THE RELATIVE TOXICITY OF SALTS OF LEAD, ZINC AND COPPER TO THE STICKLEBACK 
(*GASTEROSTEUS ACULEATUS* L.) AND 
THE EFFECT OF CALCIUM ON 
THE TOXICITY OF LEAD 
AND ZINC SALTS

By J. R. ERICHSEN JONES

Department of Zoology, University College of Wales, Aberystwyth

(*Received 2 December 1937*)

(*With Eight Text-figures*)

A. INTRODUCTION

The lethal action of dissolved salts of heavy metals on fish has been investigated by Carpenter, who in a series of papers (1925, 1927, 1930) has shown that the death of fish placed in solutions of salts of heavy metals results, not from internal poisoning but from an interaction between the metallic ion and the mucus secreted by the gills, whereby a film of coagulated mucus is formed on the gill membranes impairing their respiratory efficiency to such a degree that the fish is asphyxiated. This conclusion has been confirmed by Behrens (1925) and Ellis (1937).

The most commonly occurring metallic polluting elements in fresh waters are lead, zinc and copper. The toxic action of all three has been investigated by Carpenter, and other workers, but little attempt appears to have been made to estimate the relative toxicity of the three metals by determination of their lethal concentration limits. Attempts to compare the toxicity of metallic salts by comparing the survival times of fish at one selected concentration are of doubtful value as at high concentrations one salt may be more rapidly fatal than an equimolar solution of another, while at low concentrations the relation may be reversed. Thus at concentrations above 0.08N the toxicity of lead nitrate solutions to *Polycelis nigra* (Müller) is greater than that of equimolar solutions of copper nitrate, but below 0.08N the latter is more rapidly fatal. Much of the work on these lines has been performed on the goldfish, *Carassius auratus* (L.) which is very resistant to the toxic action of heavy metal salts, and therefore results with this species are not as valuable for general application to pollution problems as results with species of greater sensitivity, while furthermore *C. auratus* is not an indigenous species of fresh-water streams in this country.
Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback 395

The writer's investigations have been directed to the determination of the lethal limits of concentration for lead, zinc and copper for the universally distributed three-spined stickleback (*Gasterosteus aculeatus* L.). More intensive study has been made of the respiratory distress which is one of the most characteristic features of the lethal action of salts of these metals, and the effect of the addition of salts of calcium on the toxicity of lead and zinc has also been investigated.

B. LETHAL LIMITS OF CONCENTRATION FOR THE STICKLEBACK

The writer's experiments on the effect of high concentrations of lead nitrate on the stickleback gave results very similar to Carpenter's results with the minnow, *Phoxinus phoxinus* (L.). Between 0.005 and 0.20 N survival times for *Gasterosteus* proved to be almost identical with Carpenter's data for *Phoxinus* and anti- and post-mortem symptoms were similar. Experiments at lower concentrations were then performed and it was found that 500 c.c. of solution per fish was sufficient for fatal results down to a concentration of one part lead per million of water,¹ and by periodically renewing the solution it was found possible to test the effect of still lower concentrations.

For each series of survival time experiments fish of approximately equal size were used, and furthermore all the fish used in each series were captured from the same source on the same occasion and were kept in the tank for the same time before being placed in the solutions. All the sticklebacks used were obtained from a ditch which drains an area of pasture land near the sea at Aberystwyth. Generally no food was supplied to fish under experiment as it was considered that the addition of food to the solutions might have undesirable chemical effects. Controls, similarly, were not fed and this was probably the chief cause of their relatively short life.

The range of dilute solutions studied was from three parts per million to two parts per hundred million. The solutions were prepared with pure lead nitrate and Aberystwyth tap water, which is a very soft water of a high degree of purity. The use of distilled water was avoided as ordinary distilled water contains traces of copper, and is fatal to sticklebacks in about 2 days, and glass-distilled water was not available in sufficient quantity. The results obtained with mature fish (males and females) 45-50 mm. in length are set out in Table I. Two-litre volumes of solution were used, in large wide-mouthed glass jars and four fish were placed in each solution. In the table, opposite each concentration is recorded the mean survival time of the four fish placed in the solution. A number of similar fish were placed in similar vessels containing untreated tap water, as controls, the volume of solution allowed for these being 500 c.c. per fish. During the period occupied by the investigation thirty-two controls of this size were kept in all, and the mean survival time of these was 10½ days. As will be noted later, the mean survival time of small (18-20 mm.) controls was considerably longer.

All the solutions and the control water were renewed daily. The temperature of the solutions was not controlled and varied with room temperature from 14 to

¹ All concentrations given in this paper are parts metal per parts water, not parts salt.
17° C., but when the solutions were renewed the temperature of the fresh solution was adjusted to that of the old before the fish were transferred. Reference to the table shows that down to a concentration of $1 \times 10^{-6}$ death results rapidly. At $3 \times 10^{-7}$ the survival time is much shorter than that of the controls, and even at $1 \times 10^{-7}$ some evidence of toxicity is apparent. A further series of experiments with 45–50 mm. fish using larger volumes of solution (2000 c.c. per fish renewed daily), gave a very similar result; the use of these larger volumes did not result in any appreciable shortening of the survival times, as would have been the case had the smaller volumes been inadequate.

**Table I. Survival times of mature sticklebacks in lead nitrate solutions**

Concentrations are g. lead per c.c. water. Temp. 14–17° C. pH of solutions 6·4–6·6.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Average survival time</th>
<th>Concentration</th>
<th>Average survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>$20 \times 10^{-4}$</td>
<td>6½ hr.</td>
<td>$3 \times 10^{-7}$</td>
<td>4½ days</td>
</tr>
<tr>
<td>15</td>
<td>8½ &quot;</td>
<td>2</td>
<td>7 &quot;</td>
</tr>
<tr>
<td>10</td>
<td>10 &quot;</td>
<td>1</td>
<td>8½ &quot;</td>
</tr>
<tr>
<td>5</td>
<td>12 &quot;</td>
<td>6 $\times 10^{-8}$</td>
<td>11 &quot;</td>
</tr>
<tr>
<td>3</td>
<td>13½ &quot;</td>
<td>4</td>
<td>10½ &quot;</td>
</tr>
<tr>
<td>2</td>
<td>15½ &quot;</td>
<td>2</td>
<td>11 &quot;</td>
</tr>
<tr>
<td>1</td>
<td>19 &quot;</td>
<td>Average survival time of 32 controls in tap water</td>
<td></td>
</tr>
<tr>
<td>$7 \times 10^{-7}$</td>
<td>30½ &quot;</td>
<td></td>
<td>10½ &quot;</td>
</tr>
<tr>
<td>5</td>
<td>81 &quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the case of mature fish the lethal limit of concentration for lead thus appears to be approximately one part in ten million. Experiments with small sticklebacks gave some indication of slightly higher sensitivity. Results with 18–20 mm. fish are given in **Table II**, and here it will be seen that definite evidence of toxicity was obtained at $1 \times 10^{-7}$ as at this concentration the survival time was markedly shorter than that of the controls, which in this case lived for over 4 weeks. Experiments below $1 \times 10^{-7}$, however, gave indefinite results; down to $7 \times 10^{-8}$ the survival times of the fish in the solutions were very slightly shorter than that of the controls, and at lower concentrations there was no appreciable difference.

**Table II. Survival times of small sticklebacks in lead nitrate solutions**

Length of fish 18–20 mm. Volume of each solution 500 c.c. Four fish were placed in each solution, and the solutions were renewed daily.

Concentrations are g. lead per c.c. water. Temp. 14–17° C. pH of solutions 6·4–6·6.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Average survival time days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-4}$</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>$7 \times 10^{-7}$</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>10½</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Average survival time of 8 controls in tap water</td>
<td>30</td>
</tr>
</tbody>
</table>
At high concentrations the toxicity of zinc salts is very nearly equal to that of lead; thus a 0.03N solution of zinc nitrate is fatal to *Gasterosteus* in 160 min. and an equimolar solution of lead nitrate fatal in 150 min. Experiments at low concentrations, however, showed that the lethal limit of concentration for zinc is definitely higher than that for lead. Results with dilute solutions of zinc sulphate are given in Table III, and it will be seen that the lowest concentration at which definite lethal action is apparent is $3 \times 10^{-7}$. A further experiment was made with zinc sulphate using 18–20 mm. fish, and in this case a closely graded series of solutions was employed covering the range $1 \times 10^{-6}$–$1 \times 10^{-7}$ g. per c.c. The results were sufficiently consistent to be plotted as a survival curve which is given in Fig. 1. Here each plotted point represents the mean survival time of four fish in 2000 c.c. of solution. It will be seen that a sharp decline in toxicity is evident just below $3 \times 10^{-7}$, the survival curve rising steeply to the limiting value given by the controls.

Experiments with copper solutions indicated a toxicity considerably higher than that of lead or zinc, though at high

**Table III. Survival times of mature sticklebacks in zinc sulphate solutions**

Length of fish 45–50 mm. Volume of each solution 2000 c.c. Survival times are means for four fish. All solutions were renewed daily.

Concentrations are g. zinc per c.c. water. Temp. 14–17°C. pH of solutions 6.4–6.6.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Average survival time</th>
<th>Concentration</th>
<th>Average survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3 \times 10^{-4}$</td>
<td>109 min.</td>
<td>$7 \times 10^{-7}$</td>
<td>4$\frac{1}{2}$ days</td>
</tr>
<tr>
<td>2</td>
<td>143 &quot;</td>
<td>5</td>
<td>5 &quot;</td>
</tr>
<tr>
<td>$15 \times 10^{-4}$</td>
<td>182 &quot;</td>
<td>4</td>
<td>6 &quot;</td>
</tr>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>207 &quot;</td>
<td>3</td>
<td>8$\frac{1}{2}$ &quot;</td>
</tr>
<tr>
<td>$7 \times 10^{-5}$</td>
<td>243 &quot;</td>
<td>2</td>
<td>12 &quot;</td>
</tr>
<tr>
<td>5</td>
<td>277 &quot;</td>
<td>1</td>
<td>11$\frac{1}{2}$ &quot;</td>
</tr>
<tr>
<td>3</td>
<td>5$\frac{1}{2}$ hr.</td>
<td>$5 \times 10^{-4}$</td>
<td>10$\frac{1}{2}$ &quot;</td>
</tr>
<tr>
<td>2</td>
<td>52 &quot;</td>
<td>Average survival time of 32 controls in tap water</td>
<td>10$\frac{1}{2}$ &quot;</td>
</tr>
<tr>
<td>1</td>
<td>72 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$7 \times 10^{-6}$</td>
<td>10 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16$\frac{1}{2}$ &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>34 &quot;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1. Survival curve for *Gasterosteus* in dilute solutions of zinc sulphate.**
concentrations (0.10–0.20 N) the survival times of sticklebacks in copper nitrate, 
lead nitrate and zinc nitrate are very nearly equal. Reference to Table IV shows 
that in dilute solution the toxicity of copper nitrate is well maintained, as the 
survival time of the fish in the solutions does not reach the control value until the 
2 \times 10^{-8} \text{ mark is passed. The high toxicity of copper to fresh-water animals has been } 
noted by several workers, including Ludwig (1927) who notes that \textit{Paramecium} is 
killed by copper sulphate solutions at concentrations as low as 10^{-6} M; Dilling & 
Healey (1926) who find N/500,000 fatal to tadpoles; Powers (1917) who finds 
1.88 \times 10^{-8} \text{ fatal to the goldfish, and the writer (1937), whose experiments on the } 
effect of copper salts on \textit{Gammarus pulex} (L.) indicated marked toxicity at 0.000002 N, 
while the value of copper sulphate as an algicide is well known. It is evident that 
pollution by copper salts must have a far more serious effect on fresh-water fauna 
than pollution by lead or zinc.

Table IV. \textit{Survival times of mature sticklebacks in copper nitrate solutions}

Length of fish 45–50 mm. Volume of each solution 2000 c.c. Survival times are means for four 
fish. All solutions were renewed daily.
Concentrations are g. copper per c.c. water. Temp. of solutions 14–17 °C. pH 6.4–6.6.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Average survival time</th>
<th>Concentration</th>
<th>Average survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 \times 10^{-8}</td>
<td>155 min.</td>
<td>6 \times 10^{-8}</td>
<td>4 3/4 days</td>
</tr>
<tr>
<td>3</td>
<td>210 ''</td>
<td>4</td>
<td>5 1/2 ''</td>
</tr>
<tr>
<td>2</td>
<td>270 ''</td>
<td>2</td>
<td>8 1/2 ''</td>
</tr>
<tr>
<td>15 \times 10^{-7}</td>
<td>327 ''</td>
<td>1</td>
<td>11 ''</td>
</tr>
<tr>
<td>1 \times 10^{-7}</td>
<td>6 1/2 hr.</td>
<td>Average survival time of 32 controls in tap water</td>
<td>10 1/2 ''</td>
</tr>
<tr>
<td>7 \times 10^{-7}</td>
<td>10 ''</td>
<td>1</td>
<td>11 1/2 ''</td>
</tr>
<tr>
<td>5</td>
<td>11 1/2 ''</td>
<td>3</td>
<td>11 ''</td>
</tr>
<tr>
<td>3</td>
<td>21 1/2 ''</td>
<td>2</td>
<td>11 ''</td>
</tr>
<tr>
<td>2</td>
<td>33 1/2 ''</td>
<td>1</td>
<td>11 ''</td>
</tr>
<tr>
<td>1</td>
<td>55 1/2 ''</td>
<td>8 \times 10^{-8}</td>
<td>79 ''</td>
</tr>
<tr>
<td>8 \times 10^{-8}</td>
<td>79 ''</td>
<td>5 \times 10^{-8}</td>
<td>100 ''</td>
</tr>
</tbody>
</table>

C. \textit{THE EFFECT OF CALCIUM ON THE TOXICITY OF LEAD AND ZINC}

The Aberystwyth tap water used for the preparation of the solutions used in the 
survival time experiments has a very low calcium content, of the order of 1 mg. per l. 
In 1934 the writer observed that solutions of lead nitrate of concentration 1 \times 10^{-6} g. 
per c.c., or less, were apparently harmless to minnows and sticklebacks if made up with 
a hard tap water containing approximately 50 mg. per l. of calcium. This reduction in 
toxicity was obviously related to the dissolved salts in the water, the chief of which 
is calcium bicarbonate, and the surmise that the calcium was the factor responsible 
for the reduction in toxicity was confirmed by the treatment of fish with solutions of 
lead nitrate in soft water to which were added varied quantities of calcium salts.

The survival time of sticklebacks in a 1 \times 10^{-6} g. per c.c. solution of lead is 
18–28 hr. The addition of 2 mg. per l. of calcium, as calcium chloride, results in 
a considerable lengthening of the survival time, and the addition of more and 
more calcium still further prolongs the survival time until at 50 mg. per l. the fish 
live for over 10 days, that is as long as the controls.
Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback 399

This effect is depicted by the lower graph in Fig. 2. Here each plotted point represents the mean survival time of five sticklebacks in 2000 c.c. of $1 \times 10^{-6}$ g. per c.c. lead solution to which was added quantities of calcium (calcium chloride) as indicated by the horizontal scale. The progressive lengthening of the survival time which results is clearly indicated. The upper graph in Fig. 2 depicts the result of adding similar quantities of calcium to a somewhat more dilute lead solution, in this case $4 \times 10^{-7}$ g. per c.c., and here it will be noticed that a slightly lower concentration of calcium is sufficient to annul the toxicity of the lead as the survival time reaches that of the controls at a concentration a little above 30 mg. per l. As lead chloride is a sparingly soluble salt it might be thought that the reduction in toxicity resulted from precipitation of the lead as lead chloride. At low concentrations a precipitate so formed would probably be quite invisible, but the explanation of the effect is not so simple; the amount of calcium chloride necessary to annul the toxicity is vastly in excess of what would be required were the action simply one of precipitation; secondly, lead sulphide can be precipitated from the filtered lead nitrate-calcium chloride mixture; and thirdly, exactly the same reduction of toxicity is effected by the addition of equimolar quantities of pure calcium nitrate, which obviously cannot precipitate the
lead as the addition of this salt introduces no anion with which the lead could form an insoluble compound.

It was noticed that the fish in the solutions containing 50 mg. per l. of calcium did not display any sign of the symptoms observed in the case of those treated with solutions of lead only; in particular the respiratory distress displayed by the latter was completely absent. The stickleback under normal conditions breathes at a somewhat irregular rate, five to ten rapid opercular movements alternating with periods of inertia lasting some 10-15 sec., but on an average the rate of breathing is about 120 opercular movements per min. at 17° C., falling to about 100 per min. during periods of sluggishness, and rising to about 140 per min. after periods of active swimming. A stickleback placed in a 1 \times 10^{-8} \text{g. per c.c.} lead solution soon shows signs of uneasiness, making rapid darting movements, and the respiration rate rises rapidly to reach 170 per min. in 4 hr. and in 6 hr. attains over 200 per min. The other symptoms noted by Carpenter now become evident; the gills and body surface gradually become coated with a whitish film of coagulated mucus and the opercular movements become not only more rapid but greater in amplitude. Otherwise the fish becomes sluggish, occasionally swimming in a spasmodic manner, but for the most part resting on the bottom of the vessel, propped on its tail and pelvic spines.

The respiration rate continues at 200-240 per min. for about 10 hr., rising slightly after the periods of swimming and falling a little after the periods of rest. Then it begins to decline, in all probability because a point is eventually reached when the film on the gills reduces their respiratory efficiency to such an extent that even at the increased rate of breathing the fish fails to obtain sufficient oxygen and begins to succumb. At this point the fish loses its sense of balance and swims on one side or upside down, and the pelvic spines become rigidly extended. Once begun, the decline in respiration rate is rapid, in 2 hr. it falls to 140 per min. and in the next hour to zero.

The variation in respiration rate during the survival time is recorded in Fig. 3, in which it is plotted as ordinate against time as abscissa. Observations were made at
hourly intervals, and all values are means for three fish. Values for 0–12 hr. were taken with three fish placed in the solution at 10 a.m. and values for 13–18 hr. with a further three fish placed in another vessel of solution 12 hr. earlier. Thus in all six fish were employed, and by this means the difficulty of observation during the night was avoided. The fish usually remained sufficiently still for the opercular movements to be counted for at least half a minute, but in some cases it was found neces-

![Graph](image)

**Fig. 4. Gasterosteus; respiration graphs for high concentrations of lead nitrate.**

sary to hold the fish gently in a blunt pair of forceps while making the count. Two-litre pneumatic troughs were used, to hold the solutions in order that this could be done without removing the fish from the solutions. In this and other experiments on respiration rate, room temperature was maintained at $17 \pm 1^\circ C$, the temperature variation of the solutions being slightly less than this.

At high concentrations the same sequence of events occurs, but each phase is of shorter duration. Respiration rate graphs for *Gasterosteus* in lead nitrate solutions of concentrations 0.20, 0.10 and 0.05 $N$ are shown in Fig. 4. It will be seen that at these high concentrations the rise in respiration rate is very steep, that the maximum
rate attained is the same, but is maintained for a few minutes only and is followed by a very rapid decline. Thus at 0.20N it falls from over 240 to zero in 12 min. Each graph in Fig. 4 is the result of observations on one fish only.

If 50 mg. per l. of calcium, as chloride or nitrate, is added to the $1 \times 10^{-5}$g. per c.c. lead solution the respiration rate does not increase in this way but remains fluctuating somewhat irregularly around the normal level, finally falling when the fish eventually die, that is in about 10 days. Respiration is not laboured, the gills remain clear of mucous film and the body of the fish remains clean and shining.

The respiratory symptoms of *Gasterosteus* in zinc sulphate solutions are essentially similar to those observed in the case of lead, as will be seen from Fig. 5, which records the respiration rate variation of a stickleback in a $10 \times 10^{-6}$g. per c.c. solution of zinc. It was shown by Carpenter that in the case of lead solutions, if the fish were removed from the solution a reasonable time before death and placed in a supply of well-aerated water the film of coagulated mucus was shed off and recovery took place. Recovery takes place on removal from zinc solutions also, and the way in which the respiration rate returns to normal is illustrated by Fig. 6. Three sticklebacks were placed in a $2 \times 10^{-4}$g. per c.c. solution of zinc sulphate and the mean respiration rate recorded hourly. In 13 hr. this had risen to over 240 per min. and the fish were then removed and placed in a frequently renewed supply of well-aerated soft tap water. This point is indicated by the break in the graph, which shows that the respiration rate then fell, rapidly at first and then more slowly, until in about 45 hr. it settled down to the normal level.

The effect of calcium on the toxicity of zinc salts is similar to its effect on lead. In Fig. 7 respiration rate graphs are given for *Gasterosteus* in a $2 \times 10^{-6}$g. per c.c. solution of zinc sulphate and $2 \times 10^{-4}$g. per c.c. zinc sulphate plus 50 mg. per l. of calcium as calcium nitrate. The latter shows that, in the presence of sufficient calcium, the zinc fails to produce any symptoms of respiratory distress, the fish surviving for over 10 days with their breathing rate below, rather than above, the normal level. In both these experiments two sets of three fish were used as described previously.
Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback 403

In the experiments on minnows an apparatus was assembled, Fig. 8, in which a supply of Cambridge tap water which contains 51 mg. per l. of calcium as calcium bicarbonate (Weil & Pantin, 1931) flowed continuously at 200 c.c. per min. through a mixing bottle into which strong lead nitrate solution (made up with distilled water) dripped at 1 c.c. per min. The dilute solution which resulted from the mixture flowed on through a 2000 c.c. vessel in which two large minnows, *Phoxinus phoxinus* (L.) were placed. The apparatus was first set running with a $2 \times 10^{-4}$ g. per c.c. lead solution in the dripper, so that the solution which flowed through the vessel in which the fish were placed contained one part lead per million of water. A white precipitate of lead carbonate gradually collected in the mixing bottle and in the vessel containing the minnows and the experiment was therefore discontinued and set going with a $16 \times 10^{-5}$ solution in the dripper so that the weak solution was slightly more dilute than before ($8 \times 10^{-7}$). A perceptible precipitate collected still and the strength of the strong solution was further reduced to $14 \times 10^{-5}$ giving a $7 \times 10^{-7}$ solution. At this dilution no precipitate formed, the mixing bottle and vessel containing the minnows remaining perfectly clear after several days.

![Graph](image)  
*Fig. 6. Gasterosteus; return of respiration rate to normal on transference to water after 13 hr. immersion in $2 \times 10^{-5}$ g. per c.c. zinc sulphate.*
The original two minnows, which were still alive, were then removed and a fresh pair were placed in the apparatus. The $7 \times 10^{-7}$ g. per c.c. solution was kept running for 21 days and both fish survived this period without any apparent ill effect. The experiment was then discontinued and repeated with a further two minnows with the same result, and using the same arrangement the same result was obtained with *Gasterosteus*. In these experiments with running solution the fish were fed with "ants' eggs".

A further experiment was made with the goldfish, *Carassius auratus* (L.). As previously noted, this species is very resistant to lead and appears to survive a $1 \times 10^{-5}$ g. per c.c. solution indefinitely. A $10 \times 10^{-5}$ g. solution was therefore employed, five fish being placed each in a separate vessel containing 2000 c.c. of solution made up with Aberystwyth tap water. Another five fish were placed in a similar set of solutions containing the same amount of lead, but 50 mg. per l. of calcium, as calcium nitrate, was added. All the solutions were renewed daily and the fish, which were all 3½-4 in. in length were fed with "ants' eggs".

Those in lead only began to be uneasy in about 24 hr. and examination of the gills by lifting the operculum revealed a white film of coagulated mucus which at first appeared in patches and later became almost uniformly developed over the
Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback 405
gill membranes. Much mucus was precipitated on the body and quantities of this
were shed off rendering the solution opalescent and collecting as a distinct whitish
layer at the bottom of the vessel. Marked respiratory distress developed and the
five fish died in 60, 90, 96, 104 and 114 hr.
Those in the solutions containing calcium developed none of these symptoms.
Periodical inspection of the gills showed that they remained clean and red, no film
formed on the body and the solutions remained clear. Two fish died in 16 days, one
in 19 days, and the remaining two were alive and apparently healthy in 35 days,
when the experiment was discontinued.

Fig. 8. Apparatus for continuously flowing solution supply. The rate of flow of the tap water into
the funnel of the mixing bottle was adjusted by selecting a tube of suitable bore for the siphon, and
adjusting the height of the mixing bottle, thus varying the head of tap water in the tank. The rate
of flow of the lead solution was adjusted by varying the length of the drawn-out portion of the
dripper tube (D), and the height of the air intake. The stopcock (SC) was used to allow the escape
of the air from the dripper bottle when filling up with solution.

D. DISCUSSION

In all cases it was noticed that whereas in the experiments with lead or zinc
alone the solutions were rendered distinctly cloudy by the precipitated mucus
thrown off by the fish, in the experiments in which calcium was added the solutions
remained clear. This difference was exceptionally well marked in the case of the
goldfish. Thus it certainly does not appear that the calcium brings about a reduction
of toxicity by stimulating the secretion and dispersal of the mucus, thus enabling
the fish to keep the gills clean, as in this case more precipitated mucus would be thrown into suspension in the lead plus calcium solutions and they would be rendered more cloudy than those containing lead only. As indicated above, the reverse of this is what is actually observed, and on the contrary all the experiments seem to indicate that in the presence of sufficient calcium the reaction between the metallic ion and the mucus, which is the essential feature of the toxic process, does not take place at all. This conclusion was endorsed by some simple experiments with the mucus secreted by the eel, Anguilla anguilla (L.). About 40 c.c. of slime was removed from the body of a large eel and filtered, giving a colourless, very slimy solution. This was divided into two portions and to one 5 c.c. of 1/100 calcium nitrate solution was added. This produced no apparent effect. ½ c.c. of 1/100 lead nitrate solution was then added to both, and the mixtures were gently shaken. The mucous solution containing calcium remained clear, but in the portion to which no calcium was added the mucus was precipitated as a network of whitish strings, which quickly gathered into a yellowish white mass. Similar results were obtained with zinc sulphate.

As might be expected, the subsequent addition of calcium fails to reverse the action of the lead and bring the mucus into solution again. In agreement with this is the fact that fish removed from lead nitrate solutions and placed in 50 mg. per l. calcium nitrate solution do not recover any more rapidly than those placed in untreated soft tap water.

Ellis (1937) has observed that the toxicity of metals in solution is greatly influenced by the presence of other salts, and notes that the survival time of goldfish in a $1 \times 10^{-6}$ g. per c.c. solution of copper sulphate can be prolonged from 2½ hr. to 6 hr. 40 min. by the addition of 1/200 sodium nitrate, while the further addition of $5 \times 10^{-5}$ g. per c.c. of calcium chloride "greatly enhanced the protective action of the sodium nitrate against the lethal action of copper sulphate", the survival time now increasing to over 12 hr.

It is evident that pollution of a river with dissolved lead or zinc will have far less serious effects on the fish if the river water contains an adequate supply of calcium bicarbonate than if the water is soft. An adequate calcium bicarbonate content would result in the greater part of the lead and zinc being precipitated as insoluble carbonates, and apparently what remained in solution would be rendered innocuous. Whether the treatment of effluents from lead and zinc workings with calcium is possible is another question. Calcium carbonate, unless very finely divided, dissolves very slowly even in water containing much carbon dioxide, and the more readily soluble calcium hydroxide could not be added without disturbing the pH of the water, while in all probability treatment with calcium nitrate or calcium chloride would not be economically feasible. However, the question is worthy of further consideration, and it is clear that much work remains to be done on the effect of calcium salts and salts of other metals on the reactions that occur between heavy metal salts and the secretions of the gills and body surface of fish. How far calcium salts can reduce the toxicity of heavy metallic ions to invertebrate freshwater animals is a further problem for study, in such animals the mechanism of
Relative Toxicity of Salts of Lead, Zinc and Copper to the Stickleback 407
toxicity of metallic salts is essentially different to that in fish, as the writer (1937) has noted previously.

SUMMARY

1. Lethal limits of concentration are determined for lead, zinc and copper for the three-spined stickleback (Gasterosteus aculeatus L.).

2. The addition of calcium salts to solutions of lead nitrate or zinc sulphate reduces the toxicity of these salts to Gasterosteus. 50 mg. per l. of calcium (as nitrate or chloride) is sufficient to annul the toxicity of a $1 \times 10^{-8}$ g. per c.c. solution of lead or a $2 \times 10^{-6}$ g. per c.c. solution of zinc.

3. The reactions of the fish in the solutions with and without calcium are compared, and the respiratory symptoms are described with the aid of graphs illustrating the variation in respiration rate during the survival time.

4. It is shown that a running supply of “hard” tap water containing approximately 50 mg. per l. of calcium as calcium bicarbonate is harmless to the minnow, Phoxinus phoxinus (L.) and to the stickleback, when there is added to it the maximum amount of lead that it can hold in solution ($7 \times 10^{-7}$ g. per c.c.). This concentration of lead in soft water is fatal to Gasterosteus in $38\frac{1}{2}$ hr.

5. The same amount of calcium renders a $10 \times 10^{-6}$ g. per c.c. solution of lead harmless to the goldfish, Carassius auratus (L.).

6. It is concluded that, in the presence of sufficient calcium, the interaction between the lead, or zinc, and the mucus secreted by the fish does not take place. This conclusion was endorsed by experiments in vitro on the slime secreted by the eel, Anguilla anguilla (L.).

7. The application of these results to the pollution of natural waters by effluents from lead and zinc workings is briefly discussed.

REFERENCES