THE RELATIVE RESISTANCE OF FIFTEEN SPECIES OF FISHES TO PETROLEUM REFINERY EFFLUENT AND THE SUITABILITY OF THE SPECIES AS TEST ANIMALS

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PREFACE

Wastes from petroleum refineries contribute a considerable part of the pollution load of the waters in North America. An analysis of the effects of these wastes on the aquatic life in any particular situation requires a knowledge of the relative sensitivity of the organisms native to that environment.

A National Institutes of Health grant for a study of the resistance of fishes to petroleum refinery effluent became effective on February 1, 1960. In the study reported herein, the comparative resistance of fifteen species was established in a series of bioassays. A literature review was made of the factors affecting the use of fishes in bioassay, and a study was made on the suitability of the species tested as bioassay animals.

Professor William H. Irwin directed the research, contributing the benefits of his experience to a successful completion of the study. Doctors Roy W. Jones, Bryan P. Glass, George A. Moore, and Robert D. Morrison served on the graduate committee of the writer and made helpful suggestions in outlining the program and in editing the dissertation. Professor Moore further assisted by verifying identification of certain species. The data were analyzed statistically by Dr. David Weeks and his assistants at the O. S. U. Statistical Laboratory. Walter Whitworth, Neil Douglas, Thomas Jones, and William Gould assisted in field collection and bioassays. Data of chemical analysis of the water used in the

laboratory were provided by Mr. Lawrence Paxton of the O. S. U. Water Treatment Plant. The Oklahoma Department of Wildlife Conservation and the Arkansas Game and Fish Commission furnished a number of fishes for testing. Appreciation is extended to the above mentioned persons or agencies for their contributions, and to the National Institutes of Health for providing funds which made this study possible.

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INTRODUCTION

Bioassays are conducted to determine the effects of some agent or agents upon organisms. Currently, fishes are being used as test animals in the study of the effects of a number of groups of materials upon fish life. These materials include fish poisons, drugs and chemicals used in treating fish diseases, anesthetics used in handling and transporting fish, hormones used in spawn taking, respiratory gases in water, and pollutants that may affect aquatic life in natural waters.

The term 'pollution' as used in this paper will refer to the release of substances into natural waters in such quantities that they become detrimental to aquatic life. Major sources of pollution are mining wastes, agricultural poisons, domestic sewage, and industrial wastes. A nationwide survey by the U. S. Public Health Service (1960) listed industrial wastes as the chief cause of fish kills in 1960. A review of the literature on the toxicity of certain categories of industrial wastes to fishes is given by Doudoroff and Katz (1950 and 1953). Petroleum refinery effluents contribute a considerable, if unknown, part to industrial pollution.

Biological indices are commonly used in pollution studies. The use of indices presupposes a knowledge of the comparative resistance of the indigenous organisms. As the relative resistance to petroleum refinery effluent of many North American fishes has not been determined, the present and subsequent studies eventually will evaluate 54 fresh-water species for this quality. The present report is restricted to studies on the

guppy and 14 species native to Oklahoma: Notemigonus crysoleucas (Mitchell), golden shiner; Chrosomus erythrogaster (Rafinesque), southern redbelly dace; Notropis boops Gilbert, bigeye shiner; Notropis lutrensis (Baird and Girard), red shiner; Pimephales notatus (Rafinesque), bluntnose minnow; Campostoma anomalum (Rafinesque), stoneroller; Ictalurus punctatus (Rafinesque), channel catfish; Ictalurus melas (Rafinesque), black bullhead; Micropterus salmoides (Lacépède), largemouth bass; Lepomis cyanellus (Rafinesque), green sunfish; Lepomis microlophus (Günther), redear; Lepomis megalotis (Rafinesque), longear sunfish; Ambloplites rupestris (Rafinesque), rock bass; and Pomoxis nigromaculatus (LeSueur), black crappie.

Relative resistance was established in a series of bioassays, resistance having been measured as the 12-, 24-, 48-, and 96-hour median tolerance limits (TL_m's). Observations were made on the suitability of each species as a laboratory animal for use in bioassay.

Fishes vary in their sensitivity to a toxicant, and a particular species may be resistant to one substance and sensitive to another. The goldfish, generally considered a resistant species, is quite sensitive to some chemicals (Henderson and Tarzwell, 1957). The gizzard shad which is exceptionally sensitive to rotenone (Huish, 1959, found 0.06 to 0.14 ppm an effective lethal dose) is tolerant to high concentrations of sodium chloride (Chipman, 1959). Certain fishes (goldfish, black bullhead, and carp) may be generally classified as 'resistant', and some others (gizzard shad and salmonids) may be considered 'sensitive'. Additional information is needed on the sensitivity of fresh-water fishes to specific toxicants so that, if possible, their general sensitivity can be determined.

REVIEW OF THE LITERATURE

In the United States pioneering studies on the resistance of fishes to industrial wastes were made, and bioassay methods and procedures were developed early in the twentieth century by Marsh (1907), Shelford (1917 and 1918), Belding (1927), Carpenter (1930), and Ellis (1937). The bioassay methods widely accepted as standard and as used in the current study were largely developed by Hart, Doudoroff, and Greenbank at the Atlantic Refining Company Waste Control Laboratory (Tarzwell, 1958). Their comprehensive report considered in detail the methods and principles involved in bioassay, but was published for limited distribution and is not generally available. A more concise publication (Doudoroff, et al., 1951) derived from their work was prepared by Section III of the Subcommittee on Toxicity, Federation of Sewage and Industrial Wastes Associations. More recently, methods have been considered by Anderson (1953), Freeman (1953), Saila (1954), Henderson and Tarzwell (1957), and Tarzwell (1958). Most of the papers on methods have included discussion of the selection and use of fishes in bioassay.

The importance of petroleum-processing wastes as stream pollutants in Oklahoma has been illustrated by the results of the two following studies. Clemens and Crawford (mimeo., no date) studied the toxicity of effluents from 40 refineries. In 96-hour tests, only four of the 48 effluents tested were non-toxic to red shiners, and in 72 percent of the effluents, the TL_m was less than 30. TL_m's were as low as 0.52 for cracking-unit effluent, and 1.3 for final effluent. Ludzack, et al.,

(1957) reported on the characteristics of a stream composed of oil refinery and activated-sludge effluents. In one survey the first significant variety of pollutant-tolerant organisms was found 17 miles below the source of pollution, and clean-water organisms, including fish, were first found 30 miles below the source. A shorter recovery zone was found after heavy rains had flushed away much of the old sludge. Refinery effluent composed about one-fourth of the stream flow.

Petroleum refinery effluents are, during normal operation, an accumulation of wastes from the various processes (units) that contribute to the manufacture of petroleum products, primarily gasoline, lubricating oils, and greases. At regular intervals, and during breakdown, any number of the units may be in operation while the others are idle. The plant effluent consists of the wastes from operating units and cleaning water, and may contain relatively few toxicants, some of which may be in high concentrations. Thus a knowledge of the toxicity of the individual components found in refinery wastes may be of value in analyzing the effects of the total effluent. A study of the comparative toxicity of refinery wastes from each processing unit was made by Turnbull, et al. (1954). Wallen, et al. (1957) listed 141 chemical compounds that may appear in refinery effluents and gave the comparative toxicity of 86 compounds. Ammonia and phenol which are commonly found in lethal concentrations in petroleum-processing wastes rated among the 10 most toxic substances tested. Jenkins (unpubl.) has studied the effects of mixing among ammonia-nitrogen, sulfide, and phenol on the toxicity of each substance.

Wastes from the destructive distillation of coal contain all of the major toxicants commonly found in petroleum refinery effluents. Shelford

(1917) in his studies on the effects of coal-gas wastes on fishes stated that in a general way the relative resistance of the different species to coal-tar products is similar to the relative resistance to carbon dioxide as determined by Wells (1918). Ellis' (1937) paper included a report of the effects of 114 substances (including constituents of coal-gas wastes) to a variety of fishes, much of the data being taken from the literature. A number of the substances are constituents of petroleum refinery wastes, including ammonia and ammonium salts, lead and lead compounds, mercaptans, phenols, sodium chloride, and sulphuric acid.

Lead has been reported as a constituent of refinery wastes in quantities that are quickly lethal to fishes (Turnbull, et al., 1954). (The use of tetraethyl lead to raise octane rating is a common practice in American refineries.) Carpenter (1930) established the relative resistance of the following species to lead salts (from least resistant to most resistant): silverjaw minnow, bluntnose minnow (young), steelcolor shiner, bluntnose minnow (adult), bluegill, johnny darter, and common shiner.

Ten species common in central Oklahoma were ranked, from most resistant to least resistant, according to their toxicity thresholds to brine water from oil wells (Clemens and Jones, 1954): plains killifish, mosquitofish, white crappie, bluegill, green sunfish, channel catfish, black bullhead, red shiner, largemouth bass, and fathead minnow.

As the toxicity of petroleum refinery wastes varies greatly due to composition and concentration, a particular fish's sensitivity cannot be established by using only one species in bioassay. Conversely, if the sensitivity of the fish is unknown, the toxicity of the effluent cannot be determined. Only by using a series of fishes can sensitivity be

established. Personnel at the Atlantic Refining Company Waste Control Laboratory have been studying the effects of petroleum refinery wastes on fishes since 1935 (Turnbull, et al., 1954). Clemens and Finnell (1956) in a study of a stream polluted with refinery wastes found the plains killifish, red shiner, and fathead minnow at stations with a higher concentration of effluent than at stations where the sand shiner and green sunfish were found. Using effluent from two refineries, Douglas (1961) concluded on the basis of 10 bioassays that, of four species, the guppy was most resistant, the fathead minnow and mosquito fish were second in rank, and the plains minnow was least resistant.

METHODS AND PROCEDURES

Test Fishes

Sources. A number of farm ponds were selected during the winter of 1959-60 for use in rearing fishes for test purposes. The existing fish populations were eradicated and the ponds restocked with adults of the desired species. Three species were obtained from these ponds. Eight species were taken from other farm ponds or streams, and three were obtained from government hatcheries. Guppies were reared in the laboratory. Collection and laboratory data on each species were recorded.

Collection. All fishes except some black bullheads and those species from hatcheries were taken with nylon drag seines of Ace-style knitted construction. The netting of such seines is soft and a minimum of damage to the fishes resulted from their use. Seines were of 1/8-inch or 3/16-inch mesh, from 10 to 30 feet in length, and from 4 to 8 feet in depth. A bag seine (30 x 8 feet, with 3/16-inch mesh) was found to be especially effective in deep, clear water. Black bullheads were taken with seines or dip nets.

Transport. Fish were transported in covered tanks or in plastic bags. Hauling tanks, with usable capacity of 25 to 40 gallons, were prepared from the liners of dismantled household refrigerators by sealing the openings in the sides and bottoms. The tanks were light, of convenient size, and the inner surfaces of porcelain were easily cleaned.

A rack for holding an oxygen tank was permanently installed on a one-half-ton truck, and oxygen supplied through plastic lines. The use of flexible lines allowed the tanks to be placed in desired positions in the truck, and oxygen lines could be connected easily.

Fish were hauled in flat polyethylene bags (32 x 30 inches) by the method essentially as described by Clark (1959). A small oxygen tank (30 x 4-1/4 inches, including the valve) was found to be convenient when hauling fish in a sedan. The small tank could be filled from a regular sized (244 cu. ft. cap., and at 1200 to 1500 lbs. pressure) oxygen tank at a small fraction of the cost of having it filled at a commercial establishment. The procedure was to connect the two tanks with a special direct coupling, and to permit the pressure to slowly reach equilibrium.

Six plastic bags, each containing about 250 two-gram fish, could be hauled in one layer behind the front seat in a sedan with the rear seat removed.

Terramycin and acriflavine were used in the hauling water and were found to be effective in preventing fin rot. Irwin (1959) described the use of terramycin in the control of fin rot at Oklahoma State University.

Maintenance of guppies. Guppies were reared in a room that was heated to about 80°F. during the colder months, but no temperature control was necessary during the summer. Nine tanks similar to those used in transportation of fishes were used as brood tanks. From 50 to 150 breeders with about equal numbers of males and females were kept in each tank. Adults were confined in a nylon net bag, with the top anchored in a rectangular shape by attachment with wires to the sides of the tank. The nets had 12 meshes per inch, and were stretched 24 x 12 inches at the top. The young escaped through the net, were removed from the tank

daily, and were placed in rearing tanks according to age groups. Differential growth made it necessary to grade the young to obtain uniformity of size for tests. Fish were test-size (0.6 to 0.7 inches) at 6 to 8 weeks.

Adult fish were fed a mixture of poultry food (18 percent protein egg pellets) and meat meal (49 percent protein) which was ground through a commercial coffee grinder. Particle size could be controlled by setting the selector. Powdered egg was added to the diet for young guppies, which were fed twice daily.

Maintenance of wild fishes. Fishes were held in the laboratory in tanks, as described under the section on transport. Holding water was aged and aerated tap water. An initial treatment of terramycin and acriflavine was added to the water to deter bacterial infection.

Fish were fed once daily. The dry ration (ground mixture as described under maintenance of guppies) was fed to all species which did not require live foods. Live foods were daphnia, chironomid larvae, and young mosquito fish.

Daphnia were reared in the laboratory. A culture of chironomids developed in the fish-holding room. Apparently a few adults had accidentally entered from outdoors, and the population quickly increased in size. All containers of water were used as breeding sites; and, if no fish were present, several hundred larvae would soon be present in each tank. Immature stages were particularly abundant in the daphnia tanks where they apparently thrived on the food supplied for the daphnia. Chironomid larvae usually formed more than 50 percent of the bulk of organisms in the daphnia tanks. Effects on daphnia production were not determined.

A farm pond was stocked with mosquitofish early in the study, and young soon became abundant in the shallows along shore, where two to three thousand fish were easily captured with dip nets within a few minutes.

Bioassay

Equipment. Bioassays were conducted in a constant temperature room. Bioassay tables, with sheet metal trays for working surfaces, each held 24 test containers. Tables were equipped with racks for plastic oxygen-lines; individually controlled lines led to each container. A one-foot section of 1/8-inch I.D. rigid plastic tubing was attached to the end of each line to prevent the line from floating.

Polyethylene test containers were 11 inches in length, 7 inches in width, and 12 inches in depth, with a total capacity of 12.7 liters. Five-gallon polyethylene jugs were used for holding effluent during transport and storage.

Diluent. The dilution water (processed water), obtained from Lake Carl Blackwell, was quite consistent in hardness, alkalinity, and pH. Hardness extremes were 138 to 153 ppm, bicarbonate alkalinity extremes were 107 to 132 ppm, carbonates were 0.0, and pH extremes were 7.4 to 7.9, with values rarely outside 7.6 to 7.9. The foregoing data are based on daily chemical analysis made during the tests by personnel at the OSU water treatment plant. A more complete analysis (in parts per million unless otherwise specified) was made in July, 1960 by the U. S. Geological Survey:

Silica (SiO_2)	3.6	Fluoride (F)	1.1
Iron (Fe)	0.0	Nitrate (NO_3)	0.8
Calcium (Ca)	37.0	Percent sodium	27.0

Magnesium (Mg)	12.0	Dissolved solids (Evap. 180° C.)	218.0
Potassium (K)	25.0	Hardness (as $CaCO_3$)	142.0
Bicarbonate (HCO_3)	134.0	Specific conductance (Micromhos at 25° C)	396.0
Carbonate (CO_3)	0.0	pH	7.9
Sulfate (SO_4)	26.0		
Chloride (Cl)	39.0		

Effluent. The toxicant used was the final effluent from a petroleum refinery in central Oklahoma, taken from the discharge pipe prior to any mixing with the receiving stream.

Test Procedure

Collection of effluent. A fresh supply of effluent was taken for each bioassay. It was pumped into jugs, sealed, and transported to the laboratory. Upon arrival at the laboratory, the effluent was placed in the bioassay room and allowed to cool to testing temperature.

Exploratory tests. Because the effluent varied in toxicity, and the fishes varied in sensitivity, the approximate toxicity was determined prior to each bioassay. A wide range of concentrations was prepared, and two fish of each species being tested were placed in two liters (or four liters depending on the weight of the fish) of solution at each dilution to determine the lethal concentration. Exploratory tests were begun in the afternoon and mortality checked the following morning. Full scale bioassays were then begun, based upon the results obtained.

Temperature. Temperatures in the laboratory were maintained at 73 to 79° F., with extreme recordings of 65 to 83°. A Taylor maximum-minimum thermometer was kept on one of the bioassay tables to detect temperature variations due to power failure or other causes, that might otherwise go unnoticed. Water temperatures in containers were approximately 2° F. lower than the air temperature.

Oxygenation. Oxygen was bubbled into each container at the rate of one bubble per second. Henderson and Tarzwell (1957) reported that the addition of oxygen at the rate of 30 to 180 bubbles per minute did not greatly affect the toxicity of wastes containing volatile compounds.

Test solutions. Four dilutions of effluent, with concentration values taken from a logarithmic series (Doudoroff, et al., 1951), were prepared for each species tested at each bioassay. This group of dilutions was called the 'A' series. Duplicates of the 'A' concentrations were prepared and labeled the 'B' series. Ten liters of test solution were measured into each container, dilution being made as percent by volume. Controls were placed in dilution water.

Bioassay. Ten fish were placed in each concentration in each series (A and B), and ten fish were used as controls. The maximum fish-to-liquid ratio was two grams of fish per liter. Availability determined when a species was used. The guppy was included in every test, and each of the other species was tested on four different dates, each time with a different effluent sample.

Observations on survival were made and recorded at 1, 6, 12, 24, 48, and 96 hours after the fish were introduced. The test solution was not renewed during the 96-hour period. Dead fish were removed when survival was recorded. A primary purpose of the 1- and 6-hour survival checks was to establish a standard time to remove dead fish. As a greater part of the kill occurred early in the tests, it was desirable to remove dead fish to avoid excessive wastes from putrefaction.

Analysis of Data

The 12-, 24-, 48-, and 96-hour TL_m's were calculated on semi-log

paper by straight line graphical interpolation (Doudoroff, et al., 1951). The 24-hour TL_m's were used to determine relative resistance for all species.

A two-way classification (species x tests) for 13 species was analyzed statistically by the Doolittle Technique, using the model, $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ij}$. The mean TL_m for each species, adjusted for difference in effluents, was determined. Means were ranked and subjected to the new Duncan's 5 percent multiple range test.

Data were deleted for one bioassay in which the stoneroller and black crappie were tested, since unexplainable deaths prevented calculation of the TL_m for the reference species. To save time, statistical analysis was made on the other 13 species while a search for more specimens of the stoneroller and black crappie continued. The search was not productive, and, in order to obtain an estimate of the adjusted mean TL_m's (24-hour) for the species in question, a separate two-way classification including only the guppy, stoneroller, and black crappie was analyzed by the Doolittle Technique. It is recognized that this procedure does not necessarily make the results comparable, but the error mean squares for the two analyses were not greatly different, and the results for the reference species were approximately the same. The results of the second analysis were adjusted to the same scale as those in the first, and the positions of all 15 species on the relative resistance scale were compared graphically.

FACTORS IMPORTANT TO BIOASSAY

Chemical and Physical Factors

Among the factors that may affect toxicity, directly or indirectly, are: temperature; concentration of toxicant; light (photo-decomposition); acidity, alkalinity, and hardness of dilutant; combination, decomposition, precipitation, synergism and antagonism among chemicals; volatility; dissolved oxygen; ratio of fish weight to solution volume; and accumulation of toxic substances in the fish's body. The relationships and interrelationships among these factors are complicated and varied. Effects of any one factor may be affected by a number of the other factors. Some of the relationships have been reviewed by Henderson and Tarzwell (1957); Doudoroff and Katz (1950 and 1953); and Hart, et al. (1945).

Refinery Wastes

The chemical composition of refinery wastes is complex, variable, and incompletely known. Among the toxic substances commonly found in refinery effluents are phenols, ammonia, sulfide, mercaptans, and unidentified hydrocarbons. Dissolution of substances found in the oil-bearing strata, and the addition of chemicals or formation of compounds in the refinery process contribute to the complexity of refinery effluents. The pH is usually high (8-10). A study of the toxicity of the various components of refinery wastes has been made by Turnbull, et al.

(1954), and Jenkins (unpubl.).

Diluent

Qualities of a diluent used in reference tests are suggested by Hart, et al. (1945). Freeman (1953) gives the formulas of stock solutions to be used in preparation of the standard reference water as described by Hart, et al. Water used in the present studies varied from that recommended by Hart, et al. (1945) in the following respects:

	Standard Reference Water ppm	Water Used ppm
Total alkalinity (ppm $CaCO_3$)	60-120	107-132
Total hardness	75-150	138-153
Sulfates	20-50	26*
Dissolved solids (residue on evap.)	< 500	218*
pH		7.4-7.9

*one determination

Selection of Test Concentrations

Occasionally, all of the fish will survive in the lower, and none in the higher of the two critical concentrations (concentrations used in calculating the TL_m's). In this situation, the numerical difference between the values of the critical concentrations is termed, within this paper, the total-to-no-survival (TS to NS) interval from data. The question arises whether or not to include the data in analysis. Doudoroff (1951) suggested that for most effluents, the data may be used if the numerical interval between the critical concentrations is not much more than 25 percent of the higher value. Figure 1 includes a graphical explanation of the following discussion. The TS to NS interval from

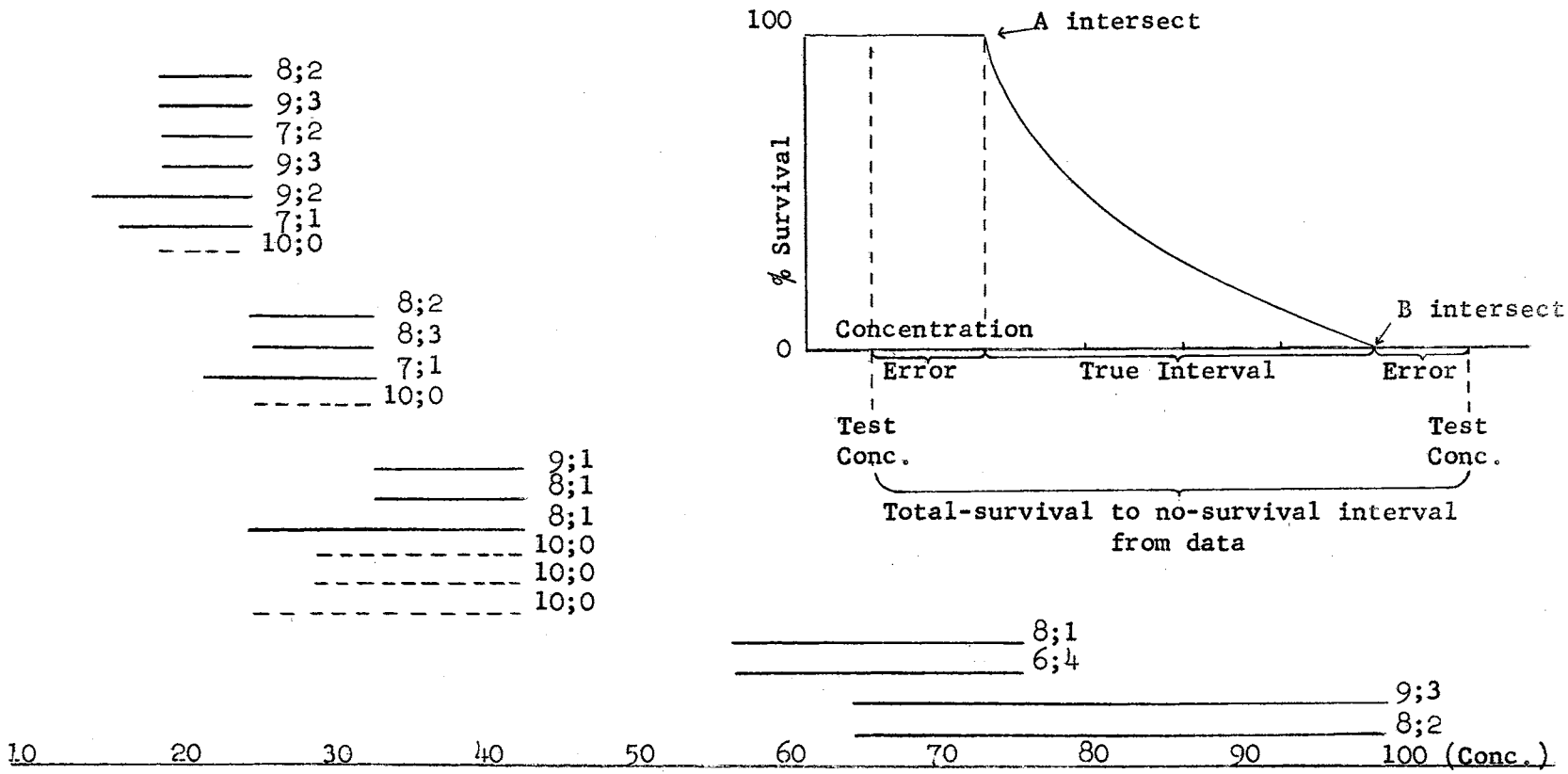

Figure 1. Comparison of data from tests in which total-survival and no-survival occurred in two critical concentrations (dotted lines) with data from other tests in which some fish died in the lowest concentration greater than that at the no-survival ordinate and some fish survived in the highest concentration less than that at the 100-percent ordinate (solid lines). Data are from 24-hour bioassays with the guppie. Each line represents the interval (the numerical difference) between a lower and a higher concentration value. The lower and higher concentrations are the best estimates obtained or the concentration values at the A and B intersects. Numbers at the ends of lines represent the number of fish (in each container of 10 fish) surviving at the lower and higher concentrations, respectively.

data is an estimate of the true interval on the survival-concentration regression curve between the point where the curve intersects the total-survival ordinate (A intersect) and the point where it intersects the no-survival ordinate (B intersect). Each such estimate of the true interval contains an error which is the difference between the true concentration value at either intersect (of the hypothetical curve with total-survival or no-survival ordinates) and the value of the critical concentration on the same ordinate.

Three factors that may affect the length of the interval are species, toxicant, and strength of the test solution. To estimate the interval for a species and a given toxicant, it is desirable to have several observations at a given concentration. In the present study, 24-hour data for the guppy were sufficient for comparison of the TS to NS intervals with the best estimate (the interval between concentrations nearest the A and B intersects of the survival-concentration curve, at which some, but not all, of the fish survived) of the true interval. The comparison is made graphically in Figure 1, which also illustrates the increase in the length of the curve between the A and B intersects with a decrease in toxicity.

The data suggest that, in setting up the test solutions of refinery effluent, the concentrations should be selected so that the interval between them would not exceed 25 percent of the higher concentration values. A close estimate of the TL_m, based upon exploratory tests, will be of value in avoiding the necessity of preparing a large number of concentrations (and the use of a proportionately large number of fish).

Test Fishes

Species. The goldfish, fathead minnow, and bluegill sunfish have been widely used as test animals. The requirements of desirable test fish have been considered briefly by Belding (1927); by Doudoroff, et al. (1951); and Hart, et al. (1945). The use of goldfish in toxicity experiments was studied by Powers (1917). Doudoroff, et al. (1951) state that fishes used in tests of pollutants of natural waters should be those species commonly found in unpolluted parts of the receiving stream, or at least species found in the same watershed. It is desirable to use fishes of direct importance to man, but of greater significance is the use of a series (of fishes) with a wide range of sensitivity. As the biological relationships of the fishes of a stream are not well understood, the sensitive species should be considered in studying the effects of pollution. The assumption is that any group may be important to the ecology of the stream.

In relative resistance studies of industrial wastes, or of complex toxicants of any kind, it is desirable to have a reference species. Chemical analysis may not disclose the nature of an effluent, and data available on the toxicity of any component to a particular species are non-conclusive in determining the toxicity of the effluent. The best measure of the toxicity of such pollutants is the reaction of a test species. If all species to be compared are not available at one time, either a reference fish must be used, or cross references to fishes previously tested must be made. When wild species are used for reference, procurement of fishes, and duplication of results become perplexing problems. The simplest solution appears to be the use of a 'standard reference fish'.

Identification. Collections of fishes which include several species or size groups should be sorted for use in bioassay. Many stream collections are of this nature. The separation of species of live fish depends on a knowledge of the fish fauna sampled, and is complicated by the resemblance of the species. Test fish cannot be handled and examined individually as are dead specimens. Glass-topped sorting tables and glass aquaria are useful. While under stress of excitement, some fishes lose their coloration, and become remarkably similar in general appearance. Such fish may be put into glass aquaria and individually removed with a small shallow dip net. Removal of fish with deep dip nets is time consuming, and unless the fish is held under water, it may be injured when the net bag is inverted. Size groups may be separated with mechanical graders. All sorting and grading should be done as early as possible after capture, so that damaged fish can recover before testing.

Sources. The fish used in a test should be from the same general source, or at least nearby from the same watershed as genetic differences may exist in populations that are isolated from each other. Hatchery fish that have been domesticated for long periods may be expected to differ from wild fish, and fish from different watersheds are not expected to react alike. Vincent (1960) found that wild brook trout were more tolerant of accumulated metabolic wastes and high water temperatures than were a domestic strain. The effects of domestication upon growth rates and survival of hatchery trout in the wilds have been extensively studied.

Test fish from polluted waters are likely to be more resistant than other fish of the same species. Clemens and Jones (1954) found the plains killifish from a brine polluted stream more resistant than those

from an unpolluted stream.

Many fishes are available from private and government-owned hatcheries, and not readily acquired elsewhere. If the fishes from hatchery are wild stock, or not far removed from wild stock, they are more satisfactory as test animals for pollution studies.

Fish may be reared in the laboratory or in outdoor ponds. Additional information is needed about the rearing of common freshwater fishes in the laboratory. Linder (1958), and Strawn (1961) have described the rearing of darters in the laboratory. The mosquitofish and stickleback have been reared in captivity. Stripping methods for small fishes have been described by Markus (1939), Strawn and Hubbs (1956), and Surber (1940) as quoted by Davis (1953). The use of laboratory-reared fishes in pollution studies may meet objections on the grounds that the effects of an artificial environment on sensitivity cannot be readily established.

Centrarchids, catfish, and many minnows have been reared in ponds. Culture methods have been described for a number of bait species (Altman and Irwin, 1957; Markus, 1939; and Dobie, et al., 1956).

Availability. Availability of wild fishes depends upon distribution, concentration, and ease of capture. Widely distributed fishes that are abundant in streams or lakes, and which may be readily captured are considered desirable. Fish may be found in abundance in one season and in smaller numbers in another season due to migratory habits, growth beyond test size, or seasonal mortality. The habitat of a fish affects its ease of capture. Fishes that live under stones, or those that seek the shelter of submerged objects may be difficult to capture. Some species may be found in a variety of habitats, the ease of capture depending on

the habitat.

Size. The size of fishes used in bioassay depends upon the purpose of the test. For species important enough to be extensively investigated, both the eggs and fish of graded sizes may be considered. Jones and Huffman (1957) discussed the use of developing fish embryos in bioassay. Belding (1927) considered yolk-sac fry more resistant than older fry to certain trade wastes. Clemens and Sneed (1958) in a study of the sensitivity of channel catfish to pyridylmercuric acetate (PMA), found yolk-sac fry more resistant than 3-inch fingerlings. The 3-inch fingerlings were more resistant than one-week-old fry. In tests with refinery effluent (OSU Aquatic Biology Laboratory), two-week-old guppies and breeding males were found to be more sensitive than were five- to eight-week-old fish.

Doudoroff, et al. (1951) state that fishes three inches or less in length are more desirable as test animals. Metabolic activity with the release of wastes into holding water is a function of fish weight. When the logarithm of the rate of oxygen consumption is plotted against the logarithm of body weight, a straight line, with a slope of roughly 0.85, is obtained (Fry, 1957). Fry states that this value is intermediate between surface area dependence and weight dependence. An 8-inch largemouth bass may weigh 50 times as much as a 2.5-inch fish, and requires considerably more space in the laboratory.

Many species of minnows, killifishes, and darters are small enough as adults to be conveniently used in bioassay. Juvenile centrarchids from 1.5 to 2.5 inches in length are suitable.

In certain species, fish of one sex grow faster and become larger than those of the opposite sex. This difference in size usually becomes

greater as the fish grow older. If larger fishes are graded according to size, there is the possibility of separating the sexes so that a predominance of fish of one sex will be tested.

Physiological condition. The physiological condition of test fishes is important as shown in a study by Clemens and Sneed (1958) on tolerance of channel catfish to PMA.

The length of time fishes are held in captivity prior to testing may indirectly affect their sensitivity to toxicants. Weiss and Botts (1957) reported that the T_{50} (time required to kill 50 percent of the test animals) for the green sunfish and fathead minnow increased with the time held in the laboratory (2 to 21 days for the sunfish and 1 to 13 days for the minnow) prior to testing. Saila (1954) found that mosquitofish became more sensitive to rotenone in proportion to the length of time (1 to 8 days) held in the laboratory. The physiological condition of fish may or may not improve after capture, depending on how accurately the investigator provided the requirements of the species and on uncontrollable factors such as diseases that may have no external manifestations during the holding time. Hormonal disturbances due to handling and injury with resulting infections are other factors that may have a detrimental influence during the acclimation period. The concentration-survival curve may become straighter after an acclimation period for the fish in the laboratory, but it does not necessarily estimate most accurately the sensitivity of the wild population of fish.

Foods. Associated with physiological condition are the foods and feeding habits of fishes. The dietary demands of the species of fishes are incompletely known, with a few possible exceptions (salmonids, carp, and goldfish). The use of natural foods, when available, alleviates the

necessity of knowing the complete nutritional requirements in physiological terms.

Additional space and facilities are required for rearing of food for predatory fishes. The space requirement for the food organisms may be greater than that for the test fishes. Dry foods, inexpensive and easy to store, were fed to all species of minnows used in these studies. Many darters and some centrarchids (black basses and crappies) require live foods.

Starvation. Fish in good condition may not be greatly affected by short periods without food. Marsh (1907) found that three largemouth bass fry lived for an average of 72 days without food at 15 to 20° C. Another study based on a small amount of data by Shelford (1917) revealed no difference in the effects of gas wastes on freshly captured fish and starved ones. Wells (1916) found rock bass slightly more resistant to potassium cyanide after 47 days of starvation, and to low dissolved oxygen after 39 days of starvation. There was an increase in sensitivity with longer periods of starvation. Carpenter (1930) found similar results (an initial increase in resistance followed by a decrease) while testing the fathead minnow with lead salts.

In an experiment by Adelman, et al. (1955), small brook trout (3.5 inches) did not survive starvation as well as larger ones (5.5 to 7.5 inches). Very young fish, after absorption of the yolk sac have a limited supply of reserve food. Certain cold water forms have a higher metabolic rate at a given temperature than some warm water forms (Fry, 1957). Carpenter (1930) found a direct correlation between metabolic rate and sensitivity to lead salts. Very young fishes and cold water fishes may not survive tests of several days duration at higher

temperatures without feeding. This would be expected in cold water forms when the reserve food supply was exceptionally low prior to testing.

Seasonal mortality. In certain species such as the fathead minnow and stoneroller, an exceptionally high mortality frequently occurs during the breeding season. The use of fish (of any species) with a large percentage of spent breeders appears to be a questionable procedure. Some species do not feed actively during spawning and the reserve foods are greatly depleted (Hoar, 1957).

Resistance to injury. Some species, including shad, crappie (juvenile), and golden shiners are unusually susceptible to injury from handling. The loss of scales and the resulting invasion of pathogenic organisms may quickly result in death unless preventive measures are taken.

Catfish and carp may be injured if their pectoral or dorsal spines become entangled in the mesh of nets.

Resistance to disease. A disease may be more prevalent in one area than in another. Bacterial gill disease, ichthyophthiriasis, fin rot, and anchor parasites occurred in fishes at the OSU laboratory. Fishes vary in their susceptibility to a particular disease, some species being more resistant to the common diseases in a given locality than are others.

Behavior in the laboratory. The habit of jumping may result in loss of fish in the laboratory. Among the species tested, the golden shiner and southern redbelly dace exhibited this trait. All fish-containers should be kept covered until the habits of the species are determined.

White bass have been observed in an apparent state of shock in

which the body is rigidly arched and the gill covers extended. Apparently no respiratory movements occur. Most fish that were in this condition when put into hauling containers did not survive.

Antagonism. With some species, antagonism between individual fish is a factor affecting test results. Cannibalism is common with many predatory fishes, and fighting between adults is particularly evident in some centrarchids. Damage may occur in either holding or test containers and may result in infection at the site of the wound. If fish are compatible, there appears to be no difference in results of bioassays whether one fish or several are put into a single container (Saila, 1954) as long as the ratio of solution-volume to fish-weight is properly maintained. Doudoroff, et al. (1951) suggest that 2 grams of fish per liter of test solution not be exceeded.

Excitability. There may be some correlation between sensitivity to toxicants and excitability (Douglas, 1961). Fishes that are exeptionally excitable are poor test fishes, and injure themselves by their frantic efforts to escape. Some species that are initially excitable may become well adjusted after being held in captivity for a few days.

PERTINENT LIFE HISTORY DATA ON SPECIES TESTED

In the following and subsequent enumerations of fishes tested, species are arranged in descending order of resistance, based on 24-hour TL_m's unless otherwise specified.

Lebistes reticulatus

Guppies are native to Trinidad and Venezuela. They are a popular aquarium fish in the United States, and may have become established in some of the southern waters. Reports of wild populations that have come to the attention of the writers have not been verified.

Guppies can be maintained in the laboratory on dry foods. Commercial foods for aquarium fishes containing dried daphnia, insects, and other natural foods can be purchased, but are expensive. Supplemental feeding of live daphnia is recommended by some authors.

Although reproduction in its native habitat is seasonal (Emmens, 1953), the guppy breeds throughout the year in captivity. Fish mature, under good conditions in 8 to 10 weeks. Gestation is about 24 days at 75° F., and separate broods are born at 30 day intervals. Four or five successive broods may be fertilized by one mating, the sperm being stored until the next crop of eggs have matured. All fish of a brood are the same age, as the egg cells of a brood mature and are fertilized at the same time (Emmens, 1953). Brood size varies with the size of the female. According to Axelrod and Schultz (1955) the brood averages about 45 fish. During the study, females varied in size and broods

were apparently much smaller than 45 as less than 30 fish were usually removed from the brood tanks at one time. The extent of predation of older fish on the young was not determined. Although adult guppies are of suitable size for testing, non-breeding fish 6 to 8 weeks old were selected.

A summary of advantages and disadvantages for use as test fishes is given for all species in Table I.

Ictalurus melas

The black bullhead is found in central United States where it is most abundant in low gradient streams of small to intermediate size, and in ponds and lakes. It is tolerant of turbidity, and is not numerous in deep, clear, open waters (Trautman, 1957). The young form dense schools (in mid-July in Oklahoma) until they are nearly 2 inches in length (Harlan and Speaker, 1956). The fish swim slowly, and can be captured with dip nets if found in clear water. They are easily taken in seines.

The diet includes insects, minnows, small mollusks, crustaceans, and vegetation. Black bullheads feed readily on dry foods when in captivity.

Black bullheads will reproduce in ponds under a wide variety of conditions. Saucer-shaped nests are prepared on sand or mud bottoms, and no special spawning sites are necessary.

Harlan and Speaker (1956) reported that the black bullhead spawns in May and early June in Iowa. The young may be of suitable size for testing by mid-July in Oklahoma. Age-group-0 fish were 1.3 to 3.5 inches by August in an Iowa pond, and one-year-old fish from Indiana averaged

TABLE I

SUITABILITY OF SPECIES FOR BIOASSAY USE

Species	Advantages	Disadvantages
L. reticulatus	Can be reared in laboratory Resistant to low oxygen tension Resistant to disease Do not jump from containers	Require grading for size Of no importance in natural waters of the United States
I. melas	Widely distributed Can be reared in ponds Do not jump from containers Can be maintained on prepared foods Resistant to low oxygen tension	Size available varies with season Spines become entangled in nets Holding water may quickly become foul
N. crysoleucas	Widely distributed Available from hatcheries Can be reared in ponds Similar size available at all seasons Can be maintained on prepared foods	Jump out of containers Susceptible to injury and infection Adults become too large for general use
N. lutrensis	Can be reared in ponds Similar size available at all seasons Adults of suitable size for testing Do not frequently jump from containers Readily eat prepared foods	Not generally available from hatcheries
L. microlophus	Produced by state and commercial hatcheries Can be reared in ponds Do not jump from containers Of recognized importance to man	Restricted distribution

TABLE I (Continued)

Species	Advantages	Disadvantages
P. notatus	Widely distributed Available from hatcheries Can be reared in ponds Similar size available at all seasons Adults of suitable size for testing Can be maintained on prepared foods	
C. anomalum	Widely distributed Test-size fish do not jump from containers Can be maintained on prepared foods	Not generally available from hatcheries Adults become too large for general use Similar sizes not available at all seasons High mortality in breeding populations
N. boops	Adults of suitable size for testing Can be maintained on prepared foods	Restricted distribution Not available from hatcheries
L. cyanellus	Widely distributed Can be reared in ponds Same size available at all seasons Do not jump from containers Can be maintained on prepared foods	Frequently require grading for size
L. megalotis	Can be reared in ponds Do not jump from containers Can be maintained on prepared foods Similar size available at all seasons	Not generally available from hatcheries Frequently require grading for size
A. rupestris	Produced by state and private hatcheries Can be reared in ponds Do not jump from containers Of recognized importance to man	Size available varies with season Do not take prepared foods readily

TABLE I (Continued)

Species	Advantages	Disadvantages
C. erythrogaster	Similar size available at all seasons Adults of suitable size for testing Can be maintained on prepared foods	Restricted distribution Not available from hatcheries Jump out of containers
I. punctatus	Widely distributed Available from hatcheries Can be reared in ponds Do not jump from containers Can be maintained on prepared foods Of recognized importance to man	Become too large for general use Spines become entangled in nets Holding water may quickly become foul
M. salmoides	Widely distributed Available from hatcheries Can be reared in ponds Of recognized importance to man	Size available varies with season Do not readily take prepared foods
P. nigromaculatus	Produced by hatcheries Can be reared in ponds Do not jump from containers Of recognized importance to man	Size available varies with season Susceptible to injury and infection Require live foods

from 3.2 to 5.2 inches (Carlander, 1950).

Notemigonus crysoleucas

The golden shiner is found east of the Rocky Mountains in lakes and sluggish streams. It frequents weedy bays and shoals, but is not restricted to such areas. A deep bag seine was found to be best for collecting, especially in deep, clear water.

The diet consists of both phyto- and zooplankters, but larger animal life is also eaten. Dobie, et al. (1956) listed the foods (in percent) as follows: insects, 35; plankton, 28.5; algae, 13.8; plants, 5.3; amphipods, 0.4; mollusks, 1.9; arachnids, 1.4; bryozoans, 1.4; rotifers and protozoans, 0.2; and crustaceans, 12. Dry meal was eagerly taken by test specimens within two or three days after capture. They were maintained on the dry meal for 30 days, without apparent effects of malnutrition.

The golden shiner is commonly reared in ponds for bait purposes. The eggs, which are adhesive, are scattered over aquatic vegetation including filamentous algae and rooted plants. Artificial spawning sites may be prepared by constructing mats of Spanish moss or straw.

Golden shiners spawn in the spring when the water warms to about 68° F., and may continue to spawn throughout the summer. Young may attain a length of 2.1 inches within 70 days without artificial feeding (Dobie, et al., 1956), and a length of 4.0 inches in the first growing season (Markus, 1939). Fishes 2.0 inches long should be suitable for most bioassays, and, in the Midwest, should be available in newly stocked ponds by mid-July. Adults may attain 8 to 10 inches in length; the females grow faster, and become larger than the males. Small fish may be

found throughout the year, but a given population may consist mostly of individuals too large for test purposes.

Golden shiners are easily damaged in handling. Scales may be removed by contact with rough or hard objects, and the damaged area becomes a site of infection. Fin rot is especially troublesome with this species, but can be controlled with a mixture of terramycin and acriflavine.

Tanks must be kept covered at all times to prevent the fish from jumping from the container. Golden shiners are excitable when first collected, and quite sensitive to vibrations. Thousands of these fish may be seen jumping from the water when a strong vibration is produced on the bank of a hatchery pond (Bishop, 1950).

Notropis lutrensis

The red shiner is common in central plains streams with sand or mud bottoms, and may become abundant in impoundments. It is quite tolerant of turbid waters. Minkley (1959) in his survey of the fishes of the Big Blue River Basin, Kansas, reported N. lutrensis as occurring in all kinds of streams and in all habitats sampled. It is easily collected by seining with a 10 to 30 foot seine, depending upon the area involved.

Natural foods include algae, insects, and crustaceans (Koster, 1957). Cross (1950) found N. lutrensis had fed heavily on Chaoborus during spring and early summer. The red shiner took dry food readily in the laboratory. As specimens used in tests were kept in the laboratory only 20 days, it was not possible to determine if the diet of dry meal was adequate.

The red shiner can be reared in ponds. In a study by W. H. Irwin (pers. comm.), better reproduction occurred in deeper ponds in central

Oklahoma. Eggs are deposited on submerged vegetation (Markus, 1939). Fully ripe fish were found in shallow riffles in Wildhorse Creek near Stillwater in the summer of 1960. Filamentous algae was abundant in these riffles. If natural vegetation is a requirement for spawning, red shiners might be induced to spawn upon submerged fiber mats.

Markus (1939) reported that N. lutrensis begins spawning in early May and continues to spawn into the summer. Adults are about 2-3/4 inches in length; the males are larger than the females. Because of the extended spawning season, a variety of sizes may be collected at any time of the year.

Red shiners dart about nervously in captivity, but apparently do not often damage themselves on the walls of the container. By using reasonable care, they can be handled with little loss.

Lepomis microlophus

The redear sunfish is found in the southeastern states where it inhabits streams, bayous, ponds, and reservoirs. Bottom materials of its habitat may be mud, sand, or gravel. Toole (1951) reported that adults are usually caught near the bottom.

Natural foods include crustaceans, insects, algae, snails, and small clams. Redear used in bioassay did not take dry foods readily. Specimens were larger than those of other centrarchids used, which may account for their refusal to take the finely ground food. Daphnia were readily eaten.

The redear is easily reared in ponds. The reproductive potential is lower than that of the bluegill, thus heavier stocking is indicated. The pond stocked for the current study did not produce fish in sufficient

numbers so that the desired size group could be used.

Young-of-the-year collected on August 24 were 1.5 to 3.0 inches in length. Jenkins, et al. (1955) in small samples from Oklahoma waters found 0-age-group fish 3.1 inches long in June, 3.0 inches long in July, 3.5 inches in August, and 5.6 inches in October. As the redear spawns throughout the summer, small specimens may be available at any season of the year.

Pimephales notatus

Pimephales notatus is found in the eastern half of the United States, where it inhabits clean waters of lakes and streams. Trautman (1957) states that the bluntnose minnow is tolerant of turbidity, but its abundance may be restricted by exceptionally turbid conditions. This minnow becomes most numerous in the upper reaches of streams with sand or gravel bottoms.

Natural foods include diatoms, algae, microcrustaceans, and small aquatic insects. On a diet consisting only of dry foods, the bluntnose minnow has been maintained in apparently good condition for a period of several weeks.

This is a common bait species, and is easily reared in ponds if suitable nesting sites are provided. In natural conditions, the eggs are attached to the under side of submerged objects such as rocks and logs, usually at depths ranging from 6 inches to 3 feet.

Pimephales notatus begins to spawn in the spring when the water warms to about 70° F., and continues to spawn into the summer. Specimens from Michigan attained 0.8 to 2.5 inches in length by October of their first year (Carlander, 1950). As the adults are only 3 to 4 inches

long, specimens may be available for bioassay at any time of the year. No reference has been found of unusually high mortality in spawning populations. Adult males are larger than the females. This difference in size becomes evident when the fish become about 2 inches in length.

In one group of fish tested on April 8, chasing in tight circles occurred between individuals, with no apparent relation to size group or sex. The activity was evident each time the fish were observed, but no contact between fish was seen, and no damage to the fish could be detected. Chasing occurred much more frequently in test containers than in holding tanks.

Campostoma anomalum

The stoneroller is found in most of the United States east of the Rocky Mountains (Moore, 1957). It inhabits creeks and small rivers, preferring swift streams, with bottoms of sand, gravel, or rubble. In the Great Smoky Mountains National Park, stonerollers inhabit areas with an average gradient of 2.7 percent (Lennon and Parker, 1960). It is easily collected by seining when found over suitable bottom.

Foods include algae, diatoms, zooplankters, and small aquatic insects. Test fish took dry food readily. Approximately one percent of the individuals became emaciated after about three weeks on this diet, but others remained in apparently excellent condition.

The stoneroller spawns in the spring. Lennon and Parker (1960) reported that it spawns in late April in the Great Smoky Mountains National Park. Metcalf (1959) found gravid females and males in breeding condition on April 2 in Cedar Creek, Kansas. On April 4, stonerollers were found in breeding condition in Fourteen Mile Creek, Oklahoma, at a

water temperature of 60° F. Reed (1958) found Campostoma spawning in Pennsylvania on May 26 in water of 70° F.

Young-of-the-year taken on June 3 were 1.1 to 1.8 inches in length. In Ohio, fish averaged 2.7 inches at 1 year, 4.0 inches (females) and 4.1 inches (males) at 2 years, and 4.7 inches (males) and 5.3 inches (females) at 3 years. Females may mature at a length of 2 inches in Ohio (Trautman, 1957). Breeding males are usually larger and may be older than females (Lennon and Parker, 1960). Lennon and Parker found that none of the 2-year-old stonerollers in their study were sexually mature. Most of the 3-year-old males were immature but most of the females were mature. Of the 4-year-old fish, more than half of the males were immature, but all of the females were mature. All 5-year-old fish were mature.

Stonerollers are known to have a very high mortality rate among spent spawners. For this reason, adults are not considered good test fish during, and immediately following the breeding season.

Notropis boops

Hubbs and Lagler (1949) reported that the bigeye shiner is generally found in streams of limestone upland. Its distribution is restricted to a comparatively small area in the east central United States. It inhabits clear streams of small to intermediate size, with bottom materials of sand, gravel, or bedrock. Finnell, et al. (1956) found N. boops abundant in the Mountain Fork River, Oklahoma, from the headwaters to its mouth which has a mean discharge of 1,400 cubic feet per second. The bigeye shiner is easily captured by seining.

Trautman (1957) reported that this shiner takes animal food including

small insects. It feeds eagerly on dry meal in the laboratory. A single specimen remained in good condition on a diet of dry meal for six months. Test fish were kept on this diet for 32 days without apparent malnutrition.

Although N. boops is abundant in the flowing waters of the Little River system, Oklahoma, it is not numerous in the cutoff lakes (Finnell, et al., 1956). This could suggest that it does not reproduce readily in standing waters, but other factors may limit its abundance in such environments.

Reports of the breeding season were not found. Trautman (1957) reported young-of-the-year collected in October as 1.0 to 1.5 inches in length. Adults in Oklahoma may attain 2.9 inches in length.

Lepomis cyanellus

The green sunfish is found in the central United States in an unusually wide range of habitats. It is associated with smallmouth bass in swift upland streams, and is found in lakes, sluggish streams, and bayous in association with the largemouth bass. Abundance is usually greatest in smaller streams and ponds. The green sunfish is tolerant of turbidity, but may become stunted if waters are highly turbid for extended periods. It frequents areas that are difficult to seine because of obstructions, but is easily taken in a seine when found over smooth bottom.

Natural foods include insects, small crustaceans, and small fishes. Microcrustaceans are important food for the young. Green sunfish (0.7 to 1.4 inches in length) used in this study fed readily on dry food. They grew rapidly in the laboratory, and appeared to be in excellent condition.

The green sunfish is prolific, and will reproduce in almost any farm pond. No special spawning sites are necessary.

Spawning occurs throughout the summer. Individuals of all sizes (small fingerlings to adult) can be obtained at any time of the year. Young-of-the-year are available by late July in Oklahoma. Test animals used were 1.0 to 1.4 inches in length by July 28. Adults are 4 to 8 inches in length.

Lepomis megalotis

The longear is found in the central United States where it inhabits small to intermediate sized streams with low to moderate gradients. It may become abundant in small ponds, but apparently does not compete successfully in some impoundments if other species normally found in association with it are present. It is not tolerant of highly turbid waters. Longear are easily caught in a seine when found over a smooth bottom.

Natural foods are insects, crustaceans, and small fishes (Harlan and Speaker, 1956). The smaller fish (2 inches or less in length) take dry meal readily, and adults may be induced to feed on pelleted trout foods.

The longear can be reared in ponds. It frequently nests over gravelly areas in shoals of streams, but its requirements for nesting sites may not be restricted to such areas.

Witt and Marzolf (1954) noted spawning of longear sunfish in Missouri on June 10 with water temperature between 74° and 77° F. In Oklahoma, spawning extends throughout the summer. One-inch fish were taken on September 27 from a stocked pond. The longear growth average

for Oklahoma is 2.7 inches in 1 year, and 4.0 inches in 2 years (Jenkins, et al., 1955). By grading, fishes of suitable size can be obtained at any time of the year. In Oklahoma, adults are from 3 to 6.5 inches in length, the males being larger than the females.

Ambloplites rupestris

The rock bass occurs in the eastern United States. It is most abundant in small to intermediate-sized streams with bottom materials of gravel, boulders, and bedrock.

Natural foods include insects, crustaceans, and small fishes. Fishes used in the present study were fed on live daphnia.

Spawning occurs in late May and early June in the northern part of its range (Eddy and Surber, 1947). In Ohio, young may grow to 2.0 inches by October. Specimens from Arkansas (hatchery) were 2.0 to 4.5 inches in October. Age-group-I fish from Michigan averaged 3.2 inches in length. Rock bass from the northern part of their range may be of suitable size for testing through their second summer. Further south, the same age group may be too large to be considered a good test fish. Adults may be 4.3 to 10.5 inches in length (Trautman, 1957).

Rock bass have been reared by several state conservation departments, and by a number of privately owned hatcheries.

Chrosomus erythrogaster

The southern redbelly dace is found in the central states of eastern United States. It inhabits cool, clear creeks with rock or gravel bottoms, and may be found in large schools with few other fishes associated with it. It is easily collected with a small seine.

Natural foods include algae and small insects (Koster, 1957). This species is easily kept in captivity where it remains in good condition on a diet of dry food. Ten specimens were maintained on dry meal for nine months without showing signs of malnutrition.

The redbelly dace, a popular aquarium fish, has been reared in captivity in Germany. Innes (1951) gave a detailed account of the method used.

Smith (1908) reported C. erythrogaster spawning in Illinois in mid-May, and by June 14 many of the males had lost their breeding colors. Markus' (1939) reference to the breeding habits of the species may have been based on the habits of C. eos, a similar form. Adults are about 2-3/4 inches in length, the males slightly larger than the females. Breeding fish are easily recognized by their bright colors.

With reasonable care C. erythrogaster can be handled without excessive loss.

Ictalurus punctatus

The channel catfish occurs in central and eastern United States. It is found in streams of intermediate to large size and in some lakes (Trautman, 1957). It is tolerant of turbidity, but is not restricted to turbid waters. Adults are usually found in the deeper pools, but the young may be abundant in shallow riffles, where they can be captured by seining. Davis (1959) was successful in capturing large numbers of channel catfish from impounded waters by baiting with dead fish.

Natural foods include insects, crayfish, snails, worms, fish, and vegetable matter (aquatic plants, seeds, and fruits). Bailey and Harrison (1948) found that insect larvae, such as midges, black flies,

mayflies, and caddis flies, were the most important food of the young. In captivity, the channel catfish feeds readily on dry foods.

Channel catfish are easily reared in ponds by providing proper nesting sites. Milk cans and nail kegs are commonly used. Brood stock may be sexed, and only as many pairs as are provided with nesting sites should be stocked. The eggs may be removed from the nests and hatched in troughs, but special facilities are required, and it is not considered necessary unless maximum production is desired. After the young have hatched, adults may be removed from ponds with large-mesh seines or gill nets. As the channel catfish is reared commercially, and at government-owned hatcheries, it may be more convenient to obtain fish from these sources.

In Kansas, spawning occurs from May through mid-July (Doze, 1925), beginning in the spring when water temperature reaches about 70° F. The optimum temperature is 80° F. (Davis, 1959). The young grow rapidly and may be too large to be considered good test animals at the end of the first year. Fish used in tests were 1.6 to 3.0 inches in length on August 2. The average growth in Oklahoma is 4 inches for 1 year; 8.5 inches for 2 years; and 11.9 inches for 3 years (Finnell and Jenkins, 1954). Fish mature at 11 to 16 inches, and may attain a length of about 30 inches (Davis, 1959).

Micropterus salmoides

The largemouth bass is found throughout the eastern United States in lakes and streams and in a wide variety of habitats. It is usually most abundant in sluggish or non-flowing waters. Small fish may be taken by seining shallow bays.

Apparently any living animal life of suitable size may be eaten by largemouth bass. Important foods are insects, crayfish, and fish. Small specimens feed on animal plankters. Fish in captivity consume a large amount of food. They may be induced to take dry food (Heiliger, 1959), but possibilities for maintaining them in good condition on such a diet are not known to the writer. Fish used in the bioassays were fed primarily on young Gambusia. Live daphnia and chironomid larvae were also fed.

The largemouth bass is easily reared in ponds and needs no artificial nesting sites as the nests are prepared on any natural surface where the eggs will not become covered with silt.

This bass spawns in the spring when water temperatures reach 63° to 68° F. The young grow rapidly and may be testing size by mid-summer in Oklahoma. By early fall, this species may not be available in sizes desired for testing. It may become sexually mature at 7 inches in length (Trautman, 1957).

Pomoxis nigromaculatus

The black crappie is found in most of the eastern half of the United States, except the north Atlantic coast. It inhabits sluggish streams, ponds, and lakes. Trautman (1957) considers submerged vegetation important in its environment. The young are frequently found in weed beds where they may be taken with a seine.

The foods of young crappie consist largely of microcrustacea, aquatic insects (particularly the larvae of Chironomids and Chaoborus if available), and various zooplankters depending upon availability. Adult crappie feed upon fish, insects, and crustaceans. Test fishes were not

induced to take dry foods; they were fed on live daphnia.

Black crappie are easily reared in ponds. Clear ponds are better than turbid ones. As crappie quickly overpopulate a small pond and become stunted, it is better to stock an unpopulated pond.

Burris (1956) reported that black crappie hatched the last week in April, 1949, at Holdenville Hatchery, Oklahoma. In his studies, fish attained a length of about 1.5 inches in 7 weeks, and about 2.0 inches in 15 weeks. First year growth may be from 3 to 5 inches in Oklahoma. Test fish collected on June 21 were between 1.4 and 2.0 inches in length. Fish of fast growing populations may be too large for test purposes by October of their first year.

Black crappie are easily damaged in collecting unless care is taken to prevent contact with hard or abrasive surfaces. They are not excitable in the laboratory, and do not often jump from containers.

RELATIVE RESISTANCE STUDIES

Problems

Certain problems are inherent in relative resistance studies. One life stage of a species is not directly comparable to another life stage of the same or a second species because the sensitivity of fishes varies with age. It is not usually practical to hold all species for a similar period prior to testing; and the ecological requirements of all species cannot be equally determined. Thus the physiological conditions would vary.

The foregoing problems were not solved in this study. The use of adult fishes of all species was not practical, and age groups that might be considered 'similar' were not available because spawning dates vary with species. The data on size of fishes, foods, and length of time held in the laboratory are given (Table II).

Relative Resistance of Species

Results of the 12-hour tests were not considered a reliable basis for establishing relative sensitivity as mortality was still occurring at a high rate. The data were analyzed to determine if the relative position of the fishes on a sensitivity scale changed. A considerable number of species changed position on the scale between 12 and 24 hours. The channel catfish moved from fourth to twelfth place in resistance (first place being the most resistant). The position of other species

TABLE II

FIELD AND LABORATORY DATA ON TEST FISHES

Species	Field			Laboratory			
	Collection date	Locality+	Water temp.	Foods	Length (in.)	Ave. wt. (gms.)	Date test began
L. reticulatus				dry meal	0.6-0.9*	0.05	All tests
I. melas	7-14-60	Boomer Pond, Payne Co.	79°F.	dry meal	1.0-1.5		7-26,28
					1.2-1.4		8-2
	6-22-61	Farm Pond, Payne Co.		dry meal	2.2-2.6	2.2	6-29
N. crysoleucas	6-9-60	Epperson's Pond, Payne Co.	80°F.	dry meal, daphnia	2.8-3.5	2.3-7.8 (range)	6-18
					2.5-4.0		6-23
	7-20-61	Epperson's Pond, Payne Co.	80°F.	dry meal	2.7-3.8	3.8	8-2
					2.5-4.2		8-16
N. lutrensis	6-9-60	Wildhorse Cr., Payne Co.		dry meal	2.0-3.0	1.8-3.6 (range)	6-18
	7-29-60	Wildhorse Cr., Payne Co.	83°F.	dry meal	2.0-2.5	1.9	8-16
	3-6-61	Boomer Cr., Payne Co.		dry meal	1.9-2.9		3-10
	4-5-61	L. Stillwater Cr., Payne Co.	53°F.	dry meal	1.7-2.7		4-8
L. microlophus	8-10-60	Smith's Pond, Payne Co.	78°F.	daphnia, midges, dry meal	2.0-3.0	3.2	8-23

TABLE II (Continued)

Species	Field: Collection date	Field: Locality	Field: Water temp.	Laboratory: Foods	Laboratory: Length (in.)	Laboratory: Ave. wt. (gms.)	Laboratory: Date test began
L. microlophus (contd.)	8-24-60	Smith's Pond, Payne Co.	85°F.	daphnia, midges, dry meal	2.0-3.0		9-6
					2.0-2.8		9-13
	9-22-60	Smith's Pond, Payne Co.		daphnia, midges, dry meal	2.1-3.0		10-4
P. notatus	6-3-60	Fourteen Mi.Cr., Cherokee Co.		dry meal	1.9-3.0		6-30
	3-4-61	Fourteen Mi.Cr., & Hulbert Cr., Cherokee Co.	58°F.	dry meal	1.5-3.0	2.0	3-10
					1.4-3.0		3-17
	4-4-61	Fourteen Mi.Cr., & Hulbert Cr., Cherokee Co.		dry meal	2.0-3.5		4-8
C. anomalum	6-3-60	Fourteen Mi.Cr., Cherokee Co.		dry meal	1.0-2.0		6-18
					1.3-1.8		6-23
					1.1-1.8		6-30
N. boops	6-3-60	Fourteen Mi.Cr., Cherokee Co.	78°F.	dry meal	1.4-2.9	0.4-3.7 (range)	6-18
	6-10-60	Fourteen Mi.Cr., & Hulbert Cr., Cherokee Co.	78°F.	dry meal	2.0-3.0		6-23

TABLE II (Continued)

Species	Field: Collection date	Field: Locality	Field: Water temp.	Laboratory: Foods	Laboratory: Length (in.)	Laboratory: Ave. wt. (gms.)	Laboratory: Date test began
N. boops (contd.)					2.0-3.0		6-30
	3-4-61	Fourteen Mi.Cr., & Hulbert Cr., Cherokee Co.	58°F.	dry meal	1.8-2.9		3-10
L. cyanellus	6-16-60	Redding's Pond, Payne Co.	80°F	dry meal	0.7-1.0	0.1	6-23
					0.7-1.0		6-30
	7-20-60	Hesser's Pond, Payne Co.	80°F.	dry meal	1.0-1.4	0.3	7-26
					1.0-1.4		7-28
L. megalotis	8-13-60	Horner's Pond, Payne Co.	76°F.	dry meal	0.9-1.3		8-23
	9-2-60	Horner's Pond, Payne Co.	80°F.	dry meal	1.0-1.5		9-6
					0.9-1.5		9-13
					1.0-1.5	10-25	
A. rupestris	10-29-60	St. Fish Hatchery, Centerton, Ark.		daphnia	1.5-3.0	2.2	11-1
					1.5-3.0		11-10
					1.5-3.0		11-19
					2.0-3.5		12-3
C. erythrogaster	10-29-60	Bidding Spr.Cr., Cherokee Co.	63°F.	dry meal	2.0-2.8	1.4	11-1

TABLE II (Continued)

Species	Field			Laboratory			
	Collection date	Locality	Water temp.	Foods	Length (in.)	Ave. wt. (gms.)	Date test began
C. erythrogaster (contd.)					2.0-2.8		11-10
					2.0-2.8		11-19
	11-28-60	Bidding Spr.Cr., Cherokee Co.		dry meal	2.0-2.8		12-3
I. punctatus	7-26-60	St. Fish Hatchery, Holdenville		dry meal	1.6-3.0	1.1	8-2
	11-19-60	St. Fish Hatchery, Holdenville		dry meal	3.2-3.8	4.7	10-25
					3.2-3.8		11-1
					3.2-3.8		11-10
M. salmoides	7-26-60	St. Fish Hatchery, Holdenville		daphnia, fish	1.8-2.1	1.3	8-23
					2.1-2.5		9-6
	8-19-60	St. Fish Hatchery, Holdenville		daphnia, fish	2.3-3.2	3.1	10-25
					2.3-3.2		11-1
P. nigromaculatus	6-21-60	Boomer Pond, Payne Co.		daphnia	1.4-2.0		6-30
	7-14-60	Boomer Pond, Payne Co.		daphnia	1.4-2.0	0.9	7-26
					1.4-2.0		7-28

+ Oklahoma unless stated otherwise.
* Less than 1% not in the 0.6 to 0.7 inch range.

moved to a lesser degree. The analysis of variance and results of the multiple range test are given in Table III.

Most of the mortality during testing occurred prior to the 24-hour observations which were considered reliable in determining relative resistance. An analysis of variance (Table IV) revealed that a significant difference at the 5 percent level occurred between the species, with an F value of 9.51 (tabulated F of 2.03).

On the basis of the new Duncan's multiple range test, the means of the 24-hour TL_m's are grouped into six statistical populations; any two species not included in the same population are significantly different (Table IV). The guppy was significantly most resistant. The black bullhead ranked second, and differed significantly from all other species. The guppy and black bullhead were considered 'resistant'. The golden shiner, red shiner, and redear sunfish were included in a population that ranked third. These three species were considered 'intermediate' in sensitivity. The sixth population included the means for the bluntnose minnow, bigeye shiner, green sunfish, longear sunfish, rock bass, southern redbelly dace, channel catfish, and largemouth bass, ranked in the order given. These were considered 'sensitive' forms. The fourth and fifth populations included some of both the 'intermediate' and 'sensitive' forms.

The 24-hour TL_m's (adjusted means) are plotted in Figure 2 along with data from other relative resistance studies that have included several of the species for which resistance to refinery wastes was established. Data from several investigators are not in general comparable, due to several variables which affect toxicity and to the many different ways of expressing sensitivity.

TABLE III

STATISTICAL ANALYSIS OF RELATIVE RESISTANCE DATA FROM 12-HOUR TL_m'S

Analysis of Variance				
Source	d. f.	S. S.	M. S.	F
Total	127	17,440.842		
Tests (Unadjusted for species)	19	11,290.729		
Species (Adjusted for tests)	12	5,087.309	423.942	14.64
Experimental error	33	955.559	28.956	
Sampling error	63	107.245	1.702	

Duncan's 5% Multiple Range Test

Species	A	B	C	D	E	F	G	H	I	J	K	L	M
Means	15.86	17.13	17.39	18.23	18.42	18.58	18.58	19.23	21.06	21.51	22.29	30.71	32.86

Identification (for common names see page 2)

A. M. salmoides
B. L. megalotis
C. N. boops
D. L. cyanellus
E. L. microlophus
F. A. rupestris
G. P. notatus
H. C. erythrogaster
I. N. lutrensis
J. I. punctatus
K. N. crysoleucas
L. I. melas
M. L. reticulatus

TABLE IV

STATISTICAL ANALYSIS OF RELATIVE RESISTANCE DATA FROM 24-HOUR TL_m'S

Analysis of Variance

Source	d. f.	S. S.	M. S.	F
Total	135	18,341.536	135.836	
Tests (Unadjusted for species)	19	10,692.432	562.751	
Species (Adjusted for tests)	12	5,667.176	472.265	9.51
Experimental error	36	1,790.768	49.744	
Sampling error	68	191.160	2.811	

Duncan's 5% Multiple Range Test

Species	A	B	C	D	E	F	G	H	I	J	K	L	M
Means	15.09	15.32	16.23	16.32	16.70	16.82	17.69	18.72	19.52	20.45	22.68	26.59	32.24

Identification (for common names see page 2)

A. M. Salmoides
B. I. punctatus
C. C. erythrogaster
D. A. ruprestris
E. L. megalotis
F. L. cyanellus
G. N. boops
H. P. notatus
I. L. microlophus
J. N. lutrensis
K. N. crysoleucas
L. I. melas
M. L. reticulatus

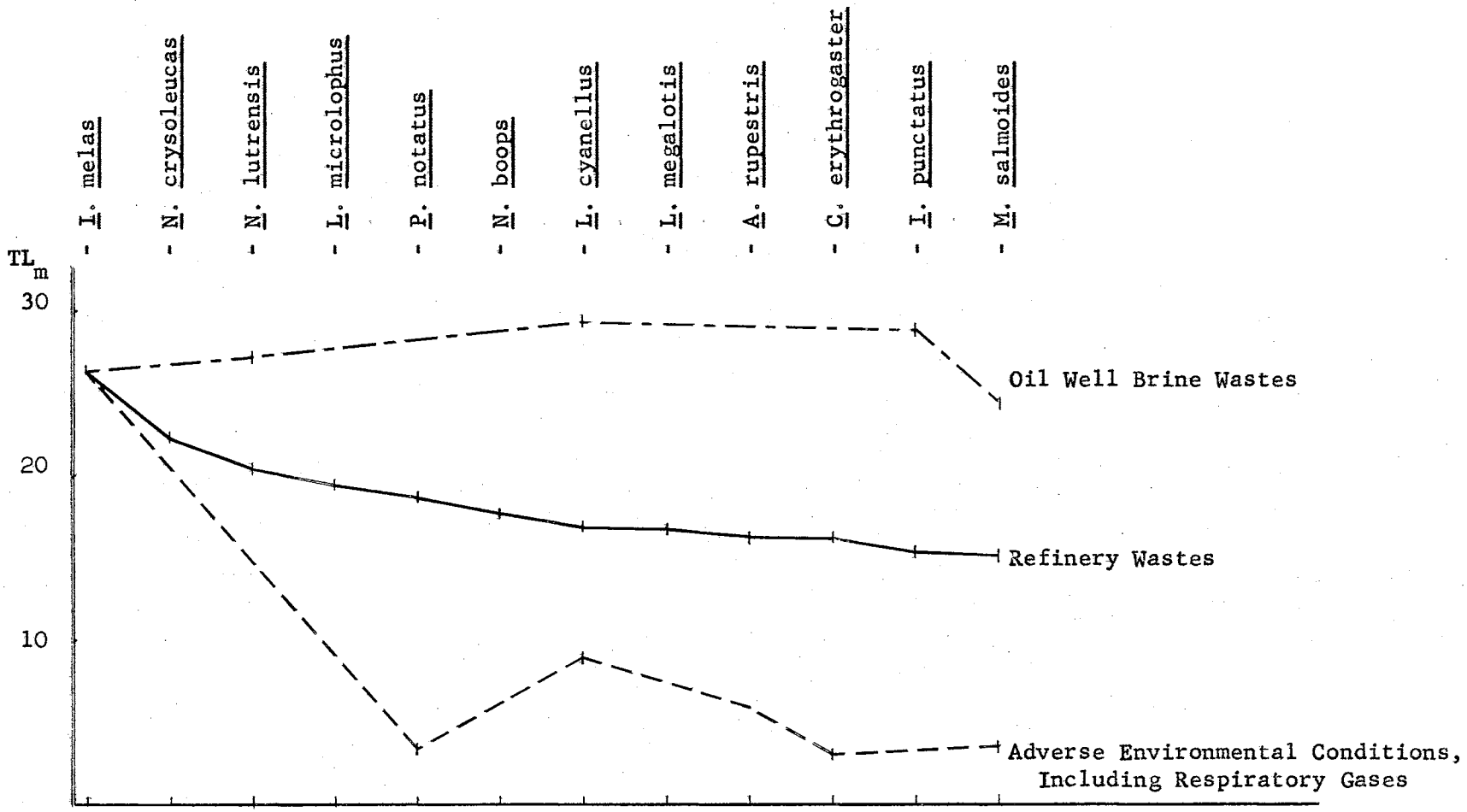

Figure 2. Comparison of relative resistance of fishes to oil well brine wastes (Clemens and Jones, 1954); refinery wastes; and adverse environmental conditions, including low dissolved oxygen and high carbon dioxide tension (Wells, 1918). Values on the left of the graph are TL_m's or adjusted values from an arbitrary scale.

Analysis of data for the 48-hour tests is shown in Table V. Some rearrangement of species on the resistance scale had occurred between 24-hour and 48-hour tests. The channel catfish moved from twelfth to fifth place in resistance (first place being most resistant). A possible explanation is given under the discussion on the 96-hour test. Rearrangement among some of the more sensitive species occurred, but changes in TL_m's were small.

Although most of the mortality occurred within 24 hours, enough mortality occurred for certain species between 24 and 96 hours to produce different results in the multiple range test. On the basis of the 96-hour tests, there were only three statistical populations (Table VI). The guppy and black bullhead were included in the first population with no significant difference between the two. The second population included the black bullhead, golden shiner, and channel catfish. The third population included the golden shiner, channel catfish, and all other species not included in populations one and two.

The channel catfish had moved from twelfth place to fourth place. One explanation for this is that the catfish in one test at 24 hours had an exceptionally low TL_m, and on the same test, the catfish mortality between 24 and 96 hours increased to the extent that no TL_m could be calculated. The deletion of data for the test resulted in an analysis of data from other tests in which the channel catfish was relatively more resistant. The same situation was true for the largemouth bass which moved from thirteenth to ninth place. The two species were from the same source, and had been brought to the laboratory only six days prior to the test. Both species from this collection were relatively more resistant in subsequent tests, and it is suspected that they were

TABLE V

STATISTICAL ANALYSIS OF RELATIVE RESISTANCE DATA FROM 48-HOUR TL_m'S

Analysis of Variance				
Source	d. f.	S. S.	M. S.	F
Total	129	17,155.265		
Tests (Unadjusted for species)	19	10,965.619		
Species (Adjusted for tests)	12	4,963.234	413.602	12.65
Experimental error	34	1,111.692	32.696	
Sampling error	64	114.720	1.781	

Duncan's 5% Multiple Range Test

Species	A	B	C	D	E	F	G	H	I	J	K	L	M
Means	14.90	15.26	15.66	16.10	16.21	16.78	17.81	18.16	19.66	20.08	20.67	25.37	31.06

Identification (for common names see page 2)

A. L. megalotis
B. M. salmoides
C. L. cyanellus
D. N. boops
E. A. rupestris
F. C. erythrogaster
G. P. notatus
H. L. microlophus
I. I. punctatus
J. N. lutrensis
K. N. crysoleucas
L. I. melas
M. L. reticulatus

TABLE VI

STATISTICAL ANALYSIS OF RELATIVE RESISTANCE DATA FROM 96-HOUR TL_m'S

Analysis of Variance

Source	d. f.	S. S.	M. S.	F
Total	127	16,968.618		
Tests (Unadjusted for species)	19	11,206.173		
Species (Adjusted for tests)	12	4,765.944	397.162	13.9
Experimental error	32	914.506	28.578	
Sampling error	64	81.995	1.281	

Duncan's 5% Multiple Range Test

Species	A	B	C	D	E	F	G	H	I	J	K	L	M
Means	15.47	15.76	15.84	15.88	16.44	16.54	17.10	17.52	17.79	19.04	20.21	26.63	30.65

Identification (for common names see page 2)

A. L. megalotis
B. L. cyanellus
C. A. rupestris
D. N. boops
E. M. salmoides
F. C. erythrogaster
G. P. notatus
H. N. lutrensis
I. L. microlophus
J. I. punctatus
K. N. crysoleucas
L. I. melas
M. L. reticulatus

not in good condition at the time of Bioassay number 15. The longear moved from ninth to thirteenth place due to lower TL_m's on two tests. The green sunfish moved from eighth to twelfth place which could be explained only as a result of missing data among the other species. Erratic results are to be expected when data are missing. The relative position of the fishes on a sensitivity scale are shown in Figure 3. The position of the stoneroller and black crappie which were analyzed separately are included on the 24-hour scale in Figure 3, but should not be considered in comparing the results of the 24-hour and 96-hour tests for the other 13 species.

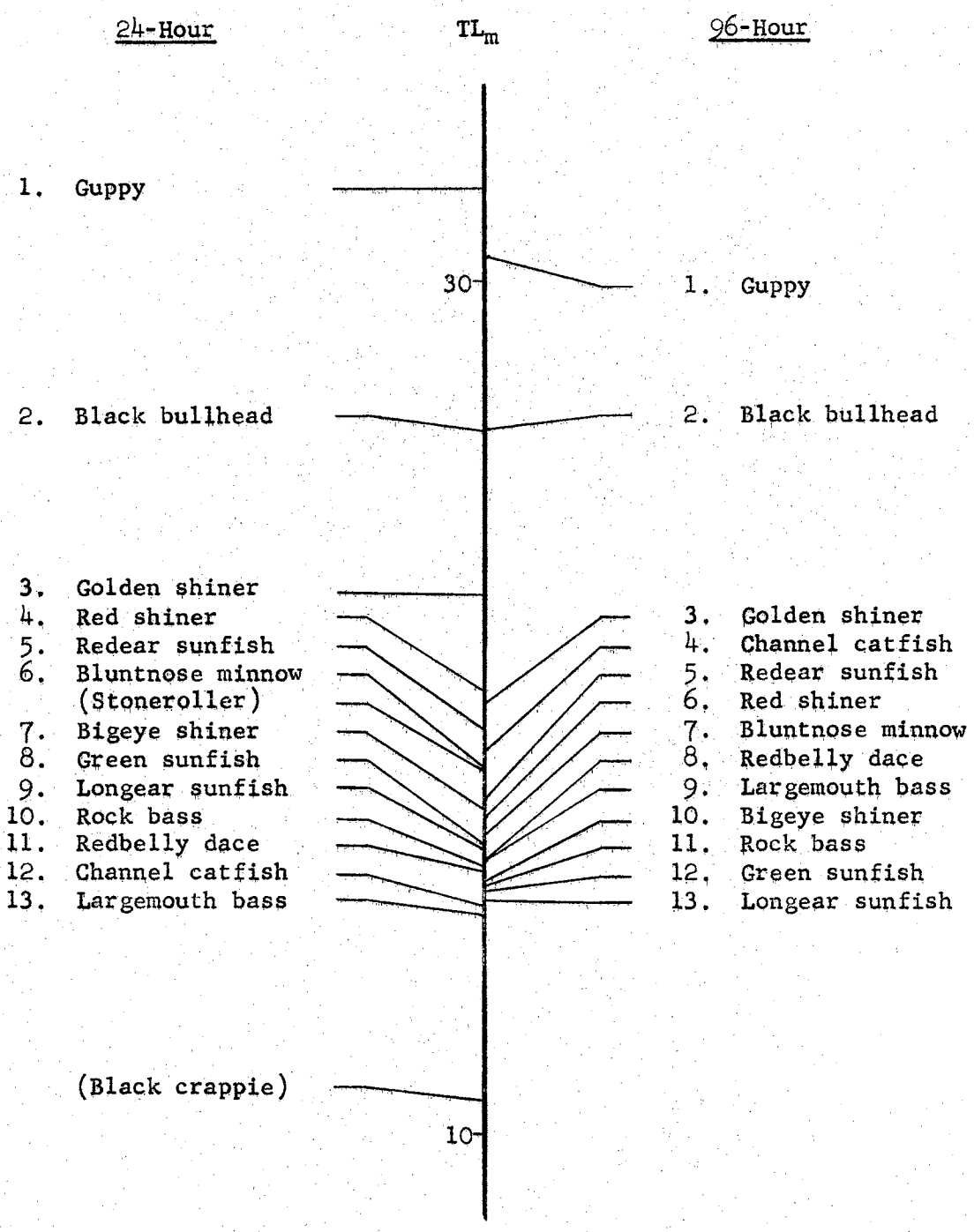

Figure 3. The sensitivity of fishes to refinery effluent based on the 24-hour and 96-hour TL_m's. The stoneroller and black crappie TL_m values were adjusted to this scale after a separate analysis.

SUMMARY

1. Fifteen species of fishes, including the guppy as a reference species, were tested in a series of twenty standing-water bioassays, using refinery effluent as a toxicant.

2. The 12-, 24-, 48-, and 96-hour TL_m's were calculated by straight-line graphical interpolation.

3. The mean 12-, 24-, 48-, and 96-hour TL_m's, adjusted for differences in effluent samples, were calculated for each species by the Doolittle Technique.

4. Relative resistance of 13 species was determined by ranking the adjusted means and subjecting them to the new Duncan's Multiple Range Test.

5. After separate analysis, the position of the other two species tested was determined for the relative resistance scale.

6. An analysis of variance was calculated by the Doolittle Technique, and the results presented in tabular form.

   a. The difference between the sensitivity of species was determined to be significant in an F test.
   b. An assumed interaction between effluent samples and species was verified.
   c. Sampling was determined to be adequate.

7. The sensitivity to refinery effluent of four age-groups of guppies was determined in two bioassays.

8. A review of the literature on the life history of each species was made, and considered in relation to the use of the species in bioassay.

9. The suitability of each species as a test fish was studied during collection, transport, acclimation, and testing.

10. The relative resistance of certain species to refinery wastes was graphically compared to their relative resistance to carbon dioxide as determined by Wells (1918), and to oil well brine wastes as determined by Clemens and Jones (1954).

11. The 24-hour relative resistance data of 13 species was compared graphically to the 96-hour data.

12. The conclusion of Doudoroff and his co-workers (1951), that total-survival-no-survival data should be used only if the difference between the two critical concentrations does not greatly exceed 25 percent of the higher value, was verified in 24-hour tests of refinery effluent with the guppy as test animal.

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APPENDIX A

TABLE 1. TWELVE-HOUR MEDIAN TOLERANCE LIMITS

Test Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Species																					
L. reticulatus	A*	21.3	18.0	37.8	32.8	64.8	34.3	28.3	33.4	38.7	20.4	22.4	--	29.1	27.7	30.5	25.2	20.2	15.5	21.8	30.5
	B	21.8	18.9	38.4	32.8	63.5	34.3	27.3	35.3	34.2	21.3	22.4	49.0	27.2	32.0	32.0	20.7	22.6	14.8	20.0	33.4
N. boops	A	7.0	9.4	15.5														7.9			
	B	7.3	9.9	15.5														8.1			
N. lutrensis	A	13.5						10.4										--		13.3	
	B	12.9						12.6										10.0		13.3	
N. chrysoleucas	A	15.6	12.8				20.0	13.6													
	B	15.6	12.8				20.0	13.8													
L. cyanellus	A		10.3	21.0	18.5	44.0															
	B		9.5	21.0	17.8	38.2															
P. notatus	A			11.3														10.7	8.7	11.2	
	B			10.8														10.0	8.2	11.4	
I. melas	A				21.8	63.1	32.6														22.1
	B				26.4	68.5	33.6														22.8
I. punctatus	A						19.2						--	24.7	19.3						
	B						18.4						--	24.3	18.8						
M. salmoides	A								15.0	16.7			21.6	19.5							
	B								12.2	18.8			24.0	21.0							
L. microlophus	A								14.7	19.3	11.3	11.6									
	B								13.5	19.3	10.8	11.6									
L. megalotis	A								15.3	16.8	10.3		--								
	B								15.0	17.8	9.6		--								
A. rupestris	A													20.0	13.8	15.3	10.8				
	B													20.5	14.8	15.6	10.8				
G. erythrogaster	A													--	16.1	15.9	10.6				
	B													--	18.0	15.3	10.3				

*A and B designate replicates

TABLE II. TWENTY-FOUR-HOUR MEDIAN TOLERANCE LIMITS

Test Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Species																					
L. reticulatus	A*	20.8	17.2	36.6	31.7	64.8	34.3	24.2	26.7	35.0	16.6	22.4	57.0	28.3	27.7	28.5	25.2	20.2	14.8	21.5	29.4
	B	21.7	18.9	36.0	32.8	63.5	34.3	24.6	27.6	33.3	19.4	22.4	43.7	27.2	32.0	30.5	20.7	21.8	13.8	19.3	33.4
N. boops	A	7.0	9.4	15.5														7.9			
	B	7.3	9.9	13.8														8.1			
N. lutrensis	A	13.5						8.7										8.7		13.3	
	B	12.9						8.7										9.2		13.3	
N. crysoleucas	A	15.4	12.8				17.2	12.1													
	B	15.6	12.8				15.9	13.1													
L. cyanellus	A		10.1	19.3	17.8	42.0															
	B		9.5	19.8	17.8	38.2															
P. notatus	A			11.3														10.7	8.7	11.2	
	B			10.8														10.0	8.2	11.4	
I. melas	A				21.8	63.1	21.0														22.1
	B				26.4	65.0	19.9														21.6
I. punctatus	A						11.2						7.5	24.3	19.3						
	B						10.8						8.3	23.4	18.8						
M. salmoides	A								15.0	16.7			12.2	19.5							
	B								10.8	18.8			10.1	19.5							
L. microlophus	A								14.7	19.3	10.8	11.6									
	B								12.2	19.3	10.3	11.6									
L. megalotis	A								12.3	16.8	9.6		20.4								
	B								10.3	17.3	5.6		17.2								
A. rupestris	A													18.8	13.8	15.3	10.8				
	B													18.0	14.8	15.3	10.8				
C. erythrogaster	A													17.2	16.1	15.6	9.9				
	B													16.2	17.2	15.3	9.4				

*A and B designate replicates

TABLE III. TWENTY-FOUR-HOUR MEDIAN TOLERANCE LIMITS (THESE DATA ANALYSED SEPARATELY FROM THOSE OF THE OTHER SPECIES TESTED)

Test Number		1	2	3	4	5
Species						
L. reticulatus	A*	20.8	17.2	36.6	31.7	64.8
	B	21.7	18.9	36.0	32.8	63.5
G. anomalum	A	11.5	9.9	18.3		
	B	11.7	9.9	16.7		
P. nigromaculatus	A			16.9	16.8	34.0
	B			16.5	16.8	34.0

*A and B designate replicates

TABLE IV. FORTY-EIGHT-HOUR MEDIAN TOLERANCE LIMITS

Test Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Species																					
L. reticulatus	A*	20.8	17.2	36.0	31.7	64.8	34.3	22.7	24.0	32.8	16.6	22.4	42.0	27.2	27.7	28.5	25.2	20.2	14.8	21.5	29.4
	B	21.7	18.9	36.0	32.8	69.5	34.3	24.6	20.7	33.2	18.0	22.4	33.6	25.7	32.0	30.5	20.7	21.8	13.8	19.3	33.4
N. boops	A	7.0	9.4	15.5														7.9			
	B	7.0	9.9	13.8														8.1			
N. lutrensis	A	13.5						--										8.7		13.3	
	B	12.6						--										9.2		13.3	
N. crysoleucas	A	15.4	12.8				15.6	8.7													
	B	15.6	12.8				15.2	8.7													
L. cyanellus	A		10.1	19.3	17.8	42.0															
	B		9.5	19.8	17.8	38.2															
P. notatus	A			11.3														10.7	8.7	11.2	
	B			10.8														10.0	8.2	11.4	
I. melas	A				21.8	63.1	--														22.1
	B				26.4	65.0	15.5														21.6
I. punctatus	A						10.8						--	23.8	19.3						
	B						10.8						--	23.4	18.8						
M. salmoides	A								10.0	16.7			--	16.8							
	B								10.0	18.8			9.3	16.8							
L. microlophus	A								14.2	19.3	5.3	11.6									
	B								11.4	19.3	5.3	11.6									
L. megalotis	A								11.4	16.8	5.3		10.1								
	B								10.0	17.3	5.3		10.1								
A. rupestris	A													15.8	13.8	15.3	10.8				
	B													15.8	14.8	13.5	10.8				
C. erythrogaster	A													15.8	16.1	15.3	9.9				
	B													16.2	17.2	15.3	9.4				

*A and B designate replicates

TABLE V. NINETY-SIX-HOUR MEDIAN TOLERANCE LIMITS

Test Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Species																					
L. reticulatus	A*	20.8	17.2	35.0	31.7	64.8	34.3	22.7	24.0	32.8	16.6	22.4	32.0	27.2	27.7	27.7	25.2	20.2	14.8	21.5	29.4
	B	21.7	18.9	35.0	32.8	63.5	34.3	24.6	19.3	33.2	18.0	21.9	32.0	25.7	32.0	30.5	20.7	21.8	13.8	19.3	33.4
N. boops	A	7.0	9.4	15.5														7.9			
	B	6.1	9.9	13.8														8.1			
N. lutrensis	A	7.0						--										8.7		13.3	
	B	6.6						--										9.2		13.3	
N. crysoleucas	A	15.4	12.8				15.2	8.7													
	B	15.6	12.1				14.8	8.7													
L. cyanellus	A		10.1	19.3	17.8	42.0															
	B		9.5	19.8	17.8	38.2															
P. notatus	A			11.3														10.7	8.7	11.2	
	B			10.8														10.0	8.2	11.4	
I. melas	A				21.8	63.1	--														22.1
	B				26.4	65.0	--														21.6
I. punctatus	A						10.8						--	23.8	19.3						
	B						10.8						--	22.8	18.8						
M. salmoides	A								9.7	16.7			--	16.8							
	B								9.4	18.8			--	16.8							
L. microlophus	A								11.4	19.3	5.3	9.6									
	B								11.4	19.3	5.3	9.6									
L. megalotis	A								10.7	16.8	5.3		10.1								
	B								10.0	17.3	5.0		10.1								
A. rupestris	A													15.8	13.8	14.6	10.8				
	B													15.6	14.2	13.5	10.8				
G. erythrogaster	A													15.6	16.1	15.3	9.9				
	B													16.2	17.2	15.0	9.4				

*A and B designate replicates

VITA

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