AN ANALYSIS OF THE BEHAVIOUR OF *LEPIDOCHITONA CINEREUS* IN RESPONSE TO CERTAIN PHYSICAL FEATURES OF THE ENVIRONMENT

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(With 3 Figures in the Text)

CONTENTS

1. INTRODUCTION

The experimental work included in this paper was undertaken to show the part played by behaviour responses in maintaining an intertidal animal, *Lepidochitona cinereus* (L.) in its characteristic position on the shore. Two questions are involved in the distribution of a shore animal such as *Lepidochitona*: firstly, how the planktonic larvae attain, and settle within, their particular zone on the shore, and secondly, how once established in that zone they maintain themselves there.

Of the first question little is known, for information concerning the settling of chiton larvae, and indeed of most planktonic larvae, is scarce. Colman (1933) has suggested that the larvae of sedentary forms reach their zone as a result of 'hit-or-miss' methods and that only those which settle, by chance, in a suitable zone, survive to reach maturity. Wilson (1937, 1948), however, has shown that in the case of certain polychaete worms metamorphosis may be delayed until a suitable substratum is found, thus increasing the chances of survival. Observations of

Moist surface

Region of maximum humidity and minimum light intensity

Stone in original position

Positively geotactic path of chiton

New region of maximum humidity and minimum light intensity

Stone overturned
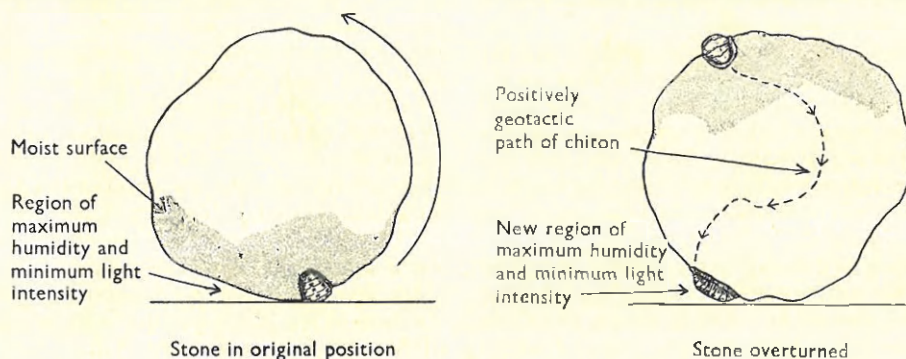
Fig. 1. Diagram of the movements of a chiton when the stone bearing it is overturned—drawn from an observed instance. Approx. × ¼.

like nature have been made on the settling of oyster larvae, which have been found (Cole & Jones, 1949) to settle more readily on shells bearing recently attached spat than on clean shells and shells devoid of spat.

It is with the second question that this paper deals, namely, the way in which the animals once established in their zone, maintain themselves there. This involves an adaptation of behaviour responses to contend with the extreme variability of shore conditions. Many intertidal forms burrow and in this way escape desiccation, others retreat into crevices and pools. *Lepidochitona* when found uncovered by the tide occurs almost invariably on the undersurfaces of stones. If such a stone, bearing beneath it chitons, is overturned on the shore on a bright sunny day, the chitons on top of the stone will be observed to begin moving and, if the surface of the stone is sufficiently moist, to collect again on its now lower surface and so resume their original orientation (Fig. 1).

The chitons in re-orientating themselves in this way resume a position of minimum light intensity, maximum humidity and maximum dorsal contact and, to reach such a position, move with gravity and away from the incident (overhead) light. The experiments which follow are designed to show the extent to which these factors influence the behaviour of the chitons and which are thereby responsible for the situation they occupy within their zone.

2. SHORE OBSERVATIONS AND SOURCES OF MATERIAL

Most of the shore observations recorded in this paper, and on which the experimental work was based, were made at Whitstable where conditions are particularly suitable for a study of this kind. A figure showing the zonation of some of the commoner littoral animals on this shore is given by Newell (1948). The chitons are limited almost entirely to a zone just over 50 yards in width extending from approximately 25 yards below the high-water neap tide level to the seaward edge of the wet part of the *Fucus* zone.

The shore here is covered with pebbles and the chitons were found, almost without exception, on the undersurfaces of the pebbles and were most abundant in the shallow pools which had not been completely drained by the retreating tide. On the landward side the distribution appeared to be limited by the length of time of exposure between tides and on the seaward side by the lack of a suitable substratum. That this factor controlled their distribution at the seaward end of the zone where the soft, sandy mud encroached upon the pebbles was indicated by the facts that on this shore occasional isolated specimens were recorded occurring on stones even down to the low tide level; and that on rocky shores in Cornwall and at Plymouth,

where there was no mud zone, they occurred down to the low-water spring tide level.

Specimens used in the laboratory experiments were usually obtained from Whitstable, but part of the work was done with chitons from Plymouth and Gerrans Bay, Cornwall. Much of this latter material was parasitized heavily with the haplosporidian parasite *Haplosporidium chitonis* Lankester. Heavily infected animals could be detected without dissection, but this was impossible in the case of less heavily parasitized material. In the experimental work both parasitized and unparasitized individuals were used indiscriminately as there were no behaviour differences apparent between them, and even the most heavily parasitized of them appeared to survive as long in the laboratory and to be as responsive as the uninfected specimens.

3. LABORATORY PROCEDURE

In the laboratory the chitons were kept in shallow dishes and transferred from one to another as little as possible. They could be moved about within a dish by a firm, gentle push, but it was found inadvisable to allow the foot to lose contact with the bottom of the dish owing to the difficulties encountered in getting the animals to resettle once they had become detached. In earlier experiments the water was periodically aerated, but this was apparently unnecessary and the animals could be kept for several months in the laboratory in a seemingly healthy condition if the water containing them was shallow and at a low temperature. Nevertheless, throughout the work it was striking how much more uniform and clear-cut were the results obtained from using freshly collected material than were those obtained by using animals which, though apparently normal, had been kept in the laboratory for some length of time.

Observations were made on the reactions of chitons in response to the influence of light, contact, gravity, and humidity stimuli, and experimental details are given in the sections which follow. Preliminary experiments were usually carried out with freshly collected material on the shore and repeated under controlled conditions in the laboratory. Before each set of laboratory experiments the chitons to be used were exposed to daylight and those, if any, which did not exhibit movement within 3 min. were discarded. The rest of the specimens were then placed in darkness for at least 30 min. before use.

The general experimental procedure throughout was arranged where possible to show:

(1) Whether or not the distribution of the animal is a random one if the stimulus is a uniform one and all the other factors are controlled.

(2) Whether or not, when the stimulus is directional, the animal orientates itself to it and moves in a

direction dependent upon the gradient of intensity (i.e. if the response is a 'taxis').

(3) If, when the stimulus is non-directional, it results in a distribution of the animal which can be shown to be dependent upon the effect of the intensity of the stimulus upon the speed or rate of change of direction of the animal (i.e. if the response is a 'kinesis').*

No study has been made of the behaviour of *Lepidochitona cinereus* in direct response to its physiological condition. The effects of hunger, for example, are unlikely to be of great significance biologically to an animal which feeds upon encrusting algae, which are always in abundance in the chiton zone. No evidence has been obtained of a difference in behaviour between the two sexes or at different stages in the reproductive cycle, though it has been recorded by Brewin (1942) that a New Zealand chiton, *Cryptoconchus porosus*, 1 or 2 days before spawning exhibits 'a marked tendency to rise to a position just below the water level and circumnavigate the tank (or rock) until spawning itself commences'.

4. LIGHT

Observations on chitons in their natural environment indicated that they aggregate in positions of low light intensity, and preliminary experiments suggested that the rate of movement of the chitons differs with differing light intensities. This response was investigated as also was the possibility that the animals orientate themselves to a unidirectional light stimulus in a manner similar to that demonstrated by Arey & Crozier (1919) in the Bermudan species *Chiton tuberculatus*.

It was at first hoped to investigate the responses of the chitons to a graded series of light intensities, but in practice only three different sets of conditions were employed: daylight, artificial light and darkness. This was because differences in the response of chitons could only be measured when the differences between the stimuli were great. The intensity of sunlight may be taken to have a value between 500 and 2000 lumens/cm.²/sec., whereas that of the artificial light used was approximately 7 lumens/cm.²/sec.

Most of the experimental work in which artificial light was employed was carried out in a dark-room using a Pointolite lamp. This was calibrated against a standard lamp supplied by Cryselco Ltd. by a null deflexion method using a Megatron photoelectric cell in circuit with a galvanometer. The light intensity employed was approximately 7 lumens/cm.²/sec., though some variation occurred due to mains fluctuations. Additional experiments were made in a

* The nomenclature of behaviour mechanisms used here is in accordance with that suggested by Fraenkel & Gunn (1940) modified from Kühn.

temporary dark-room in Cornwall where a similar light source was used, but the light was of a lower intensity, the current being supplied by a 12 V. car battery.

In order to make observations on the animals' behaviour in darkness a red light was employed. Most records, however, were obtained by examining the mucous tracks which were left by the animals during the experiments. These tracks were made visible by Ullyott's method (1936 a, b) of introducing a fine suspension of talc into the dishes at the end of the experiment. The results obtained by these two methods were sufficiently similar to indicate that the animals are completely unresponsive to red light. Ullyott's method was used to record automatically the tracks of the chitons in experiments in daylight and artificial light.

(a) Distribution under conditions of uniform light intensity

Sheets of glass moistened with sea water and each bearing ten chitons were subjected to overhead illumination. Two were placed in bright daylight, two in artificial light and the remaining two in darkness. After a period of 10 min. the positions of the chitons on the glass sheets were noted and the distance each had travelled was measured. The animals spread in all directions across the glass plate, and the distances travelled depended not only upon the duration of the experiment but also (Table 1) upon the light intensity. In the case of the experiments carried out in darkness, just over two-thirds of the experimental animals showed no movement at all. It may therefore be concluded that the distribution of chitons under conditions of uniform overhead illumination (i.e. a non-directional stimulus) is a random one, but that the rate of movement is greater when the light intensity is great.

Table 1. *Distances travelled by chitons under uniform light intensities*

Distance moved (cm.)	Daylight	Artificial light	Darkness
0	—	—	14
< 3	1	5	2
3–6	3	11	1
9	7	4	2
12	4	—	1
15	2	—	—
18	2	—	—
21	1	—	—
24	—	—	—
	20	20	20

(b) Response to a unilateral light stimulus

Experiments in which the animals were subjected to unilateral light stimulation were set up in the

dark-room on numerous occasions. In all cases the chitons moved apparently at random, and were never observed to orientate themselves to the incident light. The light intensities employed in these experiments were in the neighbourhood of 7 lumens/cm.²/sec. or about 113,000 ergs/cm.²/sec. Some indication was obtained that chitons orientate themselves to light of the intensity of strong sunlight and move away from it. This suggests a negatively phototactic response; but no experiments were set up to investigate this further owing to the difficulty of introducing a light of sufficient intensity into the dark-room.

The light intensity required to start movement of any kind in *Lepidochitona* under experimental conditions is far greater than that necessary to bring about movement in *Dendrocoelum*. It was shown by Ullyott (1936 a) that *Dendrocoelum lacteum* will react to a beam of light of an intensity as low as 7 ergs/cm.²/sec. Arey & Crozier (1919) showed that in a Bermudan species of chiton, *Chiton tuberculatus*, the response to light was a function of the age of the animal, and that the younger specimens were negatively phototactic whereas the older ones were positively phototactic; but no details were given of the light intensities employed in these experiments. No indications were obtained of any such behaviour differences in age groups of *Lepidochitona*, as indicated by size.

(c) *Aggregation in regions of low light intensity*

Evidence has already been given that chitons move at random across an experimental dish illuminated from overhead. If, however, a portion of the dish is shaded the chitons tend to collect in the shaded portion. This tendency to aggregate in regions of low light intensity was investigated and a large number of experiments was carried out. The most clear-cut results were obtained when using freshly collected specimens and illuminating them with sunlight. Precautions were taken to prevent any directional factor in the sun's rays affecting the results. The experiments were conducted on bright, cloudy days when the sun was overhead. The experimental dishes, moreover, were arranged in a circle with the centripetal half of each shaded so that the unshaded portions faced in different directions. The results of these experiments are shown in Table 2.

Experiments were also carried out in the dark-room under artificial light. These gave similar results but they were slower to accomplish. This was later shown to be due to the lower light intensities employed. In the case of the dark-room experiments it was necessary to change the water periodically in order to keep down the temperature. Attempts to absorb the heat by passing the light through a trough of water caused so much loss of intensity that little or no movement occurred.

Table 2. *The aggregation of chitons in regions of low light intensity*

(Three different groups (i.e. 30 chitons) observed simultaneously in dishes shaded to cut out directional factors in incident light.)

Duration of experiment (min.)	No. of chitons		No. of chitons		No. of chitons	
	Light	Dark	Light	Dark	Light	Dark
5	9	1	7	3	8	2
10	6	3*	7	3	7	3
15	5	4	7	3	6	4
20	3	6	5	5	5	5
25	3	6	4	6	5	5
30	3	6	3	7	5	5
35	3	6	3	7	5	5
40	3	6	3	7	5	5
45	2	7	3	7	4	6
50	1	8	3	7	4	6
55	1	8	2	8	4	6
1 hr.	1	8	3	7	2	8
2 hr.	1	8	1	9	1	9

* One chiton crawled from the experimental dish.

The experimental results recorded in this section confirmed the view that *Lepidochitona* tends to aggregate in regions of low light intensity. They showed, in addition, that this aggregation takes place more slowly when the light intensity in the unshaded portion of the dish is relatively low. This indicates that the rate of movement of chitons is to some extent dependent on the intensity of the illumination, and measurements of the rate of movement of chitons under different light intensities were made to investigate this relationship.

(d) *Rate of movement in different light intensities*

In order to investigate the relationship between rate of movement and light intensity, the distances travelled by chitons in (a) sunlight, (b) artificial light, and (c) darkness were measured. The intensity of the artificial light employed was approximately 7 lumens/cm.²/sec., and details of the lamp used and the method of calibration have already been given. Fifteen chitons were placed in Petri dishes filled with sea water and each was exposed in turn to the three different sets of conditions mentioned. After an exposure to a given light intensity a talc impression of the chitons' path was taken and this was then traced on to a sheet of graph paper. The plotted paths were then measured with a Philip's map measurer and the speeds of the chitons calculated. The results are given in Table 3 (compare also Table 1). These and other figures indicated that the rate of movement depends on the illumination and that the greater the light intensity the greater is the rate of movement.

Table 3. *Distances (cm.) moved in 10 min. by chitons under different conditions of illumination*

Sunlight	Artificial light	Darkness
10·6	5·7	0
5·4	3·6	0
8·0	4·6	4·0
8·5	6·3	2·6
6·3	5·0	0
11·0	4·6	0
4·7	4·1	2·0
10·3	7·3	0·9
8·6	6·0	0·5
8·0	4·3	0
4·3	2·0	0
12·3	6·2	4·5
10·6	8·7	2·0
5·4	4·2	1·1
8·6	3·9	3·1
Mean 8·17 ± 0·64	5·10 ± 0·43	1·38 ± 0·41

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean sq.
Within groups	42	160·1074	3·812
Between groups	2	347·1720	173·586
Total	44	507·2794	

$F = 45·5$. Significant at 1 % level

The duration of each of these experiments was 10 min. This period of time was chosen because on exposure to light there may be a latent period of up to 3 min. in duration before any movement occurs. On the other hand, a longer period of exposure resulted in too great a rise of temperature in those dishes exposed to artificial light. The use of a light filter was not practical as it cut down the light intensity too severely. It was found that the temperature of the water in the dishes exposed to artificial light rose from 12 to 13·5° C. There was no corresponding rise in temperature in those dishes exposed to sunlight. No allowance has been made for this rise in temperature, but in order to find out whether it affected the interpretation of Table 3 another experiment was carried out to investigate the effect of small differences in water temperature upon the rate of movement of the chitons. Two dishes, each containing ten chitons, were placed in conditions of uniform light intensity. The temperature of the water in one of the dishes was 13·5° C. and that of the water in the other dish was 10·5° C. The chitons were left exposed to these conditions for a period of 10 min. and at the end of this time the tracks were 'printed' and measured and the rates of movement calculated. Approximately every 3 min. the water in both dishes was gently agitated and more water of the requisite temperature was added. As a result the initial temperature difference was maintained throughout the experiment. In a second experiment the dishes were not interfered with and at the end of 10 min. the temperature difference between the water in the two dishes had fallen to just over 2·5° C. The pooled results from these two experiments show that there was slightly more movement in the warmer water, and that the difference only just fails to be statistically significant. Therefore, in the experiment on light intensity (Table 3) the difference in rates of movement observed in artificial light and in sunlight is likely to have been

(a)   (b)
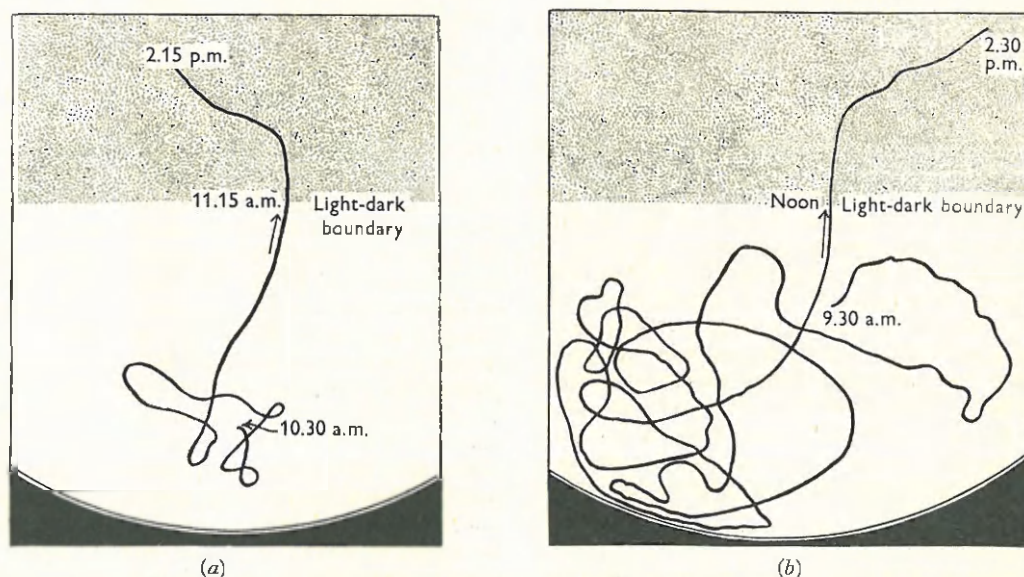
Fig. 2. Movements of two chitons placed in the illuminated halves of Petri dishes. × ⅓.

reduced rather than exaggerated by the temperature difference.

The orthokinetic response of chitons to different light intensities results in their tending to aggregate more or less automatically in the shaded regions. The tracks in Fig. 2*a*, *b* are copies of paths left by chitons placed under water in half-shaded dishes. In both experiments the chitons were placed initially in the illuminated half of the dish exposed to bright, cloudy conditions. Both followed a tortuous path at first: a fairly characteristic initial movement. The path soon straightened out somewhat and, in both instances, led by chance to the shaded area where the movements slowed down and then ceased entirely. In Fig. 2*a* the time spent in darkness was four times that spent in the sunlight; but in that time the animal travelled only one-third of the distance that it travelled when illuminated. In Fig. 2*b* the times spent in light and darkness were equal, but the distance covered in the light was over fourteen times that covered in darkness. It appears, therefore, that negative photo-ortho-kinesis is an important, if not the most important, factor in causing the aggregation of *Lepidochitona* in dark situations.

Table 4. *Relation between water temperature and rate of movement of chitons in sunlight*

Temp. (° C.) ...	13·5	10·5
No. of chitons ...	20	20
Mean distance (cm.) travelled in 10 min.	8·14 ± 0·86	6·00 ± 0·66

$t = 1·975$;   D.F. $= 38$; $P =$ just $> 0·05$.

5. DORSAL CONTACT

The fact that, typically, chitons are found beneath stones with their dorsal surfaces in contact with the surrounding pebbles or mud, and that if the stones on which they occur are displaced they tend to resume a position beneath them (see Fig. 1), suggests that they may respond to a dorsal contact stimulus. This possibility was investigated by placing chitons in shallow dishes containing raised sheets of glass supported at their corners by blocks of paraffin wax and arranged at such a height that the chitons could pass under them but in doing so would bring their dorsal surfaces in contact with the glass. Some of the experimental dishes were filled with sea water and the rest were moist, but the chitons in them were not immersed in water. In neither of these sets of experiments did the chitons show any tendency to collect under the glass and it was concluded that no thig-motaxis or allied response was present in *Lepido-chitona*.

6. GRAVITY

The influence of gravity was investigated under two sets of conditions: (*a*) A sheet of glass bearing chitons was suspended vertically in a tank of water in conditions of uniform illumination. The chitons moved in all directions across the glass plate and showed no response to the stimulus of gravity. (*b*) A similar set of experiments was set up, but in which the vertical glass sheet bearing the chitons was not immersed in water. On exposure to the air on a damp surface the majority of chitons showed a marked positively geotactic response and all those which moved any considerable distance tended to move downwards towards the bottom of the glass plate (Table 5*a* and Fig. 3*a*). This is in complete contrast with the previous experiment under identical conditions except that the glass plate was immersed in water. The conclusion is that the responses of the animals vary according to whether they are under water or exposed. Before confirming this view, however, it was necessary to determine whether or not any other factors were involved in bringing about the response. The main alternative possibilities were: (*a*) that the weight of the chiton's body out of water caused a passive drag downwards across the smooth glass plate; (*b*) that the water run from the top of the plate to keep the surface moist caused a positively rheotactic response, and it was to this and not to gravity that the chitons were responding.

That the first postulate is unlikely is shown by the fact that the characteristic convex footprints of the chitons are clearly visible in the 'printed' track. This indicates that the route taken is actively controlled and is not the result of passive sliding. A second check on this was made by repeating the experiments with chitons placed on sheets of roughened Perspex instead of smooth glass. The experimental conditions were otherwise identical. The result (Table 5*b*) confirmed the previous finding and all the animals which moved any considerable distance travelled downwards to the bottom of the plate (Fig. 3*b*). Two chitons fell off the Perspex plate when they reached its lower edge; none fell off at any other time.

In order to test whether or not the results obtained on the smooth glass and roughened Perspex surfaces were influenced by the small current which ran down the plates to moisten them, another control experiment was set up. In this the chitons were arranged on a plate as in the previous experiments, but this was placed horizontally instead of vertically. A gentle stream of water was arranged to flow across it, and the plate left for $2\frac{1}{2}$ hr. The chitons moved in all directions across the plate and showed no sign of a rheotactic response. This bears out the view that gravity is the stimulus to which the chitons respond when out of water. It has already been shown that no response to this factor occurs when the animals are totally immersed in water. A similar case of variation of response according to whether an animal is in or out of water has been described by Fraenkel (1927),

Table 5. *Distribution of chitons after $2\frac{1}{2}$ hr. on a damp vertical surface*

		No. of chitons	Orientation of head				No Movement
			Left	Right	Up	Down	
a	Glass plate	26	3	6	—	15	2
b	Perspex plate	26	1	2	1	18	4
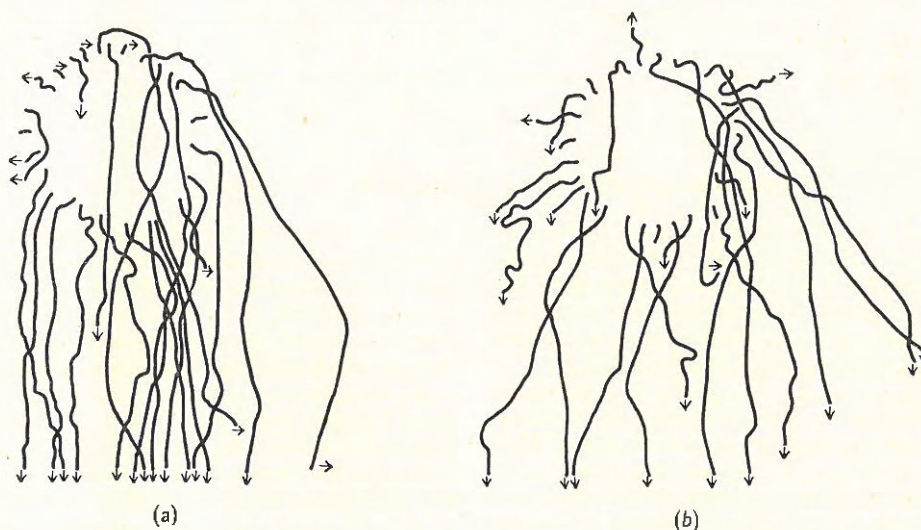
Fig. 3. Paths of chitons on vertical plates out of water (a) glass, (b) roughened Perspex. Arrows show the orientation of the head after $2\frac{1}{2}$ hr. $\times \frac{1}{4}$.

whose analysis of the behaviour of *Littorina neritoides* in relation to natural conditions showed that the response to light was dependent on two factors: whether the animal was in or out of water and whether or not it was upside down. Fraenkel showed it to be always photonegative in response when out of water, but in water to be photopositive when upside down; at other times it was photonegative. Fraenkel, in the course of his paper, correlates this response with the conditions of life of the animal. The success of *Lepidochitona* as a littoral animal may also be to a large extent dependent on the variation of response which it exhibits according to whether it is out of water or immersed in it.

7. HUMIDITY

As inhabitants of the littoral zone, chitons are covered twice daily by the tide. Between tides, however, the stones on which they occur are exposed. The upper surfaces of these stones rapidly dry but their under-surfaces tend to remain damp. It was stated in an earlier section that it is here, in a permanently damp micro-habitat, that the chitons are to be found when the tide is low. Moreover, if a stone bearing chitons

is overturned they move to that part of the stone now underneath, thus resuming a position of maximum humidity (Fig. 1). The following investigations were made to find the sensitivity of chitons to water loss and to test their responses to varying conditions of humidity.

(a) Rate of water loss in saturated and dry air

The chitons used in the following experiments were left undisturbed in an aerated aquarium tank on two weighed glass plates for 24 hr. prior to use. The plates were then removed, wiped, and the chitons on them dried with blotting-paper. The plates bearing the chitons were then weighed and each was suspended in a closed chamber. One was placed in a chamber containing calcium chloride, and in which no moisture could be detected in the air, the other contained a dish of water and the air in it was saturated. Weighings were made at intervals during the experiment. The glass plate from the humid chamber was always carefully blotted before weighing in order to prevent any water which might have condensed upon it from falsifying the results.

Table 6. *The rate of water loss of chitons in wet and dry air*

		Number of chitons		Total weight (g.) of chitons	
	Time	Dry chamber	Wet chamber	Dry chamber	Wet chamber
Wed.	11 a.m.	14	14	1·407	1·444
	1 p.m.	13*	14	1·148	1·377
	6 p.m.	13	12*	1·146†	1·128
Thur.	9.30 a.m.	13	12	0·623‡	1·128
	11.30 a.m.	13	12	—	1·128
Av. weight per chiton at beginning	—	—	—	0·100	0·103
Av. weight per chiton at end	—	—	—	0·048	0·094
Percentage loss of weight per chiton	—	—	—	52	8·7

* **Chitons** dropped off plate.   † 2 chitons dead.   ‡ All chitons dead.

Table 6 shows that all the chitons in the dry chamber died but that those in the chamber containing saturated air all survived. These results show the sensitivity of chitons to water loss and are in full accordance with the fact that under natural conditions the animals always occur in damp situations.

Yet more striking were the results of experiments carried out to show the rates of desiccation of chitons in moving and dry air. Two batches of chitons were allowed to settle on dry stones and then gently blotted to remove all surplus water. Half of the stones were then removed to a closed chamber containing dry air and the rest were placed in a position exposed to wind and sunshine. The criterion used in assessing the results of the experiment was the length of time taken for the animals to reach a degree of desiccation from which recovery was impossible. Although these conditions could not be accurately controlled it was found that an exposure of 60 min. was sufficient to desiccate the largest chitons used, whilst chitons of 1 cm. in length and less were usually desiccated in under 30 min. These results show that the drying power of the sun and the wind to which the chitons may be exposed when on top of a stone is sufficient to bring about the death of the largest specimen within an hour. Owing to the fact that, at Whitstable, *Lepidochitona* is restricted almost entirely to a zone above half tide level where the average exposure per tide is approximately 9 hr., these animals stand but little chance of surviving these conditions unless they spend this time in a sheltered position beneath the stones where they are protected from the desiccating power of the sun and the wind.

(b) *Distribution in a humidity gradient*

In order to investigate whether chitons respond to differences in the humidity of the air a humidity chamber was set up. This consisted of a cylindrical glass chamber 18 in. in length and containing at one end a dish of calcium chloride and at the other end a dish of water. A plate of perforated zinc was placed over these dishes and the entrance to the chamber was at the damp end and consisted of a sheet of vaselined glass. This chamber was placed in conditions of uniform light and temperature, and left overnight in order to establish a steady humidity gradient. Ten chitons, previously kept in a tank in a dark-room for 2 hr., were inserted into the chamber on a sheet of glass. The glass was carefully dried and surplus water removed from the surface of the chitons. It was hoped to see whether or not the distribution of the animals was influenced by the humidity gradient. After 30 min. the animals had scattered about the centre at which they were originally placed, but after this, movement soon ceased. The girdles of the animals were applied firmly to the glass and no further movement occurred. In other experiments using this apparatus similar results occurred. There was movement initially but this was not maintained (Table 7).

Table 7. *Distribution of chitons after 30 min. in a humidity chamber*

(Each division is 3 cm., and animals were introduced into the central one.)

	Drier side		Central 3 cm.	Damper side	
	1–3	3–6	6–9	9–12	12–15
Exp. 1	*	3	3	4	*
Exp. 2	*	3	5	1	1
Exp. 3	1	5	2	1	3
Total	1	11	10	6	4

The chitons in this experiment were removed directly from an aquarium tank in the dark-room and their introduction into the humidity chamber must have resulted in a considerable increase in the humidity of the air in their immediate vicinity. In an endeavour to prevent this, further experiments were carried out prior to which the chitons were left in the dark-room out of water on a dry glass plate for 30 min. These chitons kept their girdles firmly applied to the glass and did not move even when placed in a chamber

where the air was fully saturated. When placed in a gradient of humidity they either died at the dry end or remained alive and motionless at the damp end. It was therefore concluded that *Lepidochitona* is not sensitive to a humidity gradient. As the chitons traversed smooth glass, ground glass or stone surfaces with apparently equal facility when the surfaces were moist, but did not move across them when dry, it is concluded that dry surfaces provide a mechanical bar to progress.

(c) *The rate of movement under different conditions of humidity*

It has been concluded from the results of the humidity chamber experiments recorded in the last paragraph that on a dry surface, even when the air is saturated, the chitons do not move. However, the following experiments were conducted before those in the humidity chamber and they bring to light the important fact that under very moist conditions the chitons move across a damp surface faster than when actually under water. Chitons were placed, one to each Petri dish, in the dark-room for a period of 60 min. At the end of this time the water was poured from the dishes without disturbing the attached chitons and the bottoms of the dishes were wiped to remove existing mucous tracks. The individual observations in this set of experiments were not made simultaneously but over a period of 2 days. The results are given in Table 8. The general experimental procedure was as follows: one-third of the Petri dishes (Group A) were completely refilled with water, another third were covered with a surface layer of water which did not completely immerse the contained chitons (Group B) and the remainder were dried as completely as possible (Group C). Three dishes from each group were then taken and placed in bright overhead sunlight. They were left for 10 min., and then the chitons were removed and their mucous tracks were 'printed' and their length measured with a Philip's map measurer. The results indicated that the rate of movement was very low indeed, but that under moist conditions it was appreciably higher than when the chitons were under water. Similar results were obtained when the groups of chitons were placed (after an hour in darkness) in artificial light and in darkness.

These results confirm those for the responses of chitons to light, namely, that the rate of movement is highest in a high light intensity. In addition they show that it is also influenced by the degree of exposure of the animals. In complete dryness, even when in bright sunlight, movement soon completely ceases. In all cases the chitons move appreciably faster when in very moist conditions than they do in surroundings that are otherwise identical apart from the fact that they are immersed in water. The speed of the chitons moving in moist surroundings, even in

Table 8. *Distances moved in 10 min. under different conditions of humidity*

	Distances (cm.) moved by individual chitons in 10 min.		
Illumination	Under water, Group A	Damp, Group B	Dry, Group C
Sunlight	8·3	10·2	1·7
	5·8	8·5	2·5
	10·8	19·4	o
Av.	**8·3**	**12·7**	**1·4**
Artificial light	4·0	6·7	o
	4·6	6·5	o
	4·7	6·3	o
Av.	**4·4**	**6·5**	**o**
Darkness	o	4·4	o
	o	o	o
	o	o	o
Av.	**o**	**1·4**	**o**

low light intensities, is relatively high. Table 8 indicates that under the conditions considered the rate of movement decreases thus:

> Bright sunlight, moist conditions
> ↓
> Bright sunlight, completely immersed
> ↓
> Artificial light, moist conditions
> ↓
> Artificial light, completely immersed

and that in completely dry conditions movement is almost negligible however intense the illumination. The movement recorded under completely dry conditions in the experiments in this section is probably due in the main to the impossibility of thoroughly drying the animals used in the experiments.

8. DISCUSSION

Lepidochitona cinereus is in almost all respects a typical member of the intertidal fauna, and its behaviour in response to certain simple physical features of its environment is adapted to its life on the shore. Light, gravity and humidity all influence its behaviour. The possibility of a response to a dorsal contact stimulus was also considered but no such response was found. *Lepidochitona* possesses an orthokinetic response to a non-directional light stimulus which results in its aggregation in regions of low light intensity. Some indication was also found that the animal possesses a negatively phototactic response to light of very high intensity. It does not move across a dry surface even when the air above it is completely saturated with water vapour. Although no movement occurs under these conditions, the rate of movement of chitons out of water but on a damp surface was appreciably higher than that of chitons completely immersed in water. Chitons are extremely

sensitive to desiccation and are unable to recover from an exposure of 1 hr. in windy, sunny conditions. This implies that if they remain on top of stones out of the water when the tide is low, they suffer desiccation beyond recovery. The response of *Lepidochitona* to gravity is of particular interest as it varies according to the external conditions. When completely immersed in water no gravity response is shown, but when out of water but on a damp surface there is a well-marked positive geotaxis.

The interaction of these responses results in the aggregation of chitons below stones when the tide is low. As the tide falls the stones of the chiton zone become uncovered and the chitons on them, now out of the water, react to gravity by moving downwards. While the surface of the stone is moist they travel at a maximum rate, especially if the light intensity is high. This leads them to the region beneath the stone and here, responding orthokinetically to the low light intensity, they tend to slow down and aggregate. In this way their behaviour mechanism leads them to a damp microhabitat where they are sheltered while the beach is exposed at low tide. When the chiton zone is again covered by the tide, however, the same conditions no longer hold and the behaviour of the chitons is modified accordingly. As all the surfaces of the stones are immersed in water, rate of movement above and below the stones becomes approximately equal, the difference in light intensity above and below the stones becomes much reduced and the geotactic response, so marked when the chitons are out of water, is lost. Hence the chitons under these conditions, where there is no risk of desiccation, though tending to remain below the stones if the water is shallow and the light intensity high, are not restricted to such situations but are free to move and feed on all the surfaces of the stones.

9. ACKNOWLEDGEMENTS

10. SUMMARY

1. *Lepidochitona cinereus* is found usually attached to the undersurfaces of stones, in the intertidal zone.

2. The density of the population falls off towards the shoreward and the seaward limit of the chiton zone on the shore at Whitstable, and is at a maximum at approximately half-tide level.

3. At any level of the shore where *Lepidochitona* occurs, the density of population is greater under stones occurring in pools than under those that are not immersed. This indicates that the microhabitat of the animals is permanently damp despite the rising and the falling of the tide.

4. The influence of certain simple physical features of the environment in bringing about this orientation is considered.

5. The animals collect in shaded areas.

6. Evidence suggests that the animals are able to orientate themselves to a strong directional light stimulus and move away from it but that their distribution is dependent to a large extent on an ortho-kinetic response to a directional light stimulus, in which the rate of movement is dependent on the light intensity involved.

7. No evidence was obtained of a response to dorsal contact stimuli.

8. *Lepidochitona* exhibits a positive geotaxis when out of water but there is no geotactic response when the animals are in water.

9. No movement occurs on a completely dry surface. The rate of movement on a damp surface, however, is appreciably greater than that of chitons under water.

10. The way in which, by the interaction of these responses, *Lepidochitona* maintains its characteristic position on the shore, is discussed.

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