The Effect of Environmental Factors on the Development and Growth of Littorina littorea. — By Frederick Ronald Hayes, B. Sc., Assistant in Histology and Embryology, Dalhousie University, Halifax, N. S.

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While engaged in a general investigation of the biology of *Littorina littorea*, the common periwinkle, at the Atlantic Biological Station, St. Andrews, N. B., during the summer of 1925, the remarkable paucity of information regarding the factors influencing the development and growth of this species led to a series of experiments designed to give some information about this subject. The effect on development of decreasing the salinity of the water, an effect that would frequently act in the natural life of the species, was worked out in as quantitative terms as equipment and time would permit. Experiments were also performed and an analysis attempted, of the relative importance to growth of the common environmental factors, light, temperature and moisture.

Reproduction and Embryological Experiments.

Very little is known in detail of the breeding habits of *Littorina*. An unidentified Gastropod egg found on the Nova Scotia coast is described by Wright (1907). Tattersall (1920), rightly identifies the egg as that of *L. littorea* L. Caullery and Pelseneer (1911) have identified by a process of elimination, as belonging to *L. littorea*, an egg found in abundance at Wimereux, France. They were not, however, able to confirm their observations by inducing the adults to breed in the laboratory. I have found eggs in their characteristic cases in large numbers in plankton tows taken near the shore at St. Andrews, and, in markedly smaller numbers, in plankton tows taken off Navy Island, farther from the shore. Very small specimens of fully developed periwinkles have also been found in plankton tows, suggesting the possibility that development might proceed to the adult body form before the individual assumed its littoral existance.

The date of commencement of the breeding season has not been determined but it is undoubtedly very early, since small fully developed specimens could be found on the rocks at low tide level early in June when the first observations were made. Individuals have been induced to copulate as late as July 24, although the number copulating was notably smaller than earlier in the season (July 7th).

Tattersall states: "The sexes in *L. littorea* are separate, and, in the breeding season, the males are readily distinguished from the females by the possession of a long, rather stout penis on the right side of the body." (The penis is an erectile organ and is situated just behind the right tentacle). "The penis appears to exhibit seasonal variations in size and only attains its full development when the testis is completely ripe. At the close of the breeding season the penis becomes reduced in size externally, and against the darkly pigmented epidermis of the body, is almost invisible.

The separation of the sexes becomes difficult except the animals are removed from their shells. When the next breeding season is approaching and the generative cells are once more active, the penis again increases in size, and, keeping pace with the maturation of the sperm cells, reaches full size with the appearance in the vas deferens of ripe spermatozoa. The testis is a diffuse organ ramifying through the hepatic coil, and giving to it a pale yellow-brown color in the breeding season. The ovary is likewise diffuse but pink in color."

L. littorea was the only species with which any embryological experiments were performed. To induce copulation, in order to obtain eggs, individuals were freshly brought in and a large number were placed in a shallow granite pan which was filled to a depth of several centimetres with fresh sea water. The animals were then watched till a pair were observed copulating, when they were removed, being disturbed as little as possible, to a quart jar filled with sea water. When copulation was over, the female was placed in a four ounce bottle filled with carefully filtered sea water, and a piece of finest bolting silk tied over the mouth of the bottle, which was then suspended from the end of the wharf over night. Next morning the bottle was taken in and the eggs, usually over 200, removed to a suitable container. The eggs rested on the bottom of the bottle in their egg cases, and could be handled with a fine pipette.

Some notes were made regarding the mechanism of copulation. It was observed that during copulation the female invariably is the individual attached to the substratum, while the male clings to the shell of the female in such a way that the erected penis may be inserted under the mantle of the female immediately above the right tentacle. The female does not walk about during copulation but remains inactive except for the extended tentacles, which move about through the water. Some pairs of *L. littorea* were timed to see how long copulation lasted. One pair of animals was observed together and nine minutes later were seen to be copulating. Three minutes later they had separated. In another case copulation lasted for a minute and a half; while another copulation observed lasted two minutes.

When the eggs were brought in, about fifteen hours after copulation, they were for the most part, in the one cell stage, a few being advanced to the two or four cell stage. The eggs were encased, one or more, in the typical egg case which looks like a Chinese hat, with the eggs in the hollow crown. As many as seven eggs have been seen in a single case, though three is the number most commonly found. The usual sizes of the characteristic structures are about as follows:

Diameter of the entire capsule	0.85 to 0.65 mm.
Width of the brim of the capsule	0.16 to 0.13 mm.
Diameter of the egg membrane	0.18 to 0.16 mm.
Diameter of the egg	0.15 to 0.12 mm.

The eggs brought in were transferred to four ounce bottles containing water of varying salinities,about twenty eggs being placed in a bottle. The salinity was reduced by adding aerated distilled water to fresh filtered sea water. The bottles were corked and hung off the breakwater, thus being restored to their natural temperature conditions. Controls, using both cork stoppers and bolting silk over the mouth of the bottles, were run, using normal sea water. The salinities used were 0, 5, 10, 15, 20, 25, 30 gms. per mille, and normal controls. Five bottles of each of the low salinities were used and a bottle was taken in periodically and the eggs examined.

In salinities of 0 and 5, no development took place. Under these conditions eggs remained at precisely the same stage as when placed there, for a few days and then died, and great enlargement in size and disintegration ensued.

No development past early segmentation stages in the capsule could be found under salinity conditions of 10. Segmentation was unequal, and while the eggs appeared capable of living indefinitely, they never developed very far.

In the water of a salinity of 15, development proceeded slowly but not normally. The early trocophore stage lacked the usual ring of circumoral cilia and was very irregularly shaped. The eggs, however, were clearly able to live and develop at this salinity although abnormally. It took ten days for a trocophore to appear. No veliger larvae were found although they might have appeared had the eggs been permitted to develop for a sufficiently long time.

In salinity of 20 or over, development appeared to progress normally, although the lowered salinity slowed the rate of development down somewhat. Segmentation was completed during the first day of development in normal sea water. By the third day the circumoral ring of cilia was complete and the embryo began to rotate in its membrane. The embro as a young veliger, broke from the capsule at the sixth day and swam about freely. These veliger larvae are so minute that they are rather difficult to find in a bottle of water. A dark background proved to be of considerable help in the study of this stage. The early veliger resembled very closely Wright's figure. An examination of the empty capsules, after the embryos had broken out, confirmed the observations of Caullery and Pelseneer that the larvae breaks through the flat side of the capsule when escaping

No success rewarded efforts made to carry development to the adult stage. The reason is doubtless that the problem of how to feed the embryos was unsolved. Reliance on the plankton naturally in the water, for service as food, was impossible since unstrained sea water proved most detrimental to development, the diatoms and protozoa multiplying so rapidly that the water soon became foul.

Experiments to Determine Rate of Growth.

In the growth experiments small specimens of *L. littorea* were used, ranging in size from 2.4 mm. to 3.2 mm., although very few of the individuals used were larger than 3.0 mm. A large number of these tiny animals was collected from the crannies of a rocky ledge 1.5 metres above low tide level, near the Station. These were measured in the laboratory and divided into three groups. The single measurement recorded represents the greatest length from tip of spire to lip, as observed through a low power objective with a calibrated ocular micrometer. Each individual was placed on a slide and held with a pair of fine forceps in such a way that the greatest length could be determined. Fifty individuals were put in each of the three groups and each group was placed in a one-pint jar along with a supply of *Enteromorpha linza*, amply sufficient to provide food for a longer period than the test was expected to last. *Enteromorpha linza* was the alga selected chiefly because examinations of stomach contents had shown this form to be one very commonly eaten by *L. littorea*, and also because it appears to be a hardy form, capable of withstanding a considerable intertidal exposure. The one-pint jars used were fitted in a sort of harness made of pieces of net so that they could be suspended horizontally from a projection on the side of the Station wharf. The mouths of the jars were covered with a single layer of 1 mm.

bolting silk; so that when the jars were thus placed, as the tide rose and fell, each one would fill and empty and there would be a condition established similar to that encountered by an individual living in a sheltered cranny. The number of individuals placed in a single jar was known to be too few for there to be any danger of over-crowding, since 75 adu t *L. littorea* had remained in good physical condition after an immersion of over seven weeks in a quart jar.

The experiments were carried out in August and September, and observations indicated that the place selected on the pier from which to suspend the jars, received about five hours of sunlight each bright day. Thus when tides and sun synchronized, a jar exposed for five hours or more between high tides could receive a maximum of five hours sunlight.

A cord was hung, weighted at the lower end, which rested on the substratum, from the south side of the pier. The three jars were attached to this cord at various levels.

The following table gives an idea of the conditions to which the animals were subjected. It will be noted that the "low tide" group was placed at approximately its normal level, while the "high tide" group was at approximately the highest level at which *L. littorea* is found in the littoral zone, except in tide pools.

	Size of individuals used in milimetres.	Height above low tide-level in metres.	Average exposure of jar between tides in hours.
Low Tide Jar......	2.4 to 2.6	2	3.5
Mid. Tide Jar......	2.6 to 2.8	4	7.0
High Tide Jar.....	2.8 to 3.0	6	10.0

The jars were first immersed on August 3rd. The periwinkles were examined and again measured on August 12th.

At this time the *Enteromorpha linza* remaining was, for the most part in a healthy condition, although some small pieces were dead. After measurement a fresh supply of the alga was used and the jars cleaned and replaced over the wharf. The jars were taken up again on Sept. 16th and the animals again measured. An abundant supply of *Enteromorpha* still remained, and appeared to be alive. Some individuals examined at the September 16th reading were found to be dead and exhibiting only a very slight increase in size. It was assumed that these had died early in the experiment and they were therefore discarded and not considered in calculating the average size of the group.

The results of the measurements given are for the average of each group of fifty in a jar. They are as follows:—

(Average sizes in millimetres).

	Aug. 3	Aug. 12	Gain. 9 days	Sept. 16	Gain 44 days.
Low Tide	2.59	2.82	0.23	4.73	2.14
Mid. Tide	2.74	2.87	0.13	4.72	1.98
High Tide	2.90	2.92	0.02	3.20	0.30

From the results of these observations, conducted with a sufficiently large number of individuals to render an average reasonably accurate, it can be seen clearly that immersion is a more important factor in growth than is warmth and sunlight gained during intertidal exposure. The only other possible interpretation of the facts is that smaller individuals grow more rapidly, and this is rendered unreasonable by an observation of the size differences.

Some preliminary experiments conducted with *L. littorea* by Dr. A. B. Klugh indicate that they grow extremely rapidly at high temperatures. He kept some individuals in small vials on a window ledge exposed to full sunlight and found very rapid growth, but the animals were only able to survive the high temperature for a few days.

Summary and Conclusions.

Littorina littorea exhibits a generalized type of Gastropod development, is oviparous and may make several depositions of eggs after one copulation. The eggs are enclosed, several together, in a very characteristic capsule. Under normal conditions the developing embryo passes through the trocophore stage inside the capsule, breaking forth as an early veliger about the sixth day after fertilization. Eggs or embryos are incapable of existing in water of salinity under 10 gm. per liter. In salinities of 10 to 15 an egg can remain alive for some time, but cannot develop. Development can proceed if the salinity is 15 or over, but the salinity must be 20 or over for normal development. Salinities ranging from normal down to 20 retard development.

Experiments conducted with a view to determining the rate of growth showed that a small individual can double its length in less than two months under favorable conditions. Animals at low tide level grow much more rapidly than those at higher levels, showing that immersion is a more important factor in growth than is warmth and sunlight gained during exposure.

Literature Cited.

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