

## Responses of Freshwater Animals to Sodium Cyanide Solutions.

### III. Tadpoles of *Rana temporaria*

by

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(With three text figures)

The writer has already described experiments with different species of fish and *Gammarus pulex* to see how these animals react to sodium cyanide solutions under a variety of environmental conditions (Costa 1965 a, b). Tadpoles are more like fish, being free-swimming forms and, like fish, they are subjected to fluctuating conditions in the water. It would be interesting therefore to see whether they show similar reactions to cyanide solutions.

#### MATERIALS AND METHODS

The frog spawn was collected from a pond in the first weeks of spring 1963 and was brought to the laboratory. The whole mass was then washed carefully with water and transferred to an aquarium containing well aerated, circulating freshwater maintained at 10°C. The hatched out tadpoles were regularly given minced meat on which they were found to feed well. Before starting the experiments, the tadpoles were transferred to a dish containing water at room temperature and were kept at least two days before being tested. For the experiments, tadpoles measuring 35 - 39 mm. were used.

The experimental apparatus was similar to that employed for studying the reactions of *Gammarus pulex* (Costa 1965b). The movements of single tadpoles were copied on vertical charts after the method of Shelford (1917).

As with fish and *Gammarus*, the reaction experiments were conducted over the range 0.005 - 0.00005N NaCN. A series of survival experiments was also made.

#### EXPERIMENTAL RESULTS

The behaviour of tadpoles in concentrated solutions such as 0.001N NaCN was rather similar to that of *Gammarus*. Sudden immobilization, more or less immediately on introduction, was followed later by a bout of vigorous swimming after which the tadpoles came several times to the surface to breathe air. Later, they came to rest either on or near the surface or at the bottom with occasional swimming; the death point was not very definite. In dilute solutions such as 0.0005N, swimming is continued for a long time till they come to rest on the surface or at the bottom. Violent behaviour is not seen, the death points are very indefinite.

At 0.005N NaCN the tadpoles show a response similar to that shown by fish. The tadpoles quickly swim back to the water zone but they return to the cyanide zone apparently producing a positive reaction. This behaviour gradually gives way to a withdrawal when

in 10–12 min., with considerable effort, the tadpoles drag themselves out, (Fig. 1A); reversals however produce pseudopositive reactions.

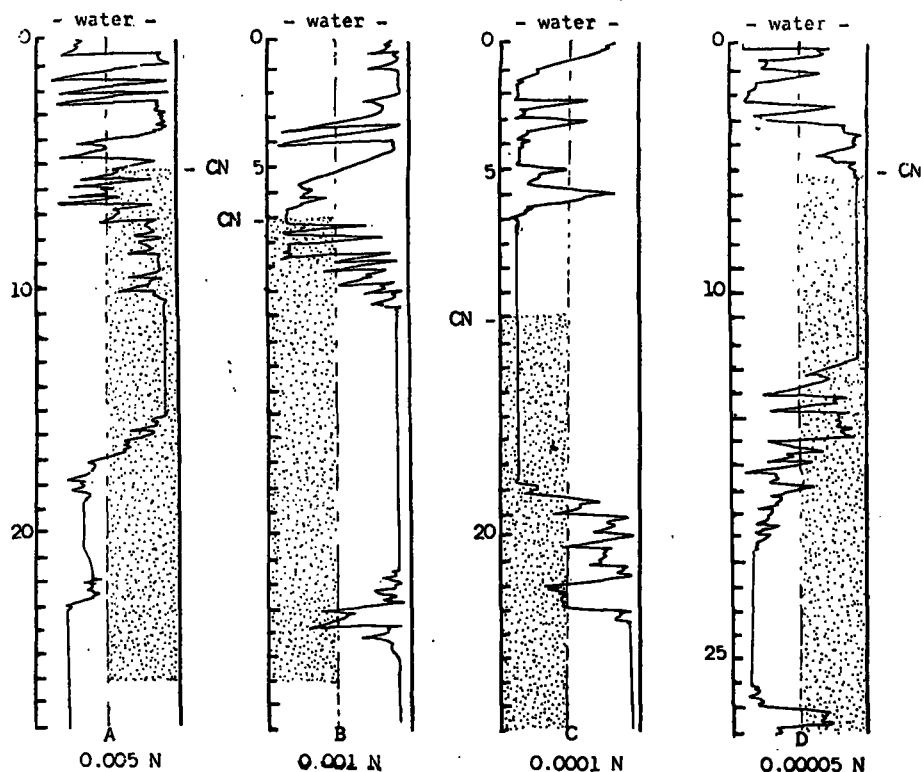


Fig. 1. The reactions of single frog tadpoles to 0.005, 0.001, 0.0001 and 0.00005 N sodium cyanide solutions. pH of solutions 8.7, 8.1, 7.3 and 7.15.

The reaction at 0.001N is clear cut, definite and prompt and is complete within 2 or 3 min. (Fig. 1B). The tadpoles survive in this solution for more than an hour in a survival time experiment.

Slow or very slow reactions are shown by tadpoles in response to coming in contact with 0.0005N, 0.0001N (Fig. 1C), 0.00005N (Fig. 1D) and 0.00001N NaCN solutions, the reaction times progressively increasing with dilution. The general behaviour is that if the solution is admitted on the preferred side, they remain stationary, till irritation by the cyanide solution stimulates them to swim to the water zone. When the NaCN solution is admitted on the opposite side, the tadpoles keep swimming across until they show signs of exhaustion; for example, the reaction time at 0.00001N is about 25–30 minutes.

The experiments conducted at 0.000004N and 0.000005N gave results which showed that tadpoles behave indifferently towards these concentrations. They occasionally show recognition movements but ultimately they may crowd in the toxic zone. During the 60 min. of the experiment, there was no tendency to avoid the toxic zone.

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

The effect of  $\text{Na}_2\text{S}_2\text{O}_3$  on the reactions of fish and *Gammarus* has already been described. As with fish and *Gammarus*, equal volumes of 0.05N  $\text{Na}_2\text{S}_2\text{O}_3$  and cyanide solutions ranging from 0.001–0.00001N were mixed so that the actual concentrations in the mixture were 0.025N for the  $\text{Na}_2\text{S}_2\text{O}_3$  and for the cyanide, half the concentration used to make the mixture.

Initially experiments were conducted to see whether  $\text{Na}_2\text{S}_2\text{O}_3$  has any repellent effect.  $\text{Na}_2\text{S}_2\text{O}_3$  solution was placed in one aspirator and offered as an alternative to tap water. Like *Gammarus*, the tadpoles at first reacted negatively but this was of short duration and later the tadpoles were swimming indifferently in both zones.

The experimental results are given in Table 1. At 0.001N and 0.0005N NaCN plus sodium thiosulphate the tadpoles display avoiding reactions which are smooth, rather rapid, and very conspicuous (reaction time 2–3 min.). However, with a 0.0001N NaCN plus "antidote", they show rather a delayed avoidance, although sudden and intense activity is displayed at first. At a still lower concentration (0.00005N NaCN +  $\text{Na}_2\text{S}_2\text{O}_3$ ), complete preference to the water zone is greatly delayed. The movements to and from are continuous; slight traces of recognition are seen throughout but crossing of the boundary is frequent. The tadpoles usually take about 40–45 min. to aggregate in the water half of the tube. On further dilution (0.00001N NaCN +  $\text{Na}_2\text{S}_2\text{O}_3$ ), the tadpoles react indifferently.

The effect of addition of thiosulphate therefore gives an effect similar to that of *Gammarus*. The reaction times appear to be shortened as the tadpoles select the water zone in a faster time than with cyanide without the "antidote".

*Effect of temperature on the reactions of tadpoles to NaCN solutions.*

A series of experiments was carried out with tadpoles, aimed principally to find out the effects produced when the environment temperature is raised or lowered. The tadpoles were first acclimated to the two temperature levels tested namely 0–2°C and 26–28°C. The mean results of all the experiments are given in Fig. 2, and are compared with the results of the experiments conducted at room temperature (13–14°C.). The results indicate that very low temperatures slow down activity and associated responses considerably, while higher temperatures lead to rapid activity, with speeding up of the responses.

Experiments conducted at 0–2°C with 0.005N NaCN produced a smooth avoiding reaction in 4–5 min. but a few tadpoles crossed into the toxic zone and stayed there producing a pseudopositive reaction. At this temperature, tadpoles are quite indifferent to a solution of 0.00005N NaCN.

At higher temperatures (26–28°C), higher concentrations of cyanide cause extreme and acute distress to the tadpoles. At very high concentrations (e.g. 0.001N), most tadpoles fail to distinguish between the water and the toxic zones and cross the tube many times. In most cases, the toxic processes overcome them before they retreat into the water zone, producing pseudopositive reactions. At 0.0001 and 0.00005N, however, clear but rapid negative reactions are seen. Even at a concentration of 0.00001N, a negative reaction is evident though it is very slow, taking 35–40 minutes.

Solutions used	Actual Concentrations in the mixture	Reaction time	Concentration of NaCN	Reaction time
{ 0.001N NaCN; 0.05N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	(0.0005N NaCN + 0.025N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> )	2—5 min.	0.0005N	3—4 min.
{ 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> )	2—5 min.	—	—
{ 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> )	10.5 min.	0.00005N	11.5 min.
{ 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> )	43 min.	—	—
{ 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> )	Indifferent	0.000005N	Indifferent

TABLE 1. Reaction times obtained for tadpoles of *Rana temporaria* using 0.025N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> as antidote.

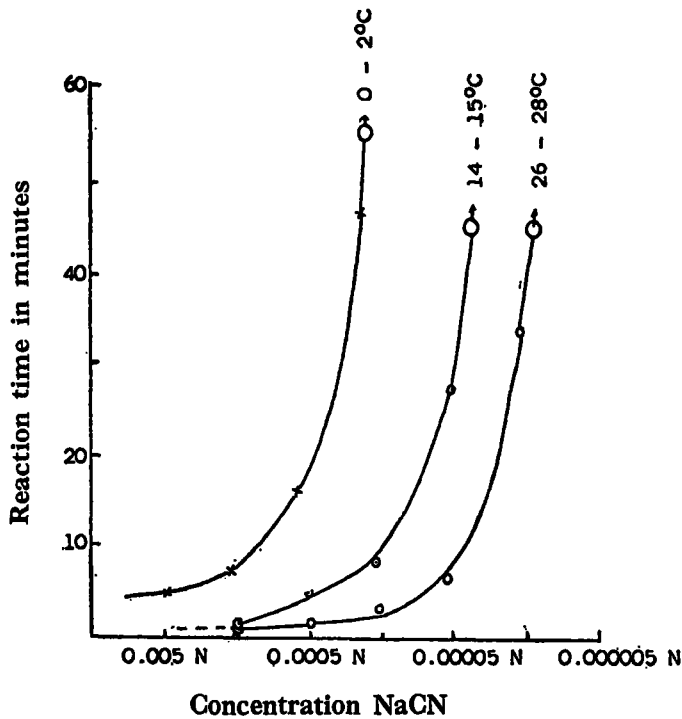


Fig. 2. The effect of temperature on the reactions of the tadpoles of *Rana temporaria* in sodium cyanide solutions.

#### *Effect of pH on the reactions of tadpoles to NaCN solutions*

It has been shown that pH plays an important part in the reaction behaviour of fish and *Gammarus* towards cyanide solutions. Tadpoles react "positively" to acidic solutions brought about by HCl (Costa 1964). It was therefore interesting to see how a change in pH would affect the reactions of tadpoles to cyanide solutions. A solution of 0.0001N NaCN was employed for these experiments. The experiments were conducted at four selected pH values: at 3.2, 5.1 (brought about by adding required quantities of HCl), 8.8 and 10.2 (brought about by adding NaOH). Tadpoles react negatively to an unmodified solution of 0.0001N NaCN in about 6-7 min. The results of the experiments are given in Figs. 3A, B, C & D.

At pH 5.1, the swimming activity was not changed; later, an aggregation of the tadpoles at the water zone was seen but the time taken is only 1-2 min., less than that taken with unmodified solution. At pH 3.2, however, the reaction is rather fast, the tadpoles, however, are not very active, lying still at first and later withdrawing into the water zone in about 4-5 minutes.

It is interesting to note that with fish and *Gammarus* a cyanide solution of this low pH would be met with a prompt reaction. At pH 8.8 the withdrawal of the tadpoles from the cyanide solution is completed in about the same time (6-7 min.) as that shown in an

experiment with an unmodified cyanide solution. The experiments conducted at pH of 10.2 produced very prompt avoidance reactions (reaction time 1–2 min.) (Fig. 3D). When the solution is admitted, the tadpoles react by showing violent, random swimming, at the same time exhibiting curling and convulsive body movements due to acute irritation. Even after retreating into the water half, marked after effects are shown for some time by the tadpoles.

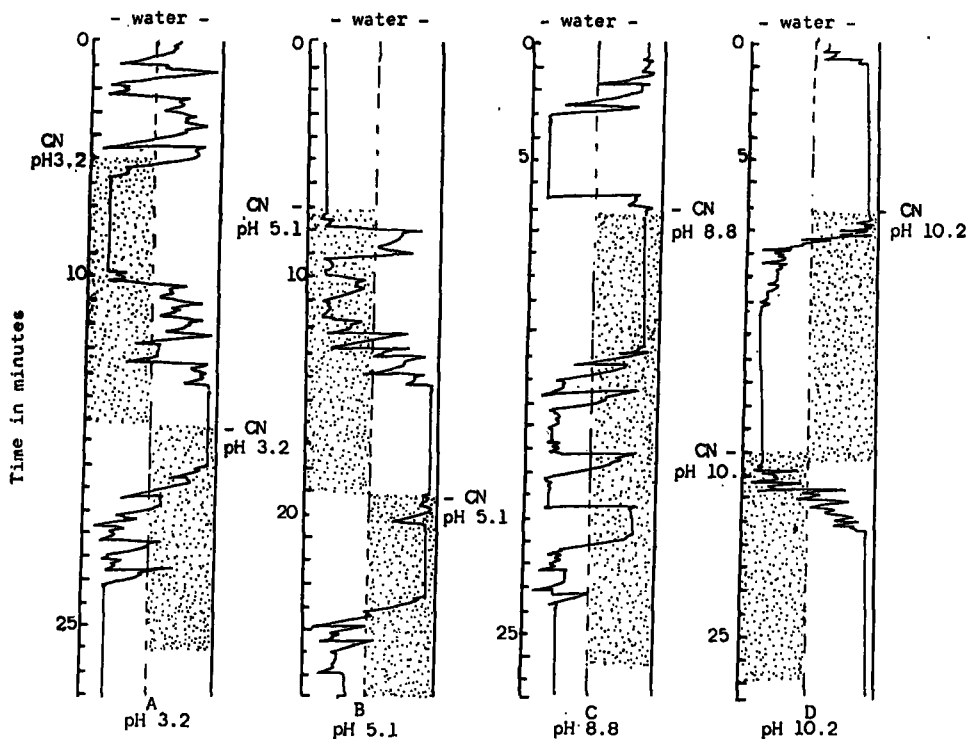


Fig. 3. The reactions of single tadpoles to 0.0001 N sodium cyanide of pH 3.2, 5.1, 8.8 and 10.2. Temperature 13–14°C.

The general behaviour of these animals towards acidified or alkaline NaCN is therefore similar to that of fish and *Gammarus*; at very low and very high pH<sup>s</sup> the avoidance reactions are accelerated.

*Effect of reduction of oxygen content on the reaction times of tadpoles to NaCN solutions.*

Tadpoles are generally sensitive to a reduction of oxygen content of water (Costa 1964). NaCN depresses respiration, and a reduction of dissolved oxygen aggravates this situation. Experiments were conducted at two levels of oxygen saturation; at 2.8 and 0.8 mg. O<sub>2</sub>/l. A 0.0001N NaCN solution was used for all the experiments and they were conducted at 13–15°C.

When the tadpoles come into contact with sodium cyanide solution in which the oxygen content has been reduced to 2.8 mg. O<sub>2</sub>/l. the tadpoles do not stop and turn back at the junction but proceed into the toxic solution zone and remain there for some time,

then gradually and smoothly they withdraw into the water half, in a time varying from 3–4 min. again to remain there. However if the tadpoles come into contact with cyanide solution containing 0.8 mg.  $O_2/l.$  the reaction exhibited is completely different; the behaviour is violent, most of the tadpoles stop abruptly at the junction and turn back, but others who proceed into the toxic zone seem to be irritated so intensely that they swim in all directions at random and rush out of the toxic zone in less than 2 min., displaying all the characteristic symptoms.

## DISCUSSION

The results of the experiments conducted with frog tadpoles show that tadpoles are more resistant to cyanide solutions than fish, although their reactions are some what similar. As with fish, a considerably longer time is taken to avoid strong cyanide solutions possibly again due to dulling of the chemoreceptors; but with moderately dilute solutions the avoidance time is proportional to the dilution; more dilute the solution is longer the reaction time. The addition of sodium thiosulphate produces effects resembling those seen with *Gammarus* rather than those described for the fishes. The overall effect appears to be to reduce the toxicity, prolong the survival times and correspondingly shorten the reaction times and increase the recognizing capabilities. The addition of thiosulphate therefore increases the sensitivity to NaCN solutions. The effect of temperature on the reactions of tadpoles to NaCN solutions appear to be similar to both fish and *Gammarus*. The writer has also shown that temperature exerts similar effects towards other toxic substances such as  $ZnSO_4$  (Costa 1964). These effects are all probably associated with the metabolic activity of the animal. Thus as with fish and *Gammarus*, seasonal changes in temperature will determine the duration, survival and intensity of reactions to NaCN solutions.

It has been shown by the writer that tadpoles react positively to very low hydrogen ion concentrations brought about by the addition of HCl. This effect appears to be reflected indirectly in the experiments conducted with cyanide solutions of low pH. These results will be of significance specially in situations where tadpoles are exposed to acidified cyanide discharges. In agreement with the previous experiments on fish and *Gammarus*, the rate of reaction to sodium cyanide by tadpoles was also found to depend on the oxygen concentration. The results indicate that cyanide affects the mechanism of oxygen uptake and oxygen transport as well as the oxygen demand. The depletion of oxygen in waters at the outfalls of effluents will therefore, aggravate pollution hazards.

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## SUMMARY

Experiments with tadpoles of *Rana temporaria* with sodium cyanide solutions gave generally similar results to those with fish and *Gammarus*. Tadpoles, however, are less sensitive than fish but more sensitive than *Gammarus*. The response limit for frog tadpoles is about 0.00001N. Changes of temperature, pH and dissolved oxygen had effects on their reactions similar to those seen with fishes and *Gammarus*.

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