

## Responses of Freshwater Animals to Sodium Cyanide Solutions.

### II. *Gammarus pulex*

by

H. H. COSTA

*Department of Zoology, University of Ceylon, Peradeniya*

( With three text figures )

Experiments have been described in which five species of fish have been used in a study of their responses to various cyanide concentrations (Costa 1965a). To have an understanding of the corresponding behaviour of an invertebrate to cyanide solutions, a similar series of experiments was carried out, using *Gammarus pulex*.

### MATERIALS AND METHODS

The material used for these experiments was obtained in large numbers from Capel Dewi Brook near Aberystwyth, U.K. In the laboratory, the animals were kept in an aquarium containing well aerated, circulating freshwater, with some stones and dead leaves, under constant temperature conditions. Adult gammarids of both sexes were used indiscriminately in the course of the experiments.

The experimental apparatus used in the present investigation was a modification of that devised by Jones (1947). The experimental tank measured 10 ins. in length, with an internal diameter of  $1\frac{1}{4}$  ins. and a capacity of about 200 ml. Only two aspirators were used. The results were recorded by the method devised by Shelford (1917).

The reaction experiments were conducted with cyanide solutions covering the concentration range 0.005–0.00001N. Survival-time experiments were also conducted to ascertain the resistance displayed by gammarids to cyanide solutions.

### EXPERIMENTAL RESULTS

When gammarids are introduced into the more concentrated solutions, they are immediately stupefied, remain stationary for some time and then begin to swim first slowly, then vigorously. After a very short period they come to rest, fall on their sides but continue beating their limbs; death was assumed to have taken place when there was no movement on tapping with a glass rod. In dilute solutions they may swim for a very long time before they come to rest on their sides.

Compared with fish, *Gammarus* shows a remarkable resistance toward cyanide solutions. In a solution of 0.005N (100 ml. of poison), the survival time is about  $1\frac{1}{2}$  hours; fish such as minnows and sticklebacks survive less than 15 min. at this concentration. In the experiment tube, the gammarids turn back promptly and avoid a solution of this concentration within a minute if they are swimming towards it, but if the animals are resting at the end at which the solution is admitted, it fails to stimulate them to swim out immediately; they fall on their sides and for a time keep on beating their limbs. Once

irritation becomes acute, they gradually begin to move out (Fig. 1A). Once out of the solution they remain in the water end without further venturing into the toxic zone. When confronted with a 0.001N solution, they withdraw from the toxic end of the experiment tube in less than 3 min. (Fig. 1B) but as in the previous series of experiments this avoidance time is not consistently displayed.

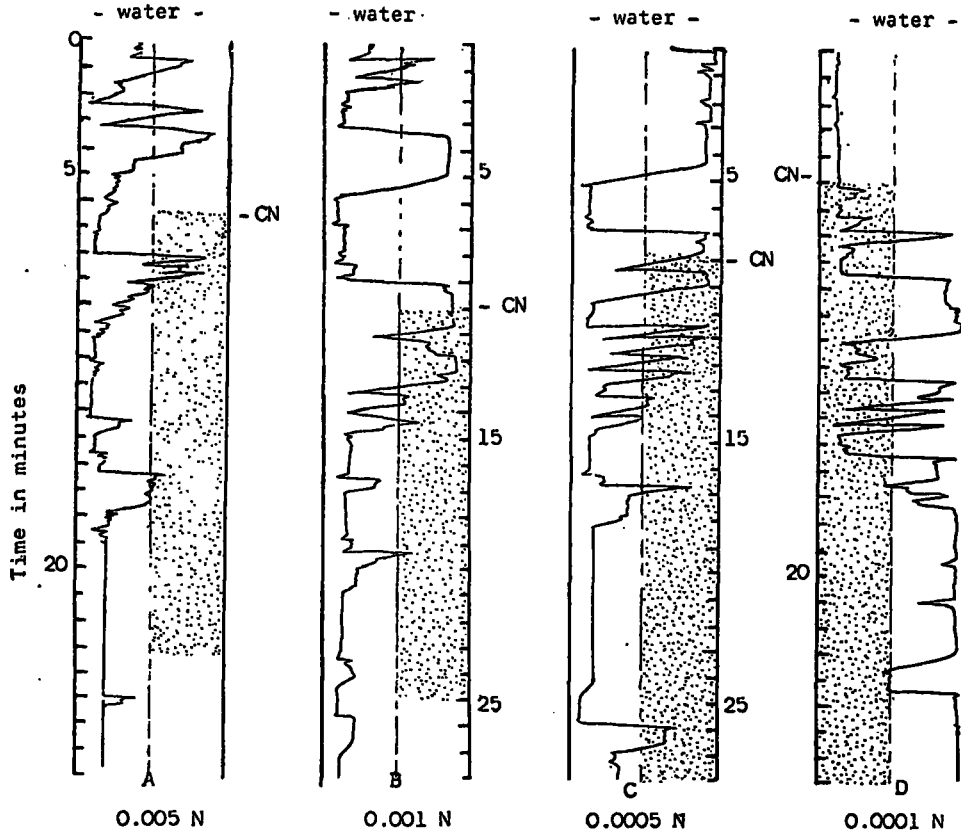


Fig. 1. Reactions of single *Gammarus* to 0.005, 0.001, 0.0005 and 0.0001 N sodium cyanide. pH of solutions 8.7, 8.1, 7.6 and 7.3. Temperature 13–14°C.

A more definite and sharper avoiding reaction is seen at 0.0005N. Although the gammarids commence to respond promptly, the reaction is not complete, as the animals are seen to swim in and out several times before a complete avoidance is seen. A time of 4–5 min. was recorded for five individual gammarids (Fig. 1C.) The response at 0.0001N is still slower. The gammarids survive about 3 hr. in this solution. At this concentration, they take about 9–11 min. to discover and restrict their movements to the water zone (Fig. 1D). Experiments conducted at 0.00005N and 0.00001N were rather surprising. The gammarids display little or no capability of detecting and avoiding these concentrations of cyanide. They venture into them, and show no tendency to avoid the solution end. In most experiments, the animals settled down at the toxic inlet, and rarely moved out of the toxic zone during the course of the experiments. It is interesting to note that fish react in 5–10 min. to this concentration of cyanide. In the survival time experiments conducted at these concentrations, immediate inhibition of swimming was not seen.

*The effect of  $\text{Na}_2\text{S}_2\text{O}_3$  on the reactions of Gammarus to NaCN solutions*

The general effect of sodium thiosulphate on the toxicity of cyanides to fish is to prolong the survival times and extend the reaction times. A generally similar effect is seen in the case of *Gammarus* but there are some striking differences.

In studying these reactions, equal volumes of 0.05N sodium thiosulphate and cyanide solutions covering the concentration range 0.005N – 0.00001N were used. The actual concentrations of the mixture were therefore 0.025N thiosulphate and half the concentration of the cyanide used. Preliminary experiments were conducted using water in one aspirator and 0.05N  $\text{Na}_2\text{S}_2\text{O}_3$  in the other aspirator to test whether *Gammarus* would react negatively to 0.05N  $\text{Na}_2\text{S}_2\text{O}_3$ . At first a sudden turning back at the water/solution junction was observed but later they showed an indifferent reaction to the thiosulphate.

The *Gammarus* reacted both vigorously and promptly to a solution of 0.001N NaCN plus thiosulphate (actual concentrations of the solutions in the mixture being 0.0005N NaCN + 0.025N  $\text{Na}_2\text{S}_2\text{O}_3$ ). The selection of the water zone was accomplished in less than a minute and it was more or less permanent, the gammarids rarely visiting the toxic zone thereafter. These results are interesting in that the animals took a considerable time to move out of 0.005N NaCN and 0.001N NaCN solutions (where the concentration of the cyanide was double or more than the cyanide in the mixture). The reaction at 0.0005N NaCN +  $\text{Na}_2\text{S}_2\text{O}_3$  was almost identical with the above.

Detection and avoidance of 0.0001N NaCN +  $\text{Na}_2\text{S}_2\text{O}_3$  is still rapid and the withdrawal is completed in about 2 – 3 min. and the gammarids lived, in a survival time experiment, in this mixture of solutions for more than 48 hrs. The responses displayed towards 0.00005N and 0.00001N NaCN plus  $\text{Na}_2\text{S}_2\text{O}_3$  are still surprisingly fast. At 0.00005N the reaction was rather rapid though initial visits to the solution were frequent; the final avoidance was completed in 5 – 6 min. A similar but delayed reaction was shown towards 0.00001N +  $\text{Na}_2\text{S}_2\text{O}_3$ . Towards 0.00005N and 0.00001N NaCN solutions, without the "antidote," indifferent reactions were shown.

It appears that the addition of thiosulphate to sodium cyanide solutions reduces the toxicity so that there is no sudden stunning of the sensory receptors, thus enabling the *Gammarus* to perceive toxic substances quickly and avoid them before any fatal reactions take place. In these experiments we see that all the reactions are accelerated and the reaction times shortened, unlike in fish where the addition of  $\text{Na}_2\text{S}_2\text{O}_3$  extend the reaction times. The results obtained in this series are given in Table 1.

*Effect of temperature on the reaction times of gammarids to NaCN solutions.*

The experiments conducted with fish have shown that the temperature is an important factor determining their behaviour and reaction time. A similar set of experiments was conducted with gammarids to see whether temperature plays any part in shortening or speeding of their reactions. The series of experiments at higher temperatures was conducted at 26 – 27°C and at lower temperatures at 0 – 2°C. All the animals used in these experiments were gradually acclimated to the required temperature for a period of 10 or more hours. The results of the experiments are summarized in Fig. 2. As with fish, experiments conducted at higher temperatures showed a speeding up of behaviour and other activities like swimming.

Solutions used	Actual Concentrations in the mixture	Reactions and reaction time in minutes	Concentration of cyanide solution	Reactions and reaction time in minutes
{ 0.001N NaCN 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	{ 0.0005 NaCN 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	0.0005N	—
		1	NaCN	4—5
{ 0.0005N NaCN 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	{ 0.00025N NaCN 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	—	—
		1—2	—	—
{ 0.0001N NaCN 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	{ 0.00005N NaCN 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	0.00005N	≠
		2—3	NaCN	> 60
{ 0.00005N NaCN 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	{ 0.000025N NaCN 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	—	—
		5—6	—	—
{ 0.00001N NaCN 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	{ 0.000005N NaCN 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	—	0.000005N	≠
		12—15	NaCN	> 60

— = Negative  
≠ = indifferent

TABLE 1. Reaction and reaction times obtained for *Gammarus pulex* using 0.025N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> as antidote.

Experiments with higher concentrations of cyanide conducted at 26–27°C generally resulted in pseudopositive reactions; thus the experiment conducted at 0.0001N shows little response at first, the *Gammarus* swimming rapidly, at random, on contact with the cyanide, then they rest motionless falling flat on their sides, but showing active respiratory movements. Later, if the experiments are continued, they try to straighten up and attempt to swim out, but in most cases a continued stay in the cyanide zone brings about complete intoxication and finally death. At concentrations greater than 0.0001N, the pseudopositive response is more common and death is more rapid.

At a slightly lower concentration (0.00005N), the gammarids begin to show clear negative reactions—although some individuals continuously stay at the toxic end without moving out—in about 5 min. The response at 0.00001N was a rapid avoiding reaction, established in about 2.5 min. As this concentration is less toxic, the animals seem to succeed in escaping before the toxic processes begin to take effect.

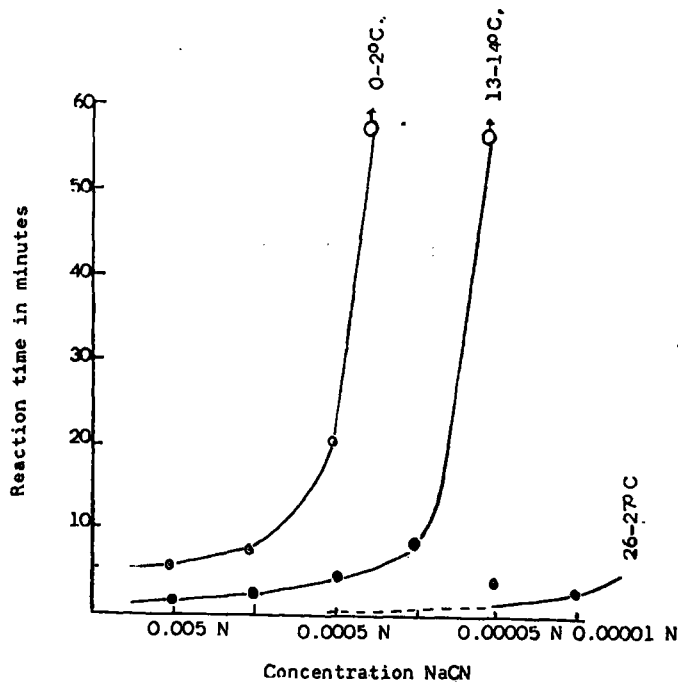


Fig. 2. The effect of temperature on the reactions of *Gammarus* to sodium cyanide solutions.

Experiments conducted with gammarids acclimated to low temperatures (0–2°C) showed tolerances to the cyanide solutions similar to those shown by the fishes. Five experiments with groups of five gammarids for each test, at 0.001N, showed that all the processes of activity and behaviour were slowed down. Towards a 0.001N solution at room temperature, the gammarids showed a negative reaction in 2.5 min. At very low temperatures, the animals reacted rarely in the first 5–6 min.: then gradually they commenced to crawl around the experiment tube, and then move to and fro in the toxic half. Finally, at 9 min., they gradually moved out into the water zone where they stopped all swimming activity. Two experiments were conducted at 0.005N to see whether at a higher

concentration they would withdraw immediately, but in both experiments they took about 5–6 min. to do so (Fig. 2).

The response was even more prolonged at 0.0005N. The gammarids took a considerable time to move out (about 20–22 min.). Those at the opposite end of the toxic inlet swam in and moved out of the toxic area in the first 20 min. or so but later almost all the animals restricted their movements to the water zone. At 0.0001N, 0.00005N and 0.00001N, the gammarids showed no tendency to move out or restrict their movements to the water zone during the 60 min. duration of the experiment. At the end of the experiments they were transferred to clean, aerated water and they all survived.

*Effect of pH on the reactions of Gammarus to NaCN solutions.*

It has been shown that changes in the hydrogen ion concentration of sodium cyanide solution have a profound influence on the reaction behaviour of fish and the degree of toxicity shown by survival time experiments. An increase in the hydrogen ion concentration raises the toxicity as a result of increased hydrolysis of the NaCN. A corresponding series of experiments with *Gammarus* was carried out to test the effects of increase or decrease of pH on their reactions towards cyanide. The strength of the cyanide solution used was 0.0001N. With no pH adjustment, *Gammarus* will avoid this solution in 9–10 min. HCl and NaOH were used respectively to decrease or increase the pH. The pH was measured colorimetrically and checked with a Cambridge pH meter. The results, which are the means of five experiments, are given in Fig. 3. The pH range covered was 3.2–10.5.

The results show a picture almost identical to that shown by fish. Reduction of the pH has an immediate effect; thus at a pH 6.6, the selection of the water zone is completed in a lesser time. At still lower pH values, the reactions are very prompt; at a pH 3.2, the reactions are violent and rapid, the gammarids with coiling movements begin to swim without any hesitation into the water zone.

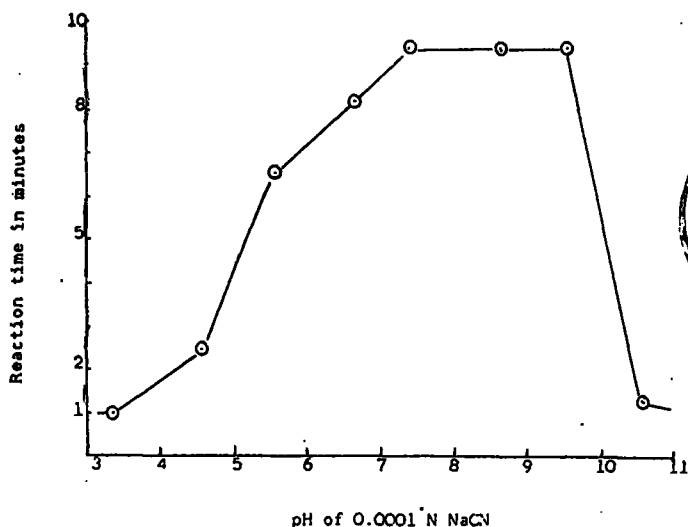


Fig. 3. The reaction times displayed by *Gammarus* to 0.0001 N sodium cyanide solutions of different pH. Temperature 13–14°C.

On raising the pH by adding sodium hydroxide, no change in the response shown by the animals is perceptible in the pH range 7-9. At pH 9.6, the gammarids exhibit an immediate response on the admission of modified cyanide, but it is short lived, as later they swim to and fro without any ill effects. This gradually gives way again at 9.5 min. to an avoidance reaction. Above this pH, the responses are immediate and the *Gammarus* withdraw with extreme rapidity and later their turnings back at the junction are quite obvious. Withdrawals into the water zone are usually accompanied by an evident display of irritation.

These experiments indicate rather definitely the effects of change of pH on the reactions of *Gammarus*; the general results are very similar to those shown by fish.

*Effects of low dissolved oxygen content on the reactions of Gammarus to NaCN solutions.*

The effect of lowering the dissolved oxygen content in NaCN on the reactions of fish has already been shown. Gammarids are sensitive to lack of dissolved oxygen as shown by the writer's experiments (Costa 1964) but avoidance of cyanide solutions is not quite rapid as expected. A combination of these two factors could be expected to bring about fatal and unfavourable conditions and to examine this, two series of experiments were conducted at two dissolved oxygen levels namely 0.8 and 2.8 mg O<sub>2</sub>/l. The concentration of cyanide solution used was 0.0001N and the selected temperature was 13-14°C.

The series conducted with cyanide solution containing 2.8 mg. O<sub>2</sub>/l. elicited rather a slow response, though the gammarids withdraw in about 4-5 min. The initial stimulus to make the gammarids swim was not strong as there was not much activity in the 1st or 2nd minutes, then a somewhat rapid increase of movement was seen and this was accompanied by rapid withdrawal. At 0.0001N NaCN with only 0.8 mg. O<sub>2</sub>/l. dissolved oxygen, the reaction was extremely prompt. Acute distress was shown initially and rapid swimming was seen from the very moment the solution was admitted; retreat into the water zone was complete in 1-2 minutes.

These experiments therefore, like those carried out with the fish, confirm that lowering the oxygen content of the cyanide solutions aggravates anoxia so that there may be an increased tendency for the animals to avoid the solution.

## DISCUSSION

The investigations carried out with *Gammarus* have shown that they avoid NaCN solutions like fish, but there are conspicuous differences in the manner of reactions, magnitude of reaction times and also in the threshold concentrations of avoidance. Survival experiments with *Gammarus* have shown that compared with fish they are highly resistant to sodium cyanide solutions. This marked resistance shown by these animals may be related to their ability to survive a longer time than fish in waters with a depleted oxygen supply. Jones (1941b) showed that with cyanide the respiratory rate of *Polycelis nigra* was heavily depressed, but the survival time was three days or longer. Although the normal respiratory rate of *Polycelis* (0.165 ml. O<sub>2</sub>/g/h) is not much less than that for trout, *Polycelis* is considerably more resistant to cyanide and this was similarly attributed to its ability to survive a longer time in oxygenated water.

*Gammarus* take a considerable time to avoid strong solutions of cyanide and in this they are different from fish. This may be possibly because strong to moderate cyanide solutions depress the respiration rate and immediately inhibit swimming (Jones 1941a). Later withdrawal is most probably due to intense irritation which stimulates them to crawl out into the water zone.

The addition of thiosulphate to cyanide solution has, with *Gammarus*, an effect somewhat different from that seen with fish. In the case of *Gammarus*, the addition of thiosulphate prolongs the survival times, but the reactions are accelerated and the reaction times considerably shortened. It is possible to conclude that the addition of thiosulphate reduces the toxicity and at the same time reduces the sudden stunning of the sensory receptors, thus preventing rapid inhibition of swimming movement and enabling the animal to move away quickly from the poison zone.

As with fish, temperature plays a similar part in the reactions of *Gammarus* to NaCN solutions. In the case of *Gammarus*, high temperatures appear to inhibit swimming activity very rapidly at high concentration levels, probably as a result of rapid suppression of respiratory processes, but with more dilute solutions, however, the normal accelerated reactions are seen. At very low temperatures all the reactions are slowed down. As with fish, these results are possibly associated with an increase or decrease in the metabolic activities of the animals.

The end results of the experiments conducted with NaCN of different pH are similar to those shown by fish. Experiments conducted by the writer with water of low pH have shown that acidic water causes more irritation than alkaline water (Costa 1964), the recognition and avoidance too is greater as a direct result of irritation. It is probable that rapid avoidance of NaCN solutions of low pH is due to the formation of more HCN. On the alkaline side of the pH range it appears that *Gammarus* will withdraw from clean water above pH 9.6. In the present experiments, the rapid avoidance of NaCN above pH 9.6 may be mostly due to the alkalinity of the solutions. *Gammarus* can exist for a certain amount of time in water partly depleted with oxygen and the results described above of experiments with NaCN solution of low oxygen tensions are therefore to be expected. However, when the oxygen content is very much diminished, the double effect of cyanide and lack of oxygen aggravates the anoxia making the animals move out within a minute or two. The apparent toleration of polluted waters by *Gammarus* provided the oxygen content is high (Hynes 1960) is therefore to be expected.

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#### SUMMARY

Experiments with *Gammarus pulex* with cyanide solutions gave generally similar results to those given by experiments with fish. These animals, however, appeared to be less sensitive than fish. The response limit for *Gammarus*, for sodium cyanide, is about

0.0001N. Addition of thiosulphate to sodium cyanide reduces the toxicity but unlike with fish, the reactions are accelerated. Increasing the temperature, increasing or decreasing the pH and lowering the oxygen content had effects on their reactions similar to those seen in fishes.

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