Generation at Carpenter Dam tends to create a deep current of cold, oxygenated water moving through Lake Hamilton. Suitable temperature ranges and sufficient oxygen levels, as were found in the channel, should sustain trout throughout the critical summer months.

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THE RELATIVE RESISTANCE OF THIRTEEN SPECIES OF FISHES TO PETROLEUM REFINERY EFFLUENT [1, 2]

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ABSTRACT

Twelve species of fishes native to Oklahoma were each tested four times in a series of twenty bioassays, and the guppy was included in each bioassay as a reference. The 24-hour median tolerance limits (TLm's) were determined. The relative sensitivity of each species was established. In a 5% multiple range test, the species were grouped into the following six statistical populations: (1); (2); (3-5); (4-10); (5-12); and (6-13). In the following ranked list, numbers in parentheses (also the rank numbers of the species) indicate the statistical populations to which the species could belong with no significant difference, while, species not included in numbers in parentheses are significantly different from the population included. Fishes rank from most resistant to least resistant as: 1. *Lebistes reticulatus* (1); 2. *Ictalurus melas* (2); 3. *Notemigonus crysoleucas* (3-5); 4. *Notropis lutrensis* (3-5), (4-10); 5. *Lepomis microlophus* (3-5), (4-10), (5-12); 6. *Pimephales notatus* (4-10), (5-12), (6-13); 7. *Notropis boops* (4-10), (5-12), (6-13); 8. *Lepomis cyanellus* (4-10), (5-12), (6-13); 9. *Lepomis megalotis* (4-10), (5-12), (6-13); 10. *Ambloplites repestris* (4-10), (5-12), (6-13); 11. *Chrosomus erythmrogaster* (5-12), (6-13); 12. *Ictalurus punctatus* (5-12), (6-13); 13. *Micropterus salmoides* (6-13).

The suitability of each species as a test animal is considered, based on information on the life histories and observations made during these tests.

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 nation-wide survey by the U. S. Public Health Service (1960) listed

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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 to considerable, if unknown, part to industrial pollution.

Biological indexes are commonly used in pollution studies. The use of indexes presupposes a knowledge of the relative resistance of the indigenous organisms. A National Institutes of Health grant for a study of the relative resistance of fishes to petroleum refinery effluent became effective on February 1, 1960. This and subsequent studies are to include 54 species of fresh-water forms. This report is restricted to studies on the guppy and 12 species native to Oklahoma which follow: *Ictalurus melas* Rafinesque, black bullhead; *Notemigonus crysoleucas* (Michill), golden shiner; *Notropis lutrensis* (Baird and Girard), red shiner; *Lepomis microlophus* (Gunther), redear; *Pimephales notatus* (Rafinesque), bluntnose minnow; *Notropis boops* Gilbert, bigeye shiner; *Lepomis cyanellus* (Rafinesque), green sunfish; *Lepomis megalotis* (Rafinesque), longear sunfish; *Ambloplites rupestris* (Rafinesque), rock bass; *Chrosomus erythrogaster* (Rafinesque), southern redbelly dace; *Ictalurus punctatus* (Rafinesque), channel catfish; and *Micropterus salmoides* (Lacepede), largemouth bass.

Relative resistance was established in a series of twenty bioassays, and measured as the 24-hour TLm's (median tolerance limits). Observations were made on the suitability of each species as a laboratory animal, and its 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 sensitive to some chemicals (Henderson and Tarzwell, 1957). The gizzard shad which is sensitive to rotenone (Huish, 1959, found 0.06 to 0.14 ppm effective) is more tolerant to sodium chloride (Chipman, 1959). Certain fishes (goldfish, black bullhead, carp) may be classified as 'resistant', and others (gizzard shad and salmonids) may be considered 'sensitive'. Because fishes vary in their reactions, a variety of species has been used in toxicity studies.

REVIEW OF THE LITERATURE

Common names used herein are those given in the list of common and scientific names published by the American Fisheries Society (1960). Marsh (1907) used fingerling largemouth bass, yellow perch, spottail shiners, salmon fry, and brook trout fry in testing the toxicity of a number of industrial wastes. The relative resistance of 18 species to carbon dioxide was listed by Wells (1918) and reviewed by Shelford (1918). Belding (1927) gave the 24-hour lethal dose for a number of chemicals for brook trout, rainbow trout, chinook salmon, carp, goldfish, and suckers. The sensitivity of several species to lead salts was studied by Carpenter (1930). Ellis (1937) reported the reactions of a number of species of fishes to individual chemical elements or compounds. Studies by Surber (1948) and Surber and Hoffman (1949) compared the resistance of several fishes to DDT. Surber and Hoffman, (1949) concluded that fingerling bluegill, smallmouth bass, and black crappie were more sensitive to DDT than were largemouth black bass, golden shiners, and trout. Lawrence (1950) reported concentrations of several insecticides which were toxic to bluegill, largemouth bass, goldfish, and fathead minnows. In aquarium tests, bass fingerlings were killed by 0.05 ppm DDT (50 percent wettable powder); bluegill fingerlings by 0.15 ppm; and goldfish by 0.2 ppm. He reported the same concentration of toxaphene dust as toxic for bass and bluegill fingerlings, and goldfish. Anderson (1953) tested five kinds of centrarchids with seven chemical compounds. From a series of 68 tests, he reported that the redear sunfish, orangespotted sunfish, and bluegill were similar in sensitivity, but found a difference between them and the largemouth bass and warmouth. There was no difference in sensitivity of the last two species. The reactions of fishes to brine water from oil wells was reported by Clemens and Jones (1954). Ten species common in central Oklahoma were ranked according to their median toxicity thresholds as follows: plains killifish, mosquitofish, white crappie, bluegill, green sunfish, channel catfish, black bullhead, red shiner, largemouth bass, and fathead minnow. In sixhour tests with constant flow apparatus, Renn (1955) found that 50 percent of white crappie survived for more than 350 minutes at 0.05 ppm N as KCN, while bluegills survived for the same period at approximately 0.07 ppm. The redbreast sunfish had about the same tolerance as bluegill. Clemens and Finnell (1956) in a study of a stream polluted with refinery effluent found the plains killifish, red shiner, and fathead minnow at stations with a higher concentration of effluent than those at which the sand shiner and green sunfish were found.

Chipman (1959) found the gizzard shad, American eel, several kinds of killifishes, three livebearers, and the spotted gar more tolerant of salt water than were sunfishes, the bowfin, and black bullhead. A unique bioassay by Weiss (1959) was based upon reduction in the amount of acetycholinesterase (AChE) in the nervous tissue of fishes after exposure to organic phosphorous insecticides. Weiss (1958) had shown that in small fish, reduction in AChE in brain tissue was proportional to concentration and exposure to anti-AChE compounds. In the study of 1959 with the golden shiner, bluegill, largemouth bass, and goldfish, the brain-enzyme activity per unit of brain weight varied with the species.

Douglas (1961) reported a study in which the relative resistance of four species of fishes to petroleum refinery effluent was determined. Using effluent from two refineries, it was concluded on the basis of 10 bioassays that of the four species, the guppy was most resistant, the fathead and mosquito fish were second in rank, and the plains minnow was the least resistant.

Personnel at the Atlantic Refining Company Waste Control Laboratory have studied the effects of refinery wastes on fishes. The studies have been in progress since 1935 (Turnbull, DeMann, and Weston, 1954). In Oklahoma, Clemens and Crawford (mineo., no date) reported on a statewide study of refinery effluents made during 1954.

METHODS AND PROCEDURES

Test Fishes

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

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 resulted from their use. Seines were of ⅛-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 were prepared from the liners of dismantled household refrigerators. The inner compartments were removed from the refrigerators, and openings in the sides and bottoms were sealed. Tank capacity (usable space) was 25 to 40 gallons. 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 polyethylene bags by the method essentially as described by Clark (1959). Flat bags (32 x 30 inches) were used. A small oxygen tank (30 x 4¼ inches, including the valve) which could be stored in the rear luggage compartment 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.) oxygen tank at a small fraction of the cost of having it filled at a commercial establishment. The procedure was to connect the smaller tank to a large one (at 1200 to 1500 pounds of pressure) 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. The mixture was 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 fish 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, and were removed from the tank daily. They 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. The mixture was the standard dry ration used for feeding all species of fishes that did not require live food. 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 prepared from old refrigerator liners 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 as a preventative measure.

Fish were fed once daily. The standard dry ration 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 accidently 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 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. The dense schools were easily captured with dip nets. Two to three thousand fish could be captured within a few minutes by this method.

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 ⅛-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, normal 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 was made in July, 1960 by the U. S. Geological Survey. (measured as parts per million except where otherwise indicated):

Silica (SiO_2)	3.6	Flouride (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	
Potassium (K)	25.0	(Evap. 180° C.)	218.0
Bicarbonate (HCO_3)	134.0	Hardness (as $CaCO_3$)	142.0
Carbonate (CO_3)	0.0	Specific conductance	
Sulfate (SO_4)	26.0	(Micromhos at 25° C)	396.0
Chloride (Cl)	39.0	pH	7.9

Effluent. The toxicant used was the final effluent from a petroleum refinery in central Oklahoma. The waste was 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 test. A wide range of concentrations were prepared, and two fish of each species were placed in two liters (or four liters depending on the weight of the fish) of solution at each concentration. Exploratory tests were made in the afternoon and 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 temperature in containers was 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 300 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. Each was tested on four different dates (four different effluent collections) except the guppy which was included in every test.

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 TLm's were calculated on semi-log paper by straight line graphical interpolation (Doudoroff, et al., 1951). The 24-hour TLm's were used to determine relative resistance.

A two-way classification (species x tests) was analyzed statistically by the Doolittle Technique. The mean TLm for each species, adjusted for differences in effluents, was determined. Means were ranked and subjected to the new Duncan's 5 percent multiple range test.

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 refining 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 studies reported herein 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.

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. The biological relationships of the fishes of a stream are not well understood. The most sensitive species should be considered in studying the effects of pollution.

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 nor the toxicity of an effluent. Thus, the lethal limits of the substance cannot be determined. The only measure of the toxicity of such pollutants is the reaction of the test fish. 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 must be sorted for use in bioassay. Many stream collections are of this nature. The difficulty of separating live fish by species depends on a knowledge of the fish fauna sampled, and the resemblance of the species. Test fish cannot be handled and examined individually as are dead specimens. Glass-top sorting tables and glass aquaria are useful. While under stress of excitement, some fishes lose their coloration, and are 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 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 from the same watershed. Genetic differences may occur 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 the 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 ourdoor 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 fish 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. A 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 thoroughly investigated, both the eggs and major size groups 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 three-inch fingerlings. The three-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 and the accumulation of wastes in holding water is a function of 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 are important. Clemens and Sneed (1958) reported on the effects of physiological condition 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-of-toxicant-to-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 conditions are the foods and feeding habits of fishes. 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 are inexpensive and easy to store. All species of minnows used in these studies were induced to eat dry foods. Many darters and some centrarchids (black basses and crappies) require live foods. 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.

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 starvation, and to low dissolved oxygen after 39 days starvation. There was a decrease of resistivity with longer periods of starvation. Carpenter (1930) found similar results (an initial increase in resistance followed by a decrease) in testing the fathead minnow with lead salts.

In an experiment by Adelman, et al. (1955), smaller 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 resulting invasion of pathogenic organisms may quickly result in death unless preventative 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. Antagonism between individual fish is a factor affecting test results with some species. 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 infections 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 (certain toxicants are absorbed by fish, and thus removed from solution). Doudoroff, et al. (1951) suggests 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 exceptionally 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.

RELATIVE RESISTANCE STUDIES

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 vary with their age. It is not usually practical to hold all species for a similar period prior to testing; and the requirements of all species cannot be equally determined. Thus the physiological condition 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 I).

Relative Resistance of Species

Most of the test mortality occurred prior to the 24-hour observations which were used in determining relative resistance. An analysis of variance (Table II) reveals that a significant difference at the 5 percent level occurs between the species, with an F value of 9.51.

On the basis of the new Duncan's multiple range test, the means of the 24-hour TLm's are grouped into six statistical populations, species included in each population are significantly different from other species tested (Table II). The guppy was significantly most resistant. The

TABLE 1.

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
	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
	4-4-61	Fourteen Mi. Cr., & Hulbert Cr., Cherokee Co.		dry meal	1.4-3.0 2.0-3.5		3-17 4-8
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
	3-4-61	Fourteen Mi. Cr., & Hulbert Cr., Cherokee Co.	58°F.	dry meal	2.0-3.0 1.8-2.9		6-30 3-10
L. cyanellus	6-16-60	Redding's Pond, Payne Co.	80°F.	dry meal	0.7-1.0	0.1	6-23 6-30
	7-20-60	Hesser's Pond, Payne Co.	80°F.	dry meal	0.7-1.0 1.0-1.4 1.0-1.4	0.3	7-26 7-28
L. megalatis	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
A. rupestris	10-29-60	St. Fish Hatchery, Centerton, Ark.		daphnia	0.9-1.5 1.0-1.5 1.5-3.0 1.5-3.0 1.5-3.0 2.0-3.5	2.2	9-13 10-25 11-1 11-10 11-19 12-3

TABLE 1. FIELD AND LABORATORY DATA ON TEST FISHES—(continued)

Species	*Field*				*Laboratory*		
	Collection date	*Locality*	*Water Temp.*	*Foods*	*Length (in.)*	*Ave. wt. (gms.)*	*Date test began*
C. erythrogaster	10-29-60	Bidding Spr. Cr., Cherokee Co.	63°F.	dry meal	2.0-2.8	1.4	11-1
					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
		Locality †			2.3-3.2		11-1

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

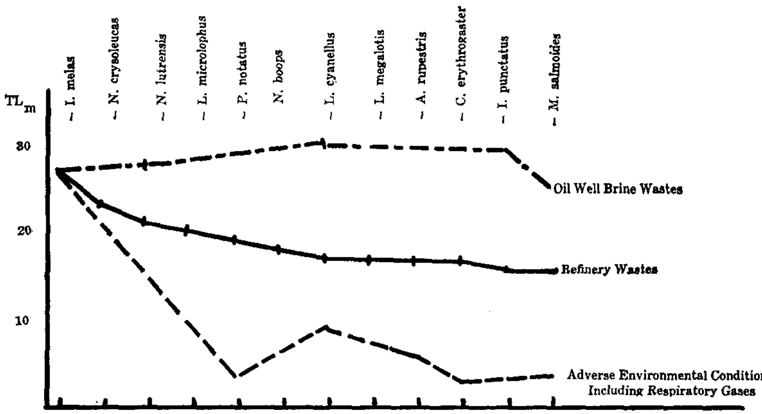

Figure 1. Comparison of relative resistance of fishes to oil well brine wastes (Clemens and Jones, 1954); refinery wastes; and adverse environmental conditions, including low dissolved oxygen and high carbon dioxide tension (Wells, 1918). Values on the left of the graph are TLm's or adjusted values from an arbitrary scale.

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 TLm's (adjusted means) are plotted in Figure 1 along with data from other relative resistance studies that have included several of the species used inthe present tests. 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.

Pertinent Life History Data on Species Tested

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 wit hthe 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.

TABLE II. STATISTICAL ANALYSIS OF RELATIVE RESISTANCE DATA FROM 24-HOUR TLm'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

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*

The chief advantage of using the guppy as a test fish is its adapability for rearing in the laboratory. Its small size is an advantage insofar as the space factor is concerned, but a disadvantage in that it is hard to handle. The guppy must be graded, it is not sensitive to refinery effluent, and it is not common in streams in the United States.

Ictalurus melas. The black bullhead 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 by seining.

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 it spawns in May and early June in Iowa. The young may be of suitable size for testing by mid-July in Oklahoma. Age-group-O fish were 1.3 to 3.5 inches in August in an Iowa pond (Carlander, 1950).

As a test fish, the black bullhead adjusts readily to laboratory conditions. It has the advantage of not jumping from containers. Water in holding containers fouls quickly (cause unknown) and the spines of the fish catch in nets, sometimes causing injury. The size available varies with the season. One-year-old fish from Indiana averaged 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 it 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.

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 two 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.

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 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 out of 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 bottom, 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. 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.

Markus (1939) reported that *N. lutrensis* begins spawning in early May and continues to spawn into the summer. Adults are about 2¾ 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.

The red shiner can be reared in ponds. Better reproduction occurred in deeper ponds in central Oklahoma. The 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 not present, red shiners might be induced to spawn upon submerged fiber mats.

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.

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 O-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.

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.

Redear are easily handled; they do not jump from containers, and are not easily injured.

Pimephales notatus. *Pimephales notatus* is found in the eastern half of the United States, where it inhabits clearer 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.

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 measured 0.8 to 2.5 inches 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 two inches in length.

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 a depth of six inches to three feet.

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.

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. Near its mouth, the Mountain Fork 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.

Reports of the breeding season were not found. Troutman (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.

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 would indicate that it does not reproduce readily in standing waters, although other factors may limit its abundance.

The bigeye shiner adjusted exceptionally well to laboratory conditions. Its relatively restricted range and habitat requirements prevented it from being rated among the best as a test fish (for refinery effluents).

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. 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.

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.

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

Green sunfish are easily handled. They adjust quickly to confinement, and remain calm under most conditions. The specimens used in tests were greedy, and crowded to the front of the tank when approached. Samples frequently include a number of size groups and must be graded.

Leopomis 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.

Witt and Marzolf (1954) noted spawning or longear sunfish in Missouri on June 10 with water temperatures 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 Oklahoma state growth average is 2.7 inches in 1 year, and 4.0 inches in two 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.

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.

The longear adjusts well in captivity, and is not susceptible to injury from handling.

Ambloplites rupestris. The rock bass occurs in the eastern United States. It is most abundant in small to intermediate size 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 & 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.

Fish used in bioassay quickly adjusted to confinement in the laboratory.

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.

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 2¾ inches in length, the males slightly larger than the females. Breeding fish are easily recognized by their bright colors.

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

With reasonable care, *C. erythrogaster* can be handled without excessive loss. It has been a popular aquarium fish.

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

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).

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.

Channel catfish adjusted readily to confinement, but required more space than other species as the water quickly became foul if they were crowded. The spines of channel catfish sometimes catch in nets and injury may result. Mortality due to *Ichthyophthirius* was high among surplus specimens.

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 writers. Fish used in the bioassays were fed primarily on young *Gambusia.* Live daphnia and chironomid larvae were also fed.

Largemouth spawn 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 mature at 7 inches (Trautman, 1957).

The largemouth 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.

Largemouth adjust readily to captivity. They are not sensitive to handling, but larger fish are active and may jump out of nets or containers. All fish were of similar size, and no antagonism was noticed.

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SAMPLING IN THE ADCLAUSTRAL ZONE OF A POWER RESERVOIR

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ABSTRACT

Spot checking the stratification pattern existing within a power reservoir generally consists of a single series of oxygen and temperature determinations taken vertically at the point of maximum depth. The point of maximum depth ordinarily is found immediately upstream from the impounding structure.

Data secured from a series of observations in the John H. Kerr Reservoir, Virginia, are presented which confirm Ellis' warning of 1936 that reservoir stratification in the immediate proximity of a power dam is very unstable and samples collected therefrom may yield entirely different results from samples taken at the same depth but beyond the limits of the adclaustral zone.

When determining the stratification pattern within a power reservoir, a series of vertical profiles is indicated. These observations should be spaced along the inundated stream bed as closely as circumstances will permit. When only a single profile is possible for determining the stratification pattern, that profile should be secured at a considerable distance from the impounding structure.

INTRODUCTION

In 1936, Ellis[1] described as the "adclaustral zone" that mass of deep water in a power reservoir lying upstream from the face of the impounding structure wherein stratification may be seriously deranged by vertical water movements. Ellis warned that water samples collected therein could yield very different results from those taken at the same depths in the reservoir beyond the limits of the adclaustral zone.

[1] Ellis, Max M., "Water Conditions Affecting Aquatic Life in Elephant Butte Reservoir". Bulletin No. 34, U. S. Department of the Interior, Bureau of Fisheries, 47 pages.