THE TOXICITIES OF COAL TAR CREOSOTE, CREOSOTE DISTILLATES, AND INDIVIDUAL CONSTITUENTS FOR THE MARINE WOOD BORER LIMNORIA LIGNORUM.

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CONTENTS.

INTRODUCTION.

With the exception of his own work on the shipworm, *Xylotrya* (*Bankia*),[1] the writer is not aware of any systematic investigations of the effects of coal tar creosote or its constituents upon marine wood borers. The present paper embodies a further study of the materials used with *Xylotrya*, together with tests on some other important constituents of creosote.

The writer has not attempted to include here any of the literature on the actions of coal tar creosote or its constituents on other living forms. Marine borers afford a special problem, and investigations into the effects of the above substances on other types of wood-destroying parasites are not likely to furnish a basis for generalizations respecting a form that lives in sea water. Nor is it likely that results with marine borers will be applicable to the prevention of, say, fungous attacks on railroad ties.

[1] Shackell, Proceedings American Wood Preservers' Association, 1915, p. 233.

MATERIALS TESTED.

These consisted of samples of a coal tar creosote and of five fractions obtained by the redistillation of another coal tar creosote. The two creosotes, however, were similar in grade and composition. In Table I are given the properties of the creosote and of the several fractions. These materials and the data on them were furnished by the Forest Products Laboratory. Other poisons tested were a series of light oils, a series of tar acids, a series of tar bases, and a series of solid aromatic hydrocarbons.

TABLE I.—*Properties of creosote and fractions.*

	Specific gravity at 60°C.	Per cent distillation (Hempel column).						Highest Temperature reached.	Residue.	Loss.	Remarks.
		To 170° C.	170 to 205° C.	205 to 255° C.	255 to 295° C.	295 to 320° C.	Above 320° C.				
Creosote	1.048	.....	11.0	35.9	14.8	12.4	.....	320	25.8	0.1	
Fraction I	.934	28.6	34.8	27.0	.....	.....	.....	225	8.1	1.5	Collected between 0 and 205° C.
Fraction II	1.003	.9	8.1	76.4	10.5	.....	.....	287	4.0	.1	Collected between 205 and 250° C.
Fraction III	1.045	.2	.5	29.2	43.8	11.3	8.8	337	5.8	.4	Collected between 250 and 290° C.
Fraction IV	1.088	.....	.....	1.4	21.7	31.5	23.1	335	22.5	.1	Collected between 290 and 320° C.
Fraction V	1.15	.....	.....	.....	2.4	7.7	38.6	360	51.2	.1	Residue above 320° C.

LIMNORIA.

Limnoria is a very small marine crustacean, 2 to 3 mm. in length and 1 to 1½ mm. in width. *Limnoria* attacks unprotected wood in great numbers. Although not as destructive as the shipworms proper, it is, nevertheless, of considerable economic importance. A short description of this animal is given to make clear the criteria of toxicity which were used.

The body of *Limnoria* consists of a number of consecutive joints or segments which are movably articulated together. The body is divided into three parts: A head, a thorax composed of seven segments, and an abdomen of six segments. Each of the thoracic and abdominal segments is provided with a pair of legs. Attached to the abdomen is a series of extremely thin leaflets—the swimmerets. The latter are respiratory as well as natatory in function. In the normal animal, whether it is quietly burrowing or crawling around, these swimmerets are in constant, rhythmic motion. The body of the borer is covered by a very thin translucent, chitinous shell.

EXPERIMENTAL METHOD AND CRITERIA OF TOXICITY.

The usual experimental procedure was as follows: About 25 *Limnoria*, freshly obtained from unprotected wood, were transferred with some sea water to each of five Stender dishes. In all but three experiments there were five experimental periods, so that about 125 *Limnoria* were used in each experiment. No animal was used more than once. Just before the addition of the toxic preparation—ordinarily 25 cc.—the sea water was drawn off as completely as possible with a capillary pipette. As soon as the animals became paralyzed, they were distributed as evenly as possible over the bottom of the dish. If the time in the poison

was to be 30 minutes or more, the dish was stirred at 15-minute intervals, and the animals again distributed over the bottom. At the end of each experimental period the poison was decanted, and the animals transferred with about 300 cc. of sea water to a finger bowl, in which they were washed at least three times with large volumes of fresh sea water. They were finally allowed to stand for about 24 hours in a large amount of sea water. The *Limnoria* in each lot were then examined individually under low magnification, and classified under one of six headings, as follows:

1. *Normal.*—The *Limnoria* grouped here apparently were unaffected by the treatment. They crawled about and swam readily.

2. *Good.*—The *Limnoria* in this group were able to crawl about actively but could not swim, though the swimmerets waved rhythmically with scarcely any intermission.

3. *Fair.*—In this group were included the borers that could not crawl about, but in which there were leg movements and occasional waving of the swimmerets.

4. *Poor.*—The borers in this class showed only sluggish leg movements, while all movements of the swimmerets had ceased.

5. *Very poor.*—These *Limnoria* were moribund and showed only an occasional slight spasmodic movement of a leg or of a single joint.

6. *Dead.*—The animals in this group were quite opaque, in contrast to the translucent appearance of those which were graded "very poor."

CREOSOTE AND ITS FRACTIONATES.

The first series of tests with the creosote and its fractions was carried out with sea-water extracts. The second series consisted of tests with emulsions.

SEA-WATER EXTRACTS.

The method of preparation of these extracts was as follows: Ten grams of the creosote preparation were intimately triturated with enough purified talc and clean sea sand to make a loose powder. To the latter were added 100 cc. of sea water; then the whole was shaken vigorously for 10 minutes and poured on a dry filter. The filtrates in all cases were perfectly clear. On these filtrates toxicity determinations were carried out in the manner already described.

The experimental data are presented in the form seen in Figures 1 to 4 in order that the reader may obtain a quick estimate of the toxicity of a preparation without resort to comparison of numerical data. In each experiment the "T" column gives the several time periods during which the respective lots, of approximately 25 animals each, were in the poison. Each asterisk (*) represents a single observed specimen of *Limnoria*.

In the upper two rows of Figure 1 the aqueous extracts have been arranged in the order of diminishing toxicities as follows: Experiments 20 (Fraction I),[2] 30 (Fraction II), 26 (creosote), 46 (Fraction III), 45 (Fraction IV), 43 (Fraction V).

[2] At the time this experiment was carried out, a separate classification of "very poor" specimens had not yet been made. In this respect experiment 20 may be compared with experiment 30 (top row, fig. 2).

The fractionates of the creosote show a steady decrease in toxicity with rise of boiling point.

That 100 cc. of sea water did not by any means exhaust the toxic substances in 10 grams of oil was shown well by toxicity determinations on extracts made from smaller quantities of the oils. In experiments 30, 34, and 35 (fig. 2) the toxicities, respectively, of 10:100, 5:100, and 2.5:100 extracts of Fraction I are compared. The toxicity of this fraction does not diminish in proportion to the weight of oil extracted. The same statement holds for creosote (experiments 39, 40, and 41). This is brought out more strikingly by comparing a 5:100 extract of Fraction II (experiment 34) with a 10:100 extract after dilution with an equal volume of sea water (experiment 36); or by comparing a 2.5:100 extract (experiment 35) with a 10:100 extract after dilution with 3 volumes of sea water (experiment 37).

Fraction II was practically solid with naphthalene at room temperature, yet a 10:100 extract of the mixed fraction (experiment 23) was not markedly less toxic than a 10:100 extract of the liquid portion only (experiment 29). But dilution of a 10:100 extract of the mixed fraction with an equal volume of sea water caused a sharp lowering of toxicity (experiment 38).

A 10:100 extract of the tarry residue of Fraction I, after the latter had been made to lose 80 per cent of its weight by spontaneous volatilization, still showed a definite toxicity (experiment 59).

EMULSIONS.

These were tried out to determine whether dispersions containing all the constituents of the several oils would yield toxicity data of the same order as those obtained with the extracts.

The method of preparation of the emulsions was as follows: Ten grams of the creosote preparation were triturated with enough finely powdered acacia to make a friable powder. This was transferred to a 250 cc. bottle and sea water added gradually, with vigorous shaking, until a total of 100 cc. was obtained. The resulting mixture was a fairly viscous, apparently homogeneous, liquid. Toxicity experiments with the emulsions were then made in the same manner as the experiments with aqueous extracts.

In the lower two rows of Figure 1 the results with the emulsions have been arranged in the order of diminishing toxicities as follows: Experiments 54 (Fraction I), 53 (Fraction II), 61 (creosote), 60 (Fraction III), 66 (Fraction IV), 73 (Fraction V). The sequence here duplicates that seen with the aqueous extracts.

It will be seen that a 10:100 emulsion of any one of these oils is more toxic than a 10:100 sea-water extract. This is to be expected, since the minute droplets of oil in the emulsion will tend to keep the surrounding water saturated with their constituents.

LIGHT OILS.

The light oils tested were benzol, toluol, and a mixture of the isomeric xylols.

In earlier experiments it had been observed that *Limnoria* would live for days when superficially dried and immersed in a nontoxic oil such as medicinal petroleum

oil or olive oil. So experiments were made using the concentrated light oils. In the first experiments of this series the animals, in a Stender dish, were freed from water as far as possible with a capillary pipette. The animals were then separated from each other and covered with 25 cc. of the oil. At the end of the period the animals were very thoroughly washed with repeated changes of fresh sea water before final transfer to sea water. The results are given in experiments 64, 68, and 62—the top row of Figure 3. It will be seen that the toxicity diminishes from benzol to xylol.

The degree of "drying"—that is, the thickness of the film of sea water surrounding each animal—was found to have a striking effect on the velocity of poisoning by the light oils. Experiments 46*y*,[3] 47*y*, and 48*y* (second row, fig. 3) illustrate this point. The method of drying the *Limnoria* for these experiments was as follows: A number of animals in a single drop of sea water were dropped upon a sheet of filter paper. When the water had been thoroughly absorbed, the *Limnoria* were dropped onto another filter paper, and from that onto a small scoop of very fine copper screen. The animals could thus be instantaneously immersed in or removed from the light oil, which was contained in a petri dish. That the period of drying—a maximum of five minutes—was not injurious to the animals was shown by control experiments.

A comparison of the results given in the top row, Figure 3 (in which the water was taken up with a pipette), with those in the second row (where drying with filter papers was used) shows the effect of more thorough drying. This shortening of the time required to effect the same grade of poisoning—virtually from minutes to seconds—was brought about by an average reduction of less than 0.5 mm. in the thickness of the water film surrounding each animal.

Experiments 55 and 65 (bottom row, fig. 3) illustrate, respectively, the poisoning of *Limnoria* by saturated sea-water solutions of benzol and xylol. The poisoning here may be compared with that produced by a 0.1 per cent solution of phenol (experiment 33). The diminution of toxicity of the light oils with rise in boiling point is probably largely a function of their respective solubilities in sea water, benzol being the most and the xylols the least soluble.

TAR ACIDS AND BASES.

TAR ACIDS.

The experiments under this head cover phenol, orthocresol, metacresol, paracresol, alphanaphthol, and betanaphthol. The results are given in the upper two rows of Figure 4. Here, in contrast with the fractions and the light oils, the toxicity of the tar acids rises with rise of boiling point—from phenol to the naphthols.

It may be pointed out here, however, that toxicity experiments with a given concentration of any purified constituent of a creosote oil will yield widely different results, depending upon the medium—sea water or neutral oil—in which the toxic substance is dispersed. Fraction IV, for instance, very probably contained high-boiling and highly toxic tar acids. But Fraction IV was only slightly toxic, due

[3] Experiments numbered with a *y* were made in 1917; those numbered *x* in 1916. All other experiments were made in 1915.

to the very low coefficients of distribution of its tar acids between it and the sea water with which it was either extracted or emulsified. This point will be considered in detail in a later paper.

If differences in toxicity exist among the isomeric cresols or between alpha-naphthol and betanaphthol, such differences are probably slight.

TAR BASES.

Four samples of tar-base distillates were furnished by S. R. Church of the Barrett Co. These fractions were obtained by the Hempel distillation of crude bases after extraction of the latter from a creosote oil with dilute sulphuric acid. The temperature limits of these distillates were, respectively, 94 to 167° C., 170 to 210°, 210 to 250°, and 250 to 315°. Experiments were made also with a sample of C. P. pyridine and of synthetic quinoline (Merck).

The experimental results with the tar bases are given in the lower two rows of Figure 4. Here, as in the case of the tar acids, there is a rise in toxicity with rise in boiling point. The point just raised in connection with the tar acids, namely, their low coefficients of distribution between their creosote-oil solutions and sea water, applies with equal force to the tar bases.

SOLID HYDROCARBONS OF CREOSOTE.

Experiments were carried out using pure samples of acenaphthene, anthracene, naphthalene, and phenanthrene. All these are wet with difficulty by sea water, so the following method was used: A quantity of the hydrocarbon, sufficient to make a layer about 5 mm. deep in the bottom of a finger bowl, was triturated with some powdered gum arabic. The powder was then stirred up with a large quantity of sea water to wash out the gum arabic. In this way the hydrocarbons settled well. Twenty-five *Limnoria* were put into a finger bowl full of sea water in the bottom of which was a layer of the hydrocarbon. The animals rapidly burrowed into this layer. Their activity was noted at 24-hour intervals. In the least toxic of these preparations, that with anthracene, a small percentage of the animals survived for five days. In a control experiment, using talc instead of a hydrocarbon, 80 per cent of the animals were found to be normal after 8 days.

Arranged in the order of diminishing toxicity, the solid hydrocarbons are naphthalene, phenanthrene, acenaphthene, and anthracene. With the exception of naphthalene, their toxicity is very low.

SUMMARY.

1. Fractionates of coal tar creosote diminish in toxicity with rise in boiling point. The same is true of the light oils—benzol, toluol, and the xylols.

2. The tar acids and the tar bases increase in toxicity with rise in boiling point.

3. Naphthalene, phenanthrene, acenaphthene, and anthracene diminish in toxicity in the order named.

4. The results with the small borer, *Limnoria*, confirm the earlier findings in the case of the ship worm, *Xylotrya*.

Experiment 21 - Aqueous Extract Fraction I.

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'		••	••••• ••••• ••••	••••• ••		••
15'			••••• ••	••••• ••••• ••		•
20'				••		••••• ••••• ••
30'						••••• ••••• •••••
40'						••••• ••••• •••••

Experiment 33 - Aqueous Extract Fraction II.

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'	••••• •••••	•••••	••••	•		
15'	•••••	••	••••• •	••••• •••••		
20'		••••	••••• •••••	•••••	•	
30'				••••• •	••••• •••••	•••
40'					••••• ••••	••••• •••••

Experiment 26 - Aqueous Extract Creosote.

T	Normal	Good	Fair	Poor	Very Poor	Dead
90'	••••• ••••• •••••	•	•	••		
120'	•	••••• •••	••••• •	•••	•	••
150'	•	•	•••••	•••••	••••• •	••
180'			•	•••••	••••• •••••	••••
210'					••••• ••••	•••••

Experiment 46 - Aqueous Extract Fraction III.

T	Normal	Good	Fair	Poor	Very Poor	Dead
240'	••••• ••••• ••••• ••••• •	••••• ••••	•••			••
300'	•••	••••• ••••• •	•	•	••	••••
360'		••••• ••••• •	••••• ••	••••• ••	••••• •	••••• ••••• •••

Experiment 45 - Aqueous Extract Fraction IV.

T	Normal	Good	Fair	Poor	Very Poor	Dead
7 Hrs.	••••• ••••• ••••• ••••• ••••• •••••					
13 Hrs.		••••• •••••	••••• •			••••• ••••• ••••• ••
22 Hrs.						••••• ••••• ••••• ••••• •••••

Experiment 43 - Aqueous Extract Fraction V.

T	Normal	Good	Fair	Poor	Very Poor	Dead
17 Hrs.	102	4	3	1	1	35

Experiment 54 - Emulsion Fraction I.

T	Normal	Good	Fair	Poor	Very Poor	Dead
5'				••••• •••• ••	••••• ••	••••• •
10'				•	••••• ••	••••• ••••• ••
15'						••••• ••••• ••••• •••••
20'						••••• ••••• ••••• •••••
25'						••••• ••••• ••••• •••••

Experiment 52 - Emulsion Fraction II. (Liquid part of Fraction II)

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'				••••• ••••	••••• ••••• •	•••••
20'					••••• •••••	••••• ••••• •••••
30'						••••• ••••• ••••• •••••
40'						••••• ••••• ••••• •••••
50'						••••• ••••• ••••• •••••

Experiment 61 - Emulsion Creosote.

T	Normal	Good	Fair	Poor	Very Poor	Dead
20'	••	••	••	•••••	••••• ••••• •••	•
30'			••	••••	••••• ••••	••••• •••••
40'					••••• ••••• ••••• •	••••• •
50'					••••• •••	••••• ••••• •
60'					••••• •	••••• ••••• ••••

Experiment 60 - Emulsion Fraction III.

T	Normal	Good	Fair	Poor	Very Poor	Dead
30'			••••	••••• •	••••• •••••	•••••
60'					••••• ••••• ••••	••••• ••••• •
90'					••••• •	••••• ••••• •••••
120'						••••• ••••• •••••
150'						••••• ••••• •••••

Experiment 66 - Emulsion Fraction IV.

T	Normal	Good	Fair	Poor	Very Poor	Dead
30'	••••• ••••• ••••• •••	•	•	••	•••	
60'		••••• ••	••	•••	••••• •••	•
90'				••	••••• ••••• ••	••••• ••
120'				.	••••• ••••• •••	••••• •••
150'					••••	••••• ••••• ••••• •

Experiment 72 - Emulsion Fraction V.

T	Normal	Good	Fair	Poor	Very Poor	Dead
90'	••••• ••••• ••	•••	••	••	•	•••••
120'	••••• •••	••••• ••••	••••	•••		•
150'	••	•••	••••• •••••	••	••••• •	••
180'	••	••••	••••• •••	•	••••	••••• •
210'	••••• ••	••	••••	•••••	•••	••••

FIG. 1.

Experiment 30 - Aqueous Extract Fraction I.

T	Normal	Good	Fair	Poor	Very Poor	Dead
5'		●●●●● ●●●	●●●●● ●●●●●	●●●●● ●	●	●
10'				●●●●● ●	●●●●● ●●●●	●●●●● ●
15'					●●	●●●●● ●●●●● ●●●●
20'						●●●●● ●●●●● ●●●●● ●●●●
25'						●●●●● ●●●●● ●●●●● ●●●●●

Experiment 34 - Aqueous Extract Fraction I (5:100).

T	Normal	Good	Fair	Poor	Very Poor	Dead
5	●●●●● ●	●●●●● ●●●●● ●●●●●	●●●	●●		
10'			●●	●●●●● ●●●●● ●●●●●	●	●●●
15'				●●●●● ●	●●●●● ●	●●●●● ●●●●● ●●●
20'					●●	●●●●● ●●●●● ●●●●● ●●●
25'						●●●●● ●●●●● ●●●●● ●●●●●

Experiment 35 - Aqueous Extract Fraction I (2.5:100).

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'	●●●●● ●	●●●●●	●●●●● ●●●●	●●●●	●	
15'		●●●	●●●●● ●●●●	●●●●● ●●●●● ●	●	●
20'			●	●●●●● ●●●	●●●●●	●●●●● ●●
25'				●●●●●	●●●●● ●●	●●●●● ●●●
30'					●●●	●●●●● ●●●●● ●●●●● ●●

Experiment 29 - Aqueous Extract Residue Fraction I. (After loss of 80% by spontaneous volatilization)

T	Normal	Good	Fair	Poor	Very Poor	Dead
20'	●●●●● ●●●●● ●●●●● ●		●●		●	●
30'	●●	●●	●●●●● ●●●●●	●●●●● ●●●	●	●●●
40'			●●●●●	●●●●● ●●●●● ●	●●●●● ●	●●●
50'				●●●●● ●●●●●	●●●●● ●●	●●●●● ●●●
60'				●	●●●●● ●●●●	●●●●● ●●●●● ●●●●●

Experiment 36 - Aqueous Extract Fraction I. (Diluted with an equal volume of sea-water)

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'	●●●●● ●●●●● ●●●●● ●●●●●					
20'		●●●●● ●●●●● ●●●●● ●	●●●●● ●●●		●	
30'				●●●●● ●●●●●	●●●●● ●●●●	●●●●●
40'					●●●●● ●	●●●●● ●●●●● ●●●●● ●●●●
50'					●●●	●●●●● ●●●●● ●●●●● ●●●●● ●●

Experiment 37 - Aqueous Extract Fraction I. (Diluted with 3 volumes of sea-water)

T	Normal	Good	Fair	Poor	Very Poor	Dead
29'	●●●●● ●●●●● ●●●●● ●●●●●					
30'	●●●●● ●●●●● ●●●●● ●●●●●					
40'	●●●●● ●●●●● ●●●●● ●●●●●					
60'	●●●●● ●●●●● ●●●●● ●●●●●					
80'	●●●●●	●●●●● ●	●●●	●●		●●●●● ●●●●

Experiment 31 - Aqueous Extract Fraction II.

T	Normal	Good	Fair	Poor	Very Poor	Dead
15'		●●●	●●●●● ●●●●● ●●	●●●●● ●●●		●●●
20'			●●●●● ●	●●●●● ●●●●● ●●●●● ●	●●	●●
30'				●●●●●	●●●●● ●●●	●●●●● ●●●●● ●●●
40'					●●●	●●●●● ●●●●● ●●●●● ●●●
50'					●	●●●●● ●●●●● ●●●●● ●●●●● ●●●

Experiment 29 - Aqueous Extract Fraction II. (Liquid part of Fraction II)

T	Normal	Good	Fair	Poor	Very Poor	Dead
5'	●●●●● ●●●●● ●●●●● ●●●●● ●●●●●					
10'		●●●●● ●●●●● ●●●●	●●●●● ●●	●●		●
15'		●●●●● ●	●●●●● ●●	●●●●● ●●●●● ●		
20'		●	●●●●● ●●	●●●●● ●●●●	●●●●● ●●●	
25'			●	●●●●● ●●●	●●●●● ●●●	●●●●● ●●●●

Experiment 38 - Aqueous Extract Fraction II. (Diluted with an equal volume of sea-water)

T	Normal	Good	Fair	Poor	Very Poor	Dead
15'	●●●●● ●●●●● ●●●●● ●●●●● ●●●●●					
30'	●●●●● ●●●●● ●●●●● ●●●●● ●●●●●					
45'	●●●●● ●●●●● ●●●●● ●●●●● ●●●●●					
60'	●●●●● ●●●●	●●●●● ●●	●●●	●		●●●
75'		●●	●●●●●	●●●	●●●●	●●●●● ●●●●●

Experiment 39 - Aqueous Extract Creosote.

T	Normal	Good	Fair	Poor	Very Poor	Dead
180'				●●	●●●●● ●●●	●●●●● ●●●●● ●
210'					●●●●●	●●●●● ●●●●● ●●●●● ●●●●●
240'						●●●●● ●●●●● ●●●●● ●●●●●
270'					●	●●●●● ●●●●● ●●●●● ●●●●
300'						●●●●● ●●●●● ●●●●● ●●●●●

Experiment 40 - Aqueous Extract Creosote (5.0:100).

T	Normal	Good	Fair	Poor	Very Poor	Dead
180'				●●●●●	●●●●● ●●●●● ●●●●●	●●●●●
210'				●●●●	●●●●● ●●●●● ●●●●	●●●●● ●●
240'				●	●●●●● ●●●●● ●●●●●	●●●●● ●●●●● ●
270'					●●●●● ●●●	●●●●● ●●●●● ●●●●● ●●
300'					●●	●●●●● ●●●●● ●●●●● ●●●

Experiment 41 - Aqueous Extract Creosote (2.5:100).

T	Normal	Good	Fair	Poor	Very Poor	Dead
180'		●	●●●●● ●●	●●●●● ●●●●	●●●●	●●●●
210'		●●●●	●●●●	●●●●● ●●●●● ●	●●●	●●●
240'			●●●●●	●●●●● ●●●●	●●●●● ●●	●●●●
270'				●●●●● ●●●●	●●●●● ●●●●●	●●●●● ●
300'				●●	●●●●● ●●●●●	●●●●● ●●●●● ●

FIG. 2.

Experiment 64 - Benzol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
5'		••••	••••	•••	••••• ••	••••• ••
10'				•	••••• ••••• ••••	••••• •••••
15'					•••	••••• ••••• ••••• ••
20'					•••	••••• ••••• ••••• ••
25'						••••• ••••• ••••• •••••

Experiment 48 - Toluol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'	••••• ••••• ••••• •	••	•	••	••	••
15'	••	••	•••	•	••••• •••	••••• ••••
20'		•		•••	••••• ••••• •	••••• •••••
25'		•		••	••••• ••••• •	••••• ••••• •
30'					••••• ••••	••••• ••••• ••••• •

Experiment 63 - Xylol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
10'	••••• ••••• ••••• ••••• •	•	•			••
20'	••••	••••• ••••	•••	•		••••• •••
30'	••	•	••••• •	•••	••••• •	••••• ••
40'		•	•		••••• •••	••••• •••••
50'			••		••••• •••••	••••• ••••• •••

Experiment 46y - Benzol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
16"					••••• •	••••• ••••
24"					•••••	••••• ••••• ••••
32"				••	•••	••••• ••••• •
40"			•		••	••••• ••••• ••
48"						••••• ••••• •••••

Experiment 47y - Toluol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
16"				•	••••• •••••	••••• ••••
24"				••	•••••	••••• ••••• •••
32"			•		••••• ••	••••• ••••• ••
40"					••••	••••• ••••• •
48"					••••	••••• ••••• ••••• •

Experiment 48y - Xylol (Concentrated).

T	Normal	Good	Fair	Poor	Very Poor	Dead
16"	•	•		•	••••• ••••• •	••••• •
24"	••				••••• ••	••••• ••••• •
32"	•				••••• ••	••••• ••••• ••
40"	•		•		••••• •••••	••••• ••••• •••
48"	•		•		••••• •••	••••• ••••• ••••

Experiment 55 - Benzol (Sat. sol. in sea-water).

T	Normal	Good	Fair	Poor	Very Poor	Dead
60'		•	••••• •	••••	••••• ••	••••• ••
90'					•••	••••• ••••• ••••• ••
120'						••••• ••••• ••••• •••••
150'						••••• ••••• ••••• •••••
180'						••••• ••••• ••••• •••••

Experiment 45 - Xylol (Sat. sol. in sea-water).

T	Normal	Good	Fair	Poor	Very Poor	Dead
180'	••••• ••••• •••	••••• •	•			•••••
240'	••••• ••••• ••	••••• •	••	•	••	••
300'	•••••	••	••		••••• •	••••• •••••
360'	•	•••		••	•••••	••••• ••••• ••••
420'	••	•	•	•	••••• •••	••••• ••••• ••

Experiment 33 - Phenol (0.1% in sea-water).

T	Normal	Good	Fair	Poor	Very Poor	Dead
180'	••••• ••••• ••••• ••••• •••••					
240'	••••• ••••• ••	•	••••• •••••			••
300'	••••• ••	•••	••••• ••••• ••••			•
360'	••••	••••	••••• ••	•••		••••• •
420'		•••••	••••• •••••	••		••••• •••

FIG. 3.

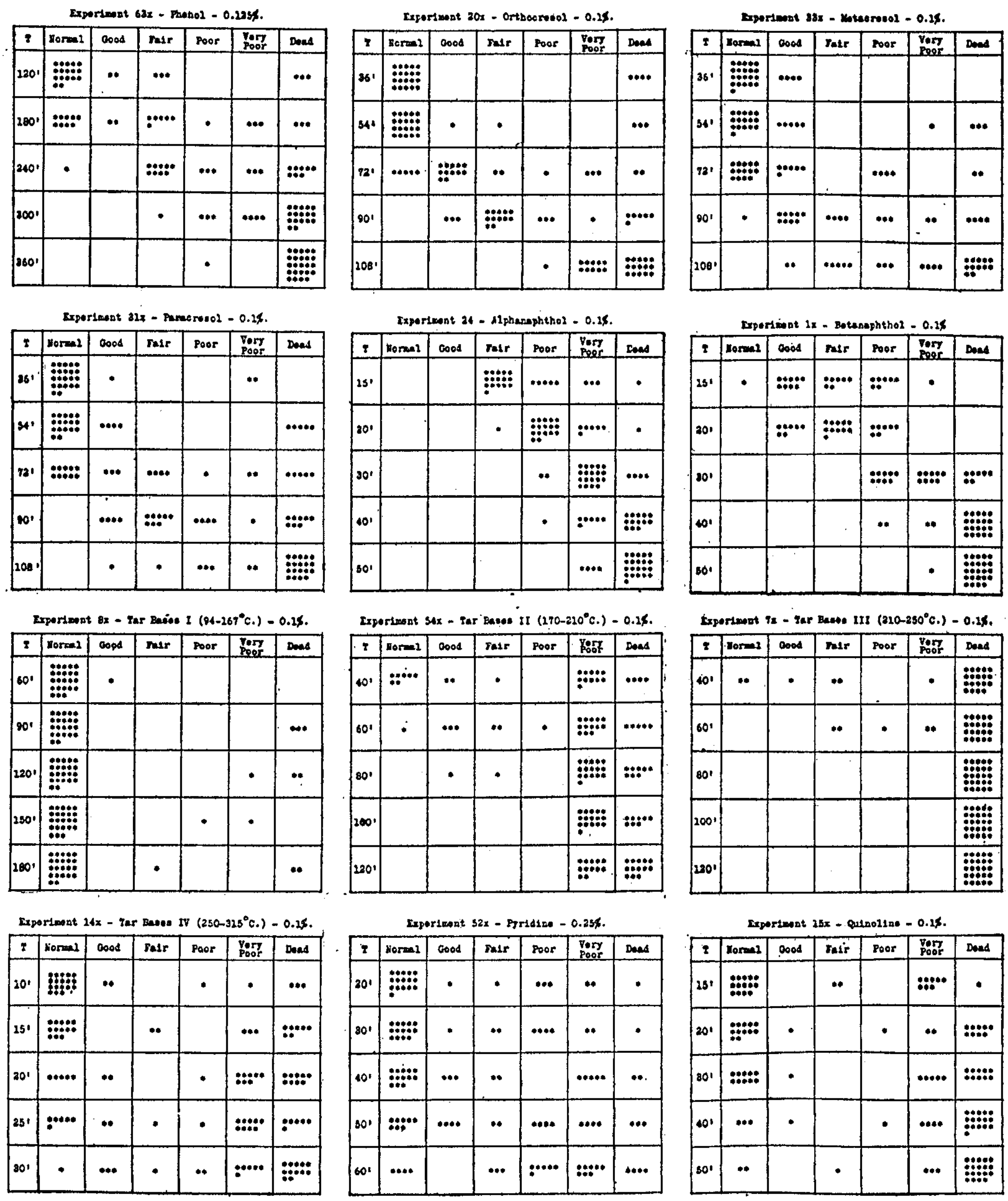
Experiment 63x - Phenol - 0.125%.
Experiment 20x - Orthocresol - 0.1%.
Experiment 33x - Metacresol - 0.1%.
Experiment 31x - Paracresol - 0.1%.
Experiment 24 - Alphanaphthol - 0.1%.
Experiment 1x - Betanaphthol - 0.1%
Experiment 8x - Tar Bases I (94-167°C.) - 0.1%.
Experiment 54x - Tar Bases II (170-210°C.) - 0.1%.
Experiment 7x - Tar Bases III (210-250°C.) - 0.1%.
Experiment 14x - Tar Bases IV (250-315°C.) - 0.1%.
Experiment 52x - Pyridine - 0.25%.
Experiment 15x - Quinoline - 0.1%.
T
Normal
Good
Fair
Poor
Very Poor
Dead

FIG. 4.

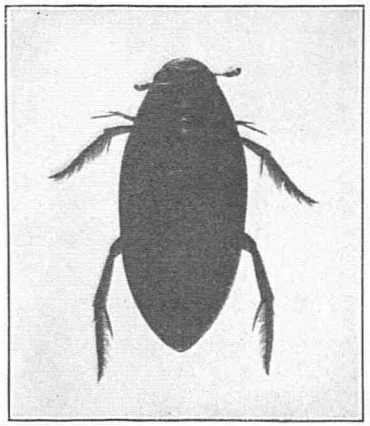

FIG. 1.—*Hydrous triangularis.*

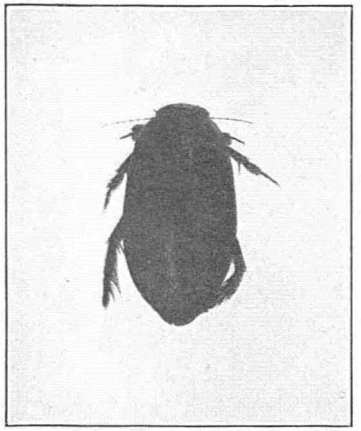

FIG. 2.—*Dytsicus hybridus.*

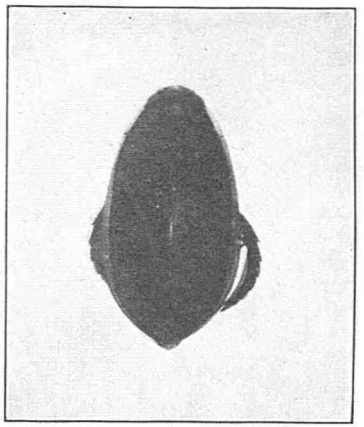

FIG. 3.—*Cybister fimbriolatus.*

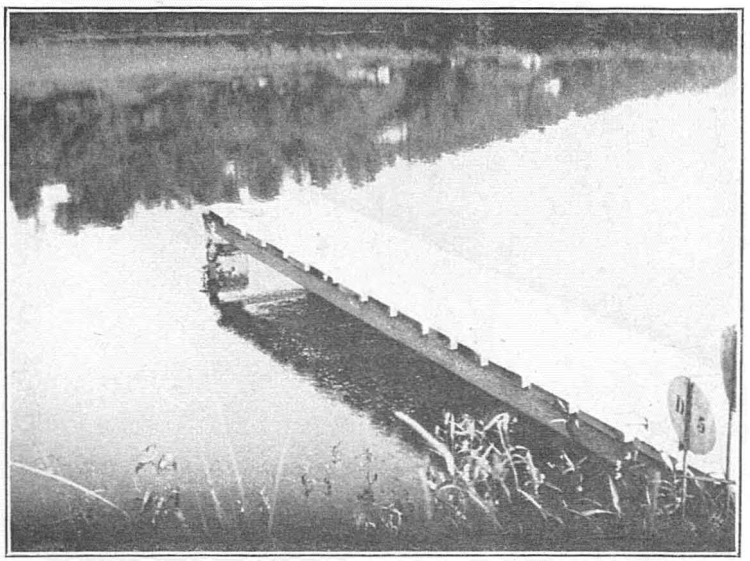

FIG. 4.—The pier of pond 5D, showing practically all the adult *Dineutes americanus* in the pond assembled in its shade.

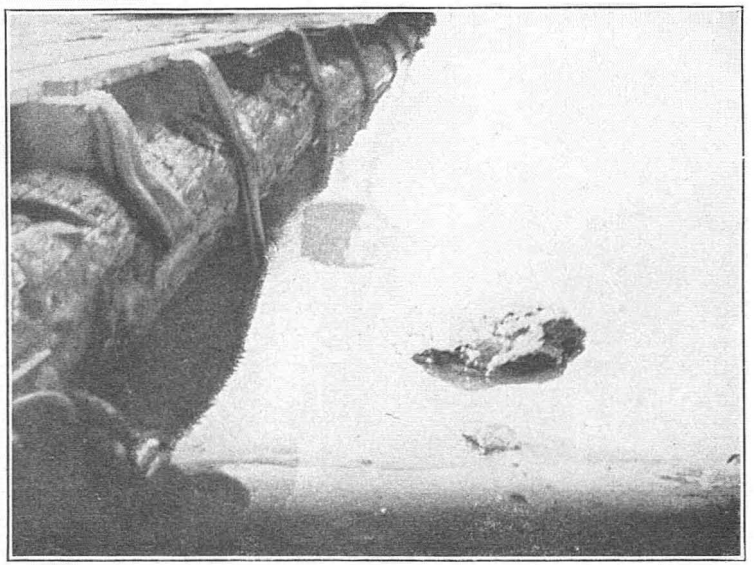

FIG. 5.—The boat wharf on the river, showing a swarm of *Gyrinus analis* on its down-river side.