The Negative Geotropism of the Periwinkle: A Study in Littoral Ecology.—By F. Ronald Hayes, B. Sc., Assistant in Histology and Embryology, Dalhousie University, Halifax, N. S.

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I. Introduction

Various investigators have interested themselves from time to time in an investigation of the anatomical and physiological features associated with the negative geotropism of snails (Parker 1911; Kanda 1916-1), and various theories have been set forth in an attempt to account for this phenomenon.

As early as 1897 Davenport and Perkins experimenting on *Limax maximus*, came to the conclusion that "the precision of orientation in the slug varies directly with the active component of gravity" and "this tendency (to go either up or down) must be ascribed to some internal condition of the individuals, for it varies in different individuals and in the same individuals at different times." It was early suspected moreover, that response to gravity might be associated with the center of gravity of the body of the individual, and Frandsen (1901), also speaking of *Limax*, makes the assertion that the essential factor is "the relative proportions of the anterior and posterior regions of the animal's body. All the conditions being the same it is this factor which determines whether the head end will be directed up or down."

These facts were demonstrated in more critical terms for *Littorina littorea* (*L*) the common periwinkle, by Kanda (1916-2), who, noticing how a periwinkle sank in water, observed that the centre ofgravity for living animals and also for shells is located in the posterior region of the shell. He determined in quantitative terms the direct correspondence between the proportion of animals exhibiting negative geotropism and the active component of gravity.

It is true also that there are pronounced anatomical modifications associated with negative geotropism. Pelseneer (1895) has shown that in the periwinkle, morphological modification and specialization in the respiratory organs has been associated with this activity. Part of the wall of the pallial cavity for example, has become vascular like that of the pulmonary chamber of the true *Pulmonata*. Thus the climbing habit would appear to be normal and characteristic of the species.

Tattersall (1920) records an attempt made in Ireland to turn the climbing habits of the common periwinkle, *Littorina littorea*, to practical use by the erection of stakes along the appropriate part of the littoral zone, on which the periwinkles might ascend and so be collected more easily for marketing. While the observations are deficient in certain respects, they demonstrate several points of interest. The number of periwinkles climbing the stakes varied from 15 to 35 per cent of the total number on the area investigated. Calm water appeared to be a necessity if any periwinkles were to climb, slight agitation of the water around a stake being detrimental to a successful ascent, loosening the slight purchase a periwinkle is able to obtain on a comparatively rough climbing surface.

Tattersall summarizes his results as follows: "From measurements made on the climbing winkles and those found on the ground at the same time, there appears to be no evidence that the former were in any way larger than the latter. In other words the climbing habit is just as strongly developed in young winkles as in older ones."

This assertion is not in agreement with that of Kanda (1916-2), who states that in his experiments on the negative geotropism of L. *littorea*, he used animals about 1.5 by 1.1 centimetres because the larger ones were noticed to be more sluggish in commencing locomotion after being handled. "The younger animals are more active and quicker to respond to stimuli." Results from a series of preliminary experiments conducted with the object of determining if possible, a relationship between size and activity, support the view of Tattersall.

A "physiological agreement" has been shown to exist in many animal groupings in nature (Shelford 1913). By this agreement species may be said to be automatically directed toward their appropriate habitat. Studies have indicated that a directive mechanism exists, consisting of a remarkable assemblage of delicate adjustments of organism to environment. This mechanism results in cases where choice is possible, in a return of the animal to its optimum habitat.

The investigations here described were designed in an attempt to analyze in quantative terms, the influence of a separate environmental factor namely gravity, in determining the natural distribution of *Littorina littorea*.

Experiments were conducted in an effort to determine in quantative terms, whether there existed in specimens of *L. littorea* collected at different levels of the littoral zone, a variation in the negative geotropism which could be directly correlated with vertical habitat. The results indicated that such a variation does exist and an analysis was then made of the effects of desiccation and immersion on negative geotropism.

Desiccation and immersion serve as clues to the study of littoral ecology since, in *Littorina* for example, the essential difference in habitat of individuals in upper and lower littoral zones is a difference in the length of time they are successively submitted to the influence of these two conditions.

Situated on an estuary of the Bay of Fundy, the Atlantic Biological Station where the greater part of the work here recorded was carried out, presents ideal conditions for a study of the littoral zone. A tidal change of over 8 metres exposes at low tide, an area of considerable width, exhibiting very clearly all the features associated with an inter-tidal graduation of fauna and flora.

The littoral zone, in the area where the greater number of collections were made, was comprised of a series of rocky ledges forming an ideal habitat for the periwinkle. *L. littorea* exists in considerable numbers from a short distance above the lowest tidal level to slightly above the mid tide level. It exists below low tide level in smaller numbers to depths of at least 20 fathoms (Huntsman, 1918), and above mid tide level in tide pools and under permanently damp conditions.

Sincere thanks must be given to Prof. J. Nelson Gowanloch of Dalhousie University, for his interest and counsel and for personal direction of a great part of the investigation. I am also indebted to Dr. A. G. Huntsman, Director of the Atlantic Biological Station for advice and assistance at every stage of the work. Certain lines of the investigation were continued throughout the winter at the Zoological Laboratories of Dalhousie University, Halifax, N. S.

II. Experimental Technique

For the experiments in negative geotropism cylindrical glass jars were used, 45 centimeters high and possessing an inside diameter of 6 centimeters. These jars were so graduated that the distance that an animal had travelled from the bottom at the time a reading was taken, could be accurately determined. In most experiments four such jars were used, two of them filled to within 5 centimeters of the top with oxygenated sea water, and two freshly rinsed out with sea water in order to dampen the inside and reduce the temperature.

Five individuals were placed in each jar and their progress upwards on the inside wall of the jar recorded every ten minutes. Thus the experimental result for each "immersed" and "dry" jar experiment is usually computed from the average performance of 10 individuals, never from less than five. As soon as any animals reached the surface of the water further readings were considered invalid and have been disregarded as far as possible in calculating results.

All calculations have been made with reference to a single periodical reading which has been carefully selected as the most typical of the series. In general the procedure has been to use the first reading recorded after any individual had reached the top of the jar. This usually has been the 20 minute reading. The height to which each "immersed" and each "dry" animal had climbed, was noted, and the total distance that all the animals of each group had ascended was found. This was divided so that a figure was obtained which represented the average performance per individual per 10 minutes in centimeters, and this is the experimental result quoted.

It will be observed that no opportunity was offered by this experimental technique for the animals to exhibit positive geotropism. In view of preliminary experiments which were in complete harmony with the results of Kanda (1916-2), who showed that on a vertical surface 100 per cent of *L. littorea* exhibit negative geotropism, this precaution was not deemed necessary.

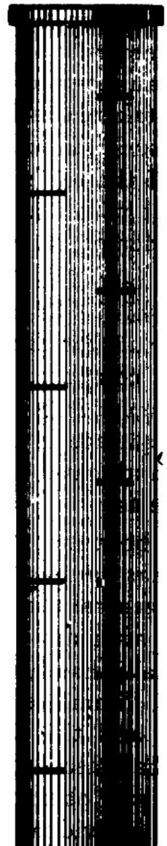

FIG. 1

A Negative Geotropism Jar. Five animals were placed in each of four such jars.

III. Correlation of Negative Geotropism with Vertical Habitat

In Table 1 will be found the figures representing the average responses of animals collected at low, mid and high tide levels.

The results for both "immersed" and "dry" groups are given. All the animals used were collected immediately before the commencement of the experiment.

Table I

TIDAL LEVEL	"IMMERSED" GROUP RESPONSE PER ANIMAL PER TEN MINUTES CENTIMETERS	"DRY" GROUP RESPONSE PER ANIMAL PER TEN MINUTES CENTIMETERS
Low	14.6	3.6
Mid	21.0	19.0
High	26.7	12.6

This table indicates that a physiological gradient exists in *L. littorea* correlated with its position in the littoral zone, and that the negative geotropic response may serve as an index to this gradient. A further discussion of these results will be found at the close of this paper.

IV. Variation in Negative Geotropism due to the Effects of Desiccation

The technique of desiccation was very simple. A number of periwinkles were placed in an open dish and exposed to the air under conditions approximating those of normal intertidal exposure.

Groups were periodically removed and subjected to a negative geotropism experiment similar to that recorded above.

For several days after the desiccation commenced the animals moved about in the dish; then the opercula became sealed with mucus and all activity ceased. Each individual used was subjected to a vital test before any experiment was performed.

It is important to note a difference in the method of calculation of results in the desiccation experiments from that used for the other experiments. Only the animals actually exhibiting a response to gravity were considered. If an individual remained motionless or active on the bottom of the jar it was disregarded from the calculations of averages; hence the twofold character of the tables showing both the percentage of animals responding and the average amount of response for those that did show any activity.

Table 2 shows the effect of a short period of desiccation on negative geotropism. This experiment was conducted in the hot sunshine of an August afternoon, the desiccation taking place in the open; thus there was an approximation of the most severe conditions under which periwinkles would find themselves during intertidal exposure.

TABLE 2.

Desiccation Time Hours	"IMMERSED" GROUP		"DRY" GROUP	
	Percentage responding	Amount of response per animal per ten minutes Centimeters	Percentage responding	Amount of response per animal per ten minutes Centimeters
0.0	70	7.2	10	5.0
1.0	70	11.2	0	...
1.5	100	14.0	20	5.0
2.0	100	6.5	0	...
2.5	70	5.4	20	7.5
3.0	100	9.2	0	...

It will be seen from the "immersed" group figures of this table that the result of desiccation for a short time is at first to increase both the percentage of individuals exhibiting negative geotropism and the amount of response.

This correlates with what might have been expected from a consideration of the face that animals living at higher tidal levels, and therefore subjected to longer periods of intertidal desiccation than individuals lower down in the littoral zone, exhibit a higher figure for their negative geotropism. Thus the effects of short periods of desiccation entirely confirm the results recorded in Table 1.

The figures in the "dry" group column are seemingly irregular and a suggested explanation is that they are in accord with the observed facts of the life of this form, where there exists a decided lack of activity when the tide is out.

Periwinkles do not move about in search of food after the substratum has thoroughly dried. They are under the necessity of conserving their supply of moisture till they are once more immersed by the incoming tide. A general consideration of all the "dry" group experiments here recorded will be found elsewhere in this paper.

In Table 3 figures are found showing the effect of a longer period of desiccation on negative geotropism. It will be observed that there are no "dry" group results given. The reason is that after a relatively short period of desiccation there was a total absence of activity of the periwinkles except under the stimulus of water. The animals whose performance is recorded in Table 3, were desiccated in a laboratory on a shelf over a large aquarium through which salt water was flowing. They were thus in an atmosphere containing a considerable quantity of moisture and at no time exposed to excessive heat.

TABLE 3

DESICCATION TIME HOURS	"IMMERSED" GROUP	
	PERCENTAGE RESPONDING	AMOUNT OF RESPONSE PER ANIMAL PER TEN MINUTES IN CENTIMETERS
0	90	16.8
7	30	15.8
27	30	9.3
45	50	6.0
91	50	0.7
118	50	0.8
141	40	0.7
172	20	2.8
262	30	0.6
335	10	1.6

From these figures it can be clearly seen that longer periods of desiccation result in a well defined decrease in amount of response and percentage of animals showing activity. No other results could be in accordance with the facts observable through a study of the natural history of the species. If, in nature the animals continued to walk uphill after they had been subjected to intertidal exposure for a few hours, they would clearly soon migrate out of the littoral zone and become subject to conditions which must finally prove to be lethal.

The figures for several of the latter readings are based on the performance of so few individuals that they do not form as exact a mathematical gradient as might have been desired, but the general trend of the results is very clearly definable.

In order to test whether the results of this series of experiments might not be due to starvation rather than to desiccation, animals which had been desiccated for varying periods of time were subjected to a simple test.

They were first regularly tested and then placed in a jar having the mouth covered by wire gauze, which permitted the passage of sea water but not of the algae which serves as food, and suspended in the sea for twenty-four hours; they were then tested again. The results showed conclusively that the effects were from desiccation and not starvation.

Apparently metabolic activity decreases greatly when the animals seal themselves up during periods of drought.

Table 4 furnishes two examples which will suffice as illustration.

TABLE 4

DESICCATION TIME HOURS		"IMMERSED" GROUP		"DRY" GROUP	
		PERCENTAGE RESPONDING	AMOUNT OF RESPONSE PER ANIMAL PER TEN MINUTES CMS.	PERCENTAGE RESPONDING	AMOUNT OF RESPONSE PER ANIMAL PER TEN MINUTES CENTIMETERS
91	Before immersion	50	0.8	0	. . .
	After immersion	60	6.7	40	2.9
335	Before immersion	10	1.6	0	. . .
	After immersion	70	4.6	0	. . .

The results are clear cut. It would seem a fair assumption that if a longer period of recovery had been permitted, the performance would have completely returned to normal.

V. The Effect Continued of Immersion on Negative Geotropism

For the experiments on immersion some 2000 adult specimens of *L. littorea* were collected at low tide level and placed in quart jars, 75 individuals to each jar, and there was added a supply of the fronds of *Fucus* with fruiting buds attached sufficient to serve as food for a long time.

The mouths of the jars were covered with carefully paraffined copper wire gauze. The jars were then suspended from the breakwater at the Biological Station, thus undergoing total immersion. No jar was closer than one meter from the surface and from this depth they ranged to three metres. It was not thought that light could have been a factor in any results.

Controls were used as follows, there being two jars of each control type:

(I) 10 animals per quart jar; no *Fucus*.

(II) 10 animals per quart jar; full supply of *Fucus* for 75 animals.

(III) 75 animals per quart jar; no *Fucus*.

The controls proved effective; no animals died in any control jar nor did any die in any experimental jar.

A geotropism experiment was conducted on a group of the animals periodically, in an attempt to find a variation due to the physiological change of the periwinkles resulting from their continued immersion.

Owing to storms which carried away a number of jars and made it necessary to replace several, the experimental results do not form as perfectly graded a series as might have been desired. Several long gaps toward the latter part indicate a time when it was impossible to obtain experimental data owing to the factor mentioned.

Table 5 records the responses showing in this series of tests
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TABLE 5

TIME OF IMMERSION HOURS	"IMMERSED" GROUP	"DRY" GROUP
	RESPONSE PER ANIMAL PER TEN MINUTES CENTIMETERS	RESPONSE PER ANIMAL PER TEN MINUTES CENTIMETERS
0 (Normal)	14.6	5.3
104	10.6	2.9
263	15.4	2.1
329	8.8	4.4
521	13.2	2.5
618	16.1	5.1
759	15.7	1.2
927	17.7	4.8
1344	13.1	1.8
1380	3.4	3.1

It seems at least a probability on the basis of these results, that there is an increase in negative geotropism resulting from continued immersion. The 1380 hour result may be discarded as totally out of harmony with the remainder. Starvation and overcrowding for 57 days had doubtless begun to show an effect, this hypothesis being sustained by the 1344 hour result, which gives a slight indication of a lowering of activity.

With the exception of the 329 hour group which varies radically, there is a clear gradient upwards to the 927 hour reading in the "immersed" group.

The "dry" group varies erratically as it does in the other experiments, a result which will be treated of elsewhere.

VI. The Effect of Temperature on Negative Geotropism

A series of field observations was initiated at Point Pleasant Park, Halifax, N. S., on November 28, 1924, with the object of determining the behaviour of *Littorina littorea* in winter, and of ascertaining if possible, whether a reversal of geotropism occurs during that season.

To this end a number of one square metre areas were marked out on the shore at typical parts of the intertidal zone and periodical observations made of the changes in their fauna. The varying numbers of *L. littorea* present on certain of these areas offers a picture of their winter habitat variations.

Periwinkles virtually disappeared from the upper areas and appeared in constantly increasing numbers just at the lowest tidal level. A table showing the variations in numbers of *L. littorea* in five areas at low tide level, each of one square metre, is instructive.

An area is pictured 5 meters long and 1 metre wide, with its greatest length parallel to the shore line, and divided into five one square metre areas. These areas were awash at low tide during neap tides but exposed by the lowest spring tides.
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TABLE 6

DATE	AREA				
	A	B	C	D	E
Nov. 28	155	170	135	215	180
Dec. 12	150	200	100	50	50
Dec. 27	415	260	125	205	240
Mar. 11	170	130	170	105	160

The wide variation in numbers over these areas at any given reading is capable of explanation by the prevailing weather conditions, coupled with the nature of the substratum of the areas. The first reading, that of November 28, reveals a reasonable uniformity of distribution over all the areas, a condition which agrees with the expectations, as there is no marked difference in the substratum of the areas such as mud flats, extensive growths of rockweed, etc. Up to the time of the start of the observations there had been no marked change to cold weather. The Dec. 12 records show a marked diminution of numbers of animals on areas "D" and "E" and a decrease also, though not so marked, in area "C". An explanation may be sought in the surface characters of the areas. Areas "A" and "B" provided sheltered crannies, while large flat rocks and gravel covered the other areas. The periwinkles were found in the most sheltered places. The season at this time was one of neap tides so that these areas were immersed almost constantly. Thus the animals in damp crannies and tiny tide pools would be less subject to the effects of cold than those in exposed places. The readings on December 27, taken at a period of spring tides following a period of severe cold, show a marked increase in the number of periwinkles which have come from the upper littoral zone.

The areas were at this time supporting a maximum of *Littorina*, every cranny being filled to capacity. The March 11 figure shows that an upward migration has begun.

The problem involved by these observations was whether it was a reversal of negative geotropism or loss of activity that caused the periwinkles to travel from the upper to the lower zones. It seemed a reasonable hypothesis that they might have been washed down, and such indeed proved to be the case. Observations showed an almost total lack of activity in cold water and there was no evidence of any measurable negative geotropism in individuals tested, nor of any power to cling to the substratum. Tested in water of a temperature near the freezing point the only evidence of activity in the partly extended animal was a feeble waving of tentacles, even the stimulus of a sharp instrument pricking the foot bringing no marked response.

VII. Summary and Conclusions

There exists in *Littorina* a clearly definable, specific, physiological gradient corresponding to the position in the littoral zone, and the variation in negative geotropism may be taken as an index of this gradient. Thus the average figure representing the negative geotropism of an individual collected in the upper littoral zone, will be higher than that for an animal collected at a lower level.

Confirmatory evidence of the existence of this gradient was found in an analysis of the effects of desiccation and immersion on negative geotropism. Desiccation for periods of a few hours might be likened to ordinary intertidal exposure of individuals from the upper zones, resulting in increased negative geotropism following subsequent immersion.

It was found that the greatest negative geotropism value was obtained after desiccation for 1.5 hours. Desiccation for longer periods resulted in a condition which may be compared to that of an animal left above high tide level by neap tides. The result was a profound diminution of geotropic response serving therefore in nature as an effective, automatic check upon the activity of animals already at the upper danger line of their habitat.

Subjection to constant immersion will produce a change in the physiological life history, and a variation can be demonstrated in the negative geotropism which acts as an index to this change. The evidence from immersion experiments is in entire harmony with the other experimental results correlating negative geotropism with tidal level, as it might be expected that constant immersion of an intertidal animal would produce a reaction designed to restore it to its original intertidal position.

Throughout all the experiments, the extreme variability of the factors under which the "dry" group was tested is, I think, quite sufficient explanation for its less consistent results than those for the "immersed" group. The "dry" jars were rinsed out and then the experiment was started. The temperature would be changing and the damp conditions would make a specimen alternately dry and wet. It has been shown (Mitsukuri—1901), that when a periwinkle, crawling over a dry or moist surface, comes to a pool of water, it almost always pauses and may change direction or cease motion.

In a general consideration of these results it should be remembered that the conditions to which *L. littorea* was subjected in an effort to modify its physiological life history, were conditions which would be very often met with in nature without the necessity of radical response. Between spring tides for example, the animals in the low tide level might remain immersed for weeks at a time without serious result. The animals used in the immersion experiments were moreover, collected at low tide level.

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