

Responses of Freshwater Animals to Sodium Cyanide Solutions

1. Fish

by

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(With nineteen text figures)

Effluents and polluting substances can be arbitrarily divided into many categories. These include metallic salts, acids, alkalis, phenolic wastes, organic matter etc. The toxic effects of these and many others have been recently reviewed by several authors. Doudoroff and Katz (1950, 1953), Hynes (1960) and Jones (1962) on fish, and Hawkes (1962) on invertebrates. Toxic substances may affect the fish in various ways, some, for instance, may kill the fish by precipitating a gill clogging film (Carpenter, 1927). Others like cyanide which is present in waste waters from gas manufacture, plating, case hardening and metal cleaning have a specific toxic action. Brinley (1927) and Wuhrmann and Woker (1948) have reported that the toxicity of cyanide solutions is due to the action of hydrocyanic acid which has a toxic action of a very specific type. The soluble cyanides hydrolyse in water to form HCN, the extent of the hydrolysis being influenced by the pH of the solutions, acids increasing this hydrolysis and alkalis depressing it. HCN enters the body through the gills etc., circulates through the body in the blood stream and renders the tissues incapable of utilizing the available oxygen by its effect upon the cytochrome complex concerned with respiration.

A number of studies has been made to obtain precise information on the toxicity of cyanide to fish (Alexander et al., 1935), Karsten (1934), Ellis (1937), Herbert and Merckens (1952) and Neil (1957). Their work has shown that cyanide concentrations as low as 0.05-0.2 p.p.m. may be lethal to some freshwater fishes. Environmental conditions may increase or decrease the lethal processes (Herbert and Merckens 1952, Downing 1954). Sodium cyanide depresses the oxygen consumption by fish, higher concentrations inhibiting respiration almost immediately (Jones, 1947a).

While there are a number of toxicity studies of cyanide on fish, little or nothing is known about their responses on contact with cyanide solutions. This problem becomes more apparent when there is an artificial zonation in rivers in respect to the cyanide content in the water. Even if the water in the main stream is polluted, the tributaries may afford a safe place for the fish and other aquatic animals if they could detect and avoid the danger before being overcome by the toxic substance. The avoidance of fatal concentrations of toxic solutions can thus favour the survival of aquatic animals which otherwise enter polluted waters.

The present investigation has therefore being undertaken to determine how fishes respond to graded concentrations of sodium cyanide and to study the effects of different environmental conditions on their reactions.

MATERIALS AND METHODS

All the fishes used for the experiments, except the young goldfish, were collected by the writer in and around Aberystwyth, Wales, U.K. *Gasterosteus aculeatus* L and *Anguilla anguilla* L elvers were collected from the backwaters of the river Rheidol, *Phoxinus phoxinus* L from a lake at Pendam, Ponterwyd and *Salmo trutta* L fry from a stream near Aberystwyth. The goldfish were purchased from a commercial supplying company. All the fish after being brought to the laboratory were kept in large aquaria containing tap water. The water was well aerated by passing in compressed air through diffusers, and it was maintained at a temperature of 17–18°C. The fish were kept under these conditions for at least a week before the experiments were begun and were not fed immediately before or during the experiments.

The apparatus used in this investigation was essentially similar to that used by Jones (1947b). Instead of four aspirators, only two were used for the present experiments. In this apparatus the fish are confined in a horizontal glass tube, half of which may be filled with flowing tap water, the other half with flowing solution. The water and solution under test are sharply differentiated, thus presenting the fish with an opportunity to discriminate between ordinary water and a definite concentration of any substance under test.

In the reaction experiments single fish or five fish were used at a time. In the experiments where single fish were used the method of recording the results followed that devised by Shelford (1917), the movements of the fish were copied on the charts and reaction times were determined. In the other experiments, where five fish were used at a time, records of their positions were plotted every half minute. The intensity of avoidance was determined later, from the records.

In the survival time experiments, 5–10 animals were transferred from the aquarium into each particular concentration of the solution tested, contained in glass aquaria, and their approximate survival times recorded. Care was taken to see that there were no considerable temperature differences involved in the transference.

In the preparation of solutions of sodium cyanide, stock normal solutions were prepared in the laboratory and the series of solutions necessary for the experiments was then prepared by progressively diluting the stock solutions with well aerated tap-water. All concentrations quoted are those estimated from the degree of dilution of the stock solutions.

PRESENTATION OF RESULTS

The selected results are given as :

(a) Graphical records obtained with the reaction tube; the movements of individual animals or the positions of a number of individuals at different times within the experiment tube are traced by hand and later copied, the vertical scale being used as the time axis.

The data presented include :—

- (1) The species of fish used.
- (2) The concentration of the test solution expressed in normality.
- (3) The pH of the test solution.
- (4) Temperature of the solution and water.

In order to obtain a quantitative estimate of the results, the intensity of percentage avoidance was calculated by the following formula

$$\frac{W - T}{N} \times 100 = A$$

Where W represents the numbers of position records in the water zone of the experiment tube (obtained by counting the position records plotted every half minute during the duration of the experiments)

T represents the numbers of position records of animals in the toxic zone of the experiment tube

N represents the total number of position records in both halves of the "experiment tank"

A is the intensity of percentage avoidance.

(b) Survival curves drawn from the survival times which indicate :—

1. Concentrations of the solutions (expressed in normality)
2. pH values.
3. mean survival times of five experiments
4. Temperature.

EXPERIMENTAL RESULTS

(1) *Responses and survival of fish in sodium cyanide solutions :—*

In the first series of experiments studies were made on the survival and reactions of five species of fish, namely *Gasterosteus aculeatus*, *Phoxinus phoxinus*, *Salmo trutta* fry, *Anguilla anguilla* elvers and young *Carassius auratus* to concentrations of sodium cyanide ranging from 0.005 N – 0.000001 N NaCN.

Gasterosteus aculeatus of average adult size behaves violently when placed in strong solutions of sodium cyanide such as a 0.005N solution. They remain motionless for a few seconds, then commence very active swimming. Within a few minutes they begin to lose their balance at the same time displaying very deep opercular movements; for example at 0.005N the average time of overturning is about 7 min. and complete cessation of all activities occurs at 12 min. After the opercular movements have reached a maximum frequency they gradually slow down and the fish display all the characteristic signs of cyanide poisoning (see Alexander, Southgate and Bassindale (1935)). When it has overturned the fish keeps swimming for a few minutes depending on the concentration, then comes to rest at the bottom, the intervals between the opercular movements gradually lengthen and they slowly come to a halt. This time is considered as the end point as fish do not revive when placed in aerated water.

The sticklebacks in dilute solutions do not display violent swimming immediately; they gradually seem to perceive the nature of the solution, and the swimming rate and opercular movement rate gradually increase. The overturning time is inordinately prolonged and when they come to rest on their sides, they remain in this condition sometimes for long lengths of time with slow breathing till the death point. The reaction behaviour of sticklebacks, when confronted with sodium cyanide solutions in the reaction tube, is

as complex as the behaviour displayed while studying the survival times. The first test solution studied had a concentration of 0.005N. Normally the fish do not encounter such high concentrations in natural waters but it was thought interesting to see how fish would react under laboratory conditions. The solution was admitted in most experiments, at the end preferred by the fish and in a few experiments at the opposite end. When the solution was admitted at the opposite end it was found that even if the fish swims towards the solution, it hastily retreats without proceeding into the cyanide solution, as if the fish has recognized it by smell; on the other hand, if the solution is admitted to the end of the tube occupied by the animal, (Fig. 1A, 2A) it struggles violently, then swims violently and wildly in all directions and dashes out speedily to rest almost motionless, gasping for breath at the water end of the tube; however, some lose all sense of recognition, swim several times up and down, ultimately producing pseudopositive reactions. Those who successfully avoid the solution, do so in 1-2 min. Mean position records in solution and water plotted for 3 experiments using five fish at a time for a 20 min. period showed a % avoidance of 77% (see Table 1 and Fig. 3).

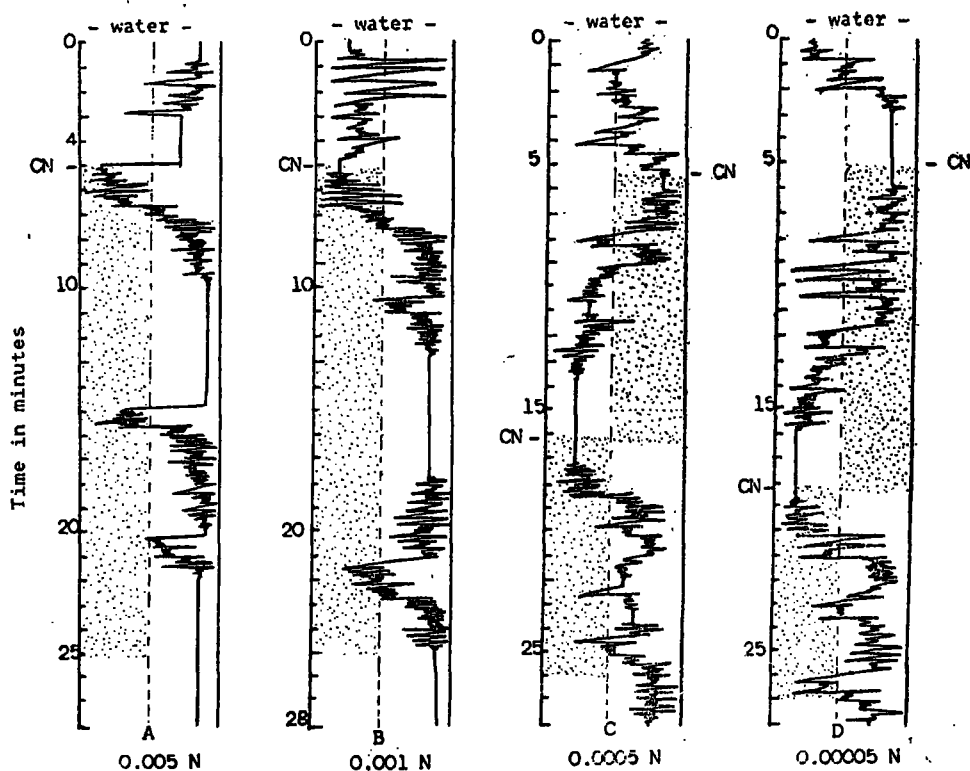


Fig. 1. The reactions of single sticklebacks to 0.005, 0.001, 0.0005 and 0.00005_N sodium cyanide. pH of the solutions 8.7, 8.1, 7.6 and 7.15. Temperature 17-18°C.

At 0.001N again a distinct and vigorous negative reaction is displayed within two minutes but the degree of avoidance is less than the above being 70% (Fig. 1B, 2B, Table 1). Those fish that venture into the toxic solution more than once are "trapped" and spend a period of stupour before dragging themselves to the water zone. It was found that when once a fish swims into the toxic zone to spend a few moments and then returns to the water

zone, the fish does not confine itself to the water zone but repeatedly swims back to spend a considerable time in the solution zone before ultimately getting back exhausted to the water zone. In the 0.0005N experiment, again a vigorous display of negative reaction is seen (Fig. 1C, 2C). The reaction time is still in the range of 3 min. The reversal reaction is as quick as in the previous experiment but the fish returns a number of times into the toxic area before ultimately selecting the water zone. At 0.0005N the sticklebacks survive nearly 14 min. in survival time experiment.

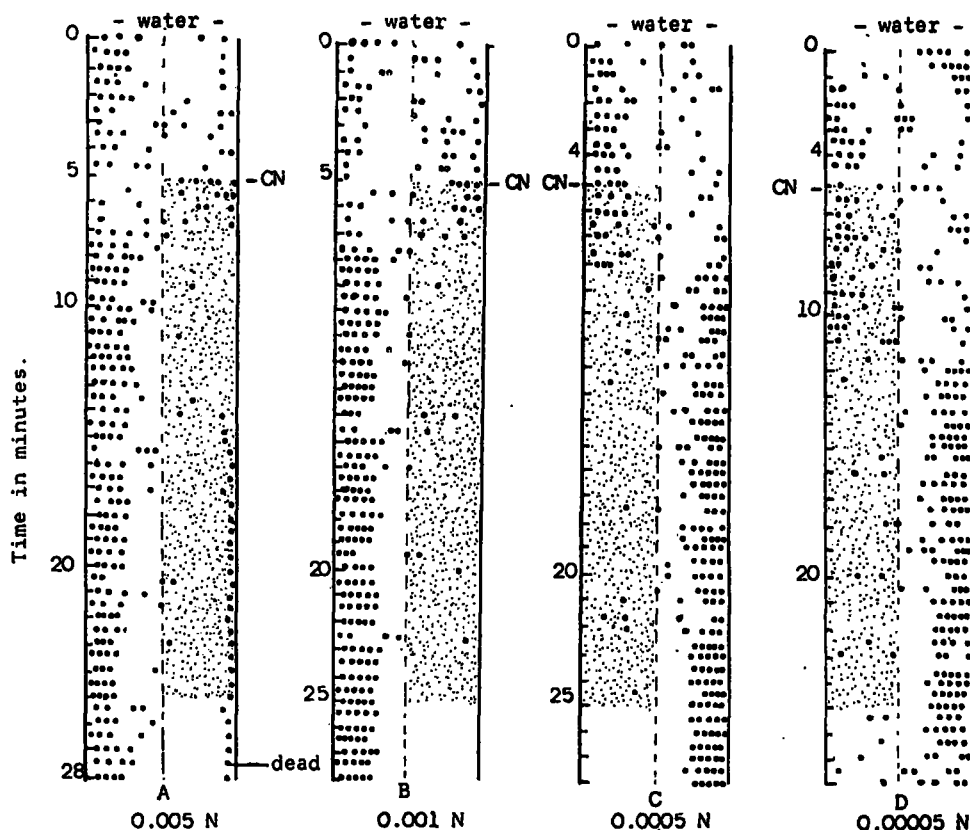


Fig. 2. The reactions of groups of five sticklebacks to 0.005, 0.001, 0.0005 and 0.00005 N NaCN. Temperature 17–18°C.

Experiments conducted at 0.0001N, 0.00005N (Fig. 1D, 2D) and 0.00001N all showed that the fish respond by swimming out of the toxic zone, the reaction times being 3–4, 5–6 and 9 min. respectively while the intensity of avoidance for these solutions was 54, 42 and 11% (Fig. 3). With all these solutions, reversals gave quicker avoidance times. With a very dilute solution (0.000005N), a negative reaction is still seen, the fish are very slow to move out though they appear to sense the unfavourable nature of the solution. In a time between 35–40 min., most of the fish select the water zone in preference to the cyanide. At a still greater dilution of 0.000001N, the water cyanide boundary is crossed without any apparent hesitation, but if the experiments are continued over an hour, an extremely vague negative reaction may be observed at about 60–70 min; later, the fishes spend more time in the water half than in the cyanide zone.

<i>Solutions tested</i>	<i>No. of fish used per experiment</i>	<i>Observation time in min.</i>	<i>Size in cm.</i>	<i>Temperature</i>	<i>Expt. I Position records</i>	<i>Expt. II Position records</i>	<i>Expt. III Position records</i>	<i>Mean Position records</i>	<i>Reaction</i>	<i>Reaction time in min.</i>	<i>% Intensity avoidance</i>
0.005N NaCN water	5	20	4—5	17-18°C	24 176	23 177	24 176	24 176	Negative sometimes Pseudo- positive	1—2	77%
0.001N NaCN water	5	20	4—5	17-18°C	34 166	31 199	27 173	30 170	Negative	1—2	70%
0.0005N NaCN water	5	20	4—5	17-18°C	38 162	42 158	34 166	38 162	Negative	2—3	62%
0.0001N NaCN water	5	20	4—5	17-18°C	48 152	46 154	45 155	46 154	Negative	3—4	54%
0.00005N NaCN water	5	20	4—5	17-18°C	57 143	59 141	59 141	58 142	Negative	5—6	42%
0.00001N NaCN water	5	20	4—5	17-18°C	82 118	98 102	88 112	89 111	Negative	8—10	11%

TABLE 1. % Intensity avoidance of *Gasterosteus aculeatus* in sodium cyanide solutions.

The survival curve for *Gasterosteusaculeatus* is drawn in Fig. 6. The graph shows that strong solutions are extremely fatal while at 0.00001N the fishes live for more than 60 hrs.—the duration of the experiment.

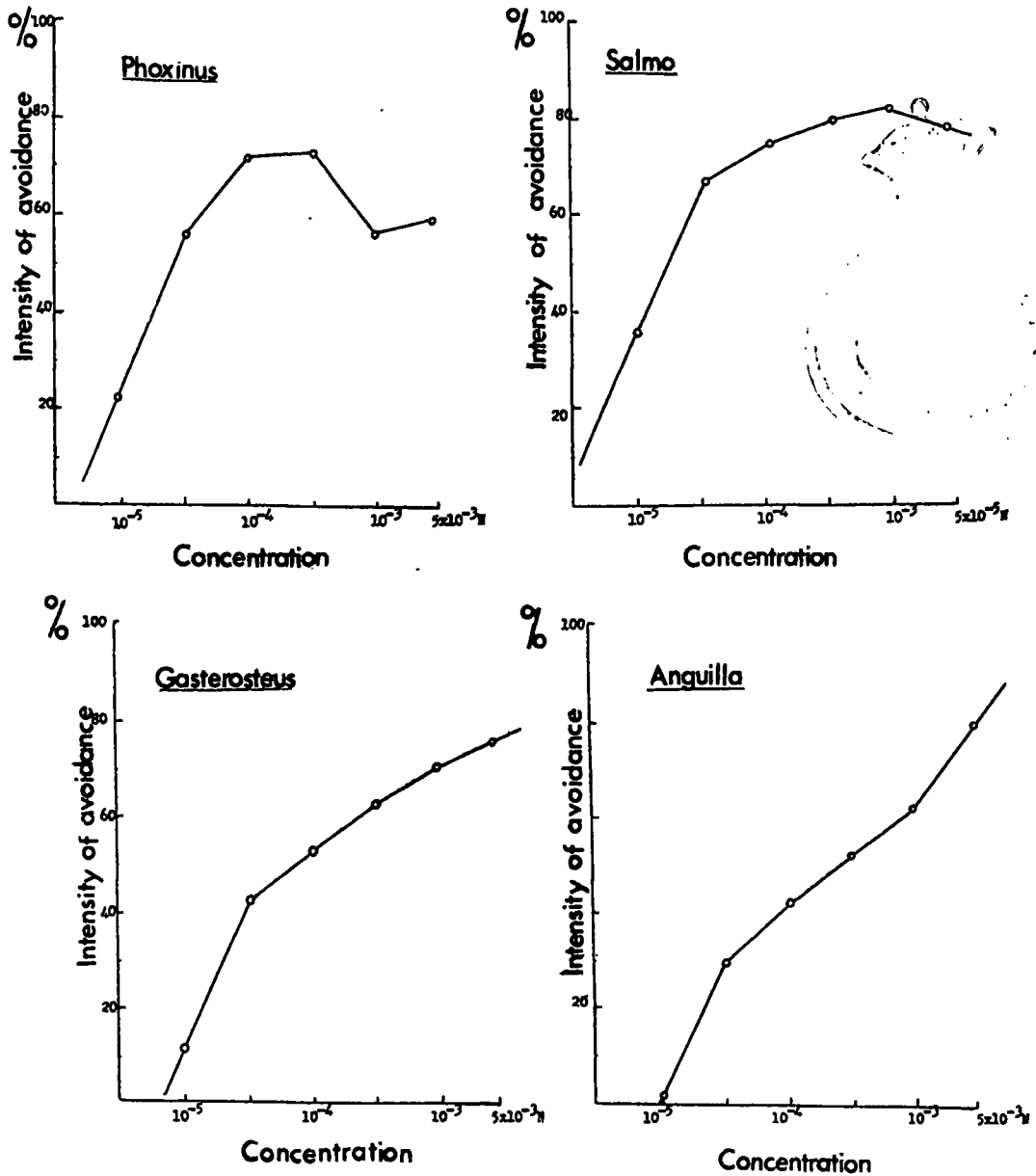


Fig. 3. The intensity of avoidance displayed by fish (*Gasterosteus*, *Phoxinus*, *Salmo* and *Anguilla*) to sodium cyanide solutions. Temperature 17—18°C.

The minnows (*Phoxinus phoxinus*) used for these experiments measured 5–6 cm. Their responses to cyanide solutions generally present a higher degree of sensitivity than

that seen with the stickleback. In concentrated solution, a violent burst of swimming is seen, the minnows losing their sense of balance in a much shorter time than that taken by sticklebacks and the characteristic symptoms of cyanide poisoning are displayed to a more marked degree. After overturning they display feeble attempts at swimming, the opercular movements then become weaker and weaker, the death point being rather definite. In weaker solutions too the minnows fall on their sides but show regular attempts at swimming till exhaustion brings them to rest at the bottom. They breathe very slowly until the opercular movements become almost imperceptible. They remain in this condition for a very long time before dying. The survival curve drawn from the survival times for a concentration range of 0.05N - 0.00005N is given in Fig. 6. The survival times show that over this range, minnows are more sensitive than the sticklebacks.

Minnows show violent and desperate activity on coming into contact with concentrated cyanide solutions in the reaction tube. A typical response to a 0.005N solution is shown in Figs. 4A, 5A. The selection of the water zone by minnows is extraordinarily delayed though the reverse might be expected. In this experiment, the cyanide solution was admitted on the left side, which the fish preferred; the immediate result is violent gulping and desperate swimming, the fish jumps and wriggles; sometimes it almost reaches the boundary but turns back, this was repeated twice before visiting the water zone but it returned to the toxic zone immediately. This was repeated many times when the fish loses its sense of balance and swims on its side to and fro in the tube until at 2.5 min. it drags itself to the water zone still wildly gulping and moving. Then it finally rests on its side motionless. It remains in this condition without further activity or swimming till it recovers and then begins to swim upright once more. If the fish later swims to the junction it hastily withdraws. Most of the fish then remained in the water but some ventured again into the cyanide. In a few experiments, the minnows were completely exhausted before they could select the water zone and lay helpless in the cyanide half till dead. Position records plotted every half minute as with sticklebacks showed an avoidance of 67% (Fig. 3). Water cyanide reversal at this concentration causes an immediate loss of balance and in many experiments it was not practised. The behaviour on the whole was similar to that described by Jones (1951) for phenol.

At 0.001N though the selection of the water zone was similar to the above in essentials, the response differed in details. The minnows seem to perceive the toxic nature of the solution as soon as it is admitted. Characteristic symptoms are displayed such as momentary loss of recognition, desperate swimming, gulping and gradual loss of balance; the fish swim up and down the tube crossing and recrossing the water cyanide junction several times before ultimately dragging themselves out of the cyanide zone to lie in a state of complete collapse. The avoidance in three series of experiments was 63%. The reaction times extending from 2 - 4 min. (see Table 2 and Figs. 4B, 5B). It is interesting to note that in the experiments with 0.005N solution, the concentration tested being high, the fish came to a halt in about 2 min. after coming into contact with the cyanide solution. In this series of experiments it seems that when the concentration of the solution is less, though still rapidly fatal (survival time 15 min. Fig. 6) the fish may swim a number of times (about 5 - 10 times) back into the cyanide zone before going into a collapse—in some experiments a collapse followed by death.

It is therefore concluded that minnows at these concentrations are so intoxicated by the cyanide that subsequent discrimination—even after a return into the water zone—is dulled, and avoidance very much delayed till either immobility or complete collapse overcame them.

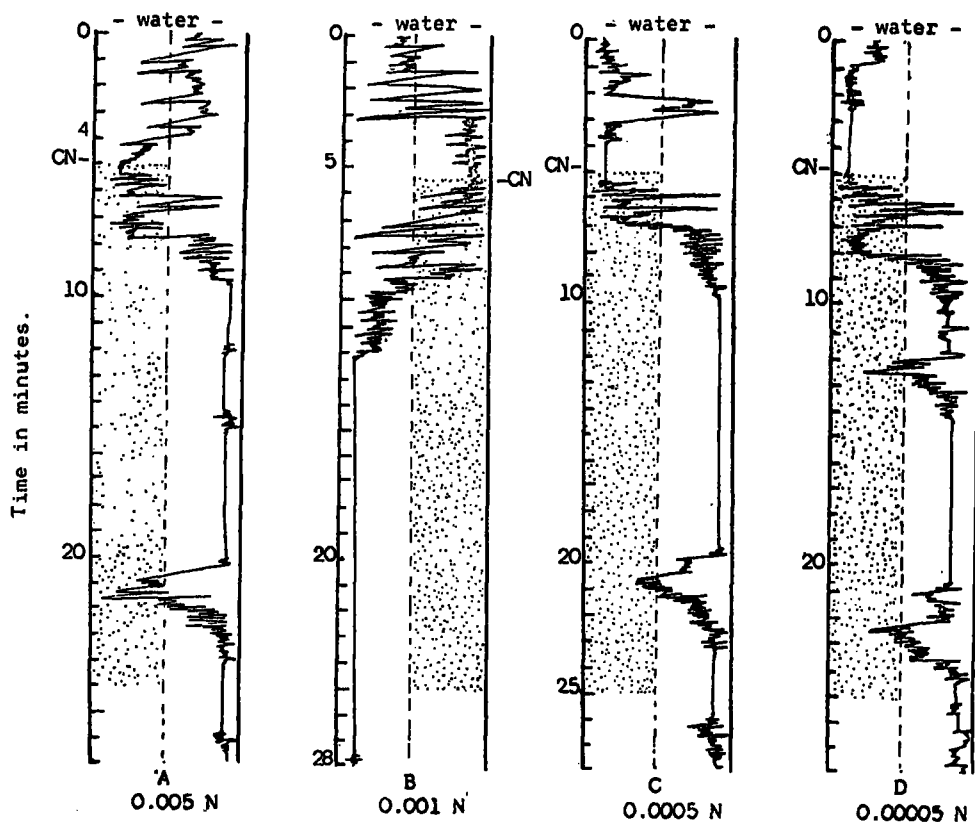


Fig. 4. The reactions of single minnows to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. pH of solutions 8.7, 8.1, 7.6 and 7.15. Temperature 17—18°C.

In contrast to the two series of experiments described above, in the experiments conducted at 0.0005N, the minnows seemed to recognize the cyanide and withdrew into the water zone in less than 2 min. the intensity of avoidance being 78% (see Table 2). Even this solution brings about death quickly, the survival time being about 15 min. The minnows appear to detect the toxic solution rather early, they swim into and out of the toxic solution a few times but reject it in time to escape fatal intoxication without themselves being exposed too long (Figs. 4C, 5C).

Minnows survive in a 0.0001N solution of sodium cyanide for a time of nearly 28 min. but in a reaction experiment they select the water and avoid the toxic solution in a time of about 2 min. (% Avoidance —76%). It is quite clear from the way the fish behave in the experiment tube that this concentration is not strong enough to produce an immediate intoxicating effect and loss of balance, but the solution is still irritating enough to stimulate the fish to produce a prompt avoiding reaction. Clear avoiding reactions, though rather more delayed, are seen at 0.00005N (Figs. 4D, 5D), 0.00001N and even at 0.000005N. The intensity of avoidance gradually decreases, for instance at 0.00005N it was 58% while at 0.00001N it was only 22%. The avoidance shown to a 0.000005N in about 35—40 min. indicates that the minnows are very sensitive to cyanide solutions, more sensitive than

Gasterosteus. It is interesting to note that like sticklebacks the minnows survive for more than 3 days (see Fig. 6) in a 0.00005N solution but establish a comparatively delayed avoiding reaction in 35–40 min.

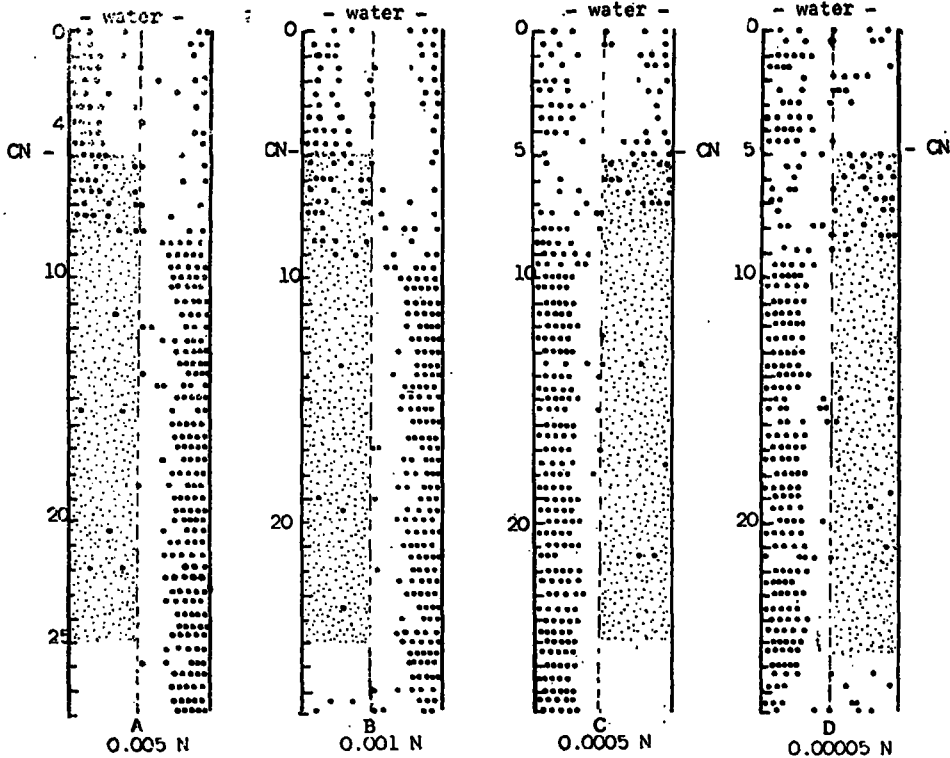


Fig. 5. The reactions of groups of five minnows to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. Temperature 17–18°C.

The *Salmo trutta* L fry used for these experiments measured between 5–6 cm. The survival curve drawn from survival times is given in Fig. 6. The survival curve shows distinctly that the trout fry are the most susceptible of the fishes so far tested. Trout fry exhibit violent behaviour in sodium cyanide solutions similar to that shown by the minnows. In concentrated solutions, an accentuated burst of swimming and immediate loss of equilibrium is the characteristic symptom. Subsequently, they fall on their sides and make a few struggling attempts to regain their normal posture at the same time showing deep and laboured respiratory movements; later, the opercular movements gradually become almost imperceptible. The death point is regarded as the time when all opercular movements cease. In dilute solutions, the loss of the sense of balance is not immediate but rather delayed; however, after a few rounds of swimming, depending upon the concentration of the solution, a gradual impairment of the power of co-ordinated movement is seen. The fish may be seen to remain in this condition for a long time, intermittently regaining its equilibrium to swim more or less normally for a short time; later, it comes to a final halt breathing helplessly. The death point is not well defined.

Solutions tested	No. of fish used per experiment	Observation time in min.	Size in cm.	Temperature	Expt. I Position records	Expt. II Position records	Expt. III Position records	Mean Position records	Reaction	Reaction time in min.	% Intensity avoidance
0.005N NaCN water	5	20	5—6	17-18°C	35 165	30 170	33 167	33 167	Negative sometimes pseudo-positive	2—3	67%
0.001N NaCN water	5	20	5—6	17-18°C	38 162	41 159	33 167	37 163	Negative	2—4	63%
0.0005N NaCN water	5	20	5—6	17-18°C	22 178	24 176	18 182	22 178	Negative	2	78%
0.0001N NaCN water	5	20	5—6	17-18°C	25 175	24 176	23 177	24 176	Negative	2	76%
0.00005N NaCN water	5	20	5—6	17-18°C	44 156	40 160	43 157	42 158	Negative	3—4	58%
0.00001N NaCN water	5	20	5—6	17-18°C	81 119	75 125	78 122	78 122	Negative	7—8	22%

TABLE 2. % Intensity avoidance of *Phoxinus phoxinus* in sodium cyanide solutions.

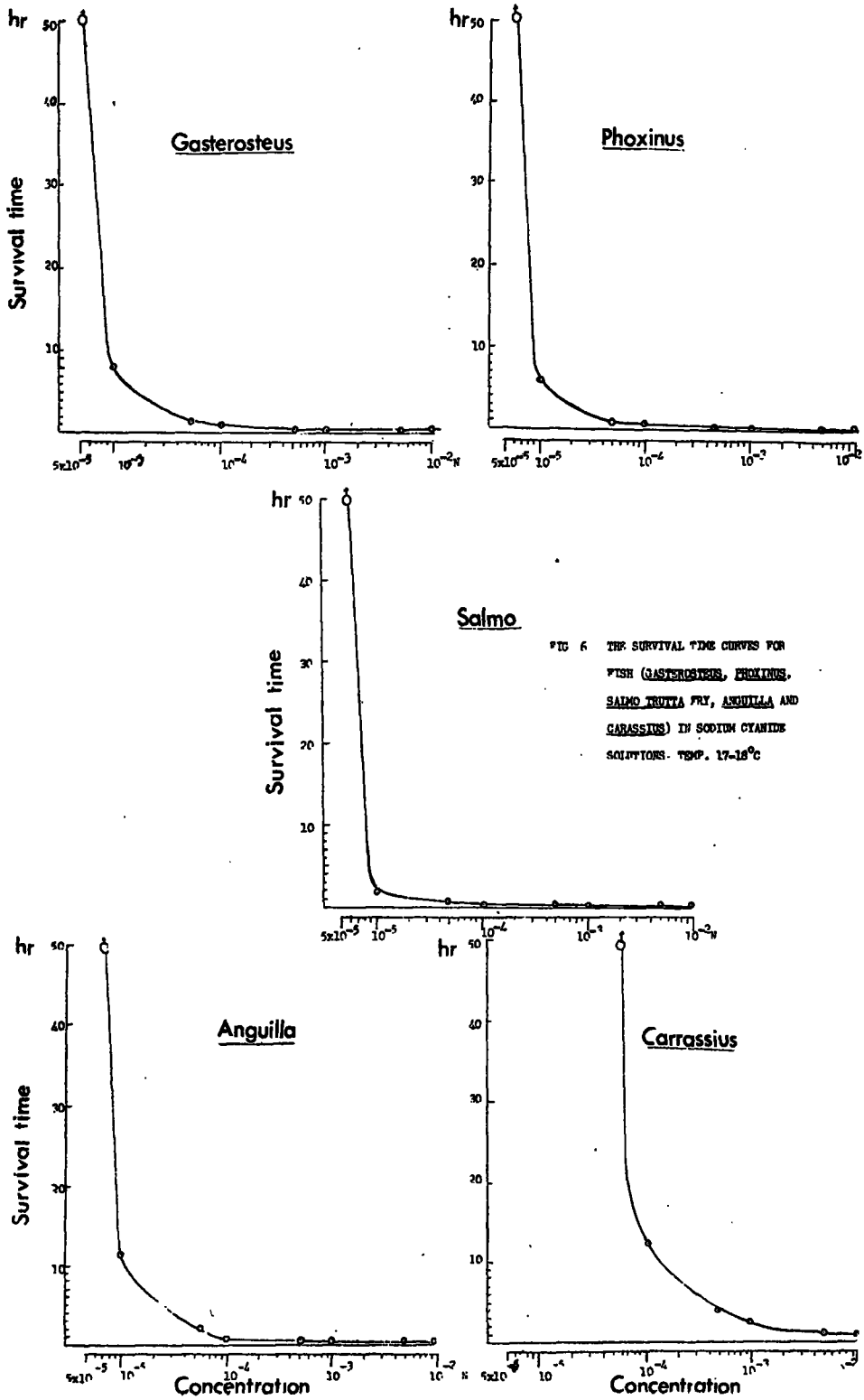


FIG. 6 THE SURVIVAL TIME CURVES FOR FISH (GASTEROSTEUS, PHOXINUS, SALMO TRUTTA FRY, ANGUILLA AND CARRASSIUS) IN SODIUM CYANIDE SOLUTIONS. TEMP. 17-18°C

The trout fry showed some ability to recognize and avoid a 0.005N sodium cyanide solution (Figs. 7A, 8A). When the solution is admitted the fry react violently, they dash in and out of the toxic zone and in a few minutes restrict their movements to the water end, resting helplessly and breathing heavily. In a few experiments, however, the fry seemed to have insufficient time to select the water zone before being overcome with the intoxicating effects, and death at the cyanide followed these pseudopositive reactions. It was also noticed that the majority of the trout fry that managed to avoid the solution died if reversals were practised, and a few died even if the experiment was stopped at the first stage. Three successful experiments conducted at this concentration gave an avoidance index of 78% (see Table 3). In a 0.001N solution the trout fry survive only for a very short period of 5 min. in a survival time experiment. Accordingly, in the reaction experiments, an exaggeratedly violent avoiding or sometimes pseudopositive reactions are seen. The negative selecting reaction seen was similar in many aspects to that observed with minnows. Fig. 7B shows the typical movements of a small trout when confronted with this concentration. The solution is admitted on the left side at 5 min; within a few seconds the fish has sensed it but instead of moving out the fish struggles and visits the inmost end of the cyanide zone; the fish turns back and reaches the water junction but turns back again into the solution, and this procedure is repeated several times before the fish lands in the water zone. The fish, half overturned, continues active movements till it gradually manages to keep within the limits of the water zone. This reaction takes place in between 1 - 2 min. with an intensity of avoidance of 82%.

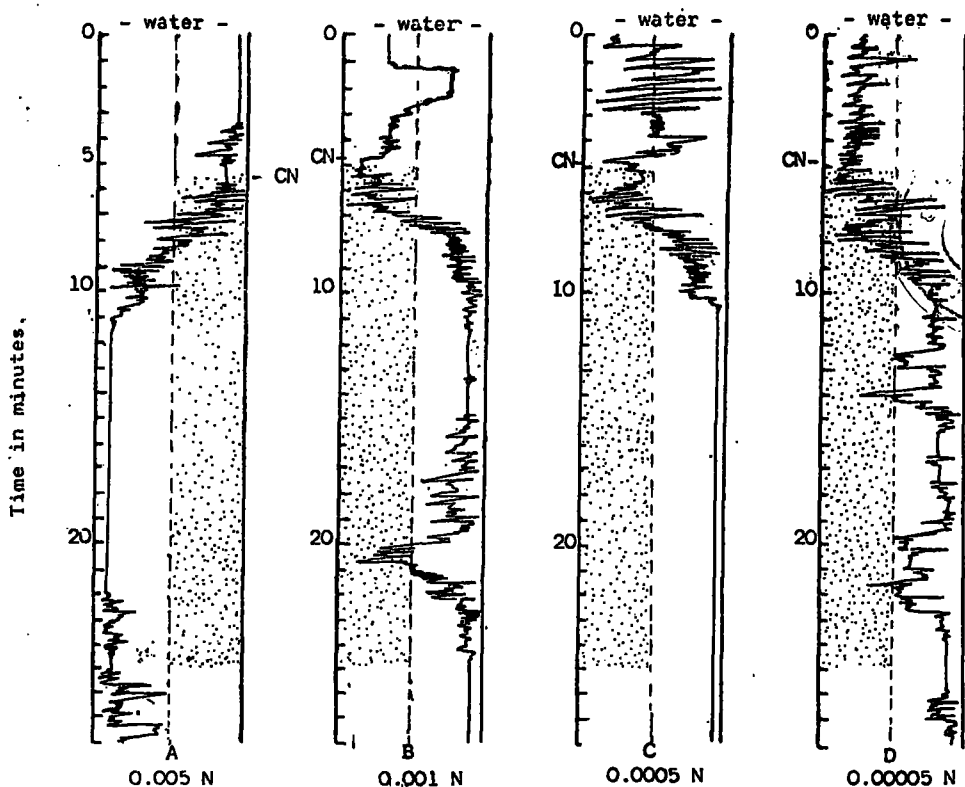


Fig. 7. The reactions of single trout fry to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. pH of solutions 8.7, 8.1, 7.6 and 7.15. Temperature 17-18°C.

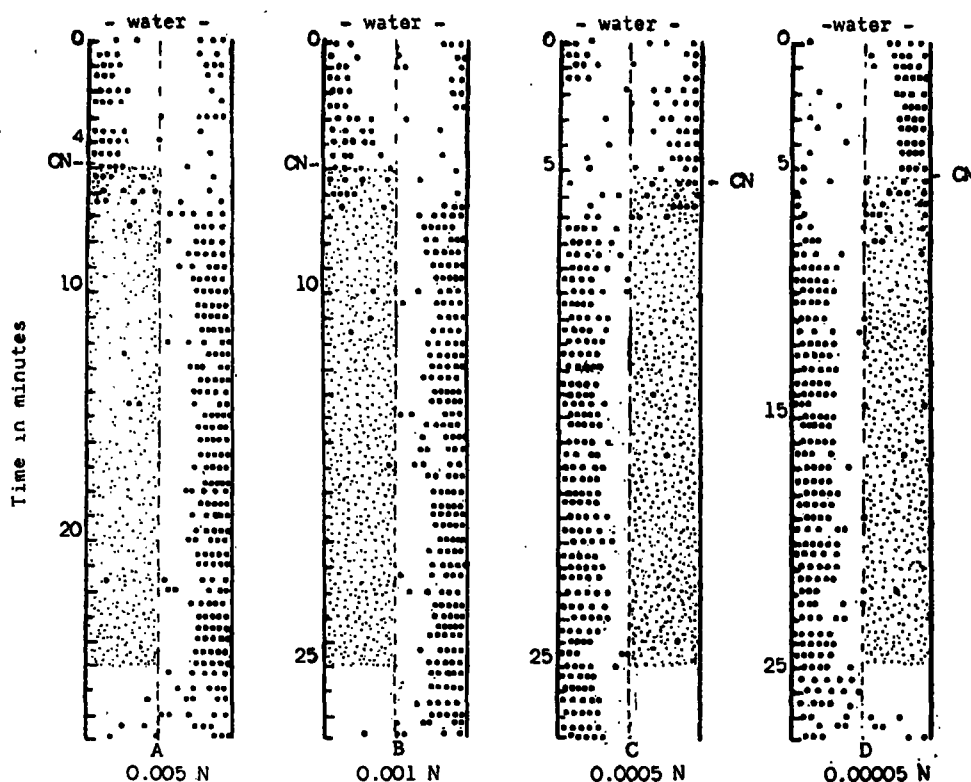


Fig. 8. The reactions of groups of five trout fry to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide solutions. Temperature 17—18°C.

At 0.0005N the avoidance reaction is still quite prompt—it should be noted that the entering solution takes about 30 secs. to fill the solution half of the experiment tube—but the tendency to return to the cyanide zone after swimming out of it is much less. This solution is still quickly lethal, bringing about the fishes' death in 7 min. As with minnows this concentration seems to be irritating enough for the trout to withdraw into the water zone before any unremediable injury could take place (Figs. 7C, 8C). At 0.0001N and 0.00005N, the avoiding reaction is still developed quickly occurring within 3 min. The intoxicating power is declining and there is a decrease in the intensity of avoidance; 74% at 0.0001N and 68% at 0.00005N, the survival times being 18 and 50 min. respectively. However, the irritating power appears to remain much the same as far as can be gathered from observation of the behaviour of the fish in the experiment tube.

In the 0.00001N, 0.000005N and 0.000001N experiments, the whole progress of the reaction is slowed down, reactions being very slow, the fish crossing the boundary between the water and the solution several times before establishing an effective negative reaction. The reaction times for the above concentrations are 6, 18 and 33 min. respectively. At higher concentrations, when once the fish has reacted negatively, it rarely ventures again into the toxic zone but in the case of dilute solutions, occasional visits to the toxic zone lasting for a few seconds or minutes are seen; however the fish prefers to remain in the water zone for most of the time. Another feature in these experiments is that reversals of the water cyanide flows can be carried out without bringing about any fatal effects.

<i>Solutions tested</i>	<i>No. of fish used per experiment</i>	<i>Observation time in min.</i>	<i>Size in cm.</i>	<i>Temperature</i>	<i>Expt. I Position records</i>	<i>Expt. II Position records</i>	<i>Expt. III Position records</i>	<i>Mean Position records</i>	<i>Reaction</i>	<i>Reaction time in min.</i>	<i>% Intensity avoidance</i>
0.005N NaCN water	5	20	5—6	17-18°C	26 174	23 177	18 182	22 178	Negative sometimes pseudo-positive	2	78%
0.001N NaCN water	5	20	5—6	17-18°C	15 185	20 180	19 181	18 182	Negative sometimes pseudo-positive	1—2	82%
0.0005N NaCN water	5	20	5—6	17-18°C	19 181	20 180	20 180	20 180	Negative	1—2	80%
0.0001N NaCN water	5	20	5—6	17-18°C	28 172	24 176	25 175	26 174	Negative	2—3	74%
0.00005N NaCN water	5	20	5—6	17-18°C	30 170	36 164	31 169	32 168	Negative	2—3	68%
0.00001N NaCN water	5	20	5—6	17-18°C	63 137	52 148	73 127	62 138	Negative	5—7	38%

TABLE 3. % Intensity avoidance of *Salmo trutta* fry in sodium cyanide solutions.

Comparing the avoiding reaction at $10^{-6}N$ for *Gasterosteus*, *Phoxinus* and *Salmo*, a striking feature noticeable is the extreme sensitivity displayed by trout fry to this solution. Trout fry react and avoid this solution in a time less than 35 min. while the reaction time for sticklebacks towards the same solution is 60–70 min. and for *Phoxinus* 35–45 min.

The *Anguilla anguilla* eelers used for the reaction and survival experiments measured on an average 7–8 cm. In the reaction experiments, the movements of single eelers in the experiment tube were copied. To study the intensity of avoidance, instead of the usual five fish per experiment, three individuals were used at a time. The observation time was however the same (i.e.) 20 min. and the positions were plotted every half minute. The behaviour of the eelers in sodium cyanide solutions was rather different from that seen with the other fishes. In the concentrated solutions no violent movements are exhibited immediately, but instead they swim smoothly at first; then in a few minutes, the smooth swimming gives way to a display of violent curling, coiling, whirling and wriggling movements. Unlike other fish which lose all power of co-ordinated movement within a few minutes, the eelers maintain equilibrium for quite a length of time (e.g. at $0.005N$ it is about 10 min.). Later they fall on their sides but do not die immediately, they remain in this condition, occasionally displaying coiling and whirling, till movements gradually cease. In dilute solutions they commence rapid swimming immediately and keep on doing so till they become exhausted, when they fall on their sides and remain in this position till the death point, except, perhaps, when there are occasional attempts to regain equilibrium. The survival curve is given in Fig. 6. A notable feature is the high degree of resistance of the eelers, the survival times at all concentrations being much longer than those seen with other fishes.

Anguilla eelers are very active and keep on swimming rapidly from the moment they are introduced into the experiment tube. The same behaviour is seen when a strong solution of $0.005N$ is admitted to the experiment tube on the right side at 5 min. (see Figs. 9A, 10A). There appears to be no immediate discomfort, the eels continue swimming into and out of the solution many times, many visits being made in the space of a minute; however, a slight recognition is seen at 7.5 min. The rapid movements by the eels are continued until a deep penetration into the cyanide zone results in wriggling and circling movements and return to the water zone at 8.5 min. The wriggling motion is still seen after withdrawal from the solution, and the eels keep on moving until they are completely exhausted.

At $0.001N$ the behaviour of the eels is almost exactly the same as above but the visits to the cyanide zone are frequent and more prolonged, though more turnings back at the water cyanide boundary are seen than in the previous experiment. The avoidance time averaged 4–5 min. for a series of five experiments. It was seen in some that the fish are so utterly exhausted on withdrawal that after a few feeble movements they become motionless and die. The intensity of avoidance at this level is 63% (Table 4). At a greater dilution of $0.0005N$ the eelers move in and out of the solution zone without any hesitation. Signs of detection begin to appear at about 4 min. (Fig. 9C) but avoidance appears to be complete in about 6.5 min. Reversals of flow give quicker results. The % avoidance of a $0.00001N$ solution by eelers is fairly low being only 43. The development of the reaction is very slow, taking about 75 min. The reaction times for five experiments ranged from 5–9 min. There was not much of a difference in the reaction times between the previous experiments and the experiment with $0.00005N$ solution (Figs. 9D, 10D) except

for the % avoidance; the elvers did not display any adverse effects on withdrawal. The experiments at 0.00001N, and 0.000005N showed that the elvers displayed avoidance at about 26 and 43 min. respectively showing only vague tendencies to prefer the water zone. At 0.000001N there was no tendency at all for the elvers to prefer any zone during the course of the experiment which lasted for 70 min. At the end of the experiment they were swimming normally the whole length of the experiment tube.

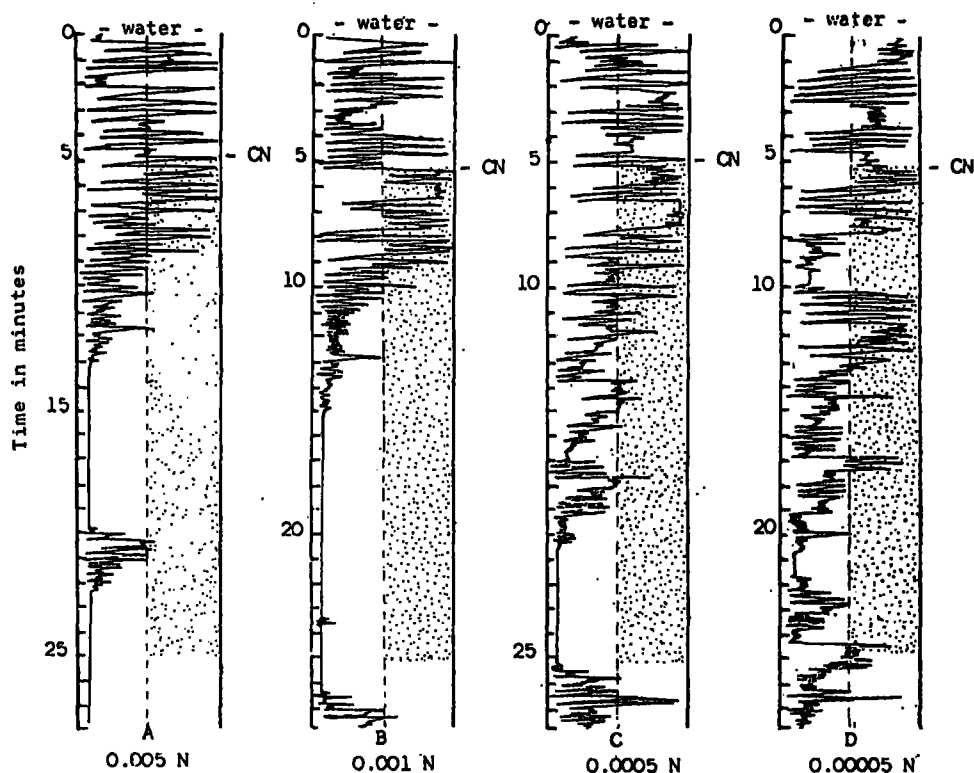


Fig. 9. The reactions of single eels to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. pH of solutions 8.7, 8.1, 7.3 and 7.15. Temperature 17—18°C.

On the whole the reaction times shown to the graded concentrations of sodium cyanide are a direct and proportional reflection of the survival times in these solutions; the eel, being a more resistant species, shows both extended survival and reaction times.

Carassius auratus. The young goldfish used for these experiments mostly ranged in size from 1½–2 ins. in length. Using goldfish of uniform size was necessary as specimens of slightly larger size showed a conspicuous difference in the survival and reaction times. The young goldfish are remarkable for their resistance shown towards sodium cyanide solutions, living even in comparatively concentrated solutions for quite a long time. When a goldfish is placed in a solution of sodium cyanide there is a sudden burst of swimming followed by the loss of sense of balance, the fish falls on its side and remains in this condition with heavy and laboured breathing; there may be a few sporadic attempts to regain its

equilibrium. In the weaker solutions they swim for a considerable time before losing all power of co-ordinated movement and after overturning they remain in this helpless condition for hours before death. In the very weak solutions they maintain power of co-ordinated movement and equilibrium for days though gills and oral lining begin to show indications of cyanide poisoning, in that the colour is redder than normal. The survival curve for young goldfish for the range of 0.05—0.00001N is given in Fig. 6. The young goldfish survive in a cyanide solution of a normality of 0.05N for about 58 min. and in a solution of 0.00001N for more than 3 days.

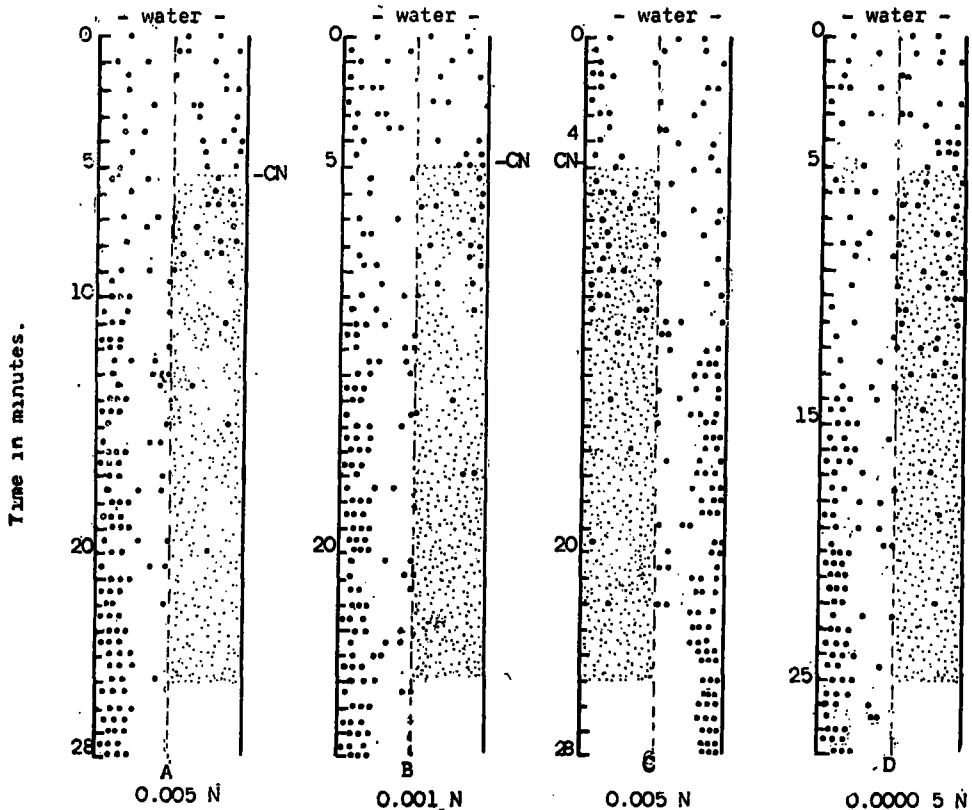
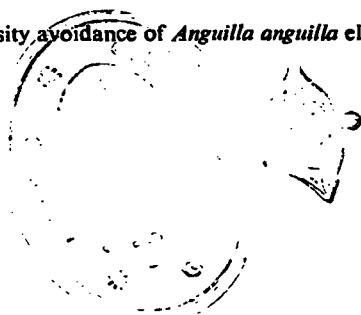


Fig. 10. The reactions of groups of three eels to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. Temperature 17—18°C.

Because of their comparatively large size it was decided to use only one goldfish at a time to study the reaction behaviour, only its movements were copied, the positions were not recorded. The behaviour of goldfish inside the experiment tube is as characteristic as those displayed by elvers of *Anguilla*. As soon as they are placed in the reaction tube they keep swimming very actively and rapidly and it is only rarely that they confine themselves to one end without showing any movements.

Solutions tested	No. of fish used per experiment	Observation time in min.	Size in cm.	Temperature	Expt. I Position records	Expt. II Position records	Expt. III Position records	Mean Position records	Reaction	Reaction time in min.	% Intensity avoidance
0.005N NaCN water	3	20	7—8	17-18°C	18 102	17 103	18 102	18 102	Negative	3—4	80%
0.001N NaCN water	3	20	7—8	17-18°C	22 98	23 97	25 95	23 97	Negative	4—5	63%
0.0005N NaCN water	3	20	7—8	17-18°C	27 93	28 92	28 92	28 92	Negative	6—7	53%
0.0001N NaCN water	3	20	7—8	17-18°C	34 86	32 88	35 85	34 86	Negative	7—8	43%
0.00005N NaCN water	3	20	7—8	17-18°C	40 80	42 78	42 78	41 79	Negative	7—8	30%
0.00001N NaCN water	3	20	7—8	17-18°C	—	—	—	—	Negative	25—28	2%

TABLE 4. % Intensity avoidance of *Anguilla anguilla* elvers in sodium cyanide solutions.

The reactions of *Carassius* to very high concentrations of sodium cyanide solutions is peculiar. A typical result with 0.005N solution is given in Fig. 11A, a similar reaction to that shown by eels is displayed in that the fish appear to show a recognition at first of the lethal qualities of the solution when it is first admitted. The fish enters the solution at once, jerks, gulps, and withdraws to the water zone only for a few seconds but then keeps on returning many times, for several minutes. This rapid up and down movement is continued, though occasional and temporary display of recognition is apparent at 9 min. till at about 13.5 min. on reaching the water solution junction they seem to begin to detect and avoid the solution, though they still continue to move about rapidly in the water zone. Though in the majority of the experiments the fish commenced recognizing and avoiding the toxic solution in about 8.5 min. in one or two preliminary experiments the goldfish took an extraordinary length of time nearly 18–20 min. before exhaustion forced it to avoid the cyanide zone. The response on reversal, however, is quick and is brought about in 3.5 min. It was also seen that reversals at this concentration do not bring about fatal results as seen with other fishes. The reactions to 0.001N by goldfish is essentially similar, the continuous swimming movements, the repeated crossings of the water cyanide junction, inability to recognize it promptly, exhaustion and final withdrawal and avoidance of the cyanide zone are more or less identical with the above except that the final avoidance takes place in a minute or so less (reaction time 7.5 min. see Fig. 11B). At 0.0005N and 0.0001N the reactions are quite alike in that fish select the water zone avoiding the solution in a time of 5.5 min. The fish do not display any frantic and desperate efforts to get out

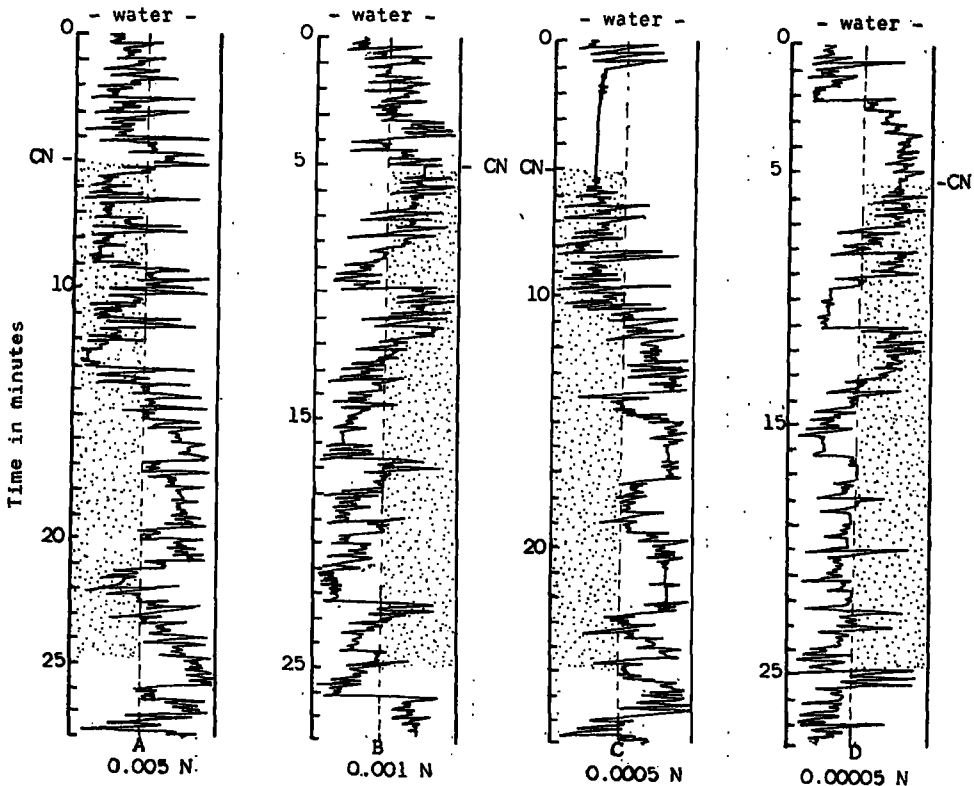


Fig. 11. The reactions of single goldfish to 0.005, 0.001, 0.0005 and 0.00005 N sodium cyanide. pH of solutions 8.7, 8.1, 7.6 and 7.15. Temperature 17–18°C.

of the toxic solutions, instead in both cases the reactions are very smooth. It is obvious from these reactions that when the concentration decreases to a certain critical concentration, the fish's capability of detecting and avoiding the solution increases progressively.

In 0.00005N (Fig. 11D) and 0.00001N solutions the goldfish live for more than 48 hours in a survival time experiment; still they react negatively and quite sharply. At 0.00005N they avoid the solution in 9 min. and at the more dilute concentration, the time taken is about 29 min. At 0.000005N however the fish seemed to be indifferent to the solution during the 60 min. duration of the experiments.

(2) *The effect of sodium thiosulphate on the reactions of fish to sodium cyanide solutions and on the survival time :—*

The effect of sodium thiosulphate upon the survival time of carp in solutions of KCN was studied by Achard & Binet (1934). They found that the toxicity of a 0.002% solution of KCN was much reduced in the presence of 0.2% of sodium thiosulphate. Sodium thiosulphate oxidizes KCN to potassium thiocyanate which reduces the toxic properties.

Since there is evidence that thiocyanate has a definite antidote effect experiments were conducted using the same species of fish, *Gasterosteus aculeatus*, *Phoxinus phoxinus*, *Salmo trutta* fry, *Anguilla anguilla* elvers and young *Carassius auratus* to see what effects $\text{Na}_2\text{S}_2\text{O}_3$ has on their responses towards NaCN solutions. At the same time survival experiments were conducted using different concentrations of cyanide to which were added $\text{Na}_2\text{S}_2\text{O}_3$.

In this series of experiments equal volumes were mixed of 0.05N thiosulphate solution and solutions of sodium cyanide of normality 0.005–0.00001. The mixture thus had a concentration of thiosulphate of 0.025N and a concentration of cyanide half that of the solution used in making up the mixture. These mixtures were used in a series of reaction experiments which are summarized in Tables 5–13. In the reaction experiments the water and the solution were run at a slightly faster rate to obtain a better separation, as the addition of the sodium thiosulphate leads to stratification.

The results obtained for *Gasterosteus* are summarized in Table 5. Sticklebacks show violent behaviour, similar to those shown towards strong cyanide solutions, on coming into contact with 0.005N NaCN and 0.001N NaCN to which equal volumes of 0.05N $\text{Na}_2\text{S}_2\text{O}_3$ have been added. The wild swimming, gulping and severe intoxication with subsequent respiratory distress resembled the reaction shown towards higher concentrations of cyanide. The reactions were very rapid the sticklebacks selecting the water zone in less than two minutes. The survival times in these solutions were 12 and 15 minutes respectively. At 0.0005N NaCN plus $\text{Na}_2\text{S}_2\text{O}_3$ a marked change in the behaviour is observed. In this mixture the cyanide is about 100 times more dilute than the thiosulphate. When this mixture is admitted into the experiment tube, instead of the usual vigorous swimming and immediate negative reaction, the sticklebacks keep on swimming to and from the toxic zone several times before retreating; the average reaction time for five experiments being 3.5 min. As the concentration of the cyanide in the mixture is further reduced, for example, at 0.0001N + $\text{Na}_2\text{S}_2\text{O}_3$ or at 0.00005N + $\text{Na}_2\text{S}_2\text{O}_3$, their behaviour become proportionately less violent and the avoidance times are more prolonged. Thus at 0.0001N the mixture is avoided in 10–12 min. while at 0.00005N the sticklebacks take about 50–55 min. to show any dislike of the toxic zone. At 0.00001N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ the sticklebacks are completely indifferent to the mixture of solutions.

Concentrations of solutions used	Actual concentration in the mixture	Reaction and reaction time in min.	Concentration of the cyanide solutions	Reaction and reaction time in minutes	Survival times	
					Mixture of solutions	Cyanide solutions
(0.005N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.0025N NaCN (0.025N Na ₂ S ₂ O ₃)	— 1—2			12 min.	
(0.001N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.0005N NaCN (0.025N Na ₂ S ₂ O ₃)	— 1—2	0.0005N NaCN	— 2	15 min.	14 min.
(0.0005N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.00025N NaCN (0.025N Na ₂ S ₂ O ₃)	— 3—4			18 min.	
(0.0001N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.00005N NaCN (0.025N Na ₂ S ₂ O ₃)	— 10—12	0.00005N NaCN	— 5—6	4 hrs. 10 min.	2 hrs. 5 min.
(0.00005N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.000025N NaCN (0.025N Na ₂ S ₂ O ₃)	— 50—55			> 3 days	
(0.00001N NaCN (0.05N Na ₂ S ₂ O ₃)	(0.000005N NaCN (0.025N Na ₂ S ₂ O ₃)	≠ >75	0.000005N NaCN	— 30—40	> 3 days	>3 days

— = Negative
≠ = Indifferent

TABLE 5. Reactions and reaction times for *Gasterosteus aculeatus* using 0.025N Na₂S₂O₃ as antidote compared with some reaction times for cyanide solutions.

Phoxinus phoxinus:— Adding thiosulphate to the more concentrated cyanide solutions brought about little change in the behaviour of the minnows, although the addition of thiosulphate cut the cyanide concentration by half. Their reactions with strong concentrations are violent and with 0.005N and $\text{Na}_2\text{S}_2\text{O}_3$ and 0.001N + $\text{Na}_2\text{S}_2\text{O}_3$ they generally show pseudopositive reactions. The addition of the 'antidote' seems to have no effect on the smoothness of the reaction or in the reduction of the toxicity of the solutions (survival times are 12 and 16 min. respectively). The same behaviour is seen at 0.0005N + $\text{Na}_2\text{S}_2\text{O}_3$. Although pseudopositive reactions are not seen, the reaction time is almost the same. However it should be noted that the concentration of the cyanide in this mixture is about 100 times less than the concentration of thiosulphate. Less irritation, smoother swimming and longer reaction and survival times are seen with a 0.0001N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ mixture. In this mixture of solutions they live for more than two hours. In a 0.00005N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ they survive for more than three days but still react negatively in 15–17 min. With cyanide still more diluted the minnows show still more longer reaction times, these are given in Table 6.

Salmo trutta fry:— The set of results obtained for brown trout fry were in distinct contrast to those obtained for sticklebacks and minnows. The results obtained are given in Table 7. In the experiment conducted with 0.005N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ the trout fry immediately dashed back from the solution to lie utterly exhausted in the water zone, the choice of the water zone is so prompt that the fish are back in a minute or so, gasping for breath; most fry were so exhausted by the contact with cyanide in the mixture that they die very soon or else the fish are so intoxicated by the solution that they produce pseudopositive reactions. In five experiments a mean withdrawal time of 1.5 min. was registered. A similar reaction is seen with 0.001N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$. Like the previous experiments the trout fry are completely and fatally exhausted on contact with the poison. With 0.0005N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ the withdrawal reactions are performed in the same manner and same hurry as above, the water zone being selected within two minutes. The survival times do not appear to be changed very much by the addition of the antidote, in that the animals die in 8 min. At 0.0001N + $\text{Na}_2\text{S}_2\text{O}_3$, however, a longer survival time is seen (23 min.) but this is not reflected in the behaviour of the fry when confronted with this solution in the experiment tube as the reaction times are still in the range of 2–3 min. Even at 0.00005N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ a striking difference in the behaviour of the fry or in the reaction time is not displayed though the fry survive for about 50 min. when immersed in this mixture. The concentration of cyanide in this mixture of solutions is only $1/100$ th that of the thiosulphate. However, a shorter reaction time of 19.5 min. (when compared to sticklebacks and minnows) is shown by trout fry to 0.00001N NaCN + $\text{Na}_2\text{S}_2\text{O}_3$ solution. The fry show a prompt recognition and swim rapidly out of the solution. The general inference from these experiments with trout fry is that the fry appear to be very sensitive and very susceptible to cyanide solutions even after a reduction of its toxic properties by the addition of thiosulphate and it appears therefore that if $\text{Na}_2\text{S}_2\text{O}_3$ has any effect as an antidote in the cyanide reaction, it will be only at very great dilution.

Anguilla anguilla elvers:— The results obtained for *Anguilla anguilla* elvers show a similarity to those obtained with trout fry in that extended and prolonged reaction times and survival times begin to appear only when the cyanide concentration is $1/1000$ of the concentration of the thiosulphate, but they are dissimilar to trout fry in that the elvers are less sensitive and less susceptible to pure cyanide solutions. At 0.005N, 0.001N, 0.0005N and 0.0001N NaCN to which equal proportions of 0.05N $\text{Na}_2\text{S}_2\text{O}_3$ has been added, there

Concentrations of solutions used	Actual concentration in the mixture	Reaction and reaction time in min.	Concentration of the cyanide solution	Reaction and reaction time in minutes	Survival times	
					Mixture of solutions	Cyanide solutions
0.005N NaCN 0.05N Na ₂ S ₂ O ₃	0.0025N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—3			12 min.	
0.001N NaCN 0.05N Na ₂ S ₂ O ₃	0.0005N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—4	0.0005N NaCN	— 2	15 min.	15 min.
0.0005N NaCN 0.05N Na ₂ S ₂ O ₃	0.00025N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—3			18 min.	
0.0001N NaCN 0.05N Na ₂ S ₂ O ₃	0.00005N NaCN 0.025N Na ₂ S ₂ O ₃	— 6—7	0.00005N NaCN	— 3—4	2 hrs. 15 min.	53 min.
0.00005N NaCN 0.05N Na ₂ S ₂ O ₃	0.000025N NaCN 0.025N Na ₂ S ₂ O ₃	— 15—17			>3 days	
0.00001N NaCN 0.05N Na ₂ S ₂ O ₃	0.000005N NaCN 0.025N Na ₂ S ₂ O ₃	— 40—50	0.000005N NaCN	— 35—40	>3 days	>3 days

— =negative

TABLE 6. Reactions and reaction times for *Phoxinus phoxinus* using 0.025N Na₂S₂O₃ as antidote compared with some reaction times for cyanide solutions.

Concentrations of the solutions used	Actual concentration in the mixture	Reaction and reaction time in minutes	Concentration of the cyanide solution	Reaction and reaction time in minutes	Survival times	
					Mixture of solutions	Cyanide solutions
0.005N NaCN 0.05N Na ₂ S ₂ O ₃	0.0025N NaCN 0.025N Na ₂ S ₂ O ₃	— 1—2			2 min.	
0.001N NaCN 0.05N Na ₂ S ₂ O ₃	0.0005N NaCN 0.025N Na ₂ S ₂ O ₃	— 1—2	0.0005N NaCN	— 1—2	7 min.	7 min.
0.0005N NaCN 0.05N Na ₂ S ₂ O ₃	0.00025N NaCN 0.025N Na ₂ S ₂ O ₃	— 1—2			8 min.	
0.0001N NaCN 0.05N Na ₂ S ₂ O ₃	0.00005N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—3	0.00005N NaCN	— 2—3	33 min.	32 min.
0.00005N NaCN 0.05N Na ₂ S ₂ O ₃	0.000025N NaCN 0.025N Na ₂ S ₂ O ₃	— 3—4				
0.00001N NaCN 0.05N Na ₂ S ₂ O ₃	0.000005N NaCN 0.025N Na ₂ S ₂ O ₃	— 19—20	0.000005N NaCN	— 18	>3 days	>3 days

— = negative

TABLE 7. Reactions and reaction times for *Salmo trutta* fry using 0.025N Na₂S₂O₃ as antidote compared with some reaction times for cyanide solutions.

appears to be little or no difference in the avoiding reactions shown to the higher concentrations of cyanide described in an earlier part (in these the concentrations of cyanide are halved because of the addition of thiosulphate). Even the survival times in these solutions are similar except in 0.0001N where they live for more than an hour. In a 0.00005N NaCN + Na₂S₂O₃, however, a clear antidote effect is seen. While the young eels live for more than 3 days in this solution without fatality yet they commence reacting negatively in about 10 min. and the avoiding reaction is complete in about 12.5 min. The eels show a very poor response to a 0.00001N + Na₂S₂O₃ and are completely indifferent to a solution of 0.000005N + Na₂S₂O₃. The reaction times are given in Table 8.

Carassius auratus:— On the whole the reactions of young goldfish to cyanide solutions with thiosulphate used as an antidote were less violent. A quicker recognition and faster avoidance is evident from the summary of results given in Table 9.

The reaction of goldfish to 0.005N NaCN + Na₂S₂O₃ is quite unlike what is seen with strong cyanide solutions. They do not display violent and rapid swimming. Though they pass the water solution junction a few times, at about 2.5 min. they seem to recognize the cyanide and do not proceed beyond the junction, subsequently avoiding it for the rest of the experiment, except for a few isolated visits. At 0.001N + Na₂S₂O₃ the behaviour shown is a close approximation to the behaviour described above. The goldfish detect and avoid the solution very quickly. On testing further with the rather dilute solution of 0.0005N NaCN + Na₂S₂O₃ the normal and expected results are seen. The solution is now rather dilute and made less toxic by the Na₂S₂O₃ solution; the goldfish appear to take less notice of the poison zone but at about 11 min. they begin to exhibit signs of discomfort, later restricting movements to the water zone.

From a comparative point of view the reaction at 0.0001N NaCN + Na₂S₂O₃ was the most significant. To this combination of cyanide and thiosulphate, sticklebacks, minnows, trout fry and young eels react within a matter of 10 min. the goldfish on the other hand take about 40–45 min., comfortably swimming in the first 45 min. or so of the experiments without any preference for either end of the tube. Goldfish, being a highly resistant species, show the least sensitivity to cyanide to which Na₂S₂O₃ has been added than any of the other species of fish tested, in other words, it could be generally assumed, that the antidote effect of Na₂S₂O₃ is more marked with goldfish than with the rest of the fishes tested.

These experiments with different species of fish indicate that the addition of thiosulphate to cyanide solutions provides more chance for the fish to escape fatal intoxication, but the effect appears to differ with the species, and the thiosulphate concentration has to greatly exceed that of the cyanide.

(3) *The effects of prior immersion of fish in sodium thiosulphate on their reactions to sodium cyanide solutions*:—

Since it is now clear that sodium thiosulphate prolongs the survival times of fish when added to the sodium cyanide solutions, and brings about prolonged and extended reaction times, it was thought interesting to see what effects prior immersion of fish in sodium thiosulphate has on the reactions of fish to sodium cyanide solutions. It is already known that immersion of fish in Na₂S₂O₃ solutions before being transferred to potassium cyanide

Concentrations of the solutions used	Actual concentration in the mixture	Reaction and reaction time in minutes	Concentration of cyanide solution	Reaction and reaction time in minutes	Survival times	
					Mixture of solutions	Cyanide solutions
0.005N NaCN 0.05N Na ₂ S ₂ O ₃	0.0025N NaCN 0.025N Na ₂ S ₂ O ₃	— 3—4			22 min.	
0.001N NaCN 0.05N Na ₂ S ₂ O ₃	0.0005N NaCN 0.025N Na ₂ S ₂ O ₃	— 4—5	0.0005N NaCN	— 6—7	24 min.	24 min.
0.0005N NaCN 0.05N Na ₂ S ₂ O ₃	0.00025N NaCN 0.025N Na ₂ S ₂ O ₃	— 6—7			26 min.	
0.0001N NaCN 0.05N Na ₂ S ₂ O ₃	0.00005N NaCN 0.025N Na ₂ S ₂ O ₃	— 7—8	0.00005N NaCN	— 7—8	4 hrs. 12 min.	2 hrs. 10 min.
0.00005N NaCN 0.05N Na ₂ S ₂ O ₃	0.000025N NaCN 0.025N Na ₂ S ₂ O ₃	— 12—13			> 3 days	
0.00001N NaCN 0.05N Na ₂ S ₂ O ₃	0.000005N NaCN 0.025N Na ₂ S ₂ O ₃	— 48—50	0.000005N NaCN	— 43	> 3 days	> 3 days

— = negative

TABLE 8. Reactions and reaction times for *Anguilla anguilla* using 0.025N Na₂S₂O₃ as antidote compared with some reaction times for cyanide solution.

Concentrations of the solutions used	Actual concentration in the mixture	Reaction and reaction time in minutes	Concentration of cyanide solution	Reaction and reaction time in minutes	Survival times	
					mixture of solutions	cyanide solutions
0.005N NaCN 0.05N Na ₂ S ₂ O ₃	0.0025N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—3			63 min.	
0.001N NaCN 0.05N Na ₂ S ₂ O ₃	0.0005N NaCN 0.025N Na ₂ S ₂ O ₃	— 2—3	0.0005N NaCN	— 5—6	3 hrs. 40 min.	3 hrs. 30 min.
0.0005N NaCN 0.05N Na ₂ S ₂ O ₃	0.00025N NaCN 0.025N Na ₂ S ₂ O ₃	— 10—12			9 hrs. 40 min.	
0.0001N NaCN 0.05N Na ₂ S ₂ O ₃	0.00005N NaCN 0.025N Na ₂ S ₂ O ₃	— 40—45	0.00005N NaCN	— 8—10	>3 days	>3 days
0.00005N NaCN 0.05N Na ₂ S ₂ O ₃	0.000025N NaCN 0.025N Na ₂ S ₂ O ₃	— 60			>3 days	
0.00001N NaCN 0.05N Na ₂ S ₂ O ₃	0.000005N NaCN 0.025N Na ₂ S ₂ O ₃	≠ >60	0.000005N NaCN	≠ >60	>3 days	>3 days

— = negative

≠ = indifferent

TABLE 9. Reactions and reaction times for *Carassius auratus* using 0.025N Na₂S₂O₃ as antidote compared with some reaction times for cyanide solutions.

solutions gives a similar protective effect, (Achard and Binet 1934). Two species of fish were used for these experiments, *Gasterosteus aculeatus* and *Phoxinus phoxinus*. These two species were selected because they gave extended reaction and survival times with $\text{Na}_2\text{S}_2\text{O}_3$ and because they were available in large numbers at the time.

The concentration of sodium cyanide solution used was 0.0005N, a hundred times more dilute than that of sodium thiosulphate (0.05N), in which the fish were immersed for varying periods of time. The same concentration of thiosulphate was used instead of water in aspirator A and offered as the alternative to the cyanide solution. The two solutions were run rather fast to obtain a sharp differentiation. Previous experiments showed that the sticklebacks and minnows survive in this concentration of NaCN for 14 and 15 min. respectively; when sodium thiosulphate is added, the survival times are extended to 18 min. in the case of both fishes. Sticklebacks and minnows withdraw and avoid this concentration of cyanide (0.0005N) in less than 2 minutes.

Minnows and sticklebacks of average size were first placed in two large aquaria containing solutions of 0.05N $\text{Na}_2\text{S}_2\text{O}_3$. The fish were removed at regular intervals, introduced into the experiment tube and were tested for their behaviour.

After two hours of immersion in 0.05N $\text{Na}_2\text{S}_2\text{O}_3$ the sticklebacks and minnows did not show any difference in the behaviour within the experiment tube; the normal violent and rapid withdrawal was the characteristic reaction with subsequent exhaustion and collapse. They recovered quickly in the $\text{Na}_2\text{S}_2\text{O}_3$ zone.

With six hours of thiosulphate treatment still no appreciable difference in the avoidance reaction was displayed, both species of fish withdrew and avoided the cyanide zone within two minutes.

Sticklebacks immersed in $\text{Na}_2\text{S}_2\text{O}_3$ for a period of 12–15 hours showed a slight delay in the selection of the thiosulphate zone and the fishes' visits to the cyanide zone were more frequent. Five sticklebacks registered a mean reaction time of 2.5 min. Minnows immersed for a similar time, on the other hand, withdrew within 2 min. showing the same symptoms as in the previous experiments.

After 18–20 hours immersion the sticklebacks commenced to show a distinct difference in the reaction times; the fish taking more than 3 min. to avoid the solution (see Fig. 12). The amount of time spent in the cyanide was definitely increased and even after their return to the $\text{Na}_2\text{S}_2\text{O}_3$ zone they continued re-entry a few times before avoiding completely. With this period of immersion minnows also began to show a greater tolerance to the cyanide remaining in it or swimming to and from it for a time of 2.5 min., a time considerably longer than in the previous experiments.

Sticklebacks and minnows kept immersed for 24–25 hours exhibited distinctly prolonged reaction times; 4.5 min. by sticklebacks and 3 min. by minnows, the fishes were seen to cross the thiosulphate cyanide junction repeatedly and by the time the fish managed to restrict their movements to the thiosulphate zone they were completely exhausted.

Experiments conducted with fish after immersion for a period of 33–35 hours gave very long reaction times, nearly 6 min. in the case of sticklebacks and 4 min. with minnows.

When the fish finally got back to the thiosulphate zone they were utterly exhausted and in a state of collapse, some of the fish died while the others took a long time to recover their normal swimming positions.

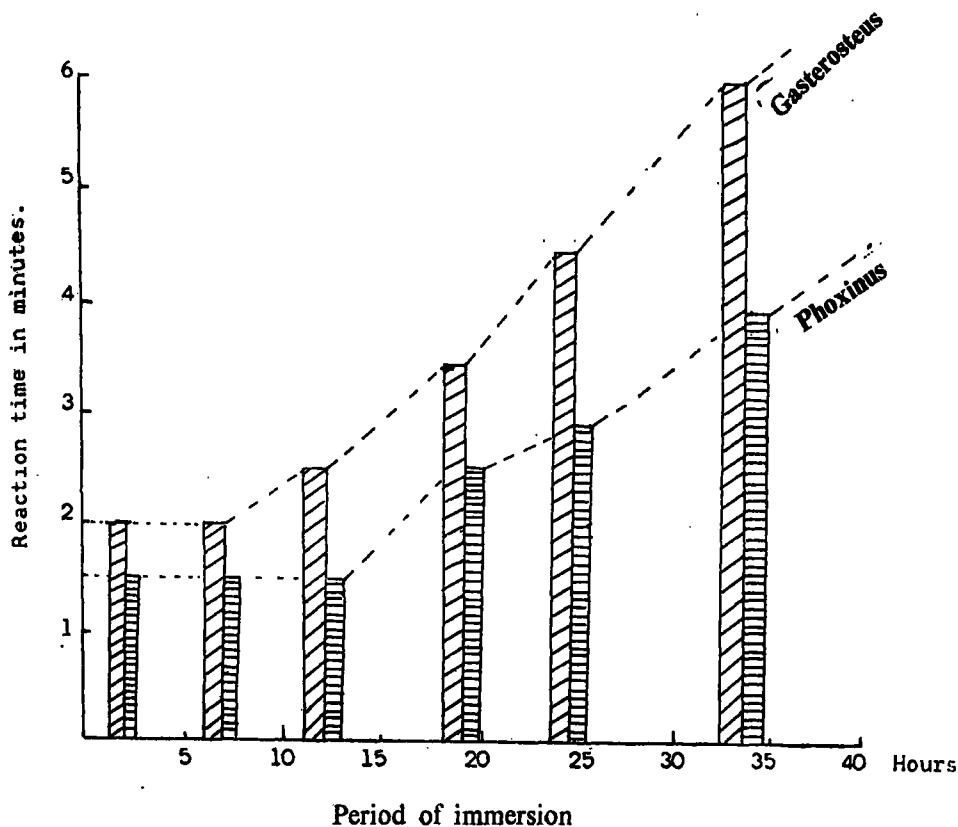


Fig. 12. Histograms drawn to show the reaction times for *Gasterosteus* and *Phoxinus* to 0.0005 N sodium cyanide after varying periods of immersion in 0.05 N sodium thiosulphate. Temperature 17—18°C.

Fig. 12 shows histograms drawn to compare the reaction times of sticklebacks and minnows shown after varying periods of immersion in $\text{Na}_2\text{S}_2\text{O}_3$.

(4) *Effect of size on the reactions of fishes to sodium cyanide solutions* :—

In the course of some preliminary experiments with NaCN it appeared very clear that the sensitivity of fishes to NaCN varied not only with the species but also considerably with size, weight and physiological condition.

Earlier, investigators have found that within a given species smaller fishes are more resistant than larger or adult individuals to CO_2 (Wells 1913), to sewage pollution (Forbes and Richardson 1913), to phenol (Gardiner 1923), to sulphite wastes (Nightingale 1931) and to potassium cyanide (Herbert and Merckens 1952). Shelford (1917) on the other hand reported that younger fish show a greater sensitivity to gas liquors and Sollmann (1906)

found that younger fishes were more sensitive to most of the drugs he tested. Shelford (1918) emphasised the importance of ascertaining injurious effects on a species of fish at the most sensitive stage of the life cycle.

In order to ascertain whether distinct differences in responses are displayed by smaller and larger fish of the same species to NaCN it was decided to investigate using sticklebacks, minnows, trout fry and eels of two different size and weight groups. 0.00005N NaCN was used for all the experiments. All the fish used were measured for size, and weighted by placing them in a previously weighed small bowl of water and noting the difference. The results of all the experiments with different fish and of different size-weight groups are summarized in Fig. 13.

The behaviour of *Salmo trutta* fry of the smaller size and weight group (4-4.5 cm. 0.9-1 gm.) was one of slow, smooth swimming, with less signs of sudden irritation. The negative reaction is very slow, developing very gradually without much violent movement. The reaction times for five experiments were 4.5, 4.5, 3.5, 4.5, 5 min. Reaction times on reversal were however quick, selecting the water zone in about 2.5 minutes.

On the other hand the responses displayed by fish of the larger size / weight group (6.5-7 cm. 2.5-2.6 gm.) contrasted greatly with the above the detection and rejection of the cyanide zone was very rapid; 2.5, 2.5, 3.5, 2.5, 3 min. were recorded in five experiments. Once the reactions are developed the fish show violent movements with wide opened mouths ending in the water zone gasping for breath and almost in a state of collapse.

Phoxinus phoxinus measuring 4.5-5 cm. (0.9-1 gm.) again showed a very slow and smooth reaction, the retreat to water zone being very gradual, the reaction times being 6.5, 6.5, 5.5, 5.5, 4.5 with a mean of 5.5 min. Minnows of the larger size/weight group also started by moving to and fro in the "tube" very slowly crossing the cyanide water zone very smoothly in the first few seconds. The reaction then developed suddenly and once developed was very intense; the fish dash out of the toxic zone with great haste to lie in a state of half collapse in the water zone. The avoidance times for five individual fish were 3.5, 2.5, 3.5, 3.5, 4.5 min. Like fish of the small size/weight group reversal reactions were performed in a faster time.

Gasterosteus aculeatus of 3.5-3.8 cm. (0.55-0.57 gm.) size registered the longest reaction times to 0.00005N, of the fishes tested,—8.5, 7.5, 8.5, 8.5, 7.5 minutes. The general behaviour was very lethargic, they would swim for a short time, stopping at either end and then swimming again. This was the general pattern. At 6.5 minutes they began to recognize the solution and the avoidance performance was completed very smoothly.

The mature adult sticklebacks started by moving about slowly but at about 3.5 min. the fish began to manifest very agitated swimming, crossing the junction several times apparently unable to distinguish the toxic zone. At 5.5 minutes however the fish seemed to make a frantic effort to restrict their movements to the water zone but by this time most of the fish were in a state of semi-collapse. Out of the five successful experiments conducted, three fishes avoided the NaCN in 5.5 minutes, one fish took 7.5 minutes while another fish withdrew in a short time of 4.5 minutes.

The very young *Anguilla anguilla* elvers measuring 7.8 cm. (0.3-0.4 gm.) displayed reactions similar to those shown by other fish, showing continuous swimming movements in the toxic and water zones. The characteristic respiratory distress develops rather late taking 7.5, 8.5, 7.5, 7.5, 7.5 minutes for five individual fish respectively.

The elvers of the larger size/weight group behaved in a very similar fashion. In the first few minutes the swimming movements were slow and they showed sluggish, serpentine movements on reaching the toxic zone; then gradually swimming became very vigorous and more rapid and at about 4.5 minutes the eels began to show acute irritation and confined themselves to the water zone, half overturned. The reaction times for these experiments ranged from 3.5-5.5 minutes.

The results of these experiments indicated rather definitely

- (1) A varying reaction time manifested by the different size groups of all the species tested
- (2) A better capacity on the part of the younger individuals of different species to withdraw from a NaCN polluted area without being affected adversely and seriously and thus enabling them to select an unpolluted area before being overcome by the toxic solution.

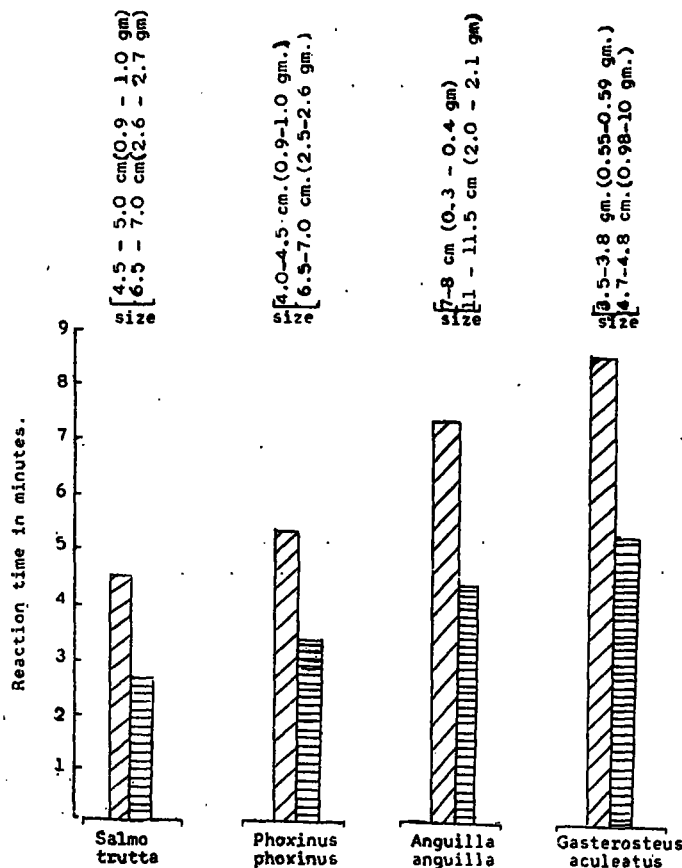


Fig. 13. Histograms drawn to show the varying reaction times displayed by different sizes of fish to 0.00005 N sodium cyanide. Temperature 17-18°C.

(5) *The effects of temperature on the reactions of *Gasterosteus* to sodium cyanide solutions :—*

Temperature plays a profound part in the survival and behaviour of fish as they are poikilothermic animals. Several investigators have stressed the importance of temperature on the survival of fish in toxic solutions—the fish dying quickly at higher temperatures. The present series of experiments was initiated to ascertain what symptoms and reactions are manifested when fish encounter cyanide solutions at low and high temperatures.

For these experiments, the material used were mature sticklebacks of average size; they were all collected from the same spot. Experiments were conducted at three sets of temperatures, at 0–2°C, at room temperature (18–19°C) and at higher temperatures of 26–28°C. The cyanide concentrations tested under these conditions ranged from 0.05N—0.00005N.

All the fish were acclimated to the test temperature for at least 24–28 hours before the experiments were begun. A constant low or high temperature was maintained in a constant temperature room and the fish were placed in large well aerated aquaria. The temperatures of the water and the solution in the two aspirators were maintained the same.

The complete results obtained are summarized in Fig. 14. At 26–28°C the whole picture was of a speeded up performance, the fish dying in most instances. At 0.05N death was almost instantaneous; the fish jumped, struggled and lost equilibrium almost immediately and begun to float towards the water solution junction. The fish, having little chance of escape, produced pseudopositive reactions in every case tested.

There was not much of a difference from the above in the reaction of sticklebacks to a solution of 0.005N NaCN; again similar violent struggles, immediate loss of balance, acute respiratory distress and in most cases a pseudopositive reaction was seen, with subsequent death in the toxic zone.

The reaction at 0.0005N, however, was a little different. In this experiment, also, violent desperate activity, acute respiratory distress and jerky gulping movements were seen in the first instance, but in most cases they succeeded in withdrawing into the water zone in the first minute or so before succumbing to the fatal effects, however they were in a state of complete collapse and subsequent recovery was extremely slow. In a few experiments the fish did not withdraw into the water, or even if they withdrew they swam back again into the toxic zone to produce pseudopositive reactions.

The violent struggling and acute respiratory movements are still displayed in the experiments conducted with 0.00005N at 26–28°C. The withdrawal and avoidance of the toxic zone is effected quickly and more smoothly, though the fish after struggling back to the water continue manifesting sporadic twitchings and other after effects of acute cyanide irritation. It is interesting to compare this reaction with that conducted at room temperature. At room temperatures the movements of the fish in the experiment tube are comparatively slow and the fish swims up and down for nearly 5.5 min. before avoiding the solution.

The fish still display a rather rapid reaction to the very dilute 0.00005N solution of NaCN, with respiratory difficulties developing almost immediately. They select the

water zone fairly quickly and avoid the solution in 6–8 min. At room temperature sticklebacks take about 35 min. to show signs of avoidance to the same concentration.

At low temperatures (0–2°C) the sticklebacks exhibit quite a contrasting behaviour, the whole performance is slowed down and violent struggling behaviour gives way to smooth gradual withdrawal. At 0.05N the fish at first moved slowly, going halfway into the toxic zone before again turning back. The fish then begin to show signs of irritation and start moving very gradually to the water zone to remain there; this time ranging between 2–3 min. In a very few cases however withdrawal was so delayed that pseudo-positive reaction was seen.

At 0.005N the sticklebacks continue to remain at the same end when the solution is admitted without showing any movements for a few minutes, then with progressive depression of respiration and increase of muscular weakness, the fish almost drags itself into the water end of the tube, in a very weakened state. The mean reaction time was 3.5 min., instead of the almost immediate avoidance shown at room temperature.

Further delay in withdrawing to the water zone is seen at 0.0005N. However the concentration of cyanide is high enough to induce the fish to commence movement and avoid the toxic solution in 5.5 min. Leisurely movements or no movement at all are seen in the 0.00005N experiment for the first 20 min. or so; then signs of irritation and recognition are developed and avoidance is displayed. At the same concentration the fish avoid this solution in 5.5 min. at room temperature, and in 1.5 min. at higher temperatures; the slowing down of all activities is clearly seen and could easily be watched.

At 0.000005N the fish would swim about very slowly or remain at the end of the toxic zone without showing any apparent signs of discomfort. During the course of the experiments (70 min.) the sticklebacks showed no tendency to go to the water zone, persistently remaining in the cyanide zone. Towards the same concentration, sticklebacks react in 3.5 min. at room temperature and 7 min. at 28°C.

All the results indicate that temperature exerts a definite influence in acceleration or deceleration of reaction behaviour. At higher temperatures vigorous swimming activity is exhibited; the lethal processes are activated ending in most cases—sooner or later—in death. At lower temperatures the reactions are all slowed down showing even an indifference to solutions which at higher temperatures they would avoid in 6 or 8 minutes.

(6) *The effects of pH on the reactions of Gasterosteus aculeatus to sodium cyanide solutions* :—

Wuhrmann and Woker (1948) have shown that the toxicity of cyanides is mainly due to undissociated HCN formed by hydrolysis and that the toxicity of cyanide solutions is therefore very dependant on their pH. Southgate (1948), however, states that the toxicity of cyanide solutions to trout is much the same over the pH range 6.0–8.5.

As no earlier work appears to be done on the responses of fish to cyanide solutions of different pH, a series of experiments was designed to find out the responses of fish, using *Gasterosteus* as experimental animal, to a 0.00005N NaCN solution of abnormally low and high pH. A pure solution of NaCN of this concentration has a pH of 7.1 and the sticklebacks react negatively in a time of 5.5 minutes.

Required hydrogen ion concentrations were obtained by adding sufficient quantities of HCl and NaOH. The pH was determined colorimetrically and also checked with a Cambridge pH meter. The amount of time the sticklebacks take to avoid the modified solutions of 0.00005N NaCN was noted and from their movements within the experiment tube the % times passed in the modified NaCN and water were determined. All experiments were conducted at 18°C and the observation time for all the experiments was limited to 25 minutes. Experiments were conducted at narrowly spaced pH levels ranging from 3.4 – 11.3. All the results are summarized in Fig. 15.

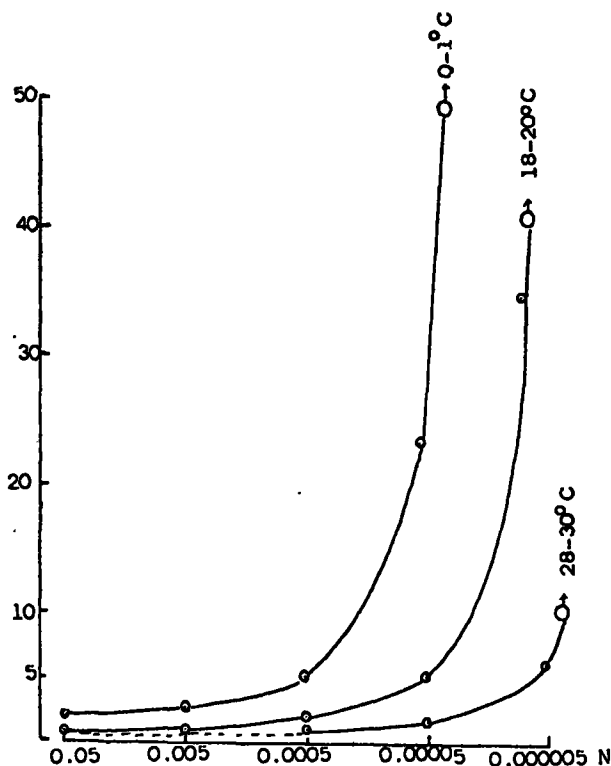


Fig. 14. Effect of temperature on the reactions of *Gasterosteus* to sodium cyanide solutions.

The avoidance time of sticklebacks to a solution of 0.00005N of pH 6.4 did not vary at all from the time taken to avoid an unmodified solution of same cyanide concentration. The time passed in the solution was not significantly less, being 40% compared to 45% in an unmodified solution.

Differences in the reactions however begin to become apparent at pH 5.5. The sticklebacks appear to be lightly irritated, swimming actively and rapidly to and fro. Only 28% of the time of 25 min. was passed in the modified zone while the fish registered an average reaction of 4.5 min. On further decrease in the pH the reactions manifested by sticklebacks become more vigorous and violent. At pH 4.1 every visit to the toxic end, produces a distinct irritable effect and the fish hurriedly retreat to the water zone usually taking a short time to recover, before swimming again only to penetrate the solution and turn back.

The survival time of sticklebacks in a solution of 0.00005N of pH 3.4 is about 1½ hours (1 hr. 32 min.) compared with over 2 hours (2 hrs. 5 min.) in an unmodified solution. In the experiment tube they exhibit violent, irritable movements and at the time they select the water zone they are in a state of semi-collapse and remain overturned for a few minutes before recovering. The reaction times are similar to above but the time spent in the toxic zone is less.

A graded increase in pH by the addition of NaOH gives at the outset a progressive increase in protection effect. In a solution of 0.00005N of pH 8.2 the sticklebacks live for more than 12 hours. A corresponding increase in the reaction times is seen in the response experiments, the fish swim about smoothly and it is only after about 11.5 min. that they begin to avoid the solution. In five experiments, of 25 min. each about 52% of the time was passed at the toxic end of the tube.

At pH 9.1 a further increase in the reaction times is seen (see Fig. 15), the sticklebacks spending about 80% of the time in the cyanide zone. The fish swim into, or persistently remain at, the cyanide end. Only at about 17 min. do they commence to select the water and begin to restrict their movements to this zone.

The sticklebacks were completely indifferent to a solution of 0.00005N of pH 10.1, during the 25 min. duration of the experiment. In three experiments the fish rarely moved from the end to which the cyanide solution was admitted, passing nearly 98% of the time in the cyanide zone.

In the experiments conducted at pH 11.3 the fish began to show rapid negative responses. It could be assumed from this that as the pH of the cyanide solution is raised, similar reactions to those shown towards solutions of very low pH could be expected.

This series of experiments confirms :

- (a) That at very low and very high pH values the reactions are accelerated though the lethal processes may become more effective
- (b) In slightly alkaline cyanide water, survival times are longer and reaction times are greater; thus in alkaline waters fish have a greater chance of escaping from the polluted area.

- (7) *The effects of a low dissolved oxygen content on reactions of fish to a solution of 0.00005N NaCN :—*

Sodium cyanide is a powerful respiratory depressant. Jones (1941) showed that even a solution of 0.00004N depresses the oxygen consumption of fish to a great degree. Downing (1954) reported that reducing the oxygen content of the solution increases the toxicity of KCN. The action of cyanides may be to limit the proportion of oxygen in the blood that can be utilized so that reducing the total amount of available oxygen aggravates anoxia and accelerates the lethal effects (Jones 1962).

The experiments were carried out with *Gasterosteus aculeatus* using 0.00005N NaCN solutions. For oxygen concentrations down to 45% the oxygen was extracted from the solutions with a suction pump. Solutions of very low oxygen content were made by diluting a very concentrated solution of 0.5N NaCN with completely deoxygenated water,

prepared by bringing the water to boiling point, and keeping it boiling for some time, also using the suction pump. As explained later only preliminary tests were made at very low oxygen tensions. The amount of oxygen in the solution was determined by the Winkler method and is expressed as $\text{mgO}_2/\text{litre}$ and % oxygen saturation. Each experiment lasted for 20 minutes. The results are given in Fig. 16 and expressed as reaction times and % times spent in the NaCN zone. The average time of avoidance with a fully oxygenated 0.00005N NaCN solution is about 5.5 minutes. The fish spends about 40% of a 20 min. experiment in the toxic zone.

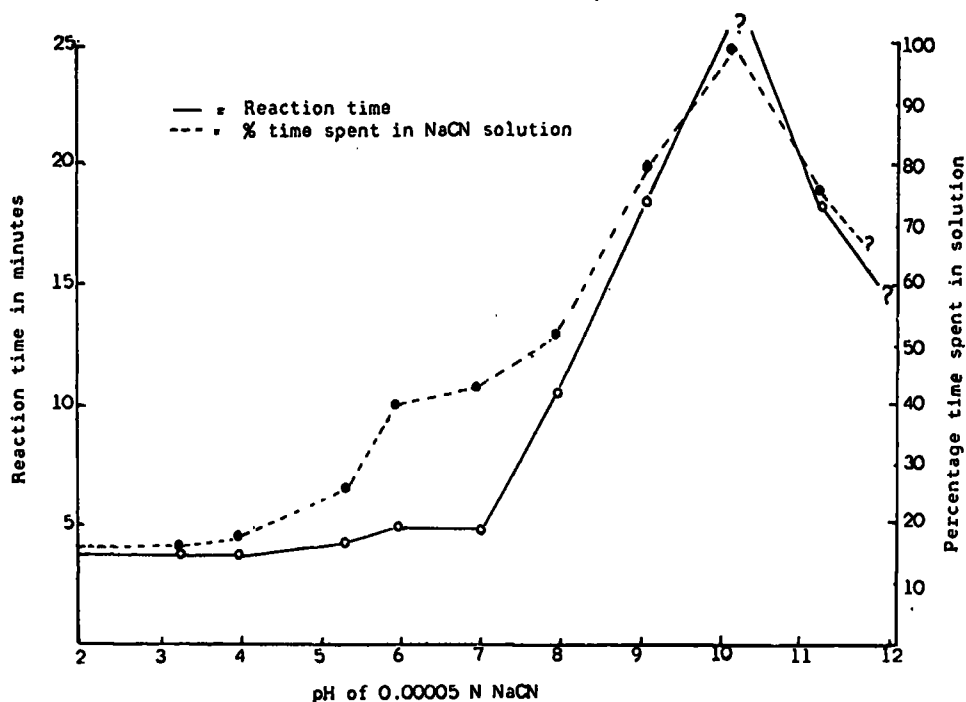


Fig. 15. Reaction times and percentage time spent by *Clasterosteus* in a 0.00005 N sodium cyanide of different pH. Temperature 17—18°C.

At 8.3 mgO_2/l , the fishes seem to show immediate perception and violent negative behaviour withdrawing in a time of 2.5 min. and spending only 12.5% of the time in the toxic solution zone. With NaCN of 76.3% and 63.3% oxygen saturation, the responses are similar to the above but the fish appear to be more affected by contact with the cyanide and the after effects persist for some time after their return to fully oxygenated water. The reactions shown at 58.3% oxygen saturation are violent and prompt. On admission, the fish remain quiet for a moment, then leave and enter the solution two or three times, then commence to swim violently ending in a collapse. In the experiments at 4.6 and 3.8 mgO_2/l , the fish develop convulsive respiratory movements almost instantaneously, "coughing" and withdrawing in haste to lie in a state of collapse in the water. At both concentrations the fish spent only 5% of the time in the cyanide zone. They lie almost upside down till they recover.

With NaCN of low oxygen content (26% O_2 saturation), the fish display acute distress, gulping violently, losing equilibrium and withdrawing into the water zone within a minute, (see Fig. 17D). By this time they are so intoxicated that they remain upturned, breathing hard, and in some cases they die. In most cases they show a negative reaction, except in one experiment where the fish showed a pseudopositive reaction.

The experiments were discontinued after testing with this concentration of dissolved oxygen as the fish showed extreme and very acute distress both in the solution and out of the solution for some time. In all these experiments the alternative given to the deoxygenated sodium cyanide solutions was completely oxygenated water.

Another series of experiments was carried out using completely oxygenated sodium cyanide solutions with water of graded oxygen content as the alternative. The purpose of these experiments was mainly to see how they would react and what selection they would make. The concentration of the cyanide solution was 0.00005N and the oxygen content was brought up to 10.0 mg O_2 /l or more by blowing in compressed air.

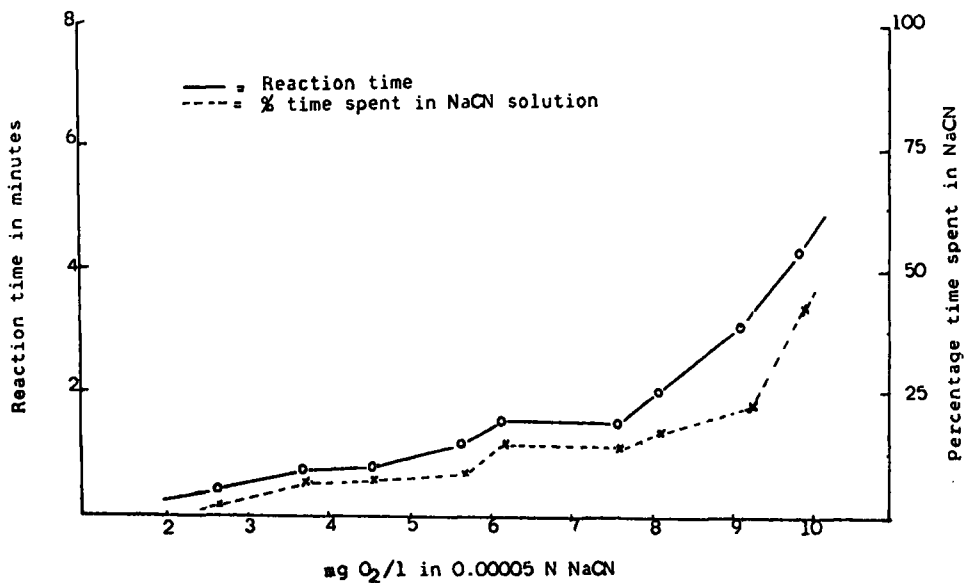


Fig. 16. Reaction times and percentage time spent by *Gasterosteus* in a 0.00005 N sodium cyanide of different oxygen tensions. Temperature 17–18°C.

The experiments were carried out in two ways. In the first series of experiments the fish were introduced into well oxygenated water in the experiment tube and kept at least 10 min. before deoxygenated water on one side and oxygenated NaCN solution of the other side were admitted. In the second set of experiments the fish were introduced into the experiment tube containing water of the selected saturation level at which the experiments were run and kept in that water for at least 10-15 minutes before the contents of the aspirators were run.

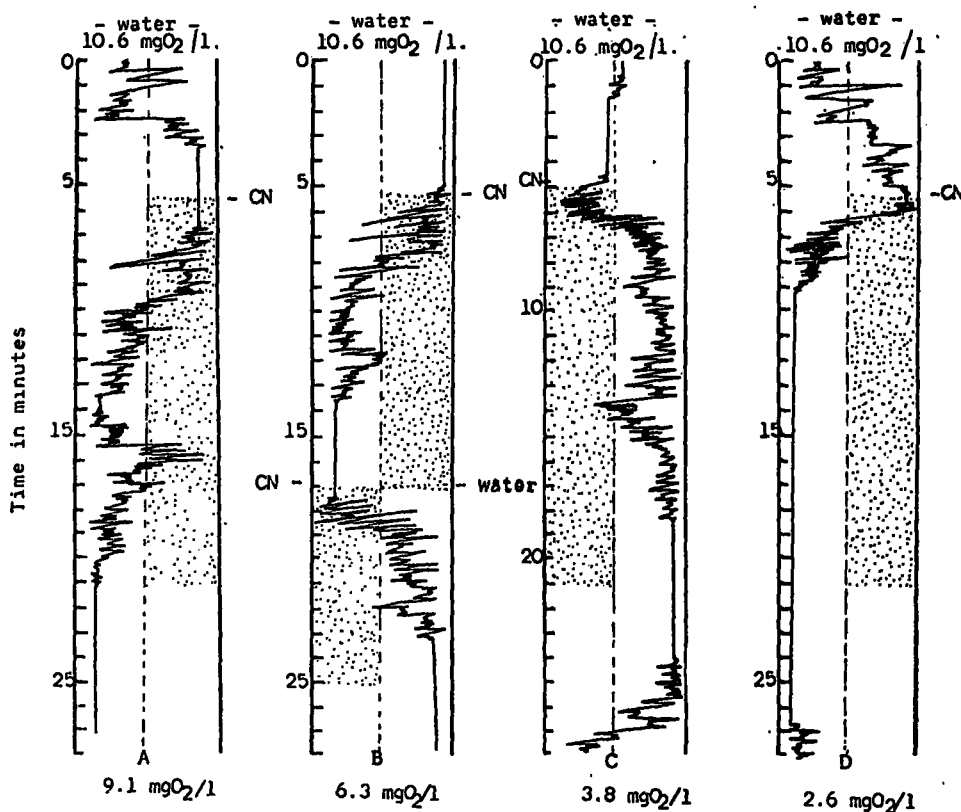


Fig. 17. Reactions of single sticklebacks to 0.00005 N sodium cyanide solutions containing 9.1, 6.3, 3.8 and 2.6 mgO_2/l .

No conspicuous differences either in the behaviour or in the reaction times were seen when fully oxygenated NaCN solution was run on one side and water of 8.2 mgO_2/l . on the other side. The reaction and selection after previous immersion in fully oxygenated and the partially oxygenated water was almost the same, the fish taking about 5–6 minutes to select the water zone.

The degree of preference for the water zone at 7.6 mgO_2 and 6.2 mgO_2 experiments, when the fish were started off in completely oxygenated water was much the same as above. However, if the fish were introduced first into water of 7.6 mgO_2 and 6.2 mgO_2 and kept in these for 15 minutes, the fish showed a slightly accelerated reaction, selecting the water zone at 3.5 minutes.

At 5.6 mgO_2/l , the fish began to show some conspicuous differences from the ones so far noted. When a fish retreats from the cyanide it hesitates at the junction, turns back into the toxic zone, swims out again and repeats this a few times; with further irritation it deliberately moves into the deoxygenated water, though showing signs of discomfort, the time taken is about 4.5 minutes. Reversing the flows in the two aspirators muddles up the whole selection behaviour. Instead of selecting the water zone quickly they swim more or less at random until they come into the water zone in 5–6 minutes.

In the experiments where the fish were started off with 5.6 mgO₂/l. water, a different picture altogether was seen. The fish swam actively from the moment they were introduced. After 15 minutes the cyanide solution was admitted; the fish with one outburst of swimming dashed into the water zone only to swim back, a few crossings of the junction were seen and the fish thereafter avoided the toxic zone. The selection of the water zone took a matter of 2—3 minutes.

With 4.3 and 3.5 mgO₂/l., with the fishes initially introduced into fully oxygenated water, and subsequently cyanide admitted, the fish showed moderate respiratory distress and agitated swimming movements. In both cases the fish preferred the deoxygenated water zone in preference to oxygenated cyanide zone in 4.5 min., but initially a hesitation at the boundary and swimming back into the toxic zone was seen. Reversals of flow brought about violent movement, the fishes swam with unusual speed crossing and recrossing the boundary several times with dyspnoea developing; after some erratic movement the fish managed to confine themselves to the water zone in about 6—7 minutes. By this time the toxic processes had taken effect and the fish lay in a very helpless state, panting heavily for breath. Normally at this point, the experiments were discontinued and oxygenated water was sent in.

With the same oxygen concentrations and the fish immersed in these for a period of 10 minutes previously to the experiments, the fish showed different behaviour. At these oxygen tensions the fish usually show slight discomfort. At 4.3 mg. when the cyanide solution is admitted the fish are so violently irritated that they swim across once or twice and then very quickly come to lie in a state of collapse; this taking place in less than 2 min. In those experiments where the fish selected the water zone before coming to a collapse, reversals of flow brought about immediate dyspnoea, paralysis and death. With 3.5 mg. the fish selected the water zone in less than 2 minutes but they stayed overturned resting on their sides almost motionless and gasping for breath.

The experiments conducted with fairly low oxygen tensions of 2.8 mgO₂/l. and 1.6 mgO₂/l. gave results which are rather peculiar. At these oxygen tensions with the fish initially immersed in fully oxygenated water, the fish took an inordinate time to select the low oxygen zone. At first it seemed that the fish preferred the completely oxygenated cyanide zone to the low oxygen water zone; the fish would swim to reach the junction and turn back to the cyanide zone, spending more time in it. Even if they penetrated into the deoxygenated water zone, they would hurriedly get back to the cyanide. However, in about 5.5 minutes in the 2.8 mg. experiments and 6.5 minutes in the 1.6 mgO₂/l. experiments, the fish eventually selected the water zone but showing acute discomfort and distress. If the experiments were continued beyond, all the fish would collapse and lose equilibrium.

On the other hand in the experiments where the fish were initially immersed in these oxygen tensions, admission of cyanide solution brings about immediate loss of balance they come to rest at the bottom of the experiment tube and for a long time show weak signs of life with flickering opercular movements. Some times even admission of oxygenated water does not bring about a recovery from this helpless condition.

The results of these experiments indicate clearly that reduction of the oxygen content increases the toxic processes and bring about loss of equilibrium and death in an earlier

time. Fish that have been earlier exposed to low oxygen tensions display acute distress and uncoordinated movements on contact with cyanide as a result of which the reactions displayed subsequently are indefinite. On the whole fish prefer water of very low oxygen content to fully oxygenated 0.00005N NaCN solution.

DISCUSSION

Shelford (1917) noted "that fishes turn away from dangerous substances which are normally found in their usual environments, but with strange and unusual substances such as those thrown into streams by gas works and other industrial plants, they frequently enter and follow up to points where concentrations are fatal, or fail to recognize the dangerous substance at all and often stay in it until they are intoxicated and finally die there"; in other words, according to Shelford, fish do not display an instinctive protective reaction to unfamiliar poisonous substances. Jones (1947*b*, 1948, 1951), in an extensive series of experiments has shown that fish react negatively to a variety of unfamiliar chemical substances and he observes "that with any particular substance the reaction of fish depends on the concentration difference maintained at the water/solution junction" and further "that the reaction displayed bears no relation to the general degree of toxicity of the salt." Jones (1947*b*, 1948) found that fish react positively to dilute ammonia and copper solutions and B. Jones et al. (1956) noted that the dark colour of the sulphite waste liquor may possibly be an attractive visual stimulus resulting in at least momentary preference for channels containing waste "in concentration not great enough to be promptly and strongly repellent chemically". Whether the fish are repelled or attracted by any given concentration may therefore depend on the dominance of the repellent, or the attractive stimulus such as pH, osmotic pressure and other properties of test solutions such as colour. The results of the experiments conducted by the writer appear to indicate that fish display an instinctive protective reaction to solutions of sodium cyanide, an unfamiliar substance up to great dilution by turning back provided the solutions are not strong enough to bring about an immediate dulling of the powers of chemoreception; the degree of reaction, however, varies with the different species.

The lethal action of cyanides has been fully discussed by Neil (1957) and Jones (1962). Survival experiments conducted with different species of fish agreed generally with the findings of other workers (Karsten 1934; Herbert & Merckens 1952; Neil 1957). Mean survival times at the same concentrations appear to be different with the different species. Of the species tested trout fry appear to be the most sensitive. From the results of the survival experiments the order of susceptibility to cyanide solutions appear to be as follows:— trout fry—minnows—sticklebacks—eels—goldfish.

The results of the survival experiments are reflected in the reaction experiments. In Fig. 18, the interpolated reaction time curves have been constructed for the various test species and are represented in one diagram for readier comparison. These indicate that the species of fish tested display differences in their sensitivity and magnitude of avoidance. Trout fry appear to be the most sensitive and if the test species are arranged in order according to their capabilities of avoidance, the order will be as follows:— trout fry—minnows—sticklebacks—eels—goldfish; the same order as that shown by the survival times.

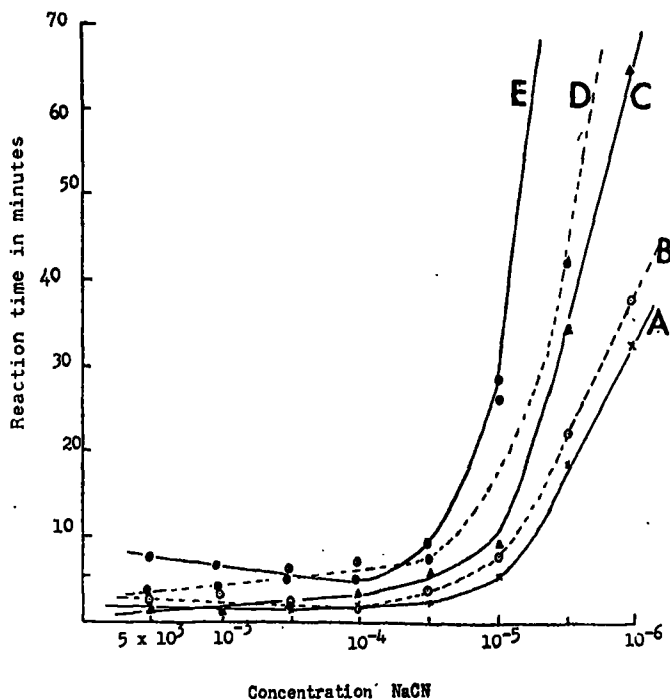


Fig. 18. Reaction time curves for A—*Salmo trutta*; B—*Phoxinus phoxinus*; C—*Gasterosteus aculeatus*; D—*Anguilla anguilla* and E—*Carassius auratus* in sodium cyanide solutions. Temperature 17—18°C.

The different fishes show, in many respects, responses which seem to be characteristic of the species, but some aspects of behaviour are common to all. Most species of fish took a longer time than expected to avoid strong concentrations of cyanide; some even exhibiting pseudopositive reactions. The delay displayed by fish in avoiding these concentrated solutions may be due to sudden paralysis of the chemoreceptors which may result in the fish swimming in and out of the toxic solution several times without recognizing it. As the solutions are progressively diluted the power of recognizing and avoiding them becomes greater, consequently moderately strong cyanide solutions are avoided in less time and before the fish are overcome by fatal exhaustion. The results of the present experiments also show that the reaction times are considerably shorter than the survival times over the whole concentration range tested.

The survival time/reaction time ratios for sticklebacks drawn for cyanide concentrations, compared with similar results calculated for zinc sulphate and lead nitrate from the data of Jones (1947b, 1948) are given in Fig. 19. The marginal area of safety for cyanide appears to be smaller in strong to moderate concentrations, and therefore the chances of escape are less, in comparison with the less toxic substances such as zinc or lead. The ratios, however, approximate closely in dilute solutions.

Neil (1957) has demonstrated the adverse effect of very small quantities of sublethal cyanide concentrations on the ability of speckled trout to perform sustained exercise; fish

which have been in contact with as little as 10 parts per billion are seriously affected. It is possible that if detrimental conditions interfere with the ability of the fish to perform work, then feeding and growth are reduced, as a result disease and predation may assume unnatural proportions. Interference may also take place in the movement of fish to breeding areas and new feeding grounds. Disability to perform these can lead to the elimination of a population. The avoidance of extremely dilute concentrations of cyanide by fish as shown by the present work are therefore of great significance. If the fish could move out before the toxic effects have come into play, the survival of a population will be more or less assured.

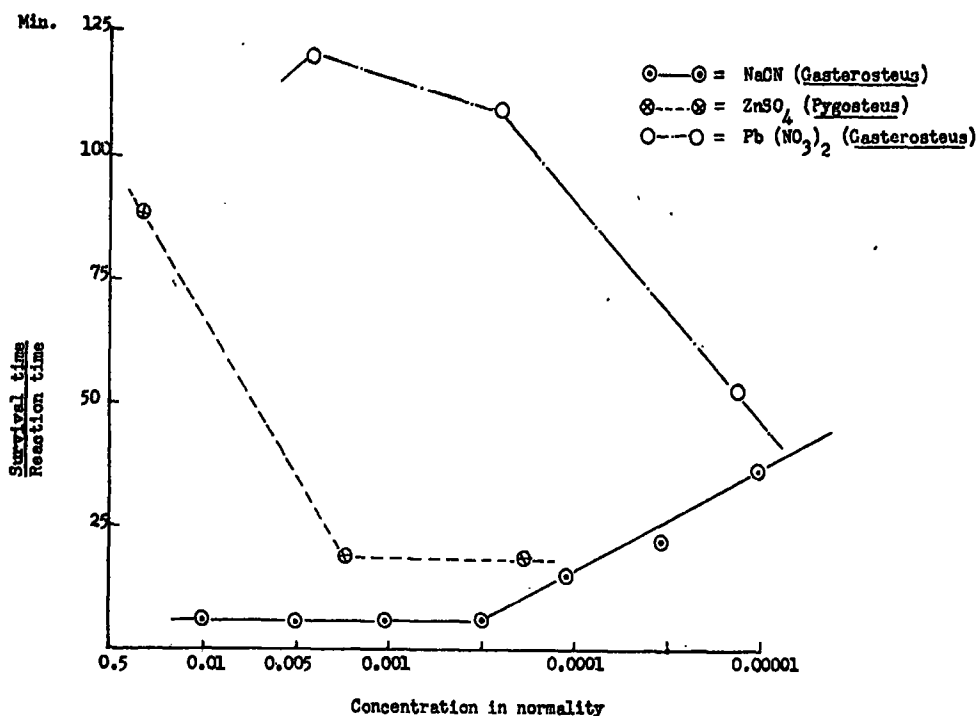


Fig. 19. The survival time/reaction time ratios for sticklebacks in sodium cyanide, zinc sulphate and lead nitrate solutions.

The addition of sodium thiosulphate to sodium cyanide solutions decidedly decreases their toxicity, as a result of which longer reaction and survival times are shown. The antidote properties of the thiosulphate is due to the conversion of the extremely lethal cyanide into less poisonous thiocyanate. The antidote effects of thiosulphate differ with the different species of fish tested; most species beginning to show a reduction of toxicity when the concentration ratio between sodium thiosulphate and cyanide is greater than hundred times; in this, the findings are in agreement with those of Achard & Binet (1934). However, the results show that thiosulphate produces no effect with concentrated cyanide solutions and any distinct reduction in toxicity is shown only with dilute cyanide solutions. From a comparative point of view the antidote effect appears to be most effective with goldfish and least with trout fry.

The present investigations also indicate that sodium thiosulphate not only gives a protective effect by its addition to cyanide solutions but also gives a similar effect when fish have been previously immersed in it. In the reaction experiments this protective effect was proportional to the period of immersion. It can be concluded from these results that if the waters in which the fish have been confined contain sufficient thiosulphate the fish could be sufficiently protected to move out of a cyanide polluted area in a quicker time without the effects being felt badly.

Investigators have reported differently about the sensitivity of fish of different sizes and ages to different toxic substances. Some investigators (Wells 1913, Forbes & Richardson 1913, Gardiner 1923, Nightingale 1931, Herbert & Merckens 1952) found that young fish are more resistant than the adults while others (Sollmann 1906, Shelford 1917) reported the contrary. The present experimental results show that younger and smaller fishes are capable of spending a longer time and making more visits to the toxic zone than the larger and adult fish. There are also marked and pronounced differences in the manner of reaction and in the length of the reaction times. These results are important from an ecological point of view in that if the younger stages could avoid a toxic substance to a greater degree than the larger forms without themselves being irreparably damaged, there is an increased chance of survival for the species, even if there is a pollution hazard.

The importance of temperature in determining the toxicity of cyanide solutions has already been stressed by Herbert & Merckens (1952). The present results show that temperature has a profound influence on the reactions of fish to NaCN solutions, the average reaction times decreasing with rising temperature and vice versa.

Changes in reaction times with changes in temperature can be presumed to result from changes in metabolic rate. As fish are poikilothermic animals, their rate of metabolism is determined to a large extent by the temperature and the effects of toxic substances will appear earlier or later as the case may be. The experimental results confirm this view. Therefore there are reasons to believe that concentrations which are not demonstrably toxic or harmless at low temperatures may be quite rapidly fatal at higher temperatures and consequently avoidance will not be possible. Seasonal temperature changes in a cyanide polluted area may be important as a result.

Investigations on the effects of change of pH on the reaction times indicate that as the pH of cyanide is lowered the toxicity and degree of avoidance increase. On the other hand no influence of pH in the alkaline range was observed except at very high values. It is possible that at extremely low pH, in addition to the HCN the hydrogen ion concentration may itself be an operative factor. Jones (1948) has shown that *Gasterosteus* will avoid water of pH below 5 in less than 5 minutes. In the present study the sticklebacks avoided cyanide solutions of similar pH in similar time. The avoidance of cyanide solutions of very high pH may be a direct response to hydrogen ion concentration. Jones (1948) also showed, for the same fish, that at pH 11.4 and onwards the reaction curve drops sharply. Similar results are seen in the present experiments.

These results may be of practical as well as theoretical importance. The pH of bodies of water may show seasonal variations due to biological activity in the water as well as to variations in rainfall. For these reasons the physiological action of cyanide solutions

may change from season to season. The present results also indicate that fish living in an alkaline river may avoid and escape the intoxicating effects of cyanide more successfully than in a less alkaline one.

The present experimental study has distinctly shown that reduction of the amount of dissolved oxygen in the cyanide solution decreases the reaction times, simultaneously producing acute distress; this may apparently be due to enhanced toxicity as a result of the reduction of the oxygen content (Downing 1954, Southgate et al. 1933). Jones (1947a) reported that even dilute solutions of NaCN bring about a considerable depression in the oxygen consumption. Hence limiting the total amount of available oxygen aggravates this situation resulting in anoxia and death. The present results also show that if a choice is given, sticklebacks would prefer almost completely deoxygenated water to oxygenated NaCN solutions indicating their great sensitivity to these solutions. The results also indicate that fish previously exposed to water of low oxygen tensions display acute distress in making this selection and if exposed to cyanide solutions subsequently, they end in death. These results of laboratory experiments have been more or less indirectly confirmed in the field work of Alabaster (1959). He showed that the distribution of fish may not be affected in dilute sewage effluents provided the oxygen content is high enough so as not to cause serious respiratory difficulties.

These experiments have provided information for cyanide under a variety of environmental conditions. Under normal conditions most or all these factors may come into effect at one and the same time. Hence it is clear that estimations of safe concentrations must be made on the basis of the most adverse conditions likely to occur—on the whole, the fish will be able to escape cyanide intoxication, if the environmental conditions are favourable and if the effluents are diluted to the maximum.

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SUMMARY

1. The present work includes the results of several hundred experiments to test the reactions of fish to sodium cyanide solutions using an apparatus which is a modification of that used by Jones (1947b).
2. Sodium cyanide solutions are highly toxic to all the species of fish tested and are detected and avoided at concentrations as low as $10^{-6}N$., the degree of sensitivity varying with the different species, the most sensitive being trout fry and least goldfish. High concentrations of this substance may stupefy the fish so rapidly that sometimes pseudopositive reactions appear.

3. The addition of thiosulphate reduces the toxicity of NaCN solutions to fish, and prior treatment with thiosulphate can give a considerable measure of protective effect. However, with thiosulphate, the fish take longer periods to establish avoiding reactions to cyanide.
4. A varying reaction time is displayed by the different size groups of all the species tested, the younger individuals manifesting a better capacity to withdraw from sodium cyanide solutions.
5. The intensity of response of fish to cyanide solutions also depends on environmental factors, such as temperature, hydrogen ion concentration and dissolved oxygen content of the cyanide solutions. High temperature, low and high pH, low dissolved oxygen content all incline to rapid and violent avoidance reactions.

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