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A STUDY OF THE EFFECT OF THE FACTORS SALINITY AND TEMPERATURE ON TWO CRUSTACEAE

A. *Balanus balanoides* (Linné)

B. *Limnoria lignorum* (Rathké)

by

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A. Effect of Temperature and Salinity on Bananus balanoides (Linn.)

These experiments were carried out at the Atlantic Biological Station, Saint Andrews, N. B., for the purpose of gaining some experience in this type of experiment, as extensive experiments of this kind were to be carried out during the latter part of the summer away from the station, in a rather remote part of Nova Scotia. It was therefore rather essential to find out what apparatus would be required, and also, how to use the apparatus to the best advantage.

The animal chosen for this preliminary work was the barnacle, Balanus balanoides, which was very abundant at Saint Andrews. It covered the rocks all around the station throughout the intertidal zone, except for a remarkably short distance below high water. Lowest low water exposed rocks on which the barnacles had not set. The tidal range is about twenty one feet - vertically.

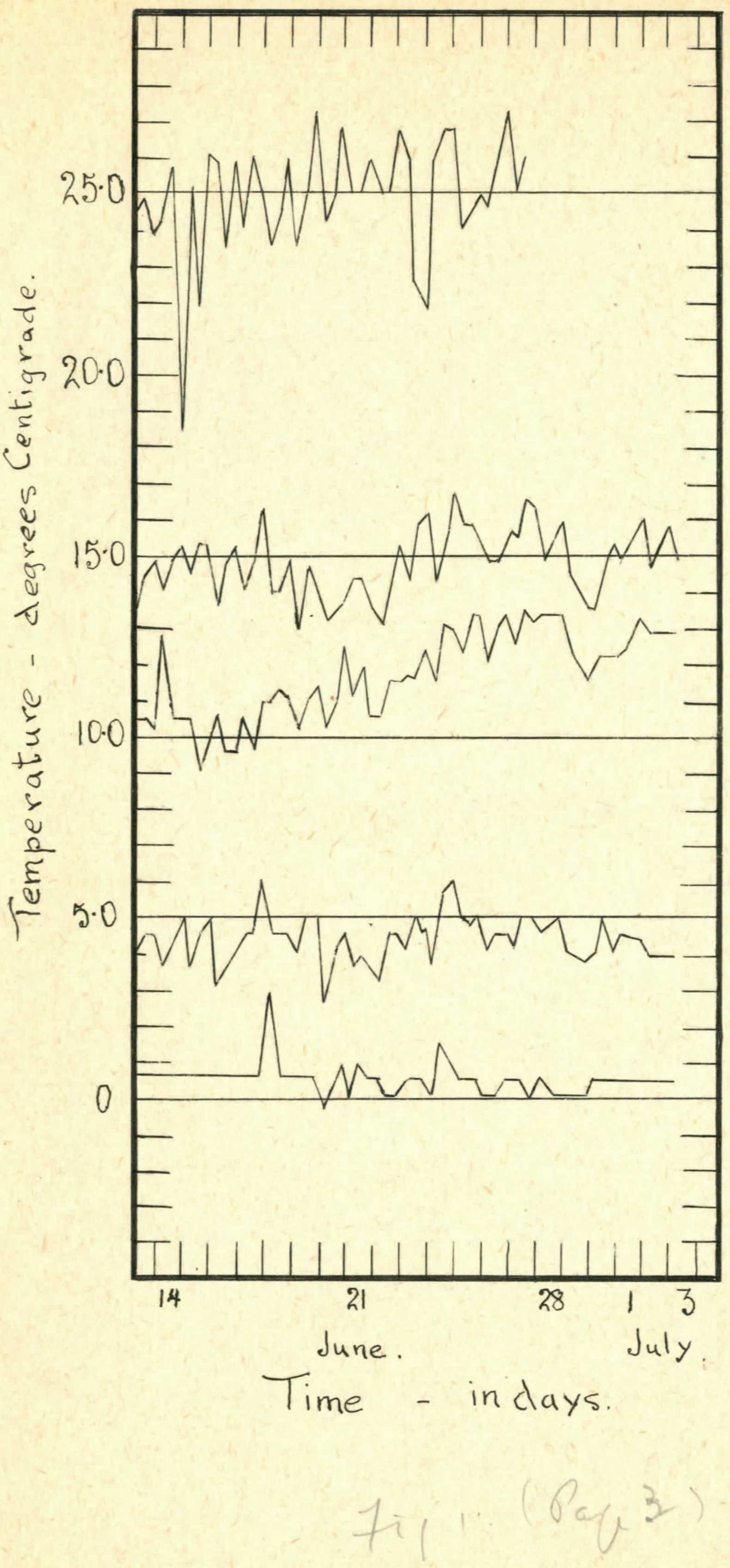
It was therefore to be presumed that this animal was a pretty hardy one, being exposed to great extremes of heat during the summer, and extremes of cold in the winter. It must also be subject to change in the salinity, as in heavy rain storms the rock pools in which great number live, must be greatly diluted, and also, under a hot sun, there must be a considerable evaporation between ebb and flood.

The plan of the work was to gather small pieces of rock, upon which the barnacles had settled pretty thickly and remove all but about six or seven of the animals. These were then each put into a wide-mouthed four ounce bottle, with forty cubic centimetres of a solution of sea-water of the desired dilution or concentration. The bottles were then placed at the desired temperature, which was attained by bath, refrigerator, or incubator. The solutions were changed daily.

The temperatures used were zero, five, ten, fifteen and twenty five degrees Centigrade. There was no suitable incubator at Saint Andrews at the time, which could be run at twenty degrees, so that the effect of that temperature, unfortunately could not be determined.

The salinities used were zero, five, ten, fifteen, twenty, twenty five, thirty, thirty five and forty parts per mille. The normal sea-water at Saint Andrews was about thirty per mille. The majorities of the solutions were therefore dilutions of this. These dilutions were made by adding fresh distilled water in the proper proportion.. The true salinity of the sea water was found by means of a hydrometer. Zero solution was plain distilled water. The solutions higher than thirty were made by evaporating sea-water to some higher strength, and then diluting to the required salinity with distilled water. This evaporation was carried out by exposing the sea-water in a wide, flat, wooden tray (which had been well water-proffed with paraffin wax) on the roof to the sun and wind. Occasionally this supply was not ample, when the water was evaporated slowly in an enamelled iron pan over a Bunsen flame.

To obtain zero, Centigrade, a large ice chest served as the refrigerator. The bottles were well corked, and packed into the ice. They probably were at zero all the time (except just when



Temperature - degrees Centigrade.
25·0
20·0
15·0
10·0
5·0
0
14
21
28
1
3
June.
July.
Time - in days.

Fig 1 (Page 3)

the solutions were changed). For five degrees, a refrigerator which was built into the ice-house was used. It ran at a fairly constant temperature, on the average a little below five degrees. The sea-water which was flowing through the trays in the laboratory was about ten degrees, and the bottles were put in the tray with a stream of water flowing over them. For fifteen degrees a bath of water was used. It was a small aquarium, and ice was added to keep the temperature at the required level. This method was not satisfactory as the graph of temperatures shows (see below). An ordinary incubator for hen's eggs, heated electrically, served for twenty five degrees. It was not intended for such a low temperature, and so was not very satisfactory. It required a lot of attention.

The temperatures were taken three times a day, and recorded. A graph at the end of this report was prepared from these figures.

Another experiment was done, and that was the thermal death-point. For this experiment stones with six or seven animals on them were put in a basin of normal sea-water. This was raised at a standard rate to a temperature above which they might be expected to live. An initial experiment was done first to ascertain at what point they would all die. The rate of heating used at the Atlantic Biological Station for these experiments is one degree every five minutes.

The preliminary experiment on the thermal death-point was done on June 13th. The initial temperature of the water was eleven degrees. The experiment began at two-fifteen in the afternoon, and continued until four-thirty, the final temperature being thirty-eight degrees.

The following observations were made during the course of the experiment:-

At 11°: All of the animals very active, opening and closing their shells rhythmically, with the protrusion of their cirri.

At 19°: Larger animals did not show cirri as frequently as before. The smaller ones (younger) did not appear affected.

At 22°: Large animals only show cirri occasionally. These were not spread as normally, but were bunched up. The younger animals did not expand as freely as normal, but protruded cirri freely.

At 26°: Large animals have not opened since 25° C. was passed. The smaller ones were still working, though less freely.

27° to 28°: Two of the large animals showed once.

At 28°: The smaller ones were moving quite stiffly.

At 30°: The smaller animals very stiff and cramped. The large ones had begun to gape open slightly.

At 31°: Most of the younger ones have stopped movement, with the cirri projecting through the closed plates. The cirri were still irritable, and were withdrawn when touched.

At 32°: The small ones were still irritable. The old ones were still gaping.

At 33°: The both sizes were irritable when stimulated.

At 35°-36°: Small ones began to lose irritability, the larger ones were still irritable.

At 37° - 38°: The large ones were not irritable.

At this point the experiment was discontinued. The water was poured off, and the animals allowed to cool. Then ordinary sea-water was poured over them. All of the animals recovered, and next morning they appeared perfectly normal.

As the death-point could not be determined by the loss of irritability, it was decided to repeat this experiment, this time removing a stone from the water at each degree above thirty-eight, until forty-two degrees was obtained. This experiment was carried out on June 21st, the experiment lasting from 2.58 p.m. until 5.30 p.m.

The following are the notes for this experiment:

At 29°: Some animals were still working, even the large ones. They did not come fully out of their shells.

At 30°: Some werestill working, though many were showing projecting cirri, which apparently were not irritable.

At 31°: Some were still working, more cirri were projecting. Most of the animals were gaping, though many of the smaller were quite tightly closed.

At 32°: One animal opened out, extruding its cirri, but most of them were passively projecting cirri which were irritable between the closed shell.

At 36°, 37°, 38°, 39°, 40°, 41° and 42° each, a stone was removed, and set aside to cool. After cooling they were put into sea-water at 11°, and allowed to stand.

On being examined the next morning (23rd) the following result was found. On each stone, the percentage of animals found to have survived was:

At 36°	100 %	40°	93 %
37°	100 %	41°	30 %
38°	80 %	42°	0 %
39°	99 %		

A diary was kept of the observations made during the course of the experiment. These observations follow:

<u>June 12th:</u> In the afternoon an experiment with <u>Balanus</u> was started. The normal rhythmic protrusion of the cirri was resumed immediately in the solutions of salinity 20, 25, 30 and 35 per mille which were at 10^{o} and 15^{o} Centigrade. Those in 30 permille solution were the most active, 25 and 20 coming next. About three hours after being put into solutions, at both 10 and 15^{o} the animals in 15 per mille began to move quite actively.

It was discovered that through some error of measurement, the actual salinity intended for 40 per mille was 57. This was left until next morning, when the proper solution was supplied.

Every day fresh solutions were prepared for the next day, and put in the refrigerator or incubator to attain the proper temperature.

<u>June 13th:</u> Animals were very active at 15^{o} C. in 20, 25 and 30 per mille. They were very slow in 35 and 40. At 10^{o} they were very active at 30, and less active at 20, and 25. They were slow in 25,35 and 40. At 5^{o} and zero they were slowly active in all salinities from 15 per mille and up. At 5^{o} they were active in a salinity of 57 per mille. At 25^{o} there were some dead in nearly all salinities, but the only active one was at 30 per mille.

<u>June 24th:</u> In the series at 5^{o}, in the 10 per mille solution, the barnacles had all their cirri projected, from their shells. (This was considered to be the sign of death, unless they showed a response on touching). But of these, three had remained irritable to touch for three days, though today only one was irritable.

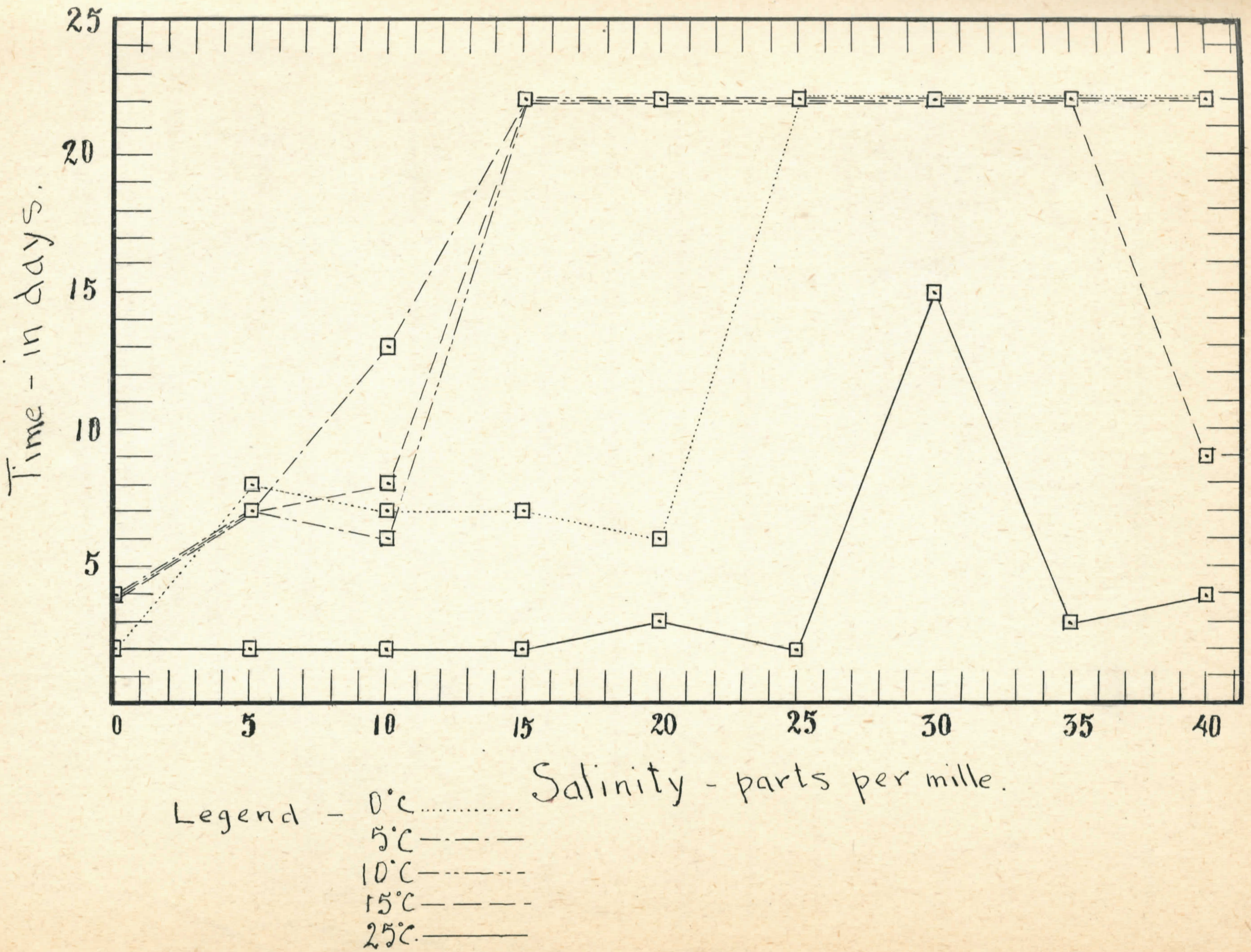
In solutions of 40 per mille, at most of the temperatures, while most of the animals remained alive, and expanded, the cirri did not unfold perfectly.

June 25th: In many of the jars today, there were discarded cirri (moults) of the barnacles. This was especially true at 10°.

July 3rd: Today the experiment was discontinued. The following observations were made of the surviving animals.

Series 0° C. at 25 per mille - moving freely though slowly.
30 " " - moving cramped
35 " " - no moving.
40 " " - moving cramped.
5° C. at 15 " " - moving slowly but freely.
20 " " - movement good.
25 " " - movement slow but limited.
30 " " - both good and limited movement was present.
35 " " - motion poor and slow.
40 " " - motion very restricted and slow.
10° C. at 15 " " - movement somewhat restricted.
20 " " - -
25 " " - moving very freely and actively.
30 " " - motion free and active.
35 " " - one moving very freely, rest restricted.
40 " " - motion very weak and restricted.
15° C.at 15 " " - no sign of motion.
20 " " - moving slowly and poorly.
25 " " - moving freely and actively.
30 " " - moving freely.
35 " " - moving fairly good but cramped.

All of the results obtained are summarized in a table which each square represents a solution in regard to temperature and salinity. The salinities are vertical and the temperatures hori-



25
20
15
10
5
0
5
10
15
20
25
30
35
40
Time - in days.
Salinity - parts per mille.
Legend - 0°C
5°C
10°C
15°C
25°C

zontal. In each square is put a number, which is the number of days that the experiment continued in that solution, or, in other words, until every animal had died, or the experiment was discontinued (twenty one days). The animals which were used at salinity 20 per mille, at 10° Centigrade, were accidently thrown out after the experiment had proceeded for seven days.

Temperature. Degrees Centigrade	Salinity - parts per mille								
	0	5	10	15	20	25	30	35	40
0	2	8	7	7	6	21	21	21	21
5	4	7	13	21	21	21	21	21	21
10	4	7	6	21	-	21	21	21	21
15	4	7	8	21	21	21	21	21	9
25	2	2	2	3	3	2	15	3	4

This table is also shown in graphic form. The ordinates represent the salinities, each unit represents one part of salt per thousand and the avscissae the length of time in days. Thus every solution has its point on the graph in respect of salinity a and length of time that the experiment runs. Then the lines representing the temperatures were drawn through the respective points concerned with that temperature (isotherms).

Conclusions.

From this experiment one would conclude that Balanus balanoides is a very hardy animal, as would be expected. It survived zero centigrade for three weeks in the higher salinities, and twenty-five degrees for two months at 30 per mille. Salinities were favourable from 15 per mille to 40, except in the highest temperature.

Favourable conditions for this animal therefore lie between five and fifteen degrees centigrade, and 15 and 35 per mille.

But apparently it can withstand unfavourable temperatures indefinitely (zero) if the salinity be above 20 permille, and there is some evidence also that in the higher salinities it can withstand such an extreme of temperature as twenty five degrees for several days. Salinities below 15 are definitely unfavourable at all temperatures, though the curiously favourable effect of five degrees should be tested. This effect appeared in other experiments.

The optimum conditions can be obtained from the observations made during the course of the experiment (page 6) June 12th, etc. The temperature was between ten and fifteen degrees, and the salinity would be 30 per mille.

From the data of July 3rd, it will be seen that 35 or 40 per mille had a marked effect on the activity of the animals. They were cramped in their expansion, even though moving freely. In this case the animals were probably affected by the fact that all the food would be killed in preparing the solutions, which were prepared by testing the water and then diluting the concentrated solutions by adding distilled water. This would induce a certain degree of starvation. These data also indicate that the optimum conditions are about 25 and 30 per mille as regards salinity, and ten and fifteen degrees centigrade.

Summary.

1. *Balanus balanoides* as surmised from its natural habitat is a very hardy animal.

2. Favourable conditions for this animal lie between five and fifteen degrees Centigrade, and 15 and 35 per mille.

3. All unfavourable temperatures or salinity can be withstood if the other condition be favourable. In other words, when both temperature and salinity are extreme the conditions are most unfavourable.

4. Optimum temperature lies between ten and fifteen degrees Centigrade.

5. Optimum salinity lies between 25 and 30 per mille.

6. Salinities of 35 and 40 per mille were unfavourable, probably due to the effect of starvation.

7. Five degrees has a curious favourable effect in the lower salinities, which is probably due to the slowing action of the lower temperatures on the rate of a biochemical reaction.

B. Effect of Temperature and Salinity on Limnoria lignorum (Rathke) with a comparison of A and B

During the previous summer (1923) Miss Jean Henderson of McGill University carried out a series of experiments on Limnoria. This previous work will serve as a check on this experiment, and will indicate the corrections of the technique employed both for Limnoria directly, and Balanus indirectly, should the results of both sets of experiments correspond, and the results on the whole have been found to correspond very well.

This animal - to a much less extent than Balanus, is an intertidal form. It does not work as far above low-water, rarely exceeding half-tide level, and it does work for a very considerable depth below it. In fact it would appear from the damage it does to pile-work that its optimum conditions were to be found below low-tide level. Therefore one would not expect this animal to be as hardy, or as resistant to extreme conditions, as Balanus. But both animals are intertidal forms in whole or part.

The same temperatures and salinities were used in this experiment as for Balanus. In fact the solutions were all made up together and the temperature also is the same, and the temperature graph for Balanus (plate 1) will apply equally in this experiment.

An important difference, however, was made in this experiment. Food was supplied to the animals in the form of thin soft-wood shavings, as produced by an ordinary carpenter's plane. At first small blocks of wood were used but it took so long for these to become waterlogged, and also the animals, when they did burrow in, were lost, and were difficult to find again.

A diary was also kept throughout the experiment. The observations recorded are as follows:

June 14th: Set up an experiment of Gribble at 5° and 15°. Three animals were put into each bottle, but they may have been injured, as they did not show much motion, but seemed to coil up in the lower salinities, and 40 per mille.

They did this throughout the series to some degree. In some of the solutions they would lie extended as well. A small piece of wood was put in each bottle.

June 17th: In nearly all of the bottles, at 15° C. the gribbles were on the wood. Some of them were in small burrows. At 5° C. they on the wood. The series at 0, 10 and 25 were set up today, those at 10° being put in the tray of running sea-water.

June 18th: The whole series of *Limnoria* up to 25° died within eighteen hours.

June 22nd: Gribble at zero were on the wood.

June 23rd: A series of *Limnoria* experiments was started to see if they would eat teak, as several references in the literature on marine borers states that teak is immune. In obtaining the animals for all these experiments it was noticed that there were large numbers of young ones in the wood. These were found in the outer superficial spongy part. The adults were always very much deeper in the compact parts of the wood.

July 3rd: The experiment was discontinued today, and the following notes were made:

Series 0° C.	15 per mille	one living, very slow movement.
	20 " "	one was moulting (?), others feeding and active.
	25 " "	two feeding and active.
	30 " "	one died.
	35 " "	-
	40 " "	two, both feeding.

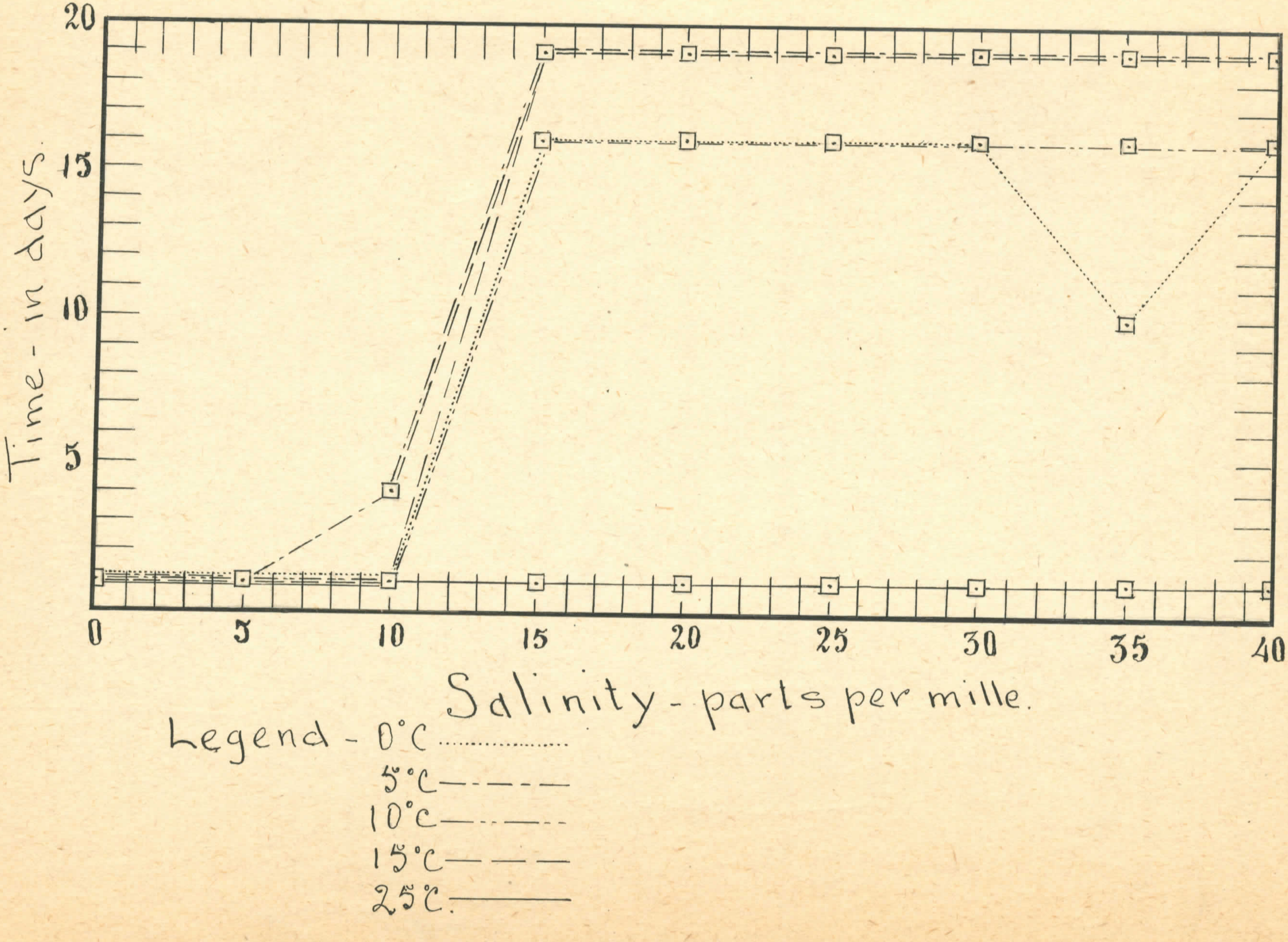
Series 5° C.	-15	per	mille	- two, both feeding.
	20	"	"	- three, two feeding.
	25	"	"	- three, two feeding.
	30	"	"	- three, one feeding, two actively swimming.
	35	"	"	- three, one feeding, two actively swimming.
	40	"	"	- three, two feeding, one actively swimming.
10°C.	- 15	"	"	- one, actively moulting, nearly complete.
	20	"	"	- three, all feeding.
	25	"	"	- two, one feeding, other swimming.
	30	"	"	- three, all feeding.
	35	"	"	- two, two feeding.
	40	"	"	- three, all very active.
15°C.	- 15	"	"	- one, feeding.
	20	"	"	- three, all feeding.
	25	"	"	- two, both feeding.
	30	"	"	- two, one feeding.
	35	"	"	- one, feeding.
	40	"	"	- two, feeding.

The experiment at 25° was repeated, using only salinities of 25, 30 and 35 per mille. The solutions were examined approximately every hour to ascertain when death occurred. Two animals were put in the solution of 25 per mille, and both were dead in about seven hours. In 35 per mille, three animals lived throughout the day, and one lived for two days. In 30 per mille, three animals were used. One died in an hour, another in six hours, but the other lived for six days. After the first day, examinations were made only once a day, when the solutions were changed. This experiment was begun on June twenty first and continued until the twenty seventh. From the tweperature chart it will be observed that during this period the temperature in the incubator fell very low, lower than any period except the initial period of adjustment. This was probably due to the fact that several were using the incubator, and it was being opened and closed a great deal. Also, as explained previously, this incubator was rather difficult to regulate. This may explain why those animals lived so much longer at the higher temperature. Of course there is another explanation, which is that in the first experiment the animals may have been so injured in removing them from their burrows that they would not have lived anyhow. The experiment should be repeated.

It has been reported that Limnoria will not attack teak, so an experiment was begun to test this out. In salinities 20, 25, and 30 per mille three animals were put in, and run at a temperature of 15°. Two series were run, one with a shaving of teak, and one with a shaving of soft wood. Fresh solutions were supplied every day, and they were watched to see if the animals attacked the teak, and if they did, was it as readily as those on the soft wood? This experiment lasted ten days only. The degree of attack on either wood was determined by the amount of excrement in the jars each day. There was probably a larger amount of soft wood eaten than teak, but apparently the animals on the teak ate it almost as readily as the others ate soft wood.

As for Balanus, the results obtained are summarized in a table in which each square represents a solution, with a number in it to represent the days each bottle was carried on.

Temperature. Degrees Centigrade	Salinity - parts per mille								
	0	5	10	15	20	25	30	35	40
0	1	1	1	16	16	16	16	10	16
5	1	1	4	19	19	19	19	19	19
10	1	1	1	16	16	16	16	16	16
15	1	1	1	19	19	19	19	19	19
25	1	1	1	1	1	1	1	1	1



20
15
10
5
Time - in days
0
5
10
15
20
25
30
35
40
Salinity - parts per mille.
Legend - 0°C
5°C
10°C
15°C
25°C

This table is also plotted graphically, just as the similar table for _Balanus_. There are two different levels at which the experiment was started, because there was not enough material available to set up all the solutions on the same day, and there is a difference of three days which will account for the difference in the level of the two sets of experiments.

Conclusions.

Limnoria survived zero temperature in a wide range of salinities from 15 to 40 per mille. It did not withstand the high temperatures, 25°, except in the second experiment with survival for six days in the solution of 30 per mille.

Salinities below 15 were very definitely unfavourable at all temperatures. This effect is very striking when compared with that for _Balanus_. The curious favouring effect of a temperature of 5° in the lower salinities, as observed and noted for _Balanus_ occurred in this experiment.

The optimum conditions cannot be ascertained from the data of this experiment. Even the notes and observations made during the course of the experiment shed no light on this point, either, as all salinities from 15 to 40 per mille in all temperatures but zero and 25 degrees appear to be favourable.

Summary.

1. _Limnoria_ survived zero from 15 and 40 per mille, but survived 25 degrees for a very short period in 30 per mille.
2. Salinities below 15 were definitely unfavourable, and from

15 to 40 quite favourable.

3. Optimum conditions cannot be ascertained from this experiment.

4. Five degrees has a curious favouring effect in the low salinities.

Comparison of the two forms.

1. Both animals find the high temperatures are unfavourable, but they are less unfavourable to Balanus, especially when the salinity is favourable.

2. Limnoria appears to be better able to resist the low temperatures. It can live in a wider range of salinities at the lower temperatures than Balanus.

3. The remarkable reaction at five degrees in the lower salinities occurs in both forms. It is apparently more favourable than both higher and lower temperatures.

4. The reaction to salinity is very much the same in both, though Balanus can withstand lower salinities better than Limnoria. The unfavourable reaction of the higher salinity on Balanus is probably not significant, as the starvation factor probably comes in to affect the results.

5. The minimal salinity for both forms is below 15 per mille and above 10.

6. Both forms have their minimal temperature below zero, but Limnoria has apparently a lower point than Balanus.

7. The optimum conditions for Limnoria were not ascertained, but a salinity of about 30 per mille, with a temperature between 10 and 15 for Balanus appears to be the optimum.