J. mar. biol. Ass. U.K. (1961) 41, 785–797
Printed in Great Britain

THE EFFECT OF SALINITY UPON THE BORING ACTIVITY AND SURVIVAL OF *LIMNORIA* (ISOPODA)

By S. K. Eltringham

Department of Zoology, The University, Southampton*

(Text-figs. 1–5)

Most of the available information about the effects of salinity upon the distribution of the marine wood-boring isopod *Limnoria* has been acquired through salinity measurements of water containing infected timber. As a result of these observations, the concept of a limiting salinity has arisen, i.e. the salinity below which *Limnoria* attack is so much reduced as to be negligible. The published field data suggest a limiting salinity between 15 and 16‰, although more extreme values are sometimes mentioned. 15‰ is the value given by Krabbe (1914) for Iceland, by Atwood & Johnson (1924) for the east coast of the U.S.A. and by Sømme (1940) for the Baltic. Miller (1926) gives an average value of 14·5‰ for San Pablo Bay, California. Kofoid & Miller (1927) quote the Danish Engineering Association's figure of 16‰. A value of 20‰ is given by van Groenou, Rischen & van Den Berge (1951) for Dutch waters, and by Chellis (1948) for the United States of America. A general figure of 16–20‰ is suggested by Calman (1936) without reference to locality.

Few experimental attempts have been made to detect the limiting salinity, and of these only those of Kofoid & Miller (1927) have been on a sufficiently large scale. These authors compared the amount of faeces deposited from *Limnoria* infected timber in water of differing salinities, for since the excavated wood is swallowed by the borer, the faecal deposit provides a suitable index of the boring activity. The quantities produced were estimated by eye and referred to a linear scale on which the amount produced in water at a salinity of 5‰ was taken as unity. This method was admittedly crude as no attempt was made to standardize the number of *Limnoria* used in each experiment, while the measurement of the faecal production was insensitive, since the unit quantity was itself very small. However, they concluded that 13‰ represented the limiting salinity.

Survival experiments to detect the limiting (not the lethal) salinity were also carried out by Kofoid & Miller (1927) who put the value between 12 and 16‰. Less reliable survival experiments are described by Henderson (1924) and White (1929) who give values of 14 and 19·5‰ respectively as the limiting salinity.

* Present address The Wildfowl Trust, Slimbridge, Gloucestershire.

The experimental method is more likely than the ecological to yield definite information about the effects of salinity upon the boring activity. Field studies suffer from the discontinuous distribution of the habitat, and the presence or absence of the borer may be due as much to the availability of timber as to the salinity of the water. Consequently, the present investigation has been made by means of a number of long-term laboratory experiments in which the boring activity has been measured at various salinities.

The cause of the reduced boring activity with lowered salinity could be ascribed to several factors. One possibility is that the extra energy required for osmoregulation is diverted from that available for boring activity. However, this hypothesis has been discounted by blood osmotic pressure determinations, which have shown that *Limnoria* has no control over the concentration of its body fluids.[1] In the absence of osmoregulation, it is likely that the reduced boring activity reflects a decline in tolerance to lowered salinities. This assumption has been tested during the present work by means of survival experiments. No recent work on the salinities lethal to *Limnoria* has been traced in the literature. Salinities reported to be lethal after 24–48 h are given as 4·7‰ (White, 1929), 11‰ (Henderson, 1924) and 6·5‰ by Kofoid & Miller (1927) who state that a value of 10‰ is 'ultimately lethal'. White (1929) investigated the effects on the survival of *Limnoria* of concentrated salinities prepared by adding sodium chloride to sea water. Lethal values for 12- and 15-day periods are given as 44·7 and 41·1‰ respectively. The experiments of White and Henderson were made on too few animals to be considered significant, and Kofoid & Miller's work ignored the number of *Limnoria* used since it was concerned with the deposit of faeces from naturally infested timber. The published evidence was of limited help, therefore, and further investigation was necessary.

MATERIAL

The *Limnoria* used were extracted by hand from naturally infected timber taken from Southampton Water where three species of *Limnoria* occur together in variable proportions. They are *Limnoria lignorum* (Rathké), *L. quadripunctata* Holthuis, and *L. tripunctata* Menzies. The mixed species were used in the activity experiments since it was not possible to maintain an adequate supply of all three species throughout the extended periods of the work. Separated species were used in the survival experiments.

The animals were kept in clean sea water for periods of at least 48 h prior to the experiments, to allow any damage resulting from the extraction technique to become apparent. Only active specimens were used.

The experimental salinities were made from distilled water and filtered Plymouth sea water. Approximate dilutions were made to the salinities required and the exact values determined by titration. The experiments were

[1] Eltringham, Ph.D. Thesis, Univ. of Southampton, 1957.

carried out in an unheated aquarium room. Daily readings of the water temperature were taken.

ACTIVITY EXPERIMENTS

METHODS

The apparatus used in these experiments consisted of a number of 400 ml. beakers containing sea water of various salinities. Each beaker contained a pair of deal test blocks measuring $45 \times 45 \times 10$ mm with the grain running parallel to the long axis. The blocks were separated by a U-shaped piece of glass rod about 3 mm in diameter, and secured by an elastic band to a glass weight which ensured that the blocks were kept below the water surface. These blocks were soaked in water of the salinity under test before use. Alterations in the salinity through evaporation were reduced by covering each beaker with a sheet of polythene through which a fine pointed narrow-bore glass tube was passed for aeration purposes. A constant air supply, controlled by individual taps for each beaker, was bubbled through the water at a slow rate. Under these conditions the increase in the salinity of the water through evaporation was shown not to exceed 2% after 7 days.

The *Limnoria* used in the experiments were rinsed in water of the test salinity and dropped into the beakers from a pipette. Two different methods were used to evaluate the boring activity. In the first, the burrow lengths were measured to the nearest mm by means of a piece of piano wire graduated in mm. Grooves produced on the wood surface were included as well as burrows but were allotted an activity value half that of burrows of equal length. The second method of measuring the boring activity was used when it was desired to follow the progressive attack by *Limnoria* upon the test blocks. For this purpose, the volume of the faeces produced each week was taken as the index of the boring activity. The faeces were freed from exuviae and removed with a pipette to a graduated centrifuge tube and their volume measured after constant treatment in a hand-operated centrifuge.

Boring activity at reduced salinities

Two experiments were conducted, each for a period of 90 days, in which nine different salinities were tested simultaneously. Fifty *Limnoria* were used in the first experiment and 80 in the second. The water was changed regularly every few days, when all dead specimens were removed and replaced by an equal number of healthy animals. Thus there was a constant number of living *Limnoria* in the beakers during the course of the experiments and all variable factors were common to each beaker except that of salinity. A wide range of salinities was tested in Expt. I with values approximately to 35‰ (full-strength sea water), 30, 25, 20, 18, 16, 14, 10 and 5‰. From the results of this experiment, it was decided to examine a narrower range in Expt. II using salinities ranging from 8 to 22‰ at intervals of 2‰ with a control at

35‰. The mean water temperature in Expt. I was 17·6° C (range 15·5–19·9° C) and in Expt. II 14·9° C (range 10·2–18·5° C). In neither case was there a violent fluctuation in temperature.

Rate of attack

This experiment was continued for a period of 20 weeks. The experiment was designed to compare the rate of attack of *Limnoria* in full-strength sea water and at a reduced salinity (30‰). A hundred *Limnoria* were added to each of two beakers containing the experimental test blocks in water of the respective salinities. The water was changed weekly when dead specimens were removed and counted but not replaced.

RESULTS

Boring activity at reduced salinities

In order to allow for the different number of animals used in each experiment, the results of these experiments are given in Table 1 as the activity per 50 *Limnoria*. The boring activity declined sharply with the salinity but the activity at comparable salinities was less in Expt. II than in Expt. I. The most likely explanation for this difference is that the second experiment was carried

TABLE 1. THE BORING ACTIVITY OF *LIMNORIA* AT REDUCED SALINITIES

Approx. salinity (‰)	Length of burrows and grooves (mm) per 50 *Limnoria*		No. of burrows per 50 *Limnoria*		No. of grooves per 50 *Limnoria*		Av. burrow length (mm)	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
35	333	270	60	39	5	8	5·5	6·7
30	271	—	45	—	11	—	5·8	—
25	271	—	48	—	8	—	5·4	—
22	—	129	—	25	—	7	—	4·8
20	202	102	46	22	22	14	4·1	4·1
18	129	85	32	18	10	19	3·6	3·5
16	129	60	32	12	12	15	3·6	3·7
14	61	24	19	6	7	6	2·1	3·3
12	—	14	—	2	—	9	—	3·3
10	12	2	5	1	3	1	1·4	2·0
8	—	0	—	0	—	0	—	—
5	0	—	0	—	0	—	—	—

out at a colder time of the year and that the reduced temperature caused a reduction in the boring intensity. There is, however, little difference between the experiments in the average burrow length at comparable salinities. On the other hand, there is a considerable preponderance of burrows in Expt. I over Expt. II (194:98), but no appreciable difference between the number of grooves in each experiment, at equivalent salinities. These results suggest that the lower temperatures during Expt. II reduced the attack (number of burrows and grooves), but not the boring activity (average burrow length) once the animals had become established within the wood.

The lowered salinity caused a definite decline in the boring activity and no boring at all was registered below 10‰. However, it is not easy to deduce from the results the value at which the salinity becomes limiting. The choice of this value must be arbitrary, but may be made objective by considering the limiting salinity to be that at which the activity is 20% of the activity in full-strength sea water. Such an attack under field conditions would be negligible and unlikely to lead to the establishment of a permanent population. The percentage attacks in Expts. I and II are plotted in Fig. 1. Regression lines have been included ($P < 0.001$ in each case). The limiting salinity, taken at the 20% activity level, is 12‰ in Expt. I and 15‰ in Expt. II. The points of zero activity shown by the regression lines differ by about 3‰ also. This figure illustrates the point not brought out in the previous analysis, that lowered temperatures bring about an increase in the value of the limiting salinity as well as a reduction in the total activity. Reduced salinities, therefore, have the greatest effect in retarding the boring activity of *Limnoria* at low temperatures. Fig. 1 suggests that boring would have been possible at a salinity in which no attack was registered under the colder conditions of Expt. II.

Similar values for the limiting salinities are obtained when the activity is examined from the standpoint of number of bores. 20% of the number formed at 35‰ is 12 in Expt. I and about 8 in Expt. II. The first figure falls halfway between the totals found at 10 and 14‰ (i.e. 12‰), while the second lies between the values recorded at 14 and 16‰ respectively.

Rate of attack

The results of this experiment are shown in Fig. 2 which includes the weekly volume of faeces produced as well as the progressive volume over the whole period of the experiment. The temperature data are the weekly averages of the daily water temperature readings. In order to allow for the death rates, the results have been expressed as the volume of faeces produced per *Limnoria* over each 7-day period. For the purposes of this calculation, all of the deaths recorded at the weekly change of water were assumed to have taken place halfway through the period.

Fig. 2 shows that the initial activity was greater than that at any subsequent period, although the water temperature was at its lowest. The activity decreased from the second to the fifth week, after which it roughly followed the fluctuations in the water temperature. Although the attack was lower at 30‰ than in full-strength sea water, the times at which changes in the rate of attack occurred were the same. From the fifth week, the relationship of activity with time did not depart significantly from a straight line ($P < 0.001$ in each case).

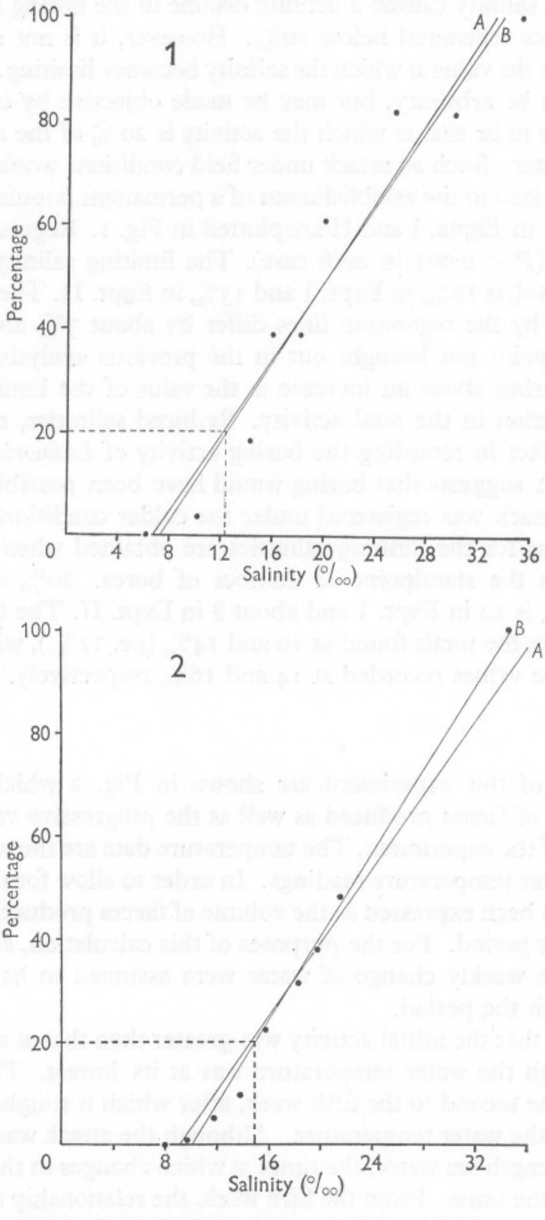

Fig. 1. The boring activity of *Limnoria* in relation to salinity: (1) Expt. I, (2) Expt. II. Each point shows the activity as a percentage of that in full-strength sea water (35 ‰). The dotted lines show the limiting salinity at the 20 % activity level. *A*, regression of *X* upon *Y*; *B*, regression of *Y* upon *X*.

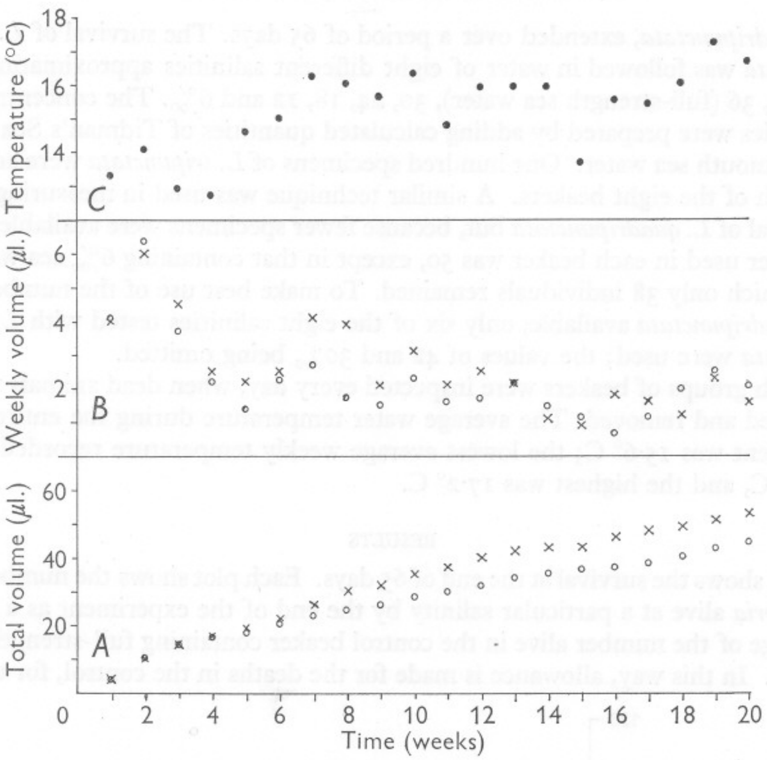

Fig. 2. The rate of attack on wood by *Limnoria*. *A*, The total volume of faeces produced per *Limnoria*; *B*, the weekly volume of faeces produced per *Limnoria*; *C*, water temperature. × faeces production at 36‰; ○, faeces production at 30‰.

SURVIVAL EXPERIMENTS

METHODS

The *Limnoria* were first washed in full-strength sea water, rinsed several times in the test solution and placed in 400 ml. beakers containing water of the experimental salinities. The top of each beaker was covered with polythene sheeting secured by a rubber band. In order to reduce evaporation, aeration was not provided, but the water was changed at least once a week. Each beaker was provided with two sheets of filter paper between which the *Limnoria* would crawl as this facilitated the counting of dead specimens at each inspection. Preliminary experiments showed that there was no significant difference in the survival between *Limnoria* with filter paper and those without. The criterion of death was complete immobility of all appendages when teased with a needle under a binocular microscope. Such individuals showed no sign of recovery when replaced in full-strength sea water.

The experiment, which compared the survival of *L. tripunctata* and

L. quadripunctata, extended over a period of 65 days. The survival of *L. tripunctata* was followed in water of eight different salinities approximating to 48, 42, 36 (full-strength sea water), 30, 24, 18, 12 and 6‰. The concentrated salinities were prepared by adding calculated quantities of Tidman's Sea Salt to Plymouth sea water. One hundred specimens of *L. tripunctata* were added to each of the eight beakers. A similar technique was used in measuring the survival of *L. quadripunctata* but, because fewer specimens were available, the number used in each beaker was 50, except in that containing 6‰ sea water, for which only 38 individuals remained. To make best use of the number of *L. quadripunctata* available, only six of the eight salinities tested with *L. tripunctata* were used; the values of 42 and 30‰ being omitted.

Both groups of beakers were inspected every day, when dead animals were counted and removed. The average water temperature during the entire experiment was 15·6° C; the lowest average weekly temperature recorded was 13·6° C, and the highest was 17·2° C.

RESULTS

Fig. 3 shows the survival at the end of 65 days. Each plot shows the number of *Limnoria* alive at a particular salinity by the end of the experiment as a percentage of the number alive in the control beaker containing full-strength sea water. In this way, allowance is made for the deaths in the control, for these

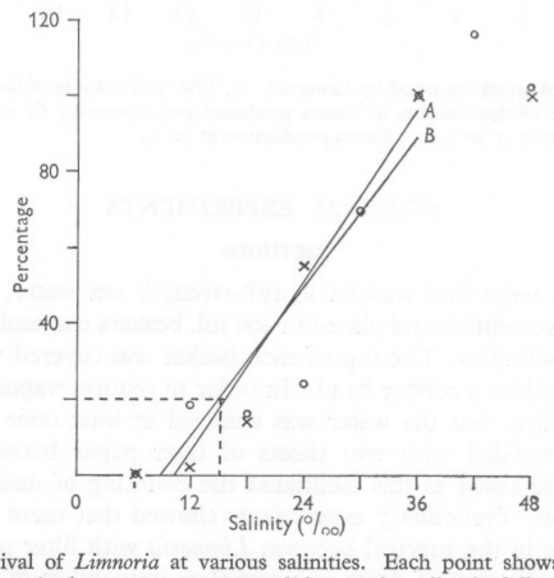

Fig. 3. The survival of *Limnoria* at various salinities. Each point shows the number of *Limnoria* alive after 65 days as a percentage of the number alive in full-strength sea water (36‰). ○, *L. tripunctata*; ×, *L. quadripunctata*. Regression lines have been drawn to the combined data excluding those for the concentrated salinities. The dotted line shows the limiting salinity at the 20% survival level. *A*, Regression of *X* upon *Y*; *B*, Regression of *Y* upon *X*.

presumably were not due to salinity effects. The number of deaths recorded in the control beakers was 26 (26 %) in the case of *L. tripunctata*, and 8 (16 %) for *L. quadripunctata*. A Student's *t* test conducted on the data for the two species (excluding the figures for the concentrated salinities) showed that there was no significant difference between the survivals of *L. tripunctata* and *L. quadripunctata*. Consequently, the data for these species have been combined in Fig. 3 in which regression lines have been included, again excluding the figures for the concentrated salinities. If the limiting salinity is taken as that at the 20 % survival level, similar to the method used in measuring the activity limiting salinity, the value is found to be about 15·5‰.

The rate at which the survival at reduced salinities decreased with time is given in Fig. 4, which shows the survival at the end of each 5-day period for the two species. Certain specific differences are seen from this analysis. The survival of *L. quadripunctata* was shown by a *t* test to be significantly higher than that of *L. tripunctata* in water of 24‰ salinity, but at 12‰ the survival was significantly higher in *L. tripunctata*. These results probably do not reflect a genuine specific difference, for it is unlikely that one species showing the higher survival at 12‰ would be less tolerant than the other of a salinity of 24‰. The survival of both species was declining rapidly at 24‰ and at this salinity range a considerable variation in the survival of the same species would not be unusual. The higher survival of *L. tripunctata* at 12‰, however, may indicate that this species is better able to withstand low salinities than *L. quadripunctata*. This is supported by the better survival of *L. tripunctata* in water of 6‰ which was the only tested salinity which proved lethal (i.e. caused 100 % deaths) within the period of the experiment.

There is no evidence to suggest that concentrated salinities up to a value of 48‰ have a deleterious effect upon the survival of *Limnoria*. The relatively high survival of *L. tripunctata* at 42‰ salinity may even indicate that increased salinity is beneficial up to a point.

DISCUSSION

It has been established that reduced salinity inhibits the boring activity of *Limnoria* and that its effects are the same on the initial attack (number of bores) as upon the boring activity within the wood (length of burrows). It has also been shown that the value of limiting salinity varies with temperature so that the concept of a limiting salinity range is more accurate than that of an absolute limiting salinity. The temperature effect is probably responsible for differences in the values of the limiting salinity reported by other workers since these were usually obtained from field observations under widely varying temperature conditions.

It is probable that there is a direct relationship between the boring activity and salinity although Kofoid & Miller (1927) drew a sigmoid curve on their plots. A statistical analysis of the present results showed that the relationship was not significantly different from a linear one, and there is no evidence of high salinity range over which the boring activity was little affected.

The experiments on the rate of attack showed that boring was continuous and varied with the temperature. This evidence supports the belief that the primary function of boring in *Limnoria* is nutritional. The animal is known to ingest the wood, and Ray & Julian (1952) have shown that it possesses a cellulase and may therefore derive at least part of its energy requirements from the

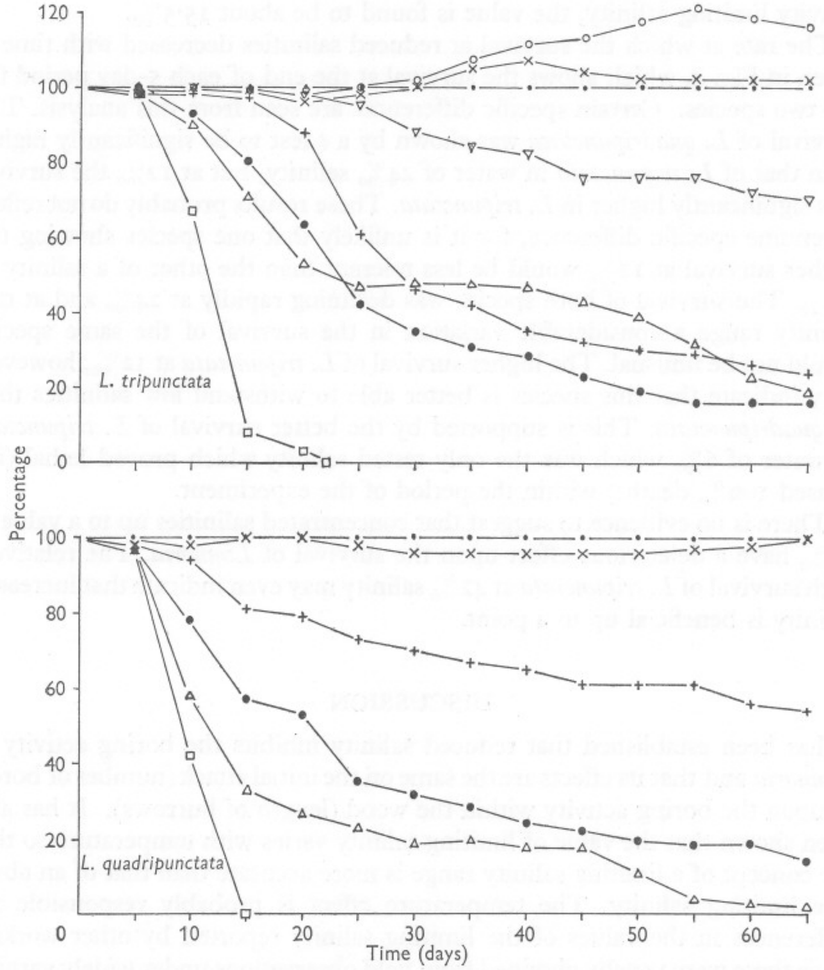

Fig. 4. The survival of *Limnoria* in waters of various salinities at the end of consecutive periods of 5 days' exposure. Each point shows the number alive as a percentage of that surviving in full-strength sea water (36‰). Key: ×, 48‰; ○, 42‰; •, 36‰; ▽, 30‰; +, 24‰; ●, 18‰; △, 12‰; □, 6‰.

wood itself. That an additional function of the boring habit is protective is suggested by the results of the experiment upon the rate of attack. The initial activity was maximal although the temperature was at its lowest. It is no doubt imperative at this stage that *Limnoria* should rapidly excavate a protective burrow before it is dislodged from the timber by wave action or destroyed by desiccation or predators.

The results of the survival experiments suggest that the reduced salinity had the same effect upon the boring activity as upon the survival. Comparison of the limiting salinities shows that the values of the survival- and activity-limiting salinities are similar at comparable temperatures (Table 2).

TABLE 2. COMPARISON OF LIMITING SALINITIES

Av. temperature (°C)	Type of limiting salinity	Value of limiting salinity (‰)
17·6	Activity	12·0
15·6	Survival	15·5
14·9	Activity	15·0

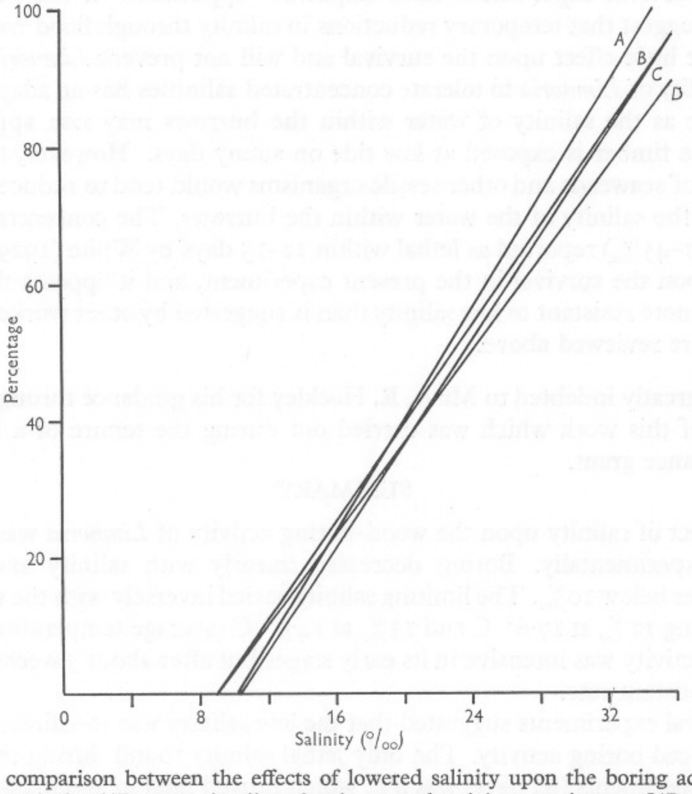

Fig. 5. A comparison between the effects of lowered salinity upon the boring activity and upon the survival. *A*/*B*, regression lines for the second activity experiment; *C*/*D*, regression lines for the survival experiment. The closeness of the lines suggests that the same factor affected the boring activity and the survival to the same extent.

A direct comparison between the effect of reduced salinity upon the boring activity and that upon the survival is given in Fig. 5. In this figure the regression lines calculated from the data for the second activity experiment and the survival experiment have been superimposed. These experiments have been selected as their average temperatures are comparable (14·9 and 15·6° C, respectively). The lines are close together indicating that the boring activity decreased with the lowered salinity in direct proportion to the lethal properties of the salinity. The comparison is not exact since the value of the survival limiting salinity varied with time as a result of differences in the death rate at the various salinities. However, these differences were considerable only during the first half of the experiment, and for the remainder of the time there were no large variations in the death rates. Consequently, the commensurate changes in the survival-limiting salinity during this period were slight. The value of the activity-limiting salinity is shown by Fig. 2 to be independent of time.

The survival experiments have important application in the field. The results suggest that temporary reductions in salinity through flood water, etc., can have little effect upon the survival and will not prevent *Limnoria* attack. The ability of *Limnoria* to tolerate concentrated salinities has an adaptive significance as the salinity of water within the burrows may rise appreciably when the timber is exposed at low tide on sunny days. However, the usual blanket of seaweeds and other sessile organisms would tend to reduce fluctuations in the salinity of the water within the burrows. The concentrated salinities (41–45‰) reported as lethal within 12–15 days by White (1929) had no effect upon the survival in the present experiment, and it appears that *Limnoria* is more resistant to low salinity than is suggested by other workers whose results are reviewed above.

I am greatly indebted to Mr A. R. Hockley for his guidance throughout the course of this work which was carried out during the tenure of a D.S.I.R. maintenance grant.

SUMMARY

The effect of salinity upon the wood-boring activity of *Limnoria* was investigated experimentally. Boring decreased linearly with salinity and ceased altogether below 10‰. The limiting salinity varied inversely with the temperature, being 12‰ at 17·6° C and 15‰ at 14·9° C (average temperatures). The boring activity was intensive in its early stages but after about 5 weeks fell to a lower constant rate.

Survival experiments suggested that the low salinity was the direct cause of the reduced boring activity. The only lethal salinity found during the period of the experiments (65 days) was 6‰ which proved fatal in about 15–20 days. The next higher salinity tested was 12‰. No reduction in survival was found in concentrated salinities up to a value of 48‰, the highest salinity examined.

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