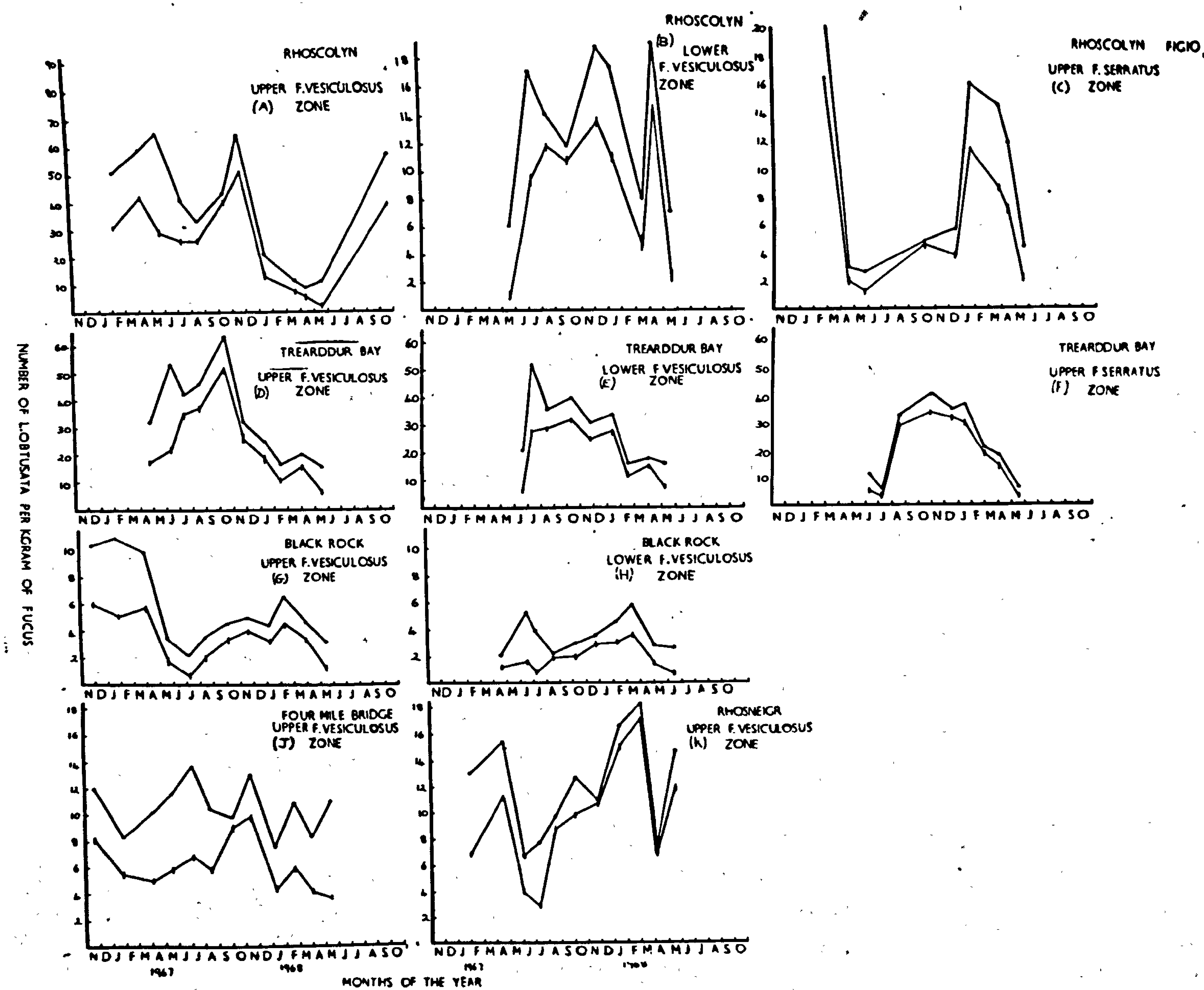
The minimum weight for an animal to be classed as large was found for each shore by calculating the weight to the nearest 0.1 gram including and above which were 90% of the population of adult animals.

Key -

Total number of large animals.
(adult and near adult) •—•—•—•—•—•—•

Total number of large adults. ┼┼─┼─┼─┼─┼┼

FIG10.


RHOSCOLYN
UPPER F.VESICULOSUS
(A) ZONE
RHOSCOLYN
(B) LOWER
F. VESICULOSUS
ZONE
RHOSCOLYN
UPPER F. SERRATUS
(C) ZONE
TREARDDUR BAY
UPPER F.VESICULOSUS
(D) ZONE
TREARDDUR BAY
LOWER F.VESICULOSUS
(E) ZONE
TREARDDUR BAY
UPPER F SERRATUS
(F) ZONE
BLACK ROCK
UPPER F.VESICULOSUS
(G) ZONE
BLACK ROCK
LOWER F.VESICULOSUS
(H) ZONE
FOUR MILE BRIDGE
UPPER F.VESICULOSUS
(J) ZONE
RHOSNEIGR
UPPER F.VESICULOSUS
(K) ZONE
N D J F M A M J J A S O N D J F M A M J J A S O
1967
1968
NUMBER OF L.OBTUSATA PER KGRAM OF FUCUS
MONTHS OF THE YEAR

Figures 15 - 24.

Cumulative frequency curves for populations of L. obtusata on five shores on Anglesey. The curves are plotted on Arithmetical probability graph paper. (see text)

Key.

Cumulative frequency of total population. . . . . . .

Cumulative frequency of adults.
(obtained by direct weighing.) x-x-x-x-x-x

Where two lines of crosses are shown, the lower of the two represents the cumulative frequency of the population of summer hatched young, obtained by graphical analysis.

The absence of any plot indicates insufficient data for a credible analysis.

Figure 15.

Black Rock.

Cumulative frequency curves for the upper F. vesiculosus zone. December 1.1966 - May 13.1968.

FIG 15 BLACK ROCK

UPPER

F. VESICULOSUS ZONE

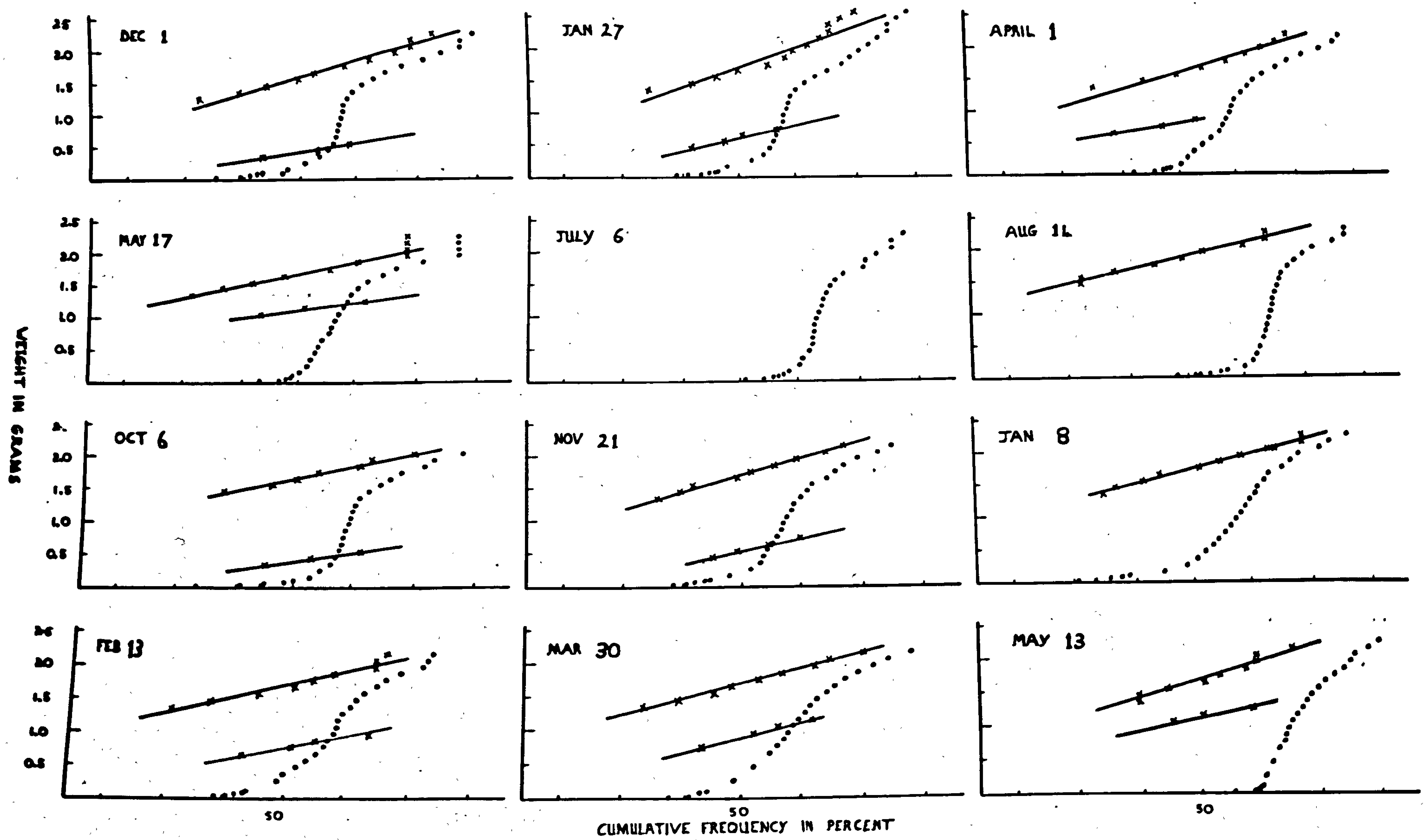


Figure 16.

Black Rock.

Cumulative frequency curves for the lower *F. vesiculosus* zone. April 18.1967 - May 13.1968.

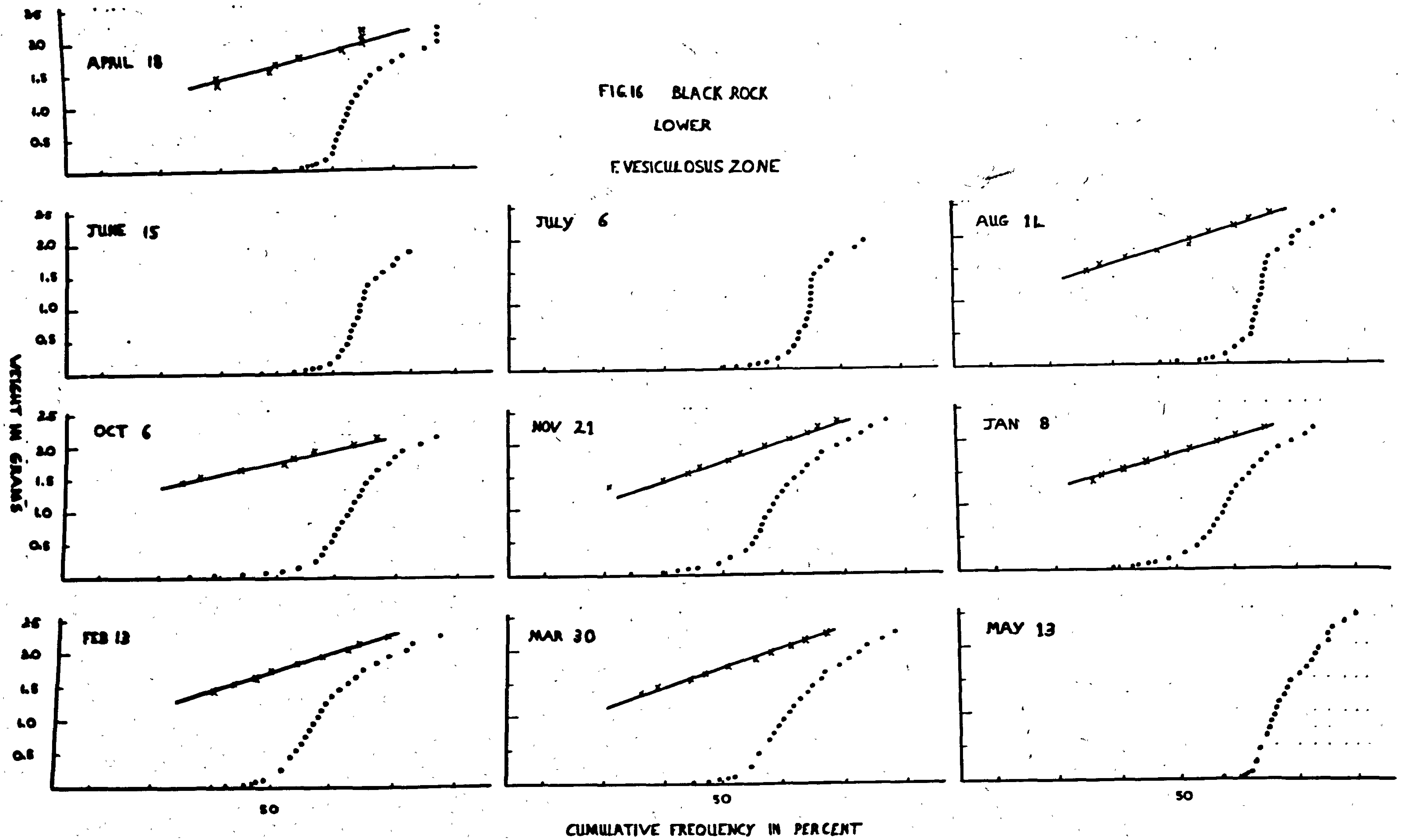


FIG 16 BLACK ROCK
LOWER
F. VESICULOSUS ZONE

Figure 17.

Four Mile Bridge.

Cumulative frequency curves for the upper
F. vesiculosus zone. December 13.1966 - May 14.1968.

FIG 17 FOUR MILE BRIDGE
UPPER
F. VESICULOSUS ZONE

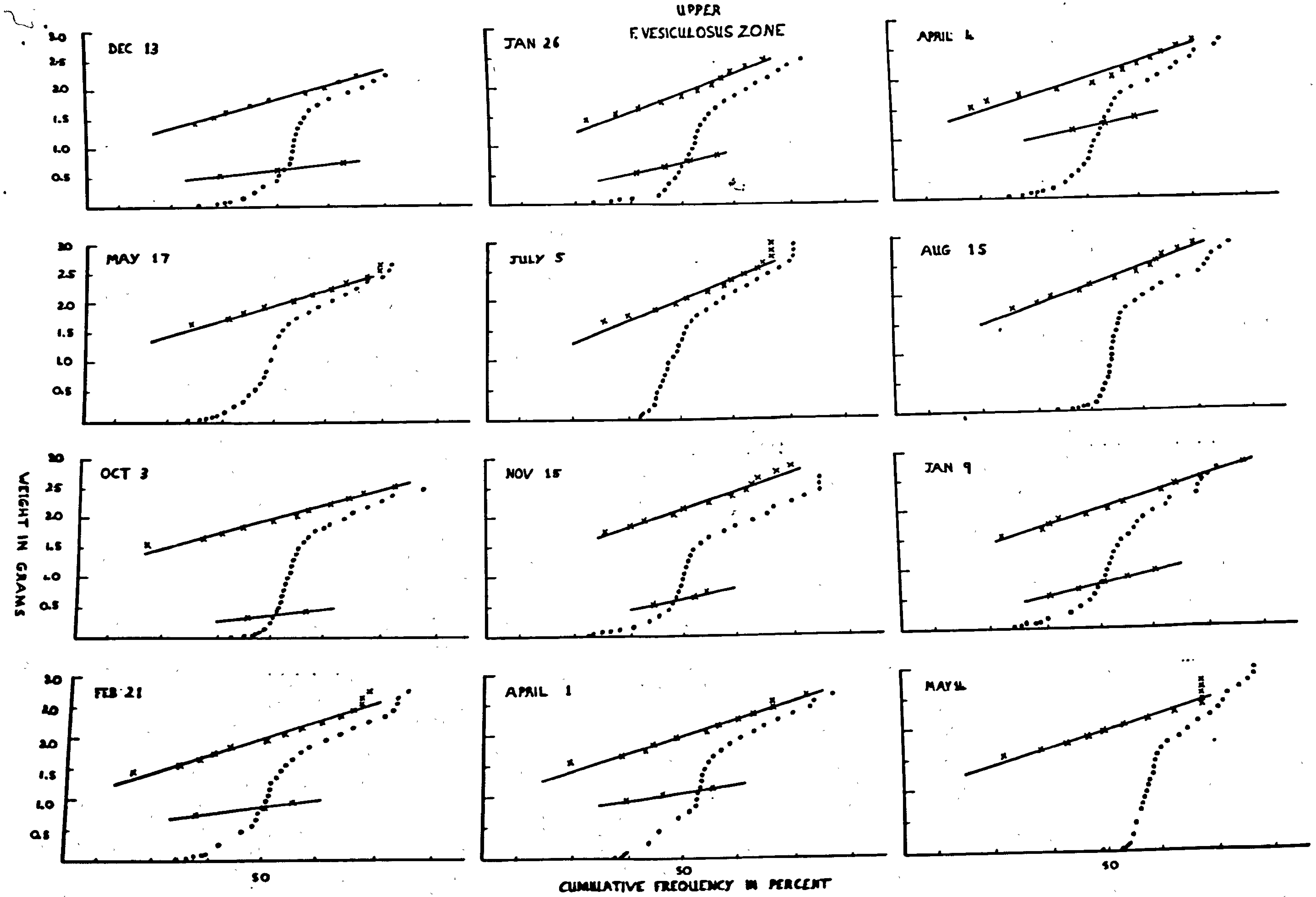


Figure 18.

Rhoscolyn.

Cumulative frequency curves for the upper

F. vesiculosus zone. February 8.1967 - May 16.1968.

FIG. 18 RHOSCOLYN

UPPER

F. VESICULOSUS ZONE

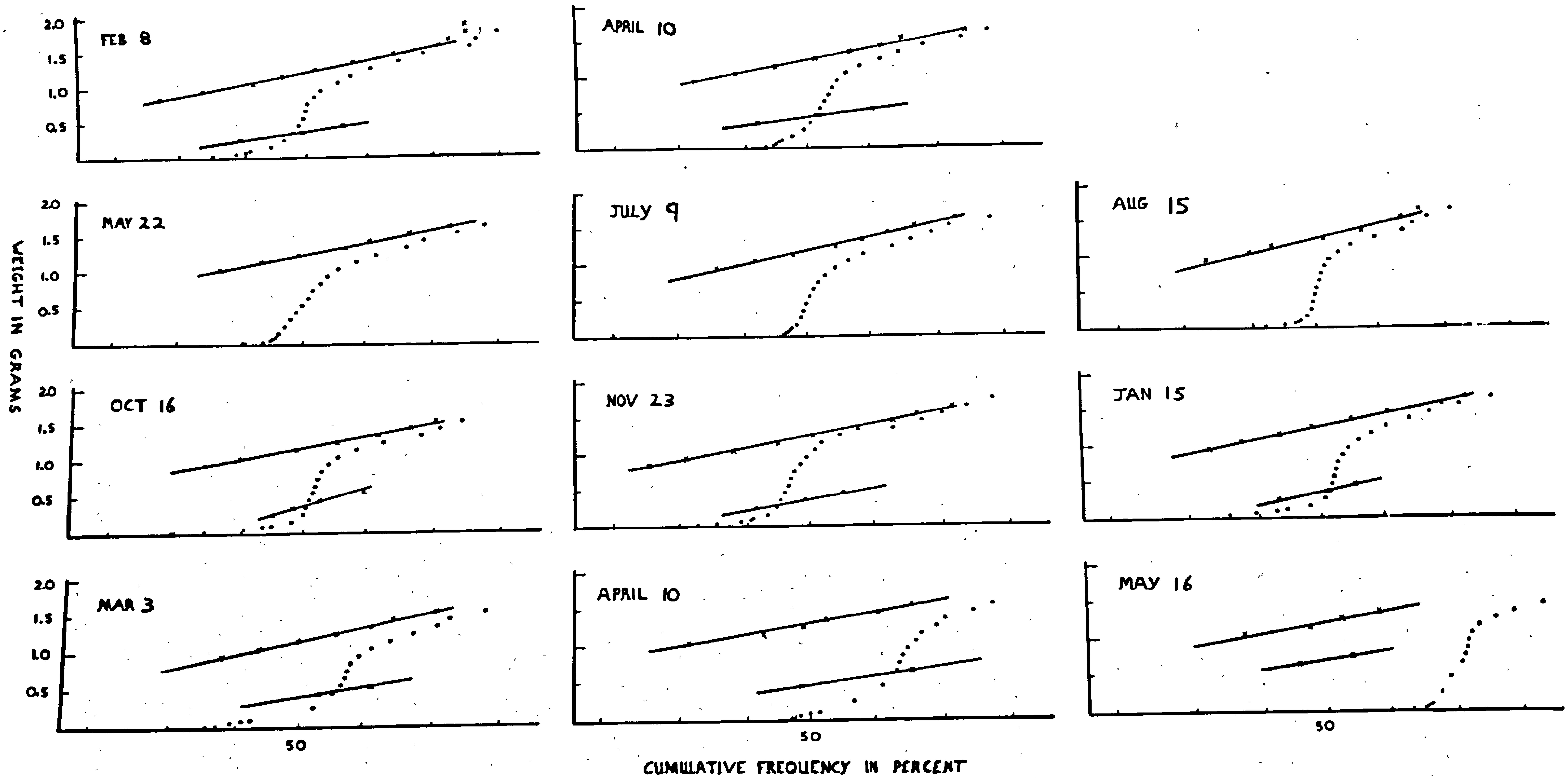


Figure 19.

Rhoscolyn.

Cumulative frequency curves for the lower *F. vesiculosus* zone. July 9.1967 - May 16.1968.

FIG.19 RHOSCOLYN

LOWER

F. VESICULOSUS ZONE

Figure 20.

Rhoscolyn.

Cumulative frequency curves for the upper *F. serratus* zone. February 8,1967 - May 16,1968.

FIG 20 RHOSCOLYN

UPPER

F. SERRATUS ZONE

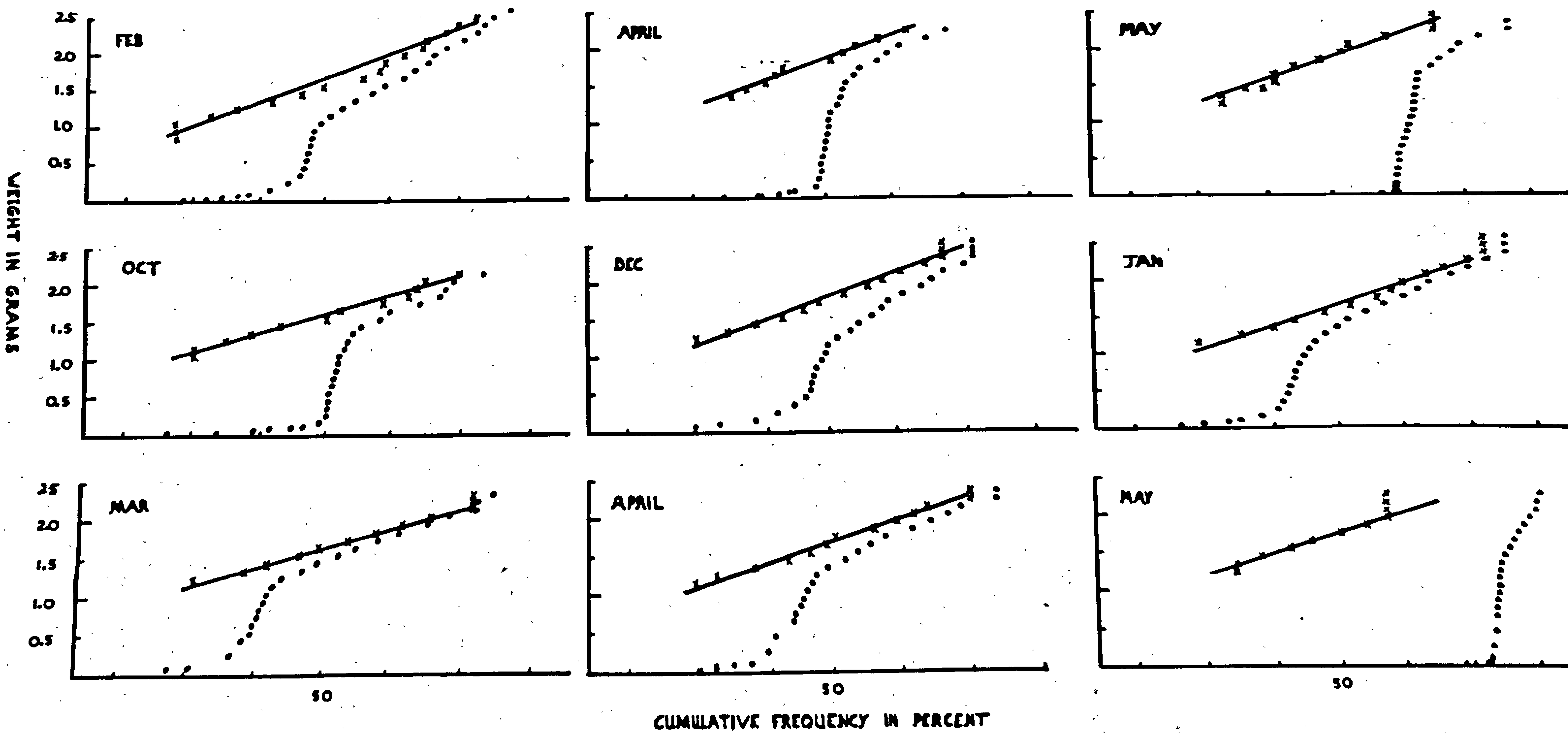


Figure 21.

Rhosneigr.

Cumulative frequency curves for the upper *F. vesiculosus* zone. January 24.1967 - May 14.1968.

FIG 21 RHOSNEIGR
UPPER
F. VESICULOSUS ZONE

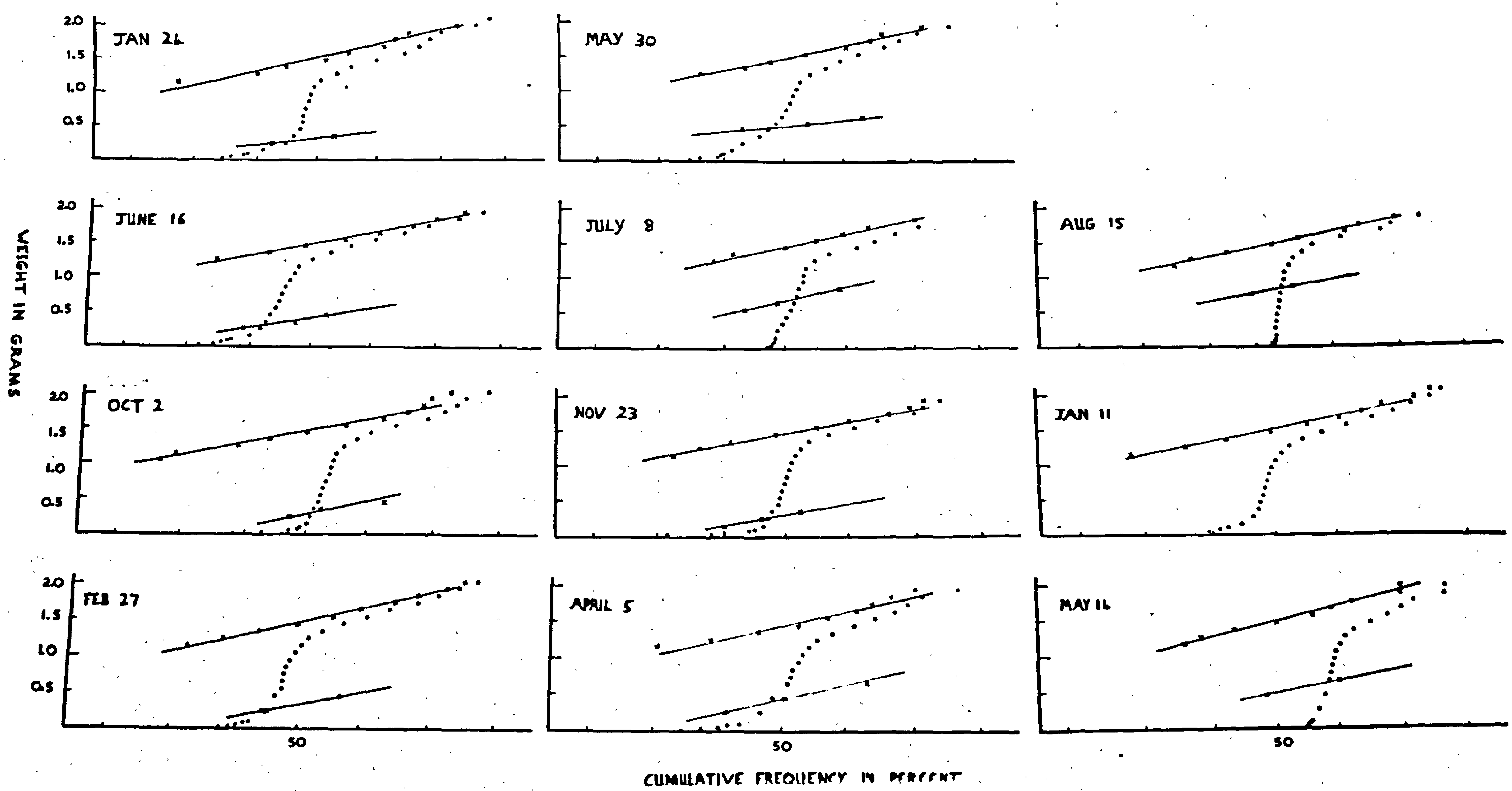


Figure 22.

Trearddur Bay

Cumulative frequency curves for the upper *F. vesiculosus* zone. July 9.1967 - May 14.1968.

FIG. 22 TREARDDUR BAY
UPPER
F. VESICULOSUS ZONE ....

Figure 23.

Trearddur Bay.

Cumulative frequency curves for the lower *F. vesiculosus* zone. June 19.1967 - May 5.1968.

FIG.23 TREARDDUR BAY

LOWER

F. VESICULOSUS ZONE

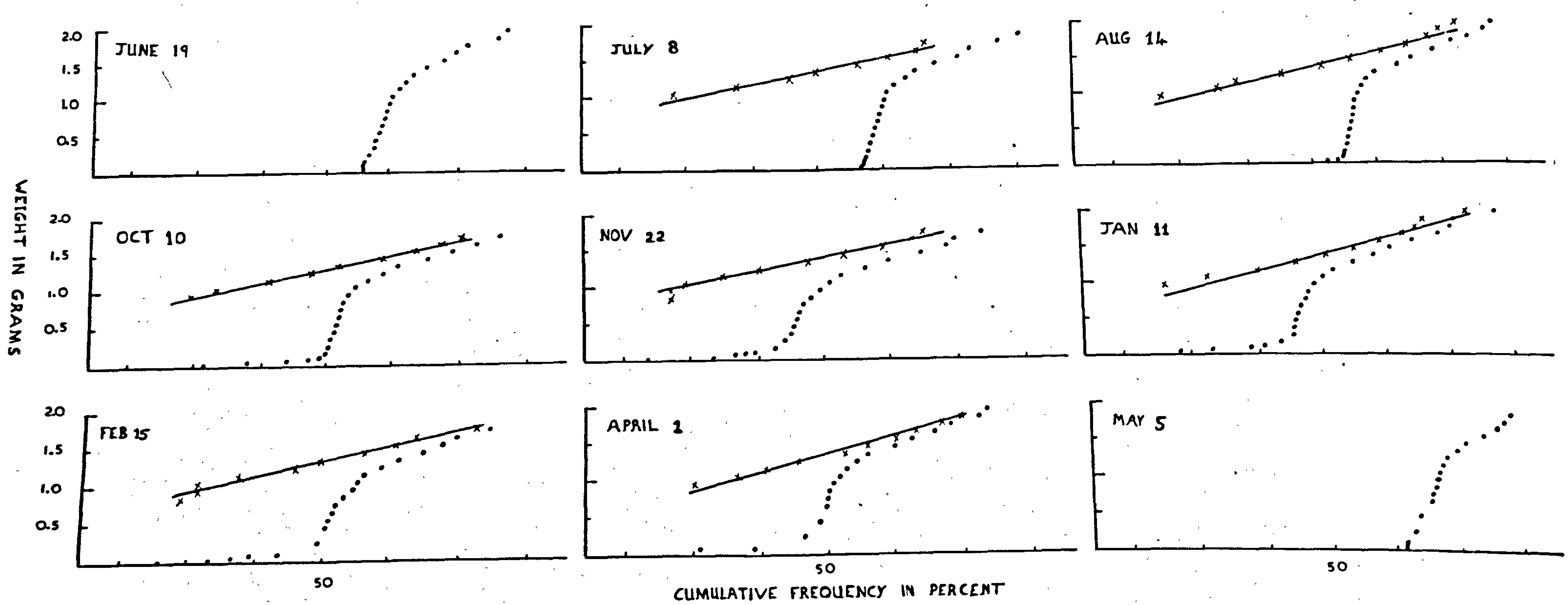


Figure 24.

Trearddur Bay.

Cumulative frequency curves for the upper F. serratus zone. June 8.1967 - May 16.1968.

FIG. 26 TREARDDUR BAY

UPPER

F. SERRATUS ZONE

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APPENDIX A.

THE EFFECT ON BEHAVIOUR OF KEEPING L. OBTUSATA IN THE LABORATORY.

Considerable disappointment was experienced initially in several laboratory experiments owing to the decline in activity and change in behaviour of *L. obtusata* which had been kept in the laboratory for longer than approximately four hours.

The following aspects of the effect of keeping *L. obtusata* under artificial conditions were investigated.

(1) The effect on the relative resistance to high speed water currents of animals collected from the extremely sheltered shore at Four Mile Bridge and the moderately sheltered shore at Red Wharfe Bay.
(2) The effect on the activity of *L. obtusata*.
(3) The effect on the orientation to currents of deep water by animals collected from Rhosneigr.

(1) The effect on the relative resistance to high speed water currents.

The apparatus used was that described in Section 1, pt. 4b, p.24. *L. obtusata* were collected from Red Wharfe Bay and Four Mile Bridge and kept in the laboratory on *F. vesiculosus* in a tank of sea water which was continuously aerated. After three days the animals were tested using the method described in Section 1, pt. 4b, p.24.

When kept for three days the resistance of the *L. obtusata* from Red Wharfe Bay was no longer greater than that of those from Four Mile Bridge. Both groups of animals differed insignificantly in their resistance (see Table 47, p.156) after they were kept for three days.

(2) The effect on the activity of L. obtusata.

As described in Section 2, pt. 4, p.53, the activity of animals was measured as the time taken to crawl from the centre to the circumference of a circle of 12 cms diameter.

Several methods of keeping the animals were tried. Some were maintained on damp *F. vesiculosus*, others in a liter beaker of aerated sea water and more in a liter beaker of aerated sea water which was continuously flushed through with sea water at the rate of one liter every fifteen seconds, thus causing considerable turbulance. Also tested were some *L. obtusata* which had been collected from Rhoscolyn and Four Mile Bridge and kept in aquarium culture tubes (see Fig. 7, p.40) for six months.

All the methods of keeping adult *L. obtusata* in the laboratory with the exception of the flushed beaker methods, resulted in a lowered activity (see Table 48, p.157).

The activity of the animals grown in the laboratory culture tubes maintained a degree of activity which did not significantly differ from the freshly collected specimens. This result gave a possible indication of the success of the culture methods.

(3) The effect on the orientation to a current of deep water.

L. obtusata were collected from Rhosneigr and kept for seven hours in the laboratory on damp F. vesiculosus. Their reaction to a current of deep water was then tested using the methods described in Section 4, pt. 2b, p.79.

The results (see Table 49a) were compared with the results of a similar experiment involving freshly collected animals using a contingency table (Table 49b, p.159). The table was analysed by the chi squared test using the Yates correction for continuity. A chi squared of 144 showed the freshly collected animals and those kept in the laboratory to differ in their orientation at the 99.9% level of probability. Those kept artifically showed little tendancy to orientate facing upstream.

Discussion

The results of the foregoing experiments underline the danger of keeping animals in the laboratory which are destined for the behaviour experiments. Most workers when referring to the laboratory behaviour of L. obtusata make no mention of the length of time they had been kept in the laboratory, a point which may well lie at the root of much of the contradiction concerning the behaviour of this species.

Table 47.

The comparative resistance of L. obtusata collected at Four Mile Bridge and Red Wharfe Bay to experimentally induced water currents in the laboratory. The animals were kept in the laboratory for twenty four hours before being tested.

First Trial.

	Dislodged	Not Dislodged	
Red Wharfe Bay	22	15	37
Four Mile Bridge	20	17	37
Total	42	32	74

Second Trial.

	Dislodged	Not Dislodged	
Red Wharfe Bay	31	23	54
Four Mile Bridge	34	20	54
Total	65	43	108

Table 48.

The effect on activity of various methods of keeping L. obtusata in the laboratory. The figures refer to the number of L. obtusata remaining in the circles after periods of six and fifteen minutes.

Table 48a.

Control: Freshly collected L. obtusata from Rhosneigr.

	Number Left In Circle	
Trial	After 6 Minutes	After 15 Minutes.
1	6	3
2	7	3
3	5	2
4	9	3
5	7	2

Table 48b.

Collected from Rhosneigr and kept for 15 hours on damp F. vesiculosus at approximately 10°C.

	Number Left in Circle	
Trial	After 6 Minutes	After 15 Minutes.
1	11	11
2	14	9
3	8	6
4	14	9
5	14	12
6	13	12
7	12	9
8	12	10
9	13	11
10	15	13

Table 48c.

Collected from Rhosneigr and kept in a liter beaker of aerated sea water at approximately 10°C., for 15 hours.

	Number Left In Circle	
Trial	After 6 Minutes	After 15 Minutes
1	13	10
2	15	13
3	15	13

Table 48d.

Collected from Rhosneigr and kept in a liter beaker of aerated and constantly flushed sea water at approximately 10°C. for 15 hours.

	Number Left in Circle	
Trial	After 6 Minutes	After 15 Minutes.
1	7	4
2	4	4

Table 48e.

Collected from both Rhoscolyn and Four Mile Bridge and kept for six months in the aquarium culture tubes.

Shore of Origin	Trial	Number Left in Circle After 6 Minutes	After 15 Minutes.
(F.M.B.)	1	10	5
(Rhosc.)	2	5	1
(Rhosc.)	3	11	7

F.M.B. = Four Mile Bridge.
Rhosc. = Rhoscolyn.

Table 49a.

The effect on orientation in a current of deep water of keeping L. obtusata in the laboratory on damp F. vesiculosus at approximately 18°C., for seven hours.

	Facing Upstream (Right)	Facing Downstream (Left)
	3	4
	4	4
	4	5
	5	5
	6	2
	5	1
	5	3
	3	3
	2	1
Total	37	28

Table 49b.

Contingency table:- comparison of the deep current orientation of freshly collected L. obtusata (see Table 23, p.81) with those kept in the laboratory for seven hours. All animals were collected from Rhosneigr.

	Facing Upstream	Facing Downstream	
Fresh	91	17	108
Kept	37	28	65
	128	45	173

$\chi^2 = 144.$

APPENDIX B.

THE RELATIVE RESISTANCE TO HIGH SPEED WATER CURRENTS OF LITTORINA MARIAE AND L. OBTUSATA

L. mariae and L. obtusata are often found inhabiting the same shores, the former species frequently extending onto surf exposed conditions not tolerated by the latter. Conversely L. obtusata is often found in conditions of extreme shelter from which L. mariae is absent.

The ability of L. mariae to resist the effects of wave action is probably due in part to small size enabling them to occupy small crevices both in the rock and in the base of seaweeds.

As it was found in Section 1 pt. 4b, p.24 that L. obtusata inhabiting moderately exposed shores were better able to resist high speed water currents than those from extremely sheltered shores, the more surf adapted species, L. mariae, would, it was thought, probably resist laboratory induced currents better than L. obtusata.

The two species were collected simultaneously from Black Rock, and only adult animals being used. They were tested immediately on returning to the laboratory using the rotating disc technique described in Section 1 pt. 4b, p.24.

The results (see Table 50, p.161) were analysed by the chi squared test using the Yates correction for continuity. A chi squared value of 12.5 for one degree of freedom showed the greater ability of L. mariae to withstand high speed water currents was significant at the 99.9% level of probability.

Though the immediate cause of the greater resistance of L. mariae was obscure, to a surf loving species it has very clear advantages.

Table 50.

Contingency table:- the relative resistance to high speed water currents of L. mariae and L. obtusata.

	Dislodged	Not Dislodged	
L. mariae	11	21	32
L. obtusata	26	6	32
	37	27	64

χ^2 for 1 degree of freedom = 12.5

Table 51.

The number of L. obtusata found in a series of thirty minute counts. L. obtusata were put out at Porth Leven March 12.1968., those at Penmon March 18.1968.

	Number Found in a Thirty Minute Count	
Date	Penmon	Porth Leven
April 9	224	-
April 20	-	495
May 28	146	590
June 18	-	543
October 28	62	385

APPENDIX C.

THE EFFECT OF CREVICES ON POPULATIONS OF L. OBTUSATA.

During the course of this work, the effect of rock crevices on populations of L. obtusata have been discussed. In general it was assumed that the presence of many crevices on a shore was advantageous in preventing the waves washing the animals onto the strand line. The conclusion was based on the observation that for a given wave fetch, the greater the dissection of the rock substrate, the more dense the population of L. obtusata.

To test the effect of crevices on the loss of L. obtusata the following transplant was performed.

From the second shore at Trearddur Bay were collected 6,000 L. obtusata. Of these 3,000 were placed on the relatively smooth limestone shore at Penmon (Anglesey) and the remaining 3,000 on the highly dissected shore at Porth Leven (Cornwall), a shore which had been denuded of its L. obtusata populations by the Torrey Canyon oil.

The wave fetch at Penmon was effectively less than at Porth Leven, being exposed only on three sides and protected from waves normal to the shore by Puffin Island. Porth Leven was however open to the full Atlantic wave fetch.

The number of animals remaining on the shore was estimated at regular intervals by counting those revealed in a fifteen minute search.

The results (see Table 51, p.161) show the loss rate from Penmon to be more rapid than that from Porth Leven despite the greater wave fetch on the latter shore. It was thought probable though not certain that the difference was mainly due to the more highly dissected nature of the Porth Leven shore.

Subsequent to this experiment, the population of L. obtusata at Penmon returned to approximately its original density, whilst that at Porth Leven was almost entirely replaced by a population of L. mariae. A winter had passed and the greater wave fetch at Porth Leven was thought to have counteracted the effect of the crevices.

APPENDIX D.

Sexual dimorphism was observed by Sacchi and Rastelli (1969) in _L. mariae_, the shells of the females being heavier than those of the males. Reference to the plot of the cumulative frequency of the adult population on five shores on Anglesey (see Figs. 15 - 24 p.132-141) showed on many occasions a highly suggestive point of inflection at about the fifty percent point, indicating a heterogeneity in the weights of the adult populations.

To investigate the possibility of sexual dimorphism the distribution of the sexes amongst the large adults and small adults was analysed. The size groups were those obtained using the set of graded sieves (see p.18). For each level on each shore those animals retained by the larger sieves were counted separately from those collected in the smaller sieves. Only unparasitised adults were included, and sex was determined by the presence of a penis in the males, and capsule and accessory gland in the females, the shell being broken open to observe these structures.

The results (see Table 52a, p.164) showed the sex ratio (female to male) to be higher amongst the large size groups than amongst the small ones in all the zones on all the shores, with the exception of the upper _F. vesiculosus_ zone at Trearddur Bay.

The results were pooled in the form of a contingency table (see Table 52b, p.164). To determine the degree of association between size and sex, the table was analysed by means of a chi squared test. The chi squared value of 24 for one degree of freedom showed highly significant association, females being larger than males.

Table 52a.

Sex ratio amongst the large and small adult L. obtusata on five shores on Anglesey to show sexual dimorphism. F.v.z. = F. vesiculosus zone and F.s.z. = F. serratus zone.

Shore	Sample Size		Sex Ratio	
	Large Adults	Small Adults	Large Adults	Small Adults
Black Rock (upper F.v.z.)	290	59	1.09	0.44
Black Rock (lower F.v.z.)	135	38	1.50	0.36
Rhoscolyn (upper F.v.z.)	781	26	1.14	0.30
Rhoscolyn (lower F.v.z.)	122	274	1.44	0.97
Rhoscolyn (upper F.s.z.)	102	139	1.62	1.05
Four Mile Bridge (upper F.v.z.)	35	599	1.33	0.99
Trearddur Bay (upper F.v.z.)	91	686	0.78	0.93
Trearddur Bay (lower F.v.z.)	114	589	1.38	1.01
Trearddur Bay (upper F.s.z.)	192	548	1.37	0.81
Total	1862	2958	1.21	0.91

Table 52b.

Contingency table based on the combined data from Table 52 a.

	Large Adults	Small Adults	Totals
Female	1020	1410	2430
Male	842	1548	2390
	1862	2958	4820

$\chi^2 = 24$ For one degree of freedom

APPENDIX E.

Data for Figs. 2 and 8 pages 11 and 41.

Table 53.

Rate of loss of L. obtusata from Black Rock and Four Mile Bridge. The figures used to compile Fig. 2 page 11.

Table 53a.

Losses from Black Rock

Period	Number present at start of period.	Number present at end of period.	Daily loss rate (m) in percent
1965			
Dec. 26 - Dec. 27	41	32	22.0
Dec. 27 - Jan. 12	48	3	16.0
1966			
April 26 - May 5	24	13	7.0
May 5 - May 16	28	12	9.7
May 16 - May 27	30	8	12.0
May 27 - June 7	18	11	4.5
June 7 - June 15	22	10	9.0
June 15 - June 22	14	12	2.0
June 22 - June 30	12	10	2.0
June 30 - July 8	15	9	6.0
July 8 - Sept. 7	10	0	-
Sept. 7 - Sept. 13	15	6	14.0
Sept. 13 - Sept. 21	8	6	3.5
Sept. 21 - Sept. 29	14	5	11.3
Sept. 29 - Oct. 11	9	3	9.0
Oct. 11 - Nov. 8	7	3	4.0
Nov. 8 - Nov. 18	35	9	14.0
Nov. 18 - Dec. 12	31	2	10.7
Dec. 12 - Jan. 16	32	5	5.0
1967			
Jan. 16 - Feb. 7	16	2	9.0

Table 53b.

Four Mile Bridge.

Period	Number present at start of period.	Number present at end of period.	Daily loss rate (m) in percent.
1966			
June 10 - June 20	44	33	2.8
June 20 - June 29	40	39	0.3
June 29 - July 5	41	37	1.6
July 5 - Sept. 6	44	25	0.8
Sept. 6 - Sept. 20	45	41	0.64
Sept. 20- Sept. 30	46	40	1.1
Sept. 30- Oct. 13	43	31	2.5
Oct. 13 - Nov. 24	34	23	0.37
Nov. 24 - Dec. 13	37	25	2.3
Dec. 13 - Jan. 18	44	43	0.04
1967			
Jan. 18 - Jan. 26	53	50	0.74
Jan. 26 - March 8	61	54	0.46
March 8 - April 4	69	49	1.37
April 4 - April 13	56	34	6.0
April 13- May 2	40	39	0.05
May 2 - June 5	45	28	1.5
June 5 - June 16	35	36	-
June 16 - July 10	37	25	1.5
July 10 - Sept. 28	27	15	0.6

Table 54.

Rate of loss at Four Mile Bridge of L. obtusata collected from Rhosneigr and Four Mile Bridge. (Data used to compile Fig. 8 p.41).

Date	Collected from Rhosneigr		Collected from Four Mile Bridge	
	Number Present	Daily Loss Rate (m) in Percent	Number Present	Daily Loss Rate (m) in Percent
May 10 1967	100	—	100	—
May 17	64	6	68	5
June 16	6	7.5	61	0.4
Sept 27 1967	100	—	100	—
Oct. 30	30	3.7	62	1.6
Dec. 12	30	0.0	60	.09
Feb. 20	22	0.46	54	0.16
Feb. 27 1968	100	—	100	—
March 7	88	1.8	98	0.16
March28	56	2.0	88	0.5
April10	45	1.8	85	0.28
May 23	11	3.2	55	1.0
May 23 1968	100	—	100	—
June 7	19	10.0	93	0.5