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STEPHEN A. FORBES, PH D., L. L. D.,
DIRECTOR

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ARTICLE VI.

AN EXPERIMENTAL STUDY OF THE EFFECTS OF
GAS WASTE UPON FISHES, WITH ESPECIAL
REFERENCE TO STREAM POLLUTION

BY

VICTOR E. SHELFORD, PH. D.

ERRATA AND ADDENDA.

- Page 50, second column, line 13 from bottom, for *Danais archippus* read *Anosia plexippus*; line 8 from bottom, for *mellifica* read *mellifera*.
- Page 51, line 11 from bottom, for *Danais* read *Anosia*.
- Page 159, at right of diagram, for *Bracon agrilli* read *Bracon agrili*.
- Page 289, second column, last line but one, for *Scalops* read *Scalopus*.
- Page 294, line 3, for *catesbeana* read *catesbiana*.
- Pages 327 and 330, line 12, for *orcus* read *oreas*.
- Page 347, line 4, for *Cecidomyidæ* read *Cecidomyiidae*.
- Page 356, line 7, for *Anthomyidæ* read *Anthomyiidae*.
- Page 368, line 18, dele second word.
- Page 373, after line 10 insert as follows: 53a, *subpruinosa* Casey, 1884, p. 38.
- Page 375, after *submucida* Le Conte, 48, insert *subpruinosa* Casey, 53a.
- Page 377, after line 7, insert as follows:—
1884. Casey, Thomas L.
Contributions to the Descriptive and Systematic Coleopterology of
North America. Part I.
- Page 379, line 11 from bottom, for *sensu lata* read *sensu lato*.
- Page 382, line 12, for VII read VIII.
- Page 408, line 2, for *the next article in* read *Article VIII of*.
- Page 410, line 6 from bottom, for = $\frac{1}{2}$ read '11.
- Page 412, line 7, for 31 read 30.
- Page 421, line 17 from bottom, insert *it* before *grows*.

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ARTICLE VI.—*An Experimental Study of the Effects of Gas Waste upon Fishes, with Especial Reference to Stream Pollution.*
BY VICTOR E. SHELFORD.

I. INTRODUCTION.

The products of destructive distillation of coal include an innumerable series of substances representing most of the important groups of organic compounds ranging from gases to solids. In the manufacture of illuminating gas all these substances are thrown into streams in varying amounts, depending upon the manner of treating by-products. The gases and volatile products are in solution in water used in washing the gas, and are often introduced into waterways. By-products, except the heavy tars, are often thrown away. This is especially true in the case of the smaller plants where the quantity is insufficient to make the further treatment of it profitable. Thus in many plants only the heavy tars are saved, the gas liquor drip from the mains and holders being dumped into waterways without the removal of even ammonia. The immense commercial value of these wasted products has been more generally appreciated since the outbreak of the European war, which cut off the large supply of foreign dyes and important organic compounds and increased the demand for such products as may be used in the manufacture of explosives. The value of these wasted products should be sufficient to prevent their wastage, but their injurious effect upon fishes and other life of streams generally is itself sufficient to justify the prohibition of pollution by this means.

II. STATEMENT OF THE FISH AND GAS-WASTE POLLUTION PROBLEM.

The gas waste problem is concerned with the effects upon fishes of the gas liquor untreated, the effect after the removal of the heavy tar, the effect of tar, the effect of gas-washing water, and that of lime, etc. from the purifiers. It is the purpose of this paper to show that essentially all the products of the distillation of coal are very toxic to fishes, some of the most toxic being those which are commonly regarded as "insoluble" in water. From the standpoint of fishes the waste problem is concerned with the reactions of fishes when encountering the pol-

luted waters. The reactions of fishes to the results of contamination with natural organic matter, such as decomposing bodies of plants and animals, are generally advantageous, as the fish turn away from the polluted area. The result of this investigation shows that in the case of gas wastes the reactions are usually disadvantageous,—the fishes swim into the polluting substances without recognizing them or turning back from them even when their toxicity is such as to cause death within a short period. The detrimental character of gas wastes is thus increased many fold.

The toxicity of waste differs for different species of fish and is greatest for the more valuable fishes as indicated by Dr. Wells' work (Article VII of this volume). It will be shown to be generally greater for the smaller and younger fishes. The writer's investigations will show that this rule holds good down to the youngest fry studied. Sollmann ('06) found some evidence that the eggs and newly hatched embryos of marine *Fundulus* are more resistant to poisons than the adults. He however seems to question his results in this respect because of the long exposure in the poison solutions and small quantity of the solution in proportion to the size and total oxygen demand and excretory output of the adult fish. Child's work with phenyl urethane which was done after long experience in the use of such poisons showed that in marine *Fundulus* the resistance declined rapidly from a maximum in the two-cell stage of the egg, to the time of hatching. In *Tautoglabris* the resistance fell from a survival time of 675 minutes soon after fertilization to 15 minutes at the time the heart began to beat and rose to 20 minutes at the time of hatching, when the experiments were discontinued. The resistance of the eggs and embryos of fresh water fishes has not been studied and compared with that of the adults, but there is every evidence that the rule reported here will hold good throughout the age and size series beginning about the time of hatching. The most sensitive period must be determined before the minimum fatal quantity can be established with any certainty. For this reason no attempt has herein been made to determine the minimum which will prove fatal to the fishes studied. Dr. Wells has found that the resistance of some fishes to various factors varies greatly with the time of year. The lowest point comes between the middle of June and the last of July when such fishes as the cyprinids can hardly be taken from the water before death sets in. From this time the resistance slowly rises until September. Then the rise becomes more rapid and reaches its highest point in March and April, when all the fishes are exceedingly resistant. With the onset of the breeding season the resistance falls, though whether or not it con-

tinues to fall until the period is well passed has not been determined. The effects on the breeding operations while of paramount importance have not been touched in this investigation. In this work no experiments were performed between June 8 and Aug. 18.

In the course of the investigation the working out of the toxicity of the different compounds has been rendered essential, first because of their general occurrence as by-products and secondly because various methods of treatment remove some compounds and not others. This toxicity is further of interest in connection with the effects of these compounds as drugs and poisons. The recent use of gold fishes, frogs, etc. as means of standardizing drugs, such as digitalis, renders these data of interest to the pharmacist and legal toxicologist. The timed killing of upwards of 1,500 fishes has, it is hoped, made clear some facts and methods which may be useful in the study of these problems with domesticated species such as gold fish.

III. MATERIAL AND METHODS.

The character of the water used is of much importance in the study of toxicity of polluting substances. The loss of oxygen and accumulation of waste matter in standing water renders experiments conducted with it open to criticism and necessitates the use of a short period to death in comparatively high concentrations of the drugs as a criterion in determining relative toxicity. It further necessitates the running of control experiments in running water. Experiments in running water are usually necessary in the case of gases. Toxicity is frequently different in distilled water and tap water.

I. THE CHARACTER OF UNIVERSITY OF ILLINOIS WATER, AND OTHER WATER PROBLEMS.

The water supply of the University comes from deep wells and the salts are nearly all present in the form of carbonates instead of a mixture of carbonates, chlorides, and sulphates as is the case in waters where fish normally occur. It also contains about twice as much magnesium and calcium and eight times as much iron as is commonly present in such waters. As it comes from the tap the university water contains no oxygen and about 18cc. per liter of carbon dioxide. The lack of oxygen alone makes it unsuitable for fishes, and the presence of so much carbon dioxide renders it wholly unfit for them. Fishes die in it quickly. The mortality among fishes brought in from streams was very great when this water was used in aquaria in which they were kept.

2. TREATMENT FOR KEEPING FISHES ALIVE.

Treatment A

The water in this case was boiled in an apparatus (Fig. 1) which continuously boils and cools it, being run through at the rate of 500 cc. per minute. This removed all of the readily precipitable iron and the excess of magnesium and calcium, thus reducing the total solids to about what one commonly finds in the average stream; but the water so treated still differed from stream water in that the salts present were nearly all carbonates, instead of a mixture of carbonates, chlorides, and sulphates, and decidedly alkaline. The water was aerated after boiling. The mortality became markedly less among fishes when they were first brought in, but on the whole it was not less than in water which received Treatment B.

Treatment B

In this treatment the water was aerated in an aerating device, so as to give air saturation. This removed nearly all the free carbon dioxide and rendered the water alkaline. In this the fishes lived fairly well but became very sluggish, so that they were not suitable for behavior experiments.

Treatment C

Thinking that the above sluggishness might be due to the absence of sulphates and the presence of carbonates only, a small quantity of sulphuric acid was added to the water. This rendered it acid by displacing some of the carbonic acid in the carbonates with the sulphate radical. This treatment proved beneficial, but the requisite manipulation was cumbersome.

Treatment D

For this treatment aerated water and direct tap water were run, half and half, into the aquaria. This rendered the fishes active and suitable for behavior experiments and the difficulties of manipulation were reduced. Later a less complete aeration in the aerating device shown in figure 1 was found to give equivalent results, and fishes lived unusually well for months without attention. In this the water was treated by running down twelve feet of incline at a rate of about two liters per minute. It then usually contained sufficient oxygen to support fishes and from 1-3 cc. of free CO_2 per liter, and had lost much of its iron and a little of its excess magnesium and calcium.

3. DIFFICULTIES TO BE GUARDED AGAINST IN FISH EXPERIMENTS.

a. Character of Water.

At the beginning of the work Dr. Wells ('15 and '15a) undertook a careful study of the relation of fishes to salts, acids, and alkalies. In general he found that carbonates do not have detrimental effects upon fishes when the water is acid. He further found many minor complications in connection with different salts which occur in some waters but none of these occurred in the water used. His findings relative to acidity, alkalinity, etc. are of general application and may be summarized as follows:

Water which is consistently slightly alkaline lessens the activity of fishes and the mortality is high. N/100 alkalinity, KOH, (56 pts. per m.) kills them in a few hours.

Neutral water also seems to be toxic to the fishes, and they become less and less active until death may occur.

An optimum acidity is obvious. 2-6 cc. of CO_2 per liter, (4-12 pts. per m.) seems to be the proper acid concentration for many fresh water fishes. Higher concentrations prove fatal very soon, though fishes will live for some time in 10-20 cc. per liter (20-40 pts. per m.) of carbon dioxide. N/10,000 H_2SO_4 , (4.9 pts. per m.) is fatal in a day or so, but N/20,000 H_2SO_4 , (2.4 pts. per m.) seems to be near their optimum as they live in this concentration for a long time.

Fishes react very definitely to exceedingly small concentrations of hydrogen and hydroxyl ions. Fresh-water fishes in a gradient which is slightly acid at one end and neutral near the middle and slightly alkaline at the other end will spend most of their time in the acid end, turning back from the alkaline end at a point just on the *acid side* of neutrality. The concentration here when tested shows that they turn back when the acid concentration falls below N/12,000 carbonic acid (3.5 pts. per m.).

In a gradient where the fishes may select between alkalinity and neutrality they avoid the neutral water to some extent and spend the greater part of the time in slightly alkaline water.

b. Quantity of Water.

In the aquaria suckers, small-mouthed and large-mouthed black bass died frequently when the flow of water was small and the depth in the aquaria more than 6 inches. This was probably due to insufficient oxygen. When the amount of water in the aquaria was small and the flow sluggish as was the case when the water was 2 or 3 inches

deep the greatest mortality was among the darters and the minnows (*Notropis* and *Pimephales*). These died in numbers in the aquaria, no darters at all being kept alive. After a number of trials a series of experiments was performed to demonstrate the cause of the death of the fishes (darters, *Etheostoma coeruleum*, and minnows, *Pimephales notatus*). The procedure was as follows:

Twenty-two 5 in. x 8 in. battery jars were set in a water bath,—the tank into which and out of which tap water flowed,—ready for filling with water modified variously, by boiling, aeration, and the addition of various substances as shown in Table I. Minnows and darters were given separate jars.

The following table shows the results.

TABLE I
AVERAGE LIFE UP TO TEN DAYS.
Two individuals in each condition except where otherwise stated.

750 cc. H ₂ O	Darters	Minnows
	Days alive	Days alive
Boiled alkaline	8	10
Boiled acid	5	8
Aerated,—alkaline to neutral	2½	10
Aerated acid	6	6 smaller died first
Iron precipitate, aerated	10	10
Iron precipitate, not aerated	6	10
Creek water, standing	10	omitted
Creek water, aerated	6 (6 fish)	6
Creek water, standing	1½ (8 fish)	..
Direct tap water	1/48	1/48

These experiments should be repeated but were sufficient to indicate the proper precaution as to quantity of water and rate of flow. As they stand they indicate that the addition of sulphuric acid in the quantity given does not improve living conditions in the boiled water for either darters or minnows. In the case of the aerated water the results are contradictory. Iron sediment does not seem to be a factor causing death. The number of fish to a given amount of water appears very important, and indicates that the losses were in part due to an insufficient flow through the aquaria. The fishes evidently add some waste products to the water which are of a non-gaseous character and thus are not removed. It appears that there should be about *one liter of water for each gram of fish* in the case of the two species studied. Less is doubtless sufficient for many species though it was thought best to use 4 liters of H₂O in which to kill a 4–6 gram sunfish in a bottle.

c. *The Transportation of Fishes.*

In collecting fishes for such experimental work they may be secured and brought to the laboratory in numbers if only a very small quantity of water is used. In general it is best to allow the dorsal fins of sunfish, basses, crappies, and suckers to protrude from the water. Minnows on the other hand, live best in about 3 inches of water. In this way many fishes may be safely brought in without the usual labor of carrying a quantity of water.

4. FISHES USED.

The fishes used in this experiment belong to the species mentioned below.

<i>Common name</i>	<i>Scientific name</i>	<i>Abundance</i>
Orange-spotted sunfish	<i>Lepomis humilis</i> Gir.	Abundant
Blue-spotted sunfish	<i>Lepomis cyanellus</i> Raf.	Common
Blue-gill	<i>Lepomis pallidus</i> Mit.	Common
Long-eared sunfish	<i>Lepomis megalotis</i> Raf.	Common
Rock bass	<i>Ambloplites rupestris</i> Raf.	Common
Small-mouthed black bass	<i>Micropterus dolomieu</i> Lac.	Common
Large-mouthed black bass	<i>Micropterus salmoides</i> Lac.	Common
Blunt-nosed minnow	<i>Pimephales notatus</i> Raf.	Very common
Steel-colored minnow	<i>Notropis whipplii</i> Gir.	Abundant
Common shiner	<i>Notropis cornutus</i> Mit.	Abundant
Golden shiner	<i>Abramis crysoleuca</i> Mit.	Abundant
Common sucker	<i>Catostomus commersonii</i> Lac.	Common
Bullheads	<i>Ameiurus nebulosus</i> LeS.	Common
Brook silverside	<i>Labidesthes sicculus</i> Cope	Occasional
Rainbow darter	<i>Etheostoma coeruleum</i> St.	Common

The small sunfish, *Lepomis humilis*, was used as a standard fish. It is only about 4" long when adult, is widely distributed in Illinois and without value as a food fish. A sufficient number of other fishes were studied to make its relative sensitiveness clear, and minnows and one of the basses were nearly always used in reaction experiments. Minnows were used also to show toxicity.

The condition of individual fishes is also a matter of importance. In a few cases fishes with obvious external protozoan parasites were killed in coal-tar products, and in every case they died sooner than the

normal fish. Thus in detailed work it is important to open and examine all fishes dying sooner than other fish of the same size.

When fishes are brought into the laboratory they do not ordinarily take food and are often not well-fed or in a semi-starved state when the experiments are performed. Wells found that in the case of salts the resistance to adverse conditions is slightly increased by starvation. To test this, fishes were kept in the aquaria from May 15 to Aug. 23. All died but six; those which died being their only source of food. On Aug. 23 fishes recently caught were compared with the starved ones. The starved fishes were from 3 to 3½ inches long (7-9 cm.) and had an average weight of 7.6 gm. while fishes of this length collected from the streams weighed twice as much. In the fresh waste the starved fish died somewhat sooner on the average though the time of some individuals of about the same length as the well-fed individuals was almost the same as the latter. In aerated waste the starved fishes lived longest. On account of the small number (six) of starved fishes available the experiment could not be carried out on a large enough scale to establish significant averages but there was nothing to indicate that any important differences existed.

IV. GAS-WASTE—ITS CHARACTER AND CONSTITUENTS.

The waste of the Champaign gas plant consists of what is known as the "drip", which accumulates in the bottom of the holders and in the pipes leading to and from them, also in the mains throughout the town. It consists of water with illuminating gases and other coal products in solution. On the surface of this water a light tar floats, while some heavy tar may rest at the bottom.

The waste is pumped from the inlet and outlet of the holder onto the ground beside the tank, and is alleged to flow into the Boneyard Creek in wet weather. The light tar is used by the gas-works people for paint, for which purpose it appears to have some value. It dries hard and rather quickly. The heavy tar is removed but as is the usual case with small plants, everything else is thrown away.

Coal-tar is an excessively complex mixture of chemical compounds many of which occur in its distillation between naphthalene on the one hand and anthracene on the other. It contains nitrogenous compounds, chiefly of a basic nature. The usual constituents of the waste and tar varies with the coal used, the temperature and the method of washing and testing the gases etc. during the process of manufacture and the amount of water gas added.

These constituents may be classified and described as follows (Lunge '00).

A. *Nitrogenized Compounds.*

Of this group ammonia and its salts are of constant occurrence. The volume of ammonia in the drip from the Champaign holder inlet is usually about 200% of the volume of liquid. The salts are abundant in all parts of an ordinary plant. Such well known compounds as ethylamine, aniline, pyridine, and quinoline belong to this group.

B. *Sulphuretted Compounds.*

To this group belong such well known compounds as hydrogen sulphide, sulphur dioxide and carbon bisulphide, and the less well known liquid thiophene, which is common as an impurity in benzene. All are very poisonous.

C. *Oxygenized Compounds.*

In this group are included such well known substances as acetone, acetic and benzoic acids, and phenol and the cresols.

D. *Hydrocarbons.*

To this group belong the solids phenanthrene, anthracene, naphthalene, and the volatile liquids, xylene, toluene, benzene, etc. The gases are numerous, including acetylene, ethylene, and methane.

E. *Carbon Oxides.*

These are the two well known gases carbon dioxide and monoxide.

Gas waste from plants which remove only the heavy tar may be regarded as containing all of these compounds. The dissolved gases of course escape into the air but are held in great quantity and given off slowly from the tarry materials.

V. TOXICITY OF WASTES FROM THE CHAMPAIGN PLANT.

The toxicity of different samples differs greatly, some samples being ten or twelve times as toxic as others. This depends upon the interval since the main was pumped and whether it comes from the inlet or the outlet to the holder. Attempts were made to determine the toxicity of waste by means of indicators and acid. There appears to be no relation between the amount of normal acid required to produce a red color with methyl orange and toxicity to fish. The same difficulty was encountered when normal alkali was used. Likewise

the amount of iodine absorbed appeared to bear no definite relation to toxicity. It is probably best to determine the toxicity of waste with fishes rather than by chemical means.

1. METHODS OF EXPERIMENTING WITH WASTE.

a. Standing water.

Battery jars 5 inches in diameter and 8 inches deep are filled to a depth of 4 inches (10 cm.) with waste diluted for use. This gave 2,000 cc. of liquid with 113 sq. cm. of exposed surface and gave conditions under which one or two fishes would live for days. This method simulated in a general way the conditions in polluted standing water. The period of toxicity determination being one or two hours the method was free from serious objections.

b. Running Water Method.

A bottle with a very wide neck, holding a liter is fitted with a rubber stopper in which are three holes (M. Fig. 1). A 12 liter aspirator bottle (Fig. 1 W) with stopper tubulature is closed at the bottom aperture and filled with waste about ten times as strong as is required for the experiment at hand. One part of the diluted waste is run into the bottle through one opening in the three holed rubber stopper, while 9 parts of water are introduced through another. The water flows from this bottle into a larger bottle holding about three liters, in which the fishes were confined. The flows were set with pinch cocks on rubber tubing and adjusted from time to time. The flows used varied from time to time but usually were between 100 to 300 cc. per minute. The object was to secure definite concentrations rather than definite flows, as all that is necessary is to change the water often and simulate the conditions in running streams. The temperature of such experiments was usually 17°C.

c. Bottle method.

For determining the exact toxicity of any sample of waste when unexposed to the air it is necessary to proceed in an entirely different way. A bottle with a wide mouth, holding a little more than four liters, is supplied with a close fitting rubber stopper. It is first filled with water to the four liter mark scratched on the outside. A definite amount of waste is then run in from a burette or Mohr's pipette. The bottle is then shaken until all of the substance is in solution. The free air space, which should not exceed 2% of the volume of the

water, serves when the bottle is laid on its side to show any undissolved substance lighter than the water, thus making the method later applicable to the light slightly soluble constituents of waste. The temperature of such experiments was usually 20°C.

2. TOXICITY OF WASTE AND TAR.

The toxicity of waste from the Champaign plant varies so that a general statement as to the toxicity can hardly be made. In general the greater the amount of tar the more toxic the waste. The most toxic sample contained much tar. Eight hundredths of a cc. of the waste was introduced from a Mohr pipette into four liters of water in a four liter bottle. The water was shaken until all the waste had gone into solution excepting a slight tarry film on the sides of the bottle near the surface of the water. It is impossible to say how much of the substance actually went into solution, but assuming that half of it did, it may be safely said that ten to twenty parts per million of this waste killed a 4-5 gram *Lepomis humilis* in an hour, while twice that amount killed such fishes in from ten to thirty-five minutes. Another sample with less tar killed fishes of the same size in five hours when 1,000 parts per million were present.

A small amount of tar was rubbed on the sides of several full grown *Lepomis humilis* and the fishes left in open aquaria; all died in from one to nineteen hours.

A small amount of tar was rubbed in the mouths and on the sides of several suckers and orange-spotted sunfishes in open aquaria. All died in from one to nineteen hours. Marsh ('07) found tar very toxic to perch and bass.

Aerating and boiling removed toxic constituents of the waste. For example, a sample of waste was treated as follows:

1. Fresh waste was added to 99 times its volume of aerated water as quickly as possible, and the small space above the water in the large bottle was filled with illuminating gas.

2. Some of the same waste was aerated by pouring from one beaker to another for three minutes. This was added to 99 parts of water and corked.

3. Some of the same waste was boiled vigorously for several minutes, until all odor of ammonia was removed, and added to 99 parts of aerated water.

The effect of these treatments is illustrated by the following experiment which is one of many. A liter of each of the three kinds of waste was put into each of three battery jars and 4-5 gm. orange-spotted sunfishes placed in them. They survived as follows:

	Fresh	Aerated	Boiled
The fishes lived . . .	23 minutes	81 minutes	150 minutes

These samples of waste were kept in loosely stoppered bottles for nearly a year and still showed differences of lesser magnitude.

The fractional distillation of tar yields various different substances. One of these distilled off at 260°–290°C., which gives heavy constituents only, was supplied by the Department of Chemistry and proved very toxic.

1000 pts. per million (by volume), not all in sol.,	killed various fishes in	5-10 min.
250 " " " " " " " " " "	" " " " " "	10-20 "
125 " " " " " " " " " "	" " " " " "	25-40 "
90 " " " " " " " " " "	" <i>Lepomis humilis</i> "	40-60 "
70 " " " " " " " " " "	" " " " " "	55-60 "

Since apparently not more than half of these amounts went into solution and tarry film adhered to the bottle, the heavier parts of the tar are very toxic and remain so for long periods.

3. TOXICITY OF ILLUMINATING GAS AND CONSTITUENT GAS-MIXTURES.

As shown by Marsh ('07), illuminating gas is very toxic to fishes. It is difficult to determine how much gas is required to produce fatal results in a sufficiently short time as the constituent gases go into solution with different rapidity and the constituents in a given sample of dissolved gas are difficult to determine, so that no attempt was made to analyze them. The illuminating gas was introduced from an inverted bottle (U) as shown in figure 1. The gas was led into a bottle by displacing water and the bottle stoppered with a two hole rubber stopper wired in place and containing one tube reaching to the bottom and another passing through the cork only. A valve leaking by drops was attached to the short arm and the water thus forced into the bottle, drop by drop. The gas was accordingly forced into the cooling and solution coils of the apparatus, much of it going into solution, but a quantity passing through to the mixing bottle and collecting, where it was allowed to escape from time to time.

A mixture of ethylene 30 cc. per liter, carbon monoxide approximately 6 cc. per liter and sulphur dioxide about 33 cc. per liter killed the standard fish in 15 minutes.

4. REACTIONS OF FISHES TO WASTE.

Much of the danger to fishes from pollution of streams, especially where the pollution is local, is determined by the reactions of the fishes

to the polluting substances. Fishes turn away from dangerous substances which are normally found in their usual environment, but with strange and unusual substances such as are thrown into streams by gas-works and other industrial plants, they frequently enter and follow up to points where the 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. (Chart II, graphs 8-11; Chart V, graph 6o.)

Conditions and Methods of Study.

The experiments were performed in a gradient tank (N), figure 1. The tank used in these experiments was 122.3 cm. long, 15 cm. wide, 13 cm. deep. The front wall was of plate glass and a plate glass top was used at times. Water of two kinds, normal and polluted, was used in the experiments. One kind was allowed to flow into one end at a definite rate and another kind into the other end at the same rate. It flowed out at the middle at the top and at the bottom so that the two kinds of water met at the center. The outflow at the center did not of course prevent the mixing of the two kinds of water and thus the middle section, equal to one half or one third of the tank was a gradient between two kinds of water. The water entered both ends at the same rate (usually 600 cc. per minute) through tees the cross-bars of which contained a number of small holes. The cross-bars of the tees were at the center of the ends of the tank behind screens. The drain openings were located at the center near the top and in the bottom. The outer openings of the drain tubes were at the level of the water in the tank. We found no evidence that fishes reacted to the slight current produced by the water flowing in at the ends and drifting toward the center and out through the drains. Since each half of the tank held about 9 liters, it required 15 minutes to fill it or to replace all of the water in one of the halves. The tank was enclosed under a dark hood. Two electric lights were fixed in the rear and above the center of the two halves, i.e., above a point midway between the screen partition and the center drain. The light was 15-20 cm. above the surface of the water which was 13 cm. deep. The experiments were observed through openings in the hood above the lights or through the glass side late at night. Fishes do not usually note objects separated from them by a light.

Water differing as little as possible from that in which the fishes usually live was used for control readings. Controls were observed and conditions in the two ends of these were the same either because the water introduced at the two ends was alike or because no water

was run into either end (standing water). In the controls (Chart I) the fishes usually swam from end to end in a rather symmetrical fashion, and thus comparing these movements with those occurring when the fishes encountered differences in water, we are able to determine the reactions of the fishes to the differences.

When the differences between the solutes at the two ends of the tank were not great we found by chemical tests that the central portion of the tank was a gradient between the characteristic waters introduced at the two ends. Usually the end thirds were essentially like the inflowing water. When the difference in concentration was great the region of the gradient was proportionally longer and the ends with the inflowing concentrations correspondingly shorter. When the difference in concentration was very great the entire tank was gradient. For an experiment a fish was placed in a dish containing enough water to barely cover it and set above the tank. When all was in readiness the fish was liberated in the center of the tank. Marks on the sides divided the tank into thirds. The fish nearly always swims back and forth, apparently exploring the tank. The movements of the fish were recorded graphically as shown in Chart I.

For this purpose sheets of ruled paper were used. Four vertical double rulings corresponded to the thirds and two ends of the tank. Distance from right to left was taken to represent the length of the tank, vertical distance to represent time and the graphs drawn to scale. The width of the tank was ignored. The graphs on the following pages are copies of the originals. The experiments were conducted with water at about 17°C.

Before or after the experiment, the headings of the sheets were filled with data regarding the kind, size, and previous history of the fish, the conditions in the tank, concentration of the solutes and other significant data. The fish was observed continuously for twenty or more minutes. Fishes are positive to waste in all concentrations tried.

Fishes are positive or indefinite to illuminating gas, and to combinations of the most important illuminating gas constituents in both acid water with 2-3 cc. of oxygen per liter and in alkaline water at oxygen saturation (Chart II, graphs 11 and 12; Chart V, graphs 53 and 54).

VI. THE TOXICITY OF ILLUMINATING GAS WASTE CONSTITUENTS.

The following table shows the relative toxicity of the chief constituents of gas-waste arranged according to the outline on p. 389. On the pages following it are given occurrence of the substance, the method of work, physiological effect, and the reactions of fishes. The

experiments were performed in the kind of water in which the fish had been living, containing about 3 cc. per liter of oxygen and 4-6 cc. per liter of CO₂. Some experiments were carried on in running water, some in jars exposed to the air, as described on page 390, and in case of volatile substances the amount required to kill a standard fish in an hour was determined in a corked four-liter bottle as described on the same page.

TABLE II

SHOWING THE RELATIVE TOXICITY OF VARIOUS PRODUCTS ASSOCIATED WITH THE MANUFACTURE OF COAL GAS. THE TOXICITIES ARE BASED ON THE AMOUNT OF THE SUBSTANCE REQUIRED TO KILL IN ONE HOUR A SMALL SUNFISH (*LEPOMIS HUMILIS*) WEIGHING 4-6 GRAMS.

The values given are approximately correct within the limits stated. Temperature 20° C except in the case of gases where it was 16-17° C.

Substance		Solid, gm. per l., Liquid, cc. per l., or Gas, cc. per l.	Parts per Million	Remarks	
A. Nitrogenized Compounds.					
Ammonia.....	NH ₃	Gas	8,000-10,000 cc.	7-8	Spasms common
Ammonium Carbonate....	(NH ₄) ₂ CO ₃	Solid	0.600-0.800 gm.	600-800	" "
Ammonium Chloride.....	NH ₄ Cl	Solid	0.700-0.800 gm.	700-800	
Ammonium Sulphate.....	(NH ₄) ₂ SO ₄	Solid	0.420-0.500 gm.	420-500	
Ammonium Sulphocyanate	(NH ₄)NCS	Solid	0.280-0.300 gm.	280-300	
Ammonium Ferrocyanide.	(NH ₄) ₄ Fe(CN) ₆	Solid	0.150-0.200 gm.	150-200	
Ethylamine.....	NH ₂ (C ₂ H ₅)	Liquid	400-800	Not accurately determined
Aniline.....	C ₆ H ₇ N	Liquid	1.000-1.100 cc.	1020-1122	
Pyridine.....	C ₅ H ₇ N	Liquid	1.500-1.600 cc.	1477-1576	
Quinoline.....	C ₈ H ₇ N	Liquid	0.048-0.052 cc.	52-56	Paralyzes; death point determined by mechanical stimulation
Isoquinoline.....	C ₉ H ₇ N	Liquid	0.060 cc.	65	
Pyrrol.....	C ₄ H ₅ N	Liquid	Not studied; unavailable
B. Sulphuretted Compounds					
Hydrogen Sulphide.....	H ₂ S	Gas	3.250-3.500 cc.	4.9-5.3	
Sulphur Dioxide.....	SO ₂	Gas	5.500-6.600 cc.	16-19	
Carbon Bisulphide.....	CS ₂	Liquid	0.090-0.100 cc.	100-127	Larger fishes die first
Thiophene.....	C ₄ H ₄ S	Liquid	0.025 cc.	27	
C. Oxygenized Compounds					
Acetone.....	C ₃ H ₆ O	Liquid	18.000-19.000 cc.	14,250-15,050	
Benzoic Acid.....	C ₇ H ₆ O ₂	Solid	0.550-0.570 gm.	550-570	
Phenol (Carbolic Acid)..	C ₆ H ₆ O	Solid	0.07-0.075 gm.	70-75	
Orthocresol.....	C ₇ H ₈ O	Solid	0.055-0.065 gm.	55-65	
Paracresol.....	C ₇ H ₈ O	Solid	0.080-0.090 gm.	80-90	
Metaresol.....	C ₇ H ₈ O	Liquid	0.120-0.130 cc.		

TABLE II—Continued

Substance		Solid, gm. per l., Liquid, cc. per l., or Gas, cc. per l.	Parts per Million	Remarks	
D. Hydrocarbons					
Phenanthrene.....	$C_{14}H_{10}$	Solid	0.001-0.002 gm.	1-2	Rated "insoluble"
Anthracene.....	$C_{14}H_{10}$	Solid	Only slightly toxic?
Naphthalene.....	$C_{10}H_8$	Solid	0.004-0.005 gm.	4-5	Rated "insoluble"
Xylene.....	C_8H_{10}	Liquid	0.055-0.056 cc.	47-48	Rated "insoluble"
Toluene.....	C_7H_8	Liquid	0.070-0.080 cc.	61-65	Rated "insoluble"
Benzene.....	C_6H_6	Liquid	0.040-0.042 cc.	35-37	Rated "insoluble"
Acetylene.....	C_2H_2	Gas	Sat. sol.	No deaths
Amylene.....	C_6H_{10}	Liquid	1.000-1.250 cc.	655-693
Ethylene.....	C_2H_4	Gas	18.00-20.00 cc.	22-25
Methane.....	CH_4	Gas	Not toxic—only one Labidesthes sicculus died
E. Carbon Oxides					
Carbon Monoxide.....	CO	Gas	16.00 cc.	75
Carbon Dioxide.....	CO ₂	Gas	See Wells' Art.
F. Mixtures					
Tar Acid.....		Liquid	0.07 cc.	70	Pts. per million by volume
Illuminating Gas.....		Gas	Amount not deter- mined
Waste.....		Liquid	0.020-0.040 cc.	20-40	Amount not deter- mined
Waste.....		Liquid	0.002 cc.	2	To kill in 24 hours
Boiled Waste.....		Liquid	Toxicity less than aerated
Aerated Waste.....		Liquid	Toxicity less than unboiled
Tar.....		Liquid	Rubbed on fishes, kills in 18 hrs.

Ammonia.

The amount of ammonia in gas-waste from which it has not been commercially removed is great. Champaign-Urbana Gas-works' waste pumped from the holder intake showed about 200% volume in waste not containing tar and 400% volume in waste containing a small amount of tar. No ammonia is recovered at this plant. The intake and outlet are pumped out daily, or two or three times per week. This waste is pumped onto the ground and finds its way into a small stream.

A solution approximately half normal (8.517 gm. per l.) was made and dilutions of this were made up quickly in 500 cc. of distilled water in 750 cc. wide-mouthed glass-stoppered bottles. Fishes were quickly dropped in and after they had died and the time to death was noted the solution was titrated with a standard acid and litmus indicator. When the killing concentration was determined approximately a solution was made up and the results verified in the 4-liter bottle.

In man ammonia causes comatose conditions or delirium and dyspnoea, death coming very suddenly (Witthaus and Becker). In frogs and mammals it causes increased reflex irritability which may be followed by tetanic convulsions (Cushny). When first placed in ammonia solution which will produce death in an hour or less, fishes are often much stimulated, the head often floating lower than the rest of the body. Erratic movements follow after a time and the fish usually turns over in convulsion and remains comparatively still with peculiar twitchings of the tail and fins until death. Ammonia is less toxic in distilled water than in water with carbonates. Five parts per million is fatal to fishes. Weigelt found that 0.1% solution killed tench in 45 minutes.

Fishes do not ordinarily turn back when they encounter ammonia, but swim into it without giving any of the avoiding reactions which characterize the movements of fishes with reference to such environmental substances as carbon dioxide. The reaction is usually indefinite or indifferent (Shelford and Allee, Wells). *Pimephales* was positive to it in alkaline water; *Lepomis humilis*, *Notropis* and *Abramis crysoleuca* were positive to fatal concentrations in acid and in alkaline water (4-6 cc. per l. of CO₂). Wells found that in very strong concentrations the fish selected a point in the gradient tank near the centre and avoided both ends but the concentration of ammonia in the part of the tank selected was such as to cause the death of the fishes in a short time (Chart II, graphs 14 and 15; Chart V, graph 55).

Ammonium carbonate.

It is present in quantity in liquors from all parts of the manufacturing plant, most abundantly from the last condenser and washers. A solution of 20 gm. per liter was prepared and its actual strength determined by titrating with standard hydrochloric acid and methyl orange indicator. Its general effects are similar to ammonia. Erratic movement occurs oftener than in the case of ammonia. Fishes are usually positive to strong concentrations, e. g., 1 gm. per liter, but they do not act with precision as in the case of other alkalies (Chart II, graph 16).

Ammonium chloride.

It is abundant in the liquor from all parts of an ordinary plant (Lunge 3d ed., 741).

A solution was made by dissolving 8 gm. per liter of water. The solution was tested by adding a definite quantity of NaOH to a known amount of the solution and boiling until the vapor did not turn litmus blue. The remaining free alkali was titrated with standard acid and

methyl orange indicator. It tends to paralyze the terminations of the motor nerves of the frog. In the case of fishes, it shows no important differences from ammonia. Reactions are usually positive to strong concentrations and negative to weak ones (Chart II, graph 17).

Ammonium sulphate.

It is present in waste in small quantities. A solution was made and tested as in the case of ammonium chloride. It is much less toxic than the carbonate. Its toxicity is greatest in the presence of carbonates (Wells '15). When all the carbonates have been transformed into sulphates it becomes less toxic. Fishes are positive in reaction to fatal concentrations and negative to weak concentrations (Chart II, graph 18).

Ammonium sulphocyanate.

It occurs in some quantities in first condensers and washers. A solution was made by weighing out the salt and dissolving it in distilled water. The strength of the solution was determined by adding a strong alkali and distilling off the ammonia into standard acid and titrating with standard alkali. It is much more toxic than the chloride. The fish showed no striking symptoms. Fishes are positive to fatal concentrations (Chart II, graphs 19 and 20).

Ammonium ferrocyanide.

It occurs in small quantities in liquor from last condensers. The general method of experimentation and determination was the same as in the case of ammonium sulphocyanate. The physiological effects are not striking on the fishes. Fishes are positive to fatal concentrations (Chart III, graph 21).

Ethylamine.

Five grams of Kahlbaum's hermetically sealed product was broken into two liters of water but with an obvious loss. Dilutions from this were made and tested. Its effect appears to be in a general way like that of ammonia. Fishes were positive in a single experiment (Chart III, graph 22).

Aniline.

A sample of Kahlbaum's, slightly brown, was used, the proper dilutions being made by adding a small quantity from a burette to 4 liters

of water. In man it causes prostration, giddiness, vomiting, and neuralgic pains. In fish it produces anesthesia. There is a considerable stimulation at first, quickly followed by trembling of the fins and some erratic movements. The fishes turn upon their sides in two minutes. They may live for hours, with their fins and gills moving slightly. Five cc. per liter of water in the open battery jar killed fishes after the mixture had been standing three days. Fishes are indefinite or positive to the concentration used (.08-.12 cc. per liter) (Chart III, graph 23).

Pyridine.

It occurs in coal-tars, crude benzene, toluene, etc. In man it causes paralysis of the motor centers and nerves, and movements become weak and unsteady. Death follows from a failure of respiration (U.S.D.). The effects on fishes are similar to those of many other substances. Heavy respiration is usually noted early and they usually die with opercles and mouth closed probably through failure of respiratory movements. Fishes are positive or indefinite in all cases studied (Chart III, graph 24).

Quinoline.

It occurs in coal-tar. It is a powerful antipyretic but causes general collapse. In .025 cc. per liter of water fish usually turn on their sides in 10-15 minutes and continue to move their fins and gills for many hours. In strong concentrations fishes are very quickly paralyzed and there is some difficulty in determining when they die, as movements of the mouth and opercles cease long before death occurs. If the fish are removed and handled roughly life may be detected by a general body movement.

A solution of it turns pink on standing and its toxicity gradually falls off on exposure to air. A solution which killed fishes in 2 minutes Nov. 1, killed them in 1 hour Nov. 13 and after several days on Dec. 10.

Fishes are usually negative in reaction to both fatal and more dilute solutions, though the standard fish was positive in two cases (Chart III, graph 25).

Isoquinoline.

Its toxic effect differs but little from quinoline. The reactions of fishes are strikingly different, being usually positive instead of uniformly negative as in the case of quinoline (Chart III, graph 26).

Pyrrrol.

This compound was not studied as none could be obtained.

Hydrogen sulphide.

This gas occurs in illuminating gas and in solution in waste. It occurs also in the decomposition of organic matter in water and forms an important part of the gas generated in connection with sewage contamination. It occurs in small quantity at the bottom of lakes (Birge and Juday, '11) but is a very important gas in salt lakes especially those with a thermocline and in thermocline arms of the sea. Marine organisms thus often encounter it, fresh water organisms only in small quantities. For experimental purposes it was generated in a Kipp generator of large size by the action of hydrochloric acid on iron sulphide. The generator afforded sufficient pressure to force it through the mixing bottle direct. It was determined with standard iodine. Fifty cc. were drawn from the mixing bottle with a 50 cc. pipette and introduced into an Erlenmeyer flask as quickly as possible and N/100 iodine quickly added until a brown color was obtained. The mixture was then carefully titrated with N/100 sodium thiosulphate which had been corrected until it was essentially the equivalent of the iodine, and the amount of thiosulphate used was deducted from the amount of iodine put into the flask. When greater accuracy was desired the determination was repeated, iodine was placed in the flask and the 50 cc. of H₂S water added to the excess of iodine beneath the surface and contact with the air essentially prevented. These determinations should be made with great care as slight differences in manipulation gave very variable results in the fish-killing experiments, with concentration which differed only in the error caused by slightly different exposures to the air in rapid manipulation.

When much diluted it produces nausea, pain in the head, and great general weakness, followed by coma. In concentrated solutions it produces loss of consciousness very quickly. In fishes the symptoms do not appear more quickly than in the case in solutions of other substances. It is more toxic when accompanied by little oxygen. Two cc. per liter are fatal to fishes. Water in a battery jar exposed to the air with 4 cc. per liter at the beginning killed fishes in 18-24 hours; meanwhile the concentration fell to less than two cc. per liter and the life of the fishes was prolonged by access to the surface. Hardy species of fish live in 1-1.8 cc. per liter without apparent injury. Weigelt found weak solutions fatal to tench.

Fishes are often positive to a weak concentration which would produce death in a few days, but are negative to strong concentrations as

a rule. When in solution the gas forms a very weak acid which would tend to cause fishes to react positively especially in alkaline water. The negative reactions are less definite than to carbon dioxide and other stimuli often encountered in natural environments of the fishes (Chart III, graphs 27 and 28).

Sulphur dioxide.

This gas was determined in the Champaign waste which showed in sample without tar 13.84 cc. per liter and sample with tar 56.21 cc. per liter. For the experimental work a tank of Kahlbaum's compressed gas was obtained from the University of Illinois chemical store room and the tank was attached to the gas introducer direct.

It is very irritating to the mucous membrane and the respiratory tract. It is generated in the burning of sulphur and its characteristic odor is familiar. In the case of fishes, gulping or other similar movements indicates its irritating character. Their respiration is usually heavy and they swim around in an intoxicated state for some time before death. A strong solution killed fishes after standing in a battery jar for over two weeks. Weigelt found that 0.0005% solution killed trout in a little more than one hour.

Fishes are usually negative to higher concentrations from 10 to 500 cc. per liter which produce death in a few minutes, but are quite generally positive to concentrations which are fatal in an hour (Chart III, graphs 29 and 30).

Carbon bisulphide.

It occurs commonly in crude benzene, and in the tars and other residuals. Known quantities were dissolved in 4 liters of water. It is a powerful poison to man, the vapor producing hysterical neurosis and the liquid taken internally produces unconsciousness quickly. In the case of fishes the symptoms are not markedly different from those caused by a number of other substances; they appear to become intoxicated rather slowly.

In .05 cc. per liter of water fishes were intoxicated but recovered after an hour and a half. The experiment was performed in a closed bottle so that evaporation could hardly be responsible. It is possible that the substance was absorbed or rendered harmless by some tissue such as fat.

Two species of sunfish were tried in the gradient tank and both were positive to fatal concentration while the minnow (*Pimephales*) was negative (Chart III, graph 29 and 30).

Thiophene.

It occurs as an impurity in commercial benzene. It is not particularly poisonous to man. In a one hour fatal concentration fishes are intoxicated showing signs of stimulation and some staggering after about 20 minutes. These symptoms were followed by intoxication. Sunfishes, basses and minnows were all positive to fatal concentrations (Chart III, graphs 33 and 34).

Acetone.

It occurs in connection with benzene (Lunge 3d ed, pp. 176). For the fish experiments it was added to the water and the final experiments performed in a four-liter bottle. It is only slightly poisonous to man and least toxic to fishes of the compounds studied. Fishes of various sorts are positive to it, particularly to the weaker concentrations (Chart IV, graph 35).

Benzoic acid.

It occurs as a residual in the manufacture of phenol, and in coal tar "oils". It is less toxic than most other coal tar products. It appears to be only slightly poisonous to man. The dry powder was weighed and dissolved in water. Fishes of various sorts are negative to it (Chart IV, graph 36).

Phenols and the Cresols.

According to Witthaus and Becker, 5% solution of any of these coagulates protein, narcotizes partially and finally paralyzes the nervous system. It occurs in coal-tar and in gas-liquor and is one of the sources of the toxicity of wastes. For the work on fishes the solid crystals were melted, one cc. was accurately measured, and added to a liter of water, and dilutions made from this solution, which was kept tightly corked.

Phenol is a powerful irritant, producing stupor and shallow irregular breathing in man when taken in small quantities. Larger quantities are rapidly fatal. Strong concentrations such as 1 cc. per liter are very rapidly fatal to fishes, but solutions of $\frac{1}{2}$ to $\frac{3}{4}$ this amount kill standard fishes only after one hour or more. In such solutions the fishes make a few erratic movements, turn on their sides and remain for a long period with faint respiratory movements and slight swimming movements of the fins. Fishes dying in it are characterized by a gaping condition of the gills which is common if

not general among fishes dying in waste. This is probably due to the irritating character of the substance. The toxicity of the water containing it is retained for several weeks, depending on the concentration at the beginning. Sixty-five parts per million will still produce fatal results after a month's exposure in the battery jars. Weigelt killed a tench in a 0.05% solution in 15 hours. Hofer ('99) obtained results which more nearly resemble mine.

Several species of fish which were tried in the gradient tank gave positive reactions to concentrations which would kill them in two or three hours (in both acid and alkaline water). Orange-spotted sunfish are indifferent to very weak concentrations (Chart IV, graph 37; Chart V, graph 57).

Cresols.

Orthocresol is less caustic than phenol but was found to be more poisonous to fishes. Fishes of various sorts were negative or indefinite to concentrations about twice that required to kill them in an hour, but were sometimes positive to weaker fatal concentrations (Chart IV, graph 38).

Paracresol is a little less toxic than phenol. A solution which killed small fishes in 20 minutes, killed similar fishes in less than 12 hours after 2 months exposure to air in a 5x8 battery jar. Fishes were uniformly positive or indifferent in both strong concentrations and very weak ones (Chart IV, graph 39; Chart V, graph 56).

Metacresol is least toxic of the four representatives of the group. The erratic convulsive movements often occur when intoxication sets in. Various fishes are variable in their reaction to fatal concentrations, but commonly when negative at the beginning of the experiment the protective reaction breaks down due to the paralyzing of the sensory nerve endings (Chart IV, graph 40).

Phenanthrene.

It is found in the last fractions of the distillation of coal tar "oils".

For the experiments on fishes a quantity of it was placed in a five gallon bottle of tap-water and warmed and shaken from time to time during several days. This solution was used for most of the fish experiments. In December, 1915, two liters of distilled water were placed in each of three bottles. Phenanthrene was added to one to make 100 mg. per liter, to another to make 5 mg. per liter, and to the third to make 2.5 mg. per liter. These were allowed to stand with occasional shaking till August, 1916. At this time there were still

crystals in the 100 mg. and 5 mg. bottles but none in the 2.5 mg. bottle. The water in the bottle containing 5 mg. per liter killed fishes in a somewhat longer time than the tap water solution but since the fishes available were larger and many substances are commonly less toxic in distilled water, the experiments were taken to indicate the approximate toxicity given in the table. One half gram of phenanthrene and one cc. of quinoline in two liters of water exposed to air proved fatal a month after being placed in solution.

Fishes are usually positive or indefinite to saturated solutions of phenanthrene (Chart IV, graph 41).

Anthracene appeared not to be toxic to fishes.

Naphthalene.

Ordinary tar contains 5–10% of naphthalene. It is commonly said to be insoluble, but evidently about 5 parts per million may be dissolved. The experiment of adding definite quantities to distilled water was conducted in the same manner as in the case of phenanthrene; no crystals were left in the bottle with 5 milligrams per liter.

In man it causes delirious intoxication. There are no violent symptoms in fishes; they are gradually intoxicated, turn on their sides and die without special symptoms or erratic movements.

It is very much more toxic in tap water containing much carbonate than in distilled water. Most fishes die in about half the time in saturated tap water as in distilled. Fishes are usually positive to naphthalene (Chart IV, graph 43, and Chart V, graph 58) but occasionally negative (Chart IV, graph 42).

Xylene.

It is present in coal-tar distillates. The experiments with fishes were performed in the four-liter bottle, which was laid on its side after the xylene was added and shaken until the surface film of the exposed portion was free from droplets of the substance.

It is more toxic to man than benzene or toluene. To fishes it is more toxic than toluene and less toxic than benzene. Fishes are gradually intoxicated in less than 50 parts per million, frequently making erratic movements, jumping up in the gradient tank, etc. They usually lie on their sides until death ensues. In nearly every case fishes are positive to it. Two species of sunfish were positive to it and remained in the stronger solution until intoxicated (Chart IV, graph 44).

Toluene.

It occurs in coal-tar and has almost the same effect upon fishes as xylene, but is a little less toxic. The reactions of fishes were almost invariably positive to fatal concentrations (Chart IV, graph 45; Chart V, graph 59).

Benzene.

Benzene occurs in coal-tar and is more toxic to fishes than either toluene or xylene. In man it causes convulsions, rapid respiration, coma and lowered temperature. In the case of fishes it intoxicates them rapidly. There is considerable erratic movement and death ensues much as in the case of toluene or xylene. Fishes are commonly negative or indifferent to benzene, their reactions to it thus differing from the reactions to the other substances. They often jump out of the gradient tank (Chart IV, graphs 46 and 47).

Acetylene.

It occurs in illuminating gas but is only slightly poisonous to man. A nearly saturated solution in running water anesthetized fishes but they recovered as soon as the amount was reduced. Fishes are usually positive to it.

Ethylene.

A determination of samples of Champaign waste showed 200–300 cc. per liter in solution. The ethylene used was made by heating ethyl alcohol and sulphuric acid and washing in alkali and water. Fishes are anesthetized, lose equilibrium gradually, and die without violent symptoms.

18 cc. per liter of ethylene (18 pts. per million), in running water, oxygen at saturation, kills 3–4 gram orange-spotted sunfish in one hour.

30 cc. per liter (30 pts. per million), and oxygen 2.25 cc. kills 3 gram orange-spotted sunfish in 24 minutes; 3 grams *Notropis* in 14 minutes; 3 gram *Pimephales* in 30 minutes.

In standing water exposed to the air in battery jars, such water killed fishes as follows:

TABLE III

Hours of exposure to the air	Fish species	Weight	Time to death
0-2	Orange-spotted sunfish	4 grams	53 minutes
0-2	Pimephales	1.8 "	16-30 "
2-4	Orange-spotted sunfish	4.2 "	90 "
2-4	Pimephales	1.4 "	22 "
4-6	Pimephales	1.3 "	38-45 "
19-24	Orange-spotted sunfish*	4.0 "
19-24	Pimephales	1.3 "	100 "

* Affected but did not die.

In nearly all the experiments tried the fishes were positive in reaction to ethylene, often being overcome in the gradient, and even dying without showing any avoidance of the ethylene water. 2, 30, and 60 cc. per liter (2, 30, and 60 pts. per million) were tried with similar results; the only suggestion of a negative reaction came in the lowest concentration (Chart V, graph 50).

Amylene.

It occurs in coal-tar but only in small quantities. Amylene was once used as an anesthetic but was found to be dangerous. It acts as an anesthetic on fishes. Various fishes are positive to it in all concentrations tried (Chart V, graphs 48 and 49).

Methane.

Methane is one of the most abundant constituents of illuminating gas. It was made by the action of soda lime on sodium acetate and washed in water. Crocker found that methane made that way was not toxic to plants while other methods yielded toxic products.

It is not toxic to man and no toxic effects were noted for fishes except in the case of a single specimen of *Labidesthes sicculus*.

VII. GENERAL DISCUSSION.

I. TOXICITY AND SIZE.

One of the very important questions arising in connection with the toxicity of substances to fish is the relation of age and size of the fishes to their resistance to the substance. In general, with the majority of compounds studied the largest fishes survived longest. While

this is true, there is, however, much variation, and some of the largest fishes died first.

A number of compounds show still further irregularity and in the cresols the larger fishes are very often first to die. In carbon bisulphide this appears to be the rule with the concentrations studied. In the case of naphthalene the larger fishes usually die first. Xylene, benzene, and toluene show considerable variation in this respect though the results with a given concentration and given weight of fish are particularly constant.

TABLE IV

Showing typical results of killing Lepomis humilis in various compounds. In sulphur dioxide the smaller fishes die first. In xylene there is some variation and in carbon bisulphide the larger appear to die first as a rule.

Compound	Concentration	Weight	Time to death in minutes
Sulphur dioxide	11cc. per l.	1.5 gm.	13
		4.3 gm.	15
		6.0 gm.	20
	37cc. per l.	3.0 gm.	8
		4.0 gm.	14
		9.4 gm.	25
Xylene	$\frac{1}{4}$ saturated Running water	4.1 gm.	30
		6.0 gm.	35
		8.3 gm.	alive at 60
	$\frac{1}{8}$ saturated Running water	2.0 gm.	90
		6.2 gm.	98
		17.0 gm.	80
Carbon bisulphide	.065 cc. per l.	7.5 gm.	25
		4.0 gm.	80

In the case of carbon dioxide and low oxygen, Wells ('13), found that the results of dividing the time to death by the weight of the fishes, secured as the time to kill a gram of fish, not a constant but a value which increased rather regularly in most cases, as the size of the fish decreased. In the case of the coal-tar products there is much more irregularity than in the case of the conditions studied by Wells.

Since the smallest individuals of given species usually die quickest the importance of contamination is greatest in connection with the younger stages. The effect upon the development of eggs is likely to be great. Gortner and Banta found that various phenolic compounds killed the eggs of frogs and other amphibians in concentration as low as 50 parts per million. The critical point for further study is in connection with the most sensitive stage. This is the weakest link in the life-history chain, and represents its strength. *It is the stage on which minimum fatal concentration must be worked out.*

2. TOXICITY AND SPECIES.

This is best determined with the use of low oxygen and carbon dioxide and the data presented by Dr. Wells in the next article in this volume shows such differences based largely on his experiments along this line. In a general way the relative resistance of the different species to coal-tar products is similar to that given in his table. According to our general experience with the orange-spotted sunfish it should be rated at 12 in Dr. Wells' table. There are, however, some outstanding exceptions. To the coal-tar products suckers are generally about as resistant as the orange-spotted sunfish, and while in most substances the green sunfish is more resistant than the orange-spotted, it is less resistant to some of the phenolic compounds.

3. FISH REACTIONS TO POLLUTING SUBSTANCES.

The study of the behavior of fishes with reference to polluting substances is a departure from the usual method of study of such relations. The graphs on the charts show that fishes are as a rule indefinite or positive to the substances which are not regularly encountered in their environments. In other words they swim into the poisonous solution without detecting it and turning back as they do in the case of low oxygen and much carbon dioxide. They may go directly into it without noting it, and after being for a brief time in the solution they very generally avoid the pure water which is identical with that in which they have been living for months even though the solution chosen caused death. In some cases fishes at the beginning tend to avoid the modified water, but soon, usually after a brief contact with the solution, begin to turn back from the pure water, having very quickly formed a preference or "habit" which keeps them in the poisonous solution. Peculiarities of *behavior* occur in some cases with reference to particular concentrations.

The behavior results are of the greatest significance to the pollution question for since fishes are positive to fatal concentrations of the vast majority of organic compounds introduced into streams by gas-works, the tendency must be for them to enter rather than avoid the portions of streams so contaminated, making the loss very much greater than it would otherwise be. The peculiarities of the reactions to the various poisons is suggestive of a possibility of investigating the physiological effects of habit-forming drugs. It is possible that detailed study of these reactions might show why habit-forming drugs produce a demand for more of the same drug, when several small quantities have been taken.

4. TREATMENT OF BY-PRODUCTS OF THE MANUFACTURE OF COAL-GAS.

The great toxicity of nearly all the representatives of the chief groups of compounds occurring as by-products of the manufacture of coal-gas render it inadvisable to permit the pollution of streams with any of these compounds. Attention is especially directed to the fact that the compounds commonly reckoned as insoluble in water by industrial chemists are among the most deadly. Further it must be noted that the volatile and gaseous products such as ethylene, carbon monoxide, benzene, xylene, etc., which doubtless go into solution in the water which is used for washing gases during manufacture, and which are least likely to be suspected of being detrimental, are among the most poisonous compounds and probably the most universally thrown into streams. Marsh ('07) has shown further that effluent from an ammonia sludge-bed, lime and iron oxide from purifiers and residuals from water-gas are very toxic.

In general the experiments leave no doubt but that earnest effort should be made to prevent the introduction of anything whatsoever in the way of coal-gas products into stream and bodies of water. No matter what method of treating the wastes may be devised the general toxicity of the entire series of compounds makes it certain that much damage will result from pollution with the residues of any form of treatment. In general however the damage to fishes will be greatest in connection with the smaller plants which ordinarily save only the heavy tar or at most tar and ammonia. If the government cannot compel gas manufacturers to make their valuable but poisonous by-products into something useful, it can at least make such an industry advisable by preventing the addition of these waste materials to streams, and if necessary conduct investigations into methods for the profitable disposal of the entire series of these by-products.

VIII. SUMMARY.

1. Illuminating gas, gas-liquor, and thirty-one out of thirty-four representatives of the chief groups of compounds found in gas and gas-liquor are very toxic to fishes. From one to fifteen hundred parts per million are fatal to an orange-spotted sunfish in one hour. (P. 391.)

2. As a rule the smaller fishes are more readily affected than the larger, down to the smallest fry studied; the minimum amount of the various substances required to kill fishes must be established by using the most sensitive stages, which are probably the smallest fry. (P. 407.)

3. Fishes usually react positively to the compounds and mixtures studied; i.e., enter the polluted water from the pure water readily and turn back into the polluted water when pure water is encountered. The danger to fishes is increased greatly thereby. Fishes often develop a "preference" for the polluted water after a number of trials of both kinds. (P. 397.)

4. On account of the extreme toxicity of gases such as CO and ethylene, and of benzene and other volatile matter, water which has been in contact with gases should not be introduced into streams. (See Article VII, also pp. 395 and 409.)

5. Various types of manufacturing and by-product recovering plants, while they remove different substances do not leave a harmless residue; on account of the fact that the very toxic substances such as carbon monoxide, benzene, and naphthalene differ widely in their properties, residues from all such plants will be almost certain to be toxic. (P. 395.)

6. The results thus far obtained may throw some light on the poisonous effect of the various compounds from the pharmaceutical standpoint, and may be of assistance in the matter of standardization of drugs with fishes.

IX. ACKNOWLEDGMENTS.

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FIGURE 1.

This figure shows the boiling and aerating device used in the experiments.

The various parts are arranged alphabetically beginning with the water supply of the water boiler, at the right side. Parts A to L on the right side make up the boiling device. The entire apparatus was built above a drain-table. Aa was the water supply pipe. Water could be passed through this directly to the float valve (Fv) or through the coil B which is a gas heater, into pipe C and thence through the float valve (Fv). From the float valve it could be passed directly into the lower tank J through G or upward through the calibrated valve F which could be set for any desired flow and into the upper boiling tank H from which it flowed into I and then J. With all these different means of introducing water into the delivery tank J any desired amount of heating or exposure to air within limits could be secured. In the tanks H, I, and J the water could be heated or boiled with gas. The apparatus would deliver about 200 cc. per minute free from oxygen and with no free carbon dioxide (decidedly alkaline to phenolphthalein). From J the water passed through the tees at k and k₁ through the three way valve (3 Wv) and through seventy-five feet of block-tin pipe to the outlets L. The gas introducer (V) could be inserted into the pipe between the two coolers and any desired gas introduced at that point. For many of the experiments with gases water was run through all three of the boilers, which usually gave from 2-3 cc. of oxygen per liter and about 5 cc. of free carbon dioxide. This is about the condition of a somewhat polluted water.

At times however this method gave very low oxygen content for some reason not obvious, and accordingly the aerating device which gave water rarely less than two and one half to three cubic centimeters per liter of oxygen and 4-5 cc. of carbon dioxide were used. In this device the water at the left end of the drain-table was brought up through a float valve (Fv) which delivered water to the corrugated incline (O) of the aerating device. From this incline the water flowed to the settling box (P) below, where most of the precipitated iron settled out. From this it flowed into the storage and withdrawal tank R. From here the water was carried downward through S and through the gas introducer (V) and through T and through the lower sixty feet of block-tin pipe in the lower cooler.

Gases were introduced under pressure as shown in the case of the bottle U. A number of valves which can be noted in the figure made possible the use of water from various sources.

The gradient tank is shown below (N), the shading representing the distribution of solutes introduced at the right. In some cases solutes were run in from the aspirator bottle (W) which was kept at water temperature by a jet of water direct from the supply pipe. In such cases the solute was added to the mixing bottle into which water was flowing.

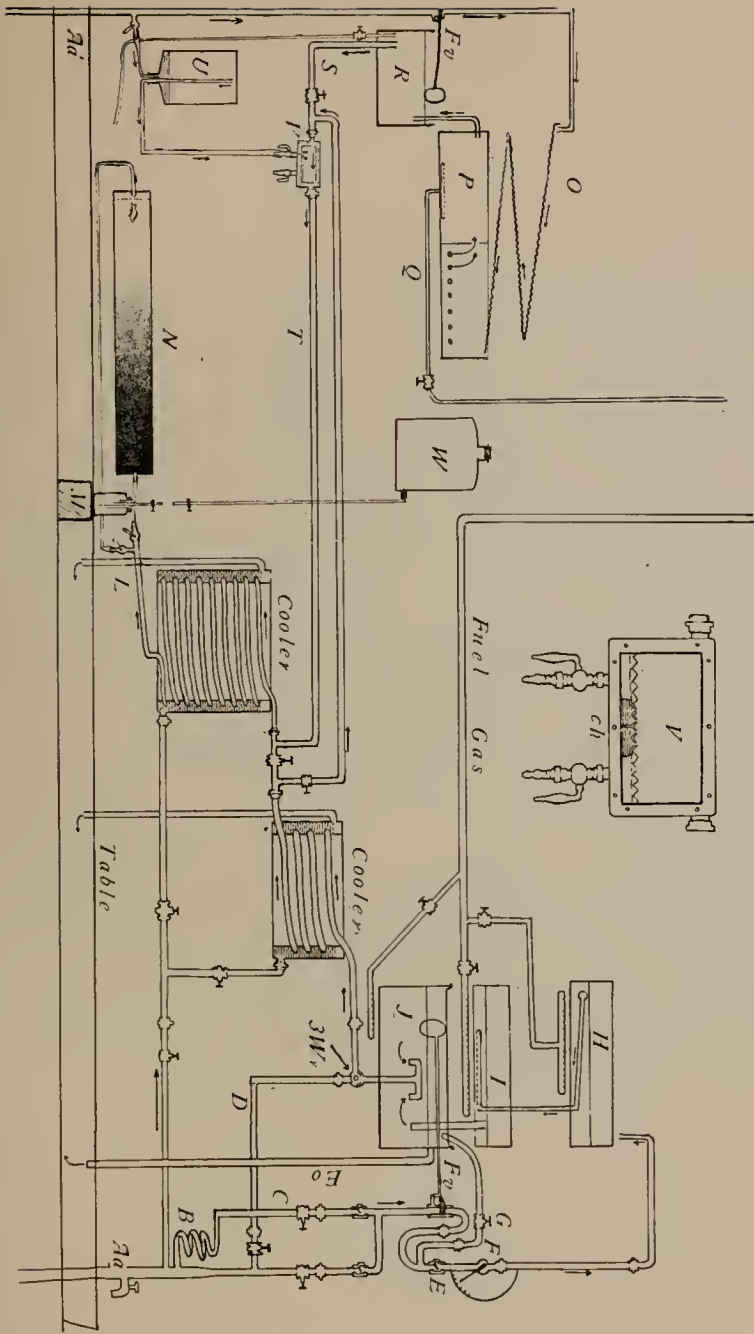


FIG. 1.

CHART I.

The graphs on this chart show the movements of fishes in the gradient tank when no contaminating substance has been added at the end, and the water is therefore of equal purity throughout. Graphs 1-5 have been previously published.

The gradient tank is shown in Figure 1, N, on preceding page. This is a diagram of a longitudinal section of the tank. The left hand end was used for the introduction of water such as the fishes were taken from and the right hand end was used for the introduction of water to which the substance being tested had been added. The water was introduced through a number of small openings in pipes which extended crosswise at each end of the tank, midway between top and bottom. The water flowed out at the center from both top and bottom. This gave pure water at the left hand end and usually in the case of dissolved solids and liquids, throughout about one third of the tank while the approximate full concentration of the polluting substance extended throughout the right hand third. The central third contained a mixture in which the concentration of the substance added at the right decreased from right to left. The central portion of the tank was accordingly a gradient between the two kinds of water introduced into the ends. The fishes introduced into the tank usually swim from end to end. The record of the movements of the fish was made by tracing their longitudinal movements in the tank on paper with reference to a time scale. Thus in Graph 1 the fish passed from the center to the left end and back to the right end during the first minute.

Graph 1 shows the longitudinal movements of a river chub made up as a composite of a number of such graphs to show that on the whole no more time was spent in one end of the tank than in the other.

Graph 2 shows the movements of two individuals of the green sunfish. Where the broken line appears, the two were moving separately. It will be noted that fishes made long stays in the ends but usually moved back and forth nearly always without turning back at the center.

Graph 3 shows the movements of a golden shiner in a uniform tank. It will be noted that the fish rarely turns around except at the end.

Graph 4 shows the movements of a specimen of *Notropis*. There was little activity and the fish turned back at the center once.

Graph 5 shows the movements of a specimen of rock bass.

Graph 6 shows the movements of a specimen of the orange-spotted sunfish. This species often turned before reaching the end of the tank but the number of turnings in the central third were the same from each direction.

Graph 7 shows the movements of a long-eared sunfish. It sometimes turned near the center, but about the same number of times from each end.

CHART I.

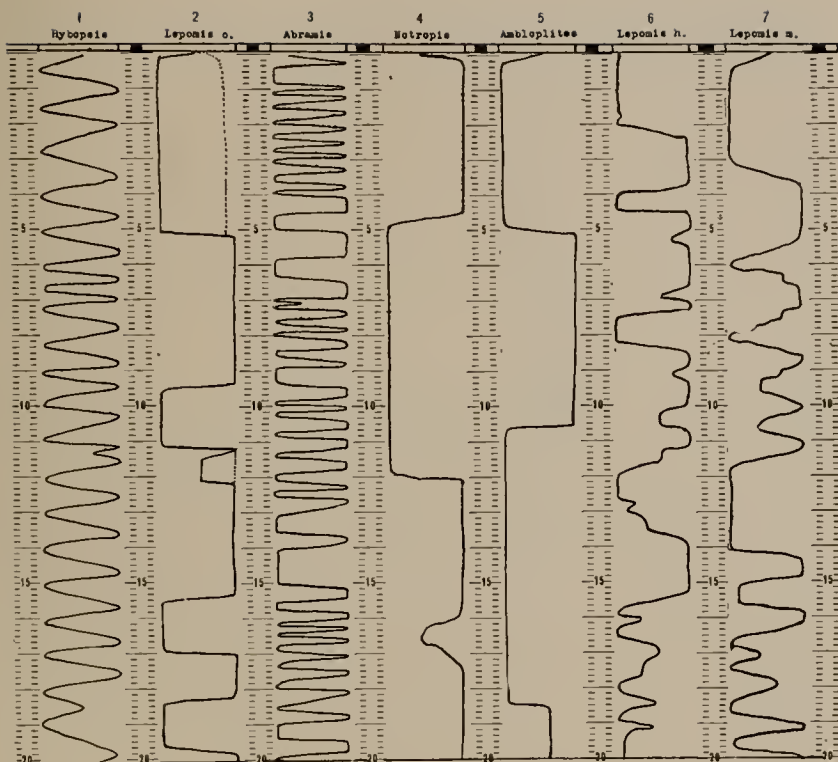


CHART II.

The relations of tank length to time scale is the same as in Chart I. In the case of this and all the charts which follow, the polluting substance was introduced into the right hand end of the gradient tank and is accordingly shown at the right side of the graphs. The vertical broken lines are intended to indicate the location of thirds of the tank length. The solid black area at the right between the two lines at the head of each graph is intended to show the part of the tank in which the polluted water is full strength and the narrowing of this black area from right to left in the middle third is intended to indicate the region of principal gradient. The unpolluted water contained about 5 cc. CO₂ per liter. X indicates that the fish became intoxicated; the arrow that it was driven.

Graph 8 shows the positive reaction of an orange-spotted sunfish to 1 part of weak waste to 25 parts of water. The fishes avoided the normal water and remained most of the time in the high concentration and gradient.

Graph 9 shows the reaction of the golden shiner to waste, 1 part in 100 of water, which killed the standard fish in a little more than one hour. In this case the fish avoided the unpolluted water and did not enter it at all until driven as indicated by the arrow.

Graph 10 shows the reaction of an orange-spotted sunfish to the same solution as was used in the case of graph 8. In this case the fish was negative, showing that there is some variation in the reaction.

Graph 11 shows the reaction of a minnow (*Pimphales*), indicated by the broken line. The fish was negative for a time but became intoxicated after a little more than two minutes and then remained positive after being driven into the strongest solution.

Graph 12 shows the reaction of an orange-spotted sunfish to illuminating gas. There was little activity and one fish remained in the clear water while the other remained in the polluted water during the period of observation. The amount of illuminating gas was not determined; much more was forced into the water than would go into solution in the pipe of the lower cooler (Fig. 1, preceding Chart I).

Graph 13 shows the reaction of a large-mouthed black bass to illuminating gas under the same conditions as in graph 12. The fish was driven into the stronger solution of gas and reacted positively thereafter.

Graph 14 shows the reaction of the golden shiner to a solution of ammonia which would prove fatal in a short time. The fish on the whole remained most of the time in the part of the tank containing a somewhat diluted solution but gave no avoiding reactions.

Graph 15 shows the positive reaction of a minnow (*Notropis*) to ammonia solution under the same conditions as graph 14. In this case the fish was clearly positive.

Graph 16 shows the positive reaction of two individuals of *Notropis* to a solution of approximately one gram per liter of NH₂(CO), which kills such fishes in less than an hour.

Graph 17 shows a decidedly positive reaction of a rock bass to approximately 1 gram per liter of ammonium chloride. The experiment continued for 10 minutes after the portion shown without change of result.

Graph 18 shows the reaction of a rock bass to 0.7 gram of ammonium sulphate per liter. The fish rested in the polluted water throughout the greater part of the time and turned back when a decreased concentration was encountered.

Graph 19 shows the reaction of a minnow (*Pimphales*) to approximately 0.25 gram of ammonium sulphocyanate (sulphocyanide) per liter. The fish moved back and forth actively and turned back regularly from a pure water.

Graph 20 shows the reaction of a full-grown rock bass to the same concentration as in Graph 19. The fish was driven into the pure water at the end of seven minutes but soon returned to the polluted portion.

CHART II.

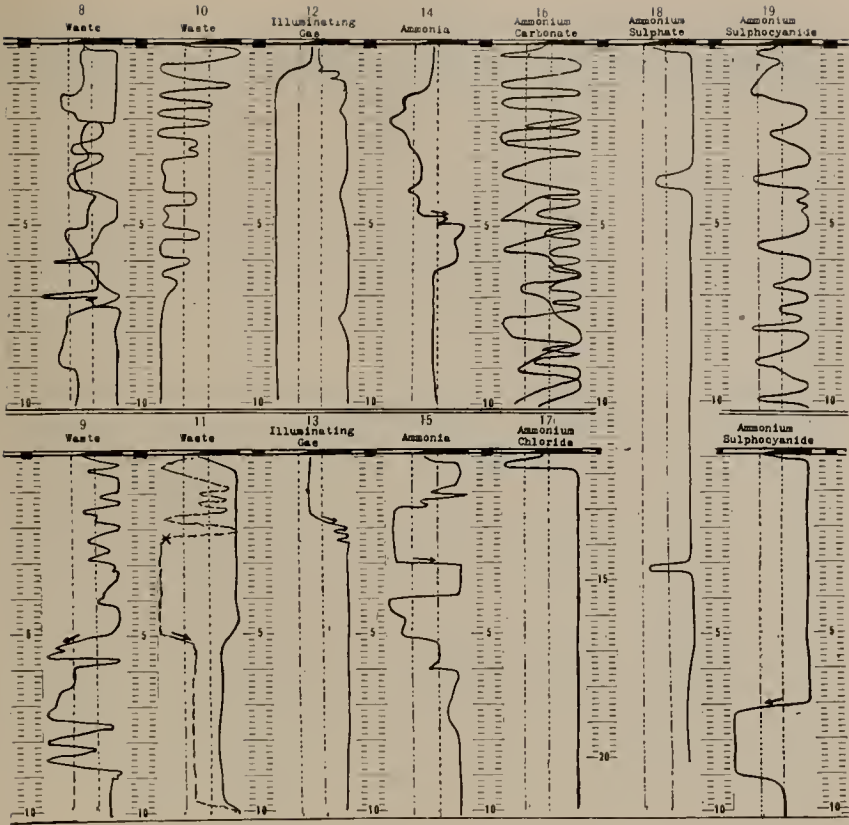


CHART III.

Graph 21 shows the reaction of a minnow (*Notropis*) to approximately 0.25 gram of ammonium ferrocyanide per liter. The fish was active and swam back and forth turning back from the pure water repeatedly.

Graph 22 shows a slight preference on the part of an orange-spotted sunfish for water containing ethylamine.

Graph 23 shows the indifference of two orange-spotted sunfishes to about one-tenth cc. of aniline per liter.

Graph 24 shows the marked activity of two individuals of *Notropis* in a pyridine gradient and their repeated avoidance of the pure water throughout the experiment.

Graph 25 shows the marked negative reaction of the minnow (*Pimephales*) and long-eared sunfish to 0.16 cc. of quinoline per liter.

Graph 26 shows a marked negative reaction of a minnow (*Notropis*) to a weak solution of isoquinoline. In the case of both quinoline and isoquinoline the fishes were active and turned back from the polluted water; the rule for the former, and the exception for the latter.

Graph 27 shows the negative reaction of a minnow (*Pimephales*) to water containing 8 cc. per liter of hydrogen sulphide. The fish became intoxicated at the end of five minutes as indicated by the X in the graph.

Graph 28 shows the positive reaction of a full-grown rock bass to two cc. per liter of hydrogen sulphide. The pure water was encountered repeatedly and repeatedly avoided.

Graph 29 shows the reaction of two orange-spotted sunfishes to approximately 500 cc. of sulphur dioxide per liter. The fishes spent the greater part of the time in the central part of the tank.

Graph 30 shows the positive reaction of two orange-spotted sunfishes to 5 cc. of sulphur dioxide per liter.

Graph 31 shows the positive reaction of a long-eared sunfish to water containing less than 1 cc. per liter of carbon disulphide.

Graph 32 shows the reaction of a minnow (*Pimephales*) under the same conditions as graph 31. The fish was positive during the first seven minutes and negative during the last three.

Graph 33 shows the positive reaction of a rock bass to a fatal concentration of thiophene.

Graph 34 shows an equally positive reaction of a long-eared sunfish.

CHART III.

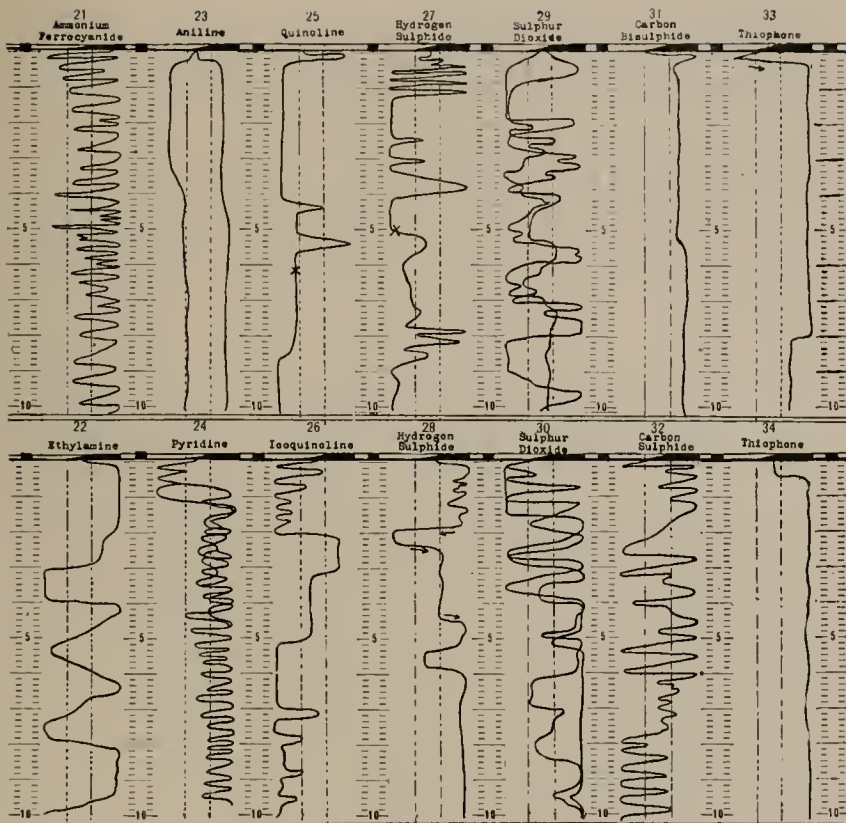


CHART IV.

Graph 35 shows the positive reaction of two minnows (*Pimephales*) to water containing $2\frac{1}{2}$ cc. per liter of acetone. The fishes turned back repeatedly from the pure water.

Graph 36 shows the decidedly negative reaction of a minnow (*Pimephales*) to water containing a fatal concentration of benzoic acid.

Graph 37 shows the reaction of a green sunfish to water containing 0.5 cc. per liter of phenol. The fish was markedly positive to the phenol during the first ten minutes, when the activity increased, probably due to its irritating effects. The greater part of the time was spent in the phenol however.

Graph 38 shows the reaction of a full-grown rock bass to approximately 0.1 cc. per liter of orthocresol, which would prove fatal to the fish in an hour or more. When the fish entered the polluted water the first time it did not recognize it at all. It gave no avoiding reaction. Later it moved toward the weaker solution and turned back again into the stronger solution. After becoming partially intoxicated, it moved into the pure water but returned to the fatal solution again and was completely overcome there.

Graph 39 shows the reaction of an orange-spotted sunfish to 0.3 cc. paraeresol per liter—about three times as much as is required to kill one of the fishes in one hour. It is to be noted in particular that the fish after trying the pure water twice, gradually avoided it more and more until it finally came to rest in the strongest solution of paraeresol.

Graph 40 shows the reaction of two orange-spotted sunfishes to 0.12 cc. of metaeresol, sufficient to kill them in an hour. One fish was negative and the other positive. The fish which happened to enter the polluted water at first became intoxicated and remained positive thereafter. Fishes are often negative to metaeresol.

Graph 41 shows the reaction of an adult rock bass to a saturated solution of phenanthrene. Fishes are often indefinite to this substance.

Graph 42 shows the negative reaction of an adult rock bass to a saturated solution of naphthalene.

Graph 43 shows the positive reaction of an individual orange-spotted sunfish to a saturated solution of naphthalene. Fishes are generally positive to this deadly substance.

Graph 44 shows the reaction of an adult rock bass to a mixture of pure water 3 parts and water saturated with xylene 1 part. The fish was decidedly positive and was soon intoxicated.

Graph 45 shows the reaction of a minnow (*Notropis*) to water containing approximately 0.08 cc. per liter of toluene. The fish is decidedly positive, though this concentration would kill it in less than an hour.

Graph 46 shows the reaction of two orange-spotted sunfishes to 0.04 cc. of benzene per liter—sufficient to kill them in an hour. In this experiment the fishes avoided the pure water and finally came to rest in the center.

Graph 47 shows the reaction of an orange-spotted sunfish and a rock bass to a slightly weaker concentration of benzene than was used in the case of graph 46. In this case the fishes both finally avoided the polluted water.

CHART IV.

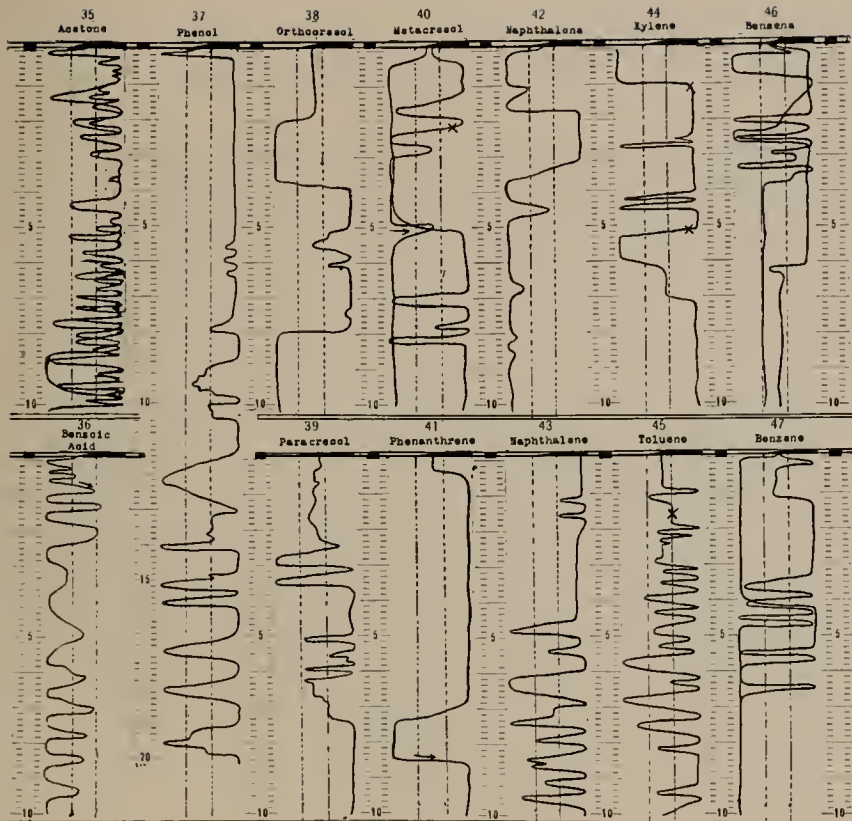


CHART V.

Graph 48 shows the positive reaction of an orange-spotted sunfish to 0.22 cc. of amylen. Reactions to this drug are usually positive.

Graph 49 shows the positive reaction of two minnows (*Notropis*) to amylen in which they appear to have selected an optimum concentration, near the center.

Graph 50 shows the reaction of a large-mouthed black bass to water containing 34.4 cc. of ethylene per liter. It is clearly positive, though this concentration would kill the fish in less than an hour.

Graph 51 shows the reaction of a minnow (*Notropis*) to water containing about ten cc. per liter of acetylene. The reaction is clearly positive though the gas is not fatal.

Graph 52 shows the reaction of an orange-spotted sunfish to about ten cc. of acetylene per liter; the reaction is clearly positive.

Graph 53 shows the reaction of three orange-spotted sunfishes to a mixture of carbon monoxide (1.4 cc. per liter) and ethylene (9.6 cc. of ethylene).

Graph 54 shows the reaction of two suckers to the same solution as in graph 53.

Graph 55 shows the reaction of an individual (*Abramis*) to ammonia in alkaline water. The fish was positive, as in acid water.

Graph 56 shows the reaction of a large-mouthed black bass to paracresol in alkaline water. The general result is the same as in acid water.

Graph 57 shows the reaction of an orange-spotted sunfish to orthocresol in alkaline water. The fish was positive, as in the acid water.

Graph 57 shows the reaction of an orange-spotted sunfish to phenol in alkaline water. This and other fishes are positive, as in acid water.

Graph 58 shows the positive reaction of an orange-spotted sunfish to naphthalene in alkaline water.

Graph 59 shows the positive reaction of a large-mouthed black bass to toluene in alkaline water.

Graph 60 shows the positive reaction of a blue-gill to gas waste in alkaline water.

CHART V.

