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## THE SUITABILITIES AND RELATIVE RESISTANCES OF TWELVE SPECIES OF FISH AS BIOASSAY ANIMALS FOR OIL-REFINERY EFFLUENTS<sup>1</sup>

By W. R. GOULD III and W. H. IRWIN  
Oklahoma State University

A grant from the National Institutes of Health was awarded to W. H. Irwin and Troy C. Dorris of Oklahoma State University for research concerning the resistances of fishes to oil-refinery effluents. The study began in February, 1960, and a supplemental grant which became effective September, 1961, was awarded to permit an increase in the number of species to be included. The study reported was done under the supplemental grant and concerns 12 species of fish as toxicity bioassay animals. Observations were made concerning collection, transportation, maintenance, and test reactions of each species. The literature was searched to provide information relating to the suitability of each species investigated. The median tolerance limit of each species to oil-refinery wastes was calculated and submitted to statistical analysis.

Some species of fish are more suitable as bioassay animals than others. A major requirement is that a species be available in adequate supply at all times

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(Henderson and Tarzwell, 1957; Warren and Doudoroff, 1958). The animals should be adaptable to laboratory temperatures, feeding, handling, and should be small specimens of uniform size (Henderson and Tarzwell, 1957). Warren and Doudoroff (1958) further suggested that a suitable test species should breed in the laboratory, be of one genetic strain, and have a known environmental history. Doudoroff *et al.* (1951) suggested using a species with a low resistance and one common in the unpolluted portion of waters receiving wastes or at least using similar species known to inhabit similar waters in the same watershed. Centrarchidae, Salmonidae, Cyprinidae, and Catastomidae were recommended (Doudoroff *et al.*, 1951). The fathead minnow, bluntnose minnow, uncolored goldfish, creek chub, suckers, stoneroller, and dace have been used successfully although the creek chub, suckers, stoneroller, and dace were not generally available in suitable sizes or numbers. Shiners and juvenile centrarchids were not so satisfactory as test animals. The non-native guppy and goldfish were desirable from the standpoint of maintenance in the laboratory but should be compared with local species (Henderson and Tarzwell, 1957). Warren and Doudoroff (1958) recommended the use of the guppy.

Douglas (1959) ranked four species by their relative resistances to petroleum-refinery effluents. *Lebistes reticulatus* was the most resistant, followed by *Gambusia affinis*, *Pimephales promelas*, and *Hybognathus placita* in order of decreasing resistance.

Ward and Irwin (1961) ranked 13 species by their resistivity to oil-refinery effluents. The guppy was the most resistant, followed by *Ictalurus melas*, *N. crysoleucas*, *N. lutrensis*, *L. microlophus*, *P. notatus*, *N. boops*, *L. cyanellus*, *L. megalotis*, *A. rupestris*, *C. erythrogaster*, *I. punctatus*, and *M. salmoides* in order of decreasing resistance.

Douglas (1962) ranked 16 species by their resistance to oil-refinery waste waters. Four were species of fish he had ranked in 1959. The guppy was the most resistant, followed by *C. auratus*, *G. affinis*, *C. carpio*, *P. promelas*, *E. inconstans*, *L. macrochirus*, *L. sicculus*, *N. percobromus*, *S. stromaculatus*, *M. dolomieu*, *H. placitus*, *C. commersoni*, *S. gairdneri*, *N. zonatus*, and *E. spectabile* in order of descending resistance.

## MATERIALS AND METHODS

The oil-refinery effluents were waste materials from a crude unit, two coking units, a catalytic cracker, a catalytic reformer, a thermal reformer, and a compound house. The effluents were obtained at intervals from June, 1961, through December, 1961. Table I contains the known extremes and average chemical characteristics of the effluents during the above period as determined by the chemists of the refinery.

TABLE I  
CHEMICAL CHARACTERISTICS OF OIL-REFINERY EFFLUENTS

	pH	NH <sub>3</sub> ppm	Phenol ppm	Sulfide ppm	C.O.D. ppm	Alk.* ppm	Alk.† ppm
Low	8.9	65.0	4.2	0.0	260	130.0	160.0
High	10.0	94.0	29.0	1.6	465	280.0	400.0
Average	9.4	76.9	11.4	0.22	326	176.9	297.4

C.O.D.—Chemical Oxygen Demand.

\* Phenolphthalein Alkalinity.

† Methyl Orange Alkalinity.

Dilution water was tap water that originated in Lake Carl Blackwell. Dechlorination was achieved by 24 hours of aeration. Tests for residual chlorine showed that it was reduced to 0.018 ppm in 12 hours. The chemical composition of the dilution water had been tested over a period of one year and had been found to be fairly constant. Table II contains an analysis of Lake Carl Blackwell water as it left the Stillwater, Oklahoma, treatment plant. The analysis was made by the U. S. Geological Survey in July of 1961. Little change took place enroute to the laboratory.

The effluent storage containers, effluent mixing vat, and the test containers were polyethylene. An oxygen tank-and-valve system were present for the addition of oxygen to test solutions. Fish-holding tanks were converted porcelain refrigerator liners.

TABLE II  
CHEMICAL CHARACTERISTICS OF DILUTION WATER

Iron .....	0.0	Silicon .....	3.6	Magnesium .....	12.0
HCO <sub>3</sub> .....	130.0	Calcium .....	37.0	Potassium .....	25.0
CO <sub>3</sub> .....	0.0	Chlorides .....	39.0	Sulfates .....	26.0
Fluoride .....	1.0	Nitrates .....	0.8	Sodium .....	58.8
pH .....	7.9	Hardness .....	142.0	Dissolved Solids .....	218.0

Chemical quantities are given in ppm.

The fishes were ranked by their ecological suitabilities as test animals. Factors considered in the rankings were ease of capture, ability to adjust to laboratory conditions, and reactions to test solutions. The literature reviewed, provided information on the seasonal availability of suitably sized test specimens, food habits, rate of growth, and size attained. Each species was ranked on a scale which ranged through poor, fair, good, and excellent.

All factors did not have the same value. If a species did not adapt to laboratory conditions sufficiently to be maintained in a healthy condition for a moderate period of time, the species was rated poor as a bioassay animal notwithstanding the rating of other factors.

Guppies, *Lebistes reticulatus* (Peters) which were used in all the tests, for reference purposes, were raised in the laboratory. Each species was compared with the guppy. Test fishes other than the guppy were obtained by seining, were purchased from bait dealers, or were donated by state or federal hatcheries. Test species other than the guppy were transported to the laboratory by one of two methods; (1) in a tank of water supplied with oxygen through lines and breaker stones, (2) in plastic bags with oxygen atmospheres.

An attempt was made to hold all specimens at least 10 days prior to use, as suggested by Doudoroff *et al.* (1951). In some instances this was impractical. During the time the test fishes were held in the laboratory any diseased, injured, abnormal, or undernourished specimens were discarded. The procedures on the feeding of test animals suggested by Doudoroff *et al.* (1951) were followed whenever possible.

Holding-tank water was treated with terramycin to prevent outbreaks of a bacterial infection, finrot (Irwin, 1959). Compressed air was applied to the water through breaker stones to provide adequate levels of dissolved oxygen and reduce carbon dioxide levels.

Oil-refinery effluents were obtained one day previous to use in a full-scale bioassay. Five-gallon polyethylene bottles were filled to the top with effluent, capped, and transported to Stillwater. Effluent was stored in the constant temperature room overnight to allow temperature adjustment.

An exploratory bioassay was made the day the effluent was collected. Each species (including the reference species) tested in the full-scale bioassay was subject to an independent exploratory test. An exploratory test consisted of several concentrations of effluent and dilution water with two fish of one species alone in a concentration. The exploratory test provided information used to determine the range of concentrations for the full-scale bioassay, required only a small part of the effluent sample, and was terminated after one day.

The full-scale bioassay for each species was made to provide at least one concentration that would kill more than 50 percent of the test animals placed in it and one concentration that would allow more than 50 percent to survive. The usual number of concentrations needed to provide the information was three, four, or five. The concentrations of each test usually differed from each other by approximately five percent of effluent by volume. The bioassay procedure as described by Doudoroff *et al.* (1951) was followed for each species tested. The amount of effluent needed per test was calculated, and the appropriate number of five-gallon effluent containers were drained into a polyethylene mixing vat and stirred to insure uniformity. The test solutions were obtained by measuring the appropriate amount of effluent into the test containers for each test concentration and diluting the effluent to 10 liters. Each test concentration had two replications.

Ten test specimens were then placed in each test container, including controls. If at any time less than 80 percent of controls survived, the test was discarded.

Four tests with four different effluent samples were used for each species except *E. lucius*, which had only three tests because of a lack of specimens.

Dissolved-oxygen and hydrogen-ion concentration determinations were begun following an interval of 15 minutes after the solutions were made. A count of surviving fish in each container was made at 1, 6, 12, 24, 48, and 96 hours. Dead fish were removed from the containers when discovered. Dissolved-oxygen concentrations were determined repeatedly throughout the bioassay to make certain that death was not due to suffocation. Observations on fish behavior were recorded throughout each test. Test concentrations were terminated upon the death of all test animals or at the end of 96 hours. Final dissolved-oxygen and hydrogen-ion readings were made at the termination of the test concentration. A Beckman battery-powered pH meter, which was checked with a Hellige comparator, was used to take hydrogen-ion determinations. Dissolved-oxygen concentrations were determined by the use of the Alsterberg modification of the Winkler method and a Bausch and Lomb Spectronic 20 spectrophotometer. All bioassays were done in a constant-temperature room at about 75° F.

All test concentrations were maintained above 2.0 ppm dissolved oxygen. In many cases the initially high dissolved-oxygen content of the dilution water and the constant diffusion of oxygen from the air into the test medium were sufficient to keep the test concentration above 2.0 ppm. Dissolved-oxygen tests were used to discover when the test concentrations approached the 2.0 ppm minimum. Test concentrations which approached the minimum dissolved-oxygen level were treated by the addition of bubbles of oxygen (Hart *et al.* 1948; Doudoroff *et al.* 1951). With every species of fish tested, specimens were shown to live under lower dissolved-oxygen levels than occurred in test concentrations.

#### CALCULATION OF RESISTANCES

A 96-hour median tolerance limit ( $TL_{m^{96}}$ ) was calculated for each species in each test. The  $TL_{m^{96}}$  values represented concentrations of effluents that would cause a 50 percent mortality to test animals (Doudoroff *et al.* 1951). The  $TL_{m^{96}}$  values were determined by plotting on semi-logarithmic graph paper, the concentrations in which more than 50 percent and less than 50 percent deaths occurred. A straight-line interpolation was used to estimate the concentration of effluents that would cause a 50 percent kill.

The median tolerance limits were statistically treated. The  $TL_{m^{96}}$  values were used in a two-way classification with unequal numbers in sub-classes (Graybill, 1961). The abbreviated Doolittle technique adjusted for effluent differences. A table was constructed which showed the analysis of variance. A matrix inversion was used to solve for independent species effects. A modification of Duncan's new five percent multiple range was used to rank the species tested.

#### SPECIES SUITABILITIES

##### *Chaenobryttus gulosus* (Cuvier)

The warmouth, *Chaenobryttus gulosus* (Cuvier), was seined from a pond near Stillwater, Oklahoma, during August and October, 1961. The fish used were the offspring of brood stock obtained from the U. S. Fish and Wildlife Service Hatchery at Tishomingo, Oklahoma, in April, 1961. The test fish averaged 1.9 gms. in wet weight and 5.1 cms. in total length.

The fish were transported without adverse effects to the laboratory in tanks of water supplied with oxygen through breaker stones. *Chaenobryttus* adjusted well to laboratory conditions. Initially they aggregated in a corner or under a thermometer in the tank. After a few days they distributed themselves throughout the tank. *Daphnia magna* Straus was fed to the fish throughout the time they were held in the laboratory and was eaten voraciously. Warmouth were held in the laboratory satisfactorily for one month.

The fish again showed a tendency to aggregate upon introduction into test solutions. After a short time they redistributed themselves throughout the test solution. The specimens hovered near the surface of lethal solutions and near the bottom of non-lethal solutions. *Chaenobryttus* withstood oxygen concentrations of 1.0 ppm without fatality. Oxygen was not applied to the test solutions since it was not needed. The test concentrations had a pH range from 8.5 to 7.4.

*Chaenobryttus gulosus* is found throughout the central and eastern United States to the Gulf Coast from Kansas and Iowa to the Mississippi River drainage in southern Wisconsin, the southern two-thirds of the Lower Peninsula of Michigan, Lake Erie, the Allegheny River tributaries southward to Florida, and westward through the Gulf States to the Rio Grande River (Larimore, 1957; Moore, 1957). Warmouth have been introduced into California, Washington, and Idaho (Larimore, 1957).

*Chaenobryttus* is found in ponds, lakes and the backwaters of rivers. It is associated with dense weed beds, brush, roots, old tree stumps, and soft bottoms. The young occur throughout the year in shallow water. Adult warmouth may spawn over a period of several months, the same individual spawning several times in a season. Specimens nest from the middle of May to July or August in Illinois (Larimore, 1957). Two-week-old fry were collected in Oklahoma on October 19, 1961.

The fish grows approximately 1.64 inches in its first year and to 3.38 in its second year in Illinois (Larimore, 1957). Individuals have reached an average total length of 3.31 inches the first year in Oklahoma (Jenkins *et al.*, 1955).

The warmouth feeds upon aquatic insects, insect larvae, cladocerans, snails, crayfish, and fish. Seldom was a motionless food item taken. *Chaenobryttus* hybridizes with a great number of sunfishes but seldom produces large numbers of hybrids in natural populations (Larimore, 1957).

Larimore (1957) described the fish as a desirable animal for laboratory experimentation because of ease of transport and maintenance in the laboratory. The species had a quiet disposition, quickly adapted to laboratory conditions, readily ate a wide variety of foods, and was tolerant to low dissolved-oxygen conditions (Larimore, 1957).

#### *Esox lucius* Linnaeus

The northern pike, *Esox lucius* Linnaeus, was obtained from a state hatchery at North Platte, Nebraska, May 3, 1961. A random sample of 20 test fish averaged 1.4 gms. in weight and 6.4 cms. in total length.

*Esox lucius* was transported to Stillwater in plastic bags with oxygen atmospheres. Transport time was one day. Approximately 30 of the 400 died in transit. At the end of a six-week holding period the remaining specimens were still easily excited. The pike darted against the sides of the holding tank in response to a sight or sound stimulus, but remained motionless near the bottom of the holding tank when not disturbed.

Small specimens of *Gambusia affinis* (Baird and Girard) were fed to the northern pike during the holding period. The pike ate by slowly moving within approximately two inches of their prey and then making a rapid dart at the food. The pike usually captured the prey crosswise to the mouth and then maneuvered the fish until it could be swallowed end first.

*Esox lucius* stayed near the bottom of the test containers. In test solutions specimens reacted to a sight or sound stimulus by short, quick, nervous dashes. A few fish jumped from test containers.

Some fish were observed to come to the surface of the test solution when the dissolved-oxygen content decreased. Oxygen was bubbled through the test solutions and controls to keep the dissolved-oxygen content above the necessary minimum. No deaths occurred in a container that had only 1.4 ppm dissolved oxygen. Test concentrations had a pH range from 8.5 to 7.2.

*Esox lucius* is found in Canada, northern United States, and northern Eurasia. The species occurs from northern New England through the Great Lakes region to eastern Nebraska and south to Missouri in the United States (Moore, 1957).

The species prefers clear waters in lakes and streams. The favorite habitat is the weed beds in sluggish currents of streams or the shallow water of a lake. In winter the larger fish go into deeper water (Van Oosten, 1946).

Pike spawn in March, April, and May in shallow, swampy, recently inundated places (Van Oosten, 1946). The young may stay in shallow water and marshes for three months (Van Oosten, 1946). Buss (1961) found that 90 percent of the young moved into deeper water in a somewhat shorter period of time, but stayed in less than one foot of water throughout the summer. The young eat waterfleas (Van Oosten, 1946), aquatic insects, and insect larvae (Harlan and Speaker, 1956; Van Oosten, 1946). They soon change their diet to fish (Van Oosten, 1946), frogs, and crayfish (Harlan and Speaker, 1956).

Fish form the major portion of the diet (Buss, 1961). Young pike can attain a length of 6 to 8 inches in length by fall (Harlan and Speaker, 1956) and 10 inches (Van Oosten, 1946) or more in the first year (Buss, 1961).

*Fundulus kansae* Garman

The plains killifish, *Fundulus kansae* Garman, was seined from a tributary of the Cimarron River, west of Lacey, Oklahoma. Collections were made during June and July, 1961. Specimens averaged 1.7 gms. in weight and 5.8 cms. in length.

The species was transported in covered water containers supplied with oxygen through a breaker stone. Transport-water temperatures ranged from 88° to 82° F. The fish survived transport well. Some tail-rot was noted after a few days of holding. Water in holding tanks was changed frequently because of "fouling." The water turned brown in color and produced a noticeable odor. *Daphnia magna* was eaten well. The smaller specimens also ate a dry food. The fish held well for one week but soon after began dying, a few each day. The majority of early deaths were of small specimens. The remaining fish appeared to be in good condition.

The plains killifish swam slowly near the surface in critical test solutions, the bodies tilted with heads higher than tails. The smaller specimens tended to remain near the surface of the non-critical test solutions and the larger fish near the bottom. Some jumping took place in strong concentrations, and cover nets were required to prevent loss.

Specimens could not survive ten fish to ten liters of a test solution or dilution water without oxygenation. Test solutions had a pH range of 8.7 to 7.2.

*Fundulus kansae* is found throughout the Great Plains region from South Dakota to the Arkansas River Basin and west to Texas and New Mexico (Moore, 1957). Simon (1951) states it is found in shallow streams of the plains from South Dakota to Oklahoma in the western tributaries of the Mississippi River.

Spawning and activities preceding spawning were observed on August 16-18, 1939, in a small tributary of the "Dry" Cimarron River in New Mexico. Hubbs and Ortenburger found that most adults taken by them in Oklahoma had spawned by July 11. The species spawned at about 82° F. water temperature. Young of the year were found by Hubbs and Ortenburger as early as June 1 (Koster, 1948). Specimens were found in spawning condition on June 16, 1961. Fry were found on June 27, and July 5, 1961. The water temperature was approximately 82°.

The plains killifish eats surface insects, floating matter, and bottom forms. They usually grow to two or three inches although some reach six inches (Simon, 1951).

*Hybognathus hankinsoni* Hubbs

The brassy minnow, *Hybognathus hankinsoni* Hubbs, was seined from a pond near Brady, Nebraska, June 3, 1961, and November 12, 1961. A random sample of ten fish averaged 1.2 gms. in weight and 5.2 cms. in length.

The species was transported in plastic bags with oxygen atmospheres. On the second date approximately 500 fish were transported. One fish died in transit. The specimens adapted to laboratory holding conditions within a few days. They remained calm in the holding tanks but preferred the darker sections of the tanks. The tanks were partly covered to reduce the light. The fish stayed fairly close together in a school. The brassy minnows were fed a coarse-ground dry food. They appeared to take food both on its descent and from the holding tank bottom. *Daphnia magna* was presented but was not eaten.

After approximately two weeks in the laboratory a few dead fish were found in each of two holding tanks. The killing agent was not tail-rot. Examination of the dead showed the fish to be extremely thin and shrunk. Before death the fish appeared to lose their equilibrium, swimming on one side, then the other.

Specimens of the brassy minnow remained fairly quiet and stayed close together in a test container. *Hybognathus hankinsoni* survived an oxygen level of 1.4 ppm without mortality. Test solutions had a pH range of 8.7 to 7.4.

*Hybognathus hankinsoni* may be found in the Great Lakes drainage, except Lake Erie in Ohio. It is present from Montana and Manitoba eastward to Quebec and New York and southward through Colorado, Kansas, and Missouri in the west. It has been introduced into British Columbia (Moore, 1957).

The species is found in small creeks, bog streams, and sometimes in lakes (Dobie *et al.*, 1956). Simon (1951) stated that it is a herbivorous species. Dobie *et al.* (1956) reported that it eats zooplankton, phytoplankton, aquatic insects, plants, and surface drift. *Hybognathus hankinsoni* grows to 2.5 to 3 inches in two years (Dobie *et al.*, 1956).

#### *Ictiobus cyprinellus* (Valenciennes)

The bigmouth buffalo, *Ictiobus cyprinellus* (Valenciennes), was obtained on September 30, 1961, from a state hatchery at Lonoke, Arkansas. Specimens averaged 2.2 gms. in weight and 5.9 cms. in length.

The fish were transported in plastic bags with oxygen atmospheres. In the laboratory the fish were fed a dry food. The holding water tended to become milky white with "soapy" foam on the surface which necessitated frequent changes of water. The condition appeared to be due to the reactions of the food, fish secretions, and excretion. A few dead fish were found each day in the holding tanks. Close examination revealed anchor worms (*Lernaea* sp.) were present on many specimens. The parasitized fish were carefully separated from the non-parasitized, and no parasitized specimens were knowingly used in any test.

Bigmouth buffalo appeared to dislike the full light intensity of the holding room. They sought the darker corners of the holding tanks until partial covers were used to reduce the light at which time they distributed themselves more evenly over the bottoms of the holding tanks. Throughout approximately five weeks of holding time the species was easily disturbed by a sight or sound stimulus.

In non-lethal test solutions fish remained inactive near the bottoms of the test containers. In the initial phases of reaction to a lethal concentration the specimens rose to the surface, head higher than tail, and hovered there. Previous to death they often exhibited a short violent dash after which they sank to the bottom. Death followed immediately or after a short period of convulsion. The vigorous dashes necessitated that nets be used over the higher test concentrations.

The bigmouth buffalo reduced oxygen levels in the test concentrations sufficiently to require oxygenation. No deaths occurred in a test concentration in which oxygen was reduced to 0.9 ppm. Test solutions had a pH range of 8.3 to 7.2.

*Ictiobus cyprinellus* is found in the Red River of the North, south through the Mississippi Valley to Alabama, Louisiana, and Texas. The species is found in western Lake Erie and some Arizona impoundments (Moore, 1957).

The bigmouth buffalo is an inhabitant of large rivers, oxbow lakes, and sloughs (Moore, 1957). Its food consists of scuds, insect larvae, small crustaceans, algae and other vegetation, occasionally small fish, fish eggs, and plant seeds, especially cottonwood seeds in the spring. The species reproduces in the latter part of April and early part of May in Iowa. It can reach seven inches at the end of the first growing season (Harlan and Speaker, 1956).

#### *Lepomis humilis* (Girard)

The orangespotted sunfish, *Lepomis humilis* (Girard), was obtained from two locations. In August of 1961, fish for two tests were seined from a pond near Boomer Lake, Stillwater, Oklahoma. They were the offspring of brood stock placed in the pond in May, 1961. The fish for the final two tests were seined in November, 1961, from Red Rock Creek and averaged 1.7 gms. in weight and 5.0 cms. in length.

The fish were transported in covered water tanks to which oxygen was applied through a breaker stone. Specimens were retained for over one month. They adapted to holding conditions easily, remained nearly motionless in the holding tank, and distributed themselves evenly throughout it. After a few days in holding containers they became accustomed to being fed by a person and at his appearance "gathered" in anticipation of food. The fish ate *Daphnia magna* voraciously.

In lethal test concentrations orangespotted sunfish tended to stay near the surface of the solution prior to death. Upon death most specimens fell to the container bottom. The fish died without a struggle. In non-lethal test concentrations the fish stayed quietly near the test container floor in a rather even distribution. They were able to withstand oxygen concentrations of 1.7 ppm

without casualty. The pH of test solutions had an initial high of 8.7 and a final low of 7.5.

*Lepomis humilis* is found from North Dakota east to western Ohio and south through western Kentucky, Tennessee, Alabama, and Louisiana. It is found south from the Dakotas through the Great Plains to Texas (Moore, 1957).

*Lepomis humilis* is well adapted to stream and pond life. It is commonly found in a sluggish stream or pond in which there is a scarcity of vegetation. Muddy water does not restrict the species (Barney and Anson, 1923).

The species has an extended breeding season, the length of which depends upon the geographical location and the weather conditions but in general extends from early spring to fall (Barney and Anson, 1923).

Orangespotted sunfish eat insects, small crustaceans, small fishes (Harlan and Speaker, 1956; Ward, 1959; Barney and Anson, 1923), insect larvae, and probably accidentally some algae (Barney and Anson, 1923). Growth data show the species can attain a length of 3.4 cms. in its first year. The two-year-old fish may range from approximately 2.5 to 4.5 cms. in length (Barney and Anson, 1923). Specimens seldom reach a length more than four inches (Ward, 1959).

#### *Menidia audens* Hay

The Mississippi silversides, *Menidia audens* Hay, was seined from Lake Texoma, Oklahoma, on August 18, 1961, and on August 22, 1961. The fish averaged 0.8 gm. in weight and 6.4 cms. in length.

Two attempts were made to transport *M. audens* in plastic bags with oxygen atmospheres. Neither attempt was successful. Specimens transported in a covered water tank to which oxygen was applied through breaker stones incurred only slight mortality. The Mississippi silversides adjusted well to laboratory holding conditions. Specimens remained nearly motionless in holding tanks. The fish were fed a dry food and *Daphnia magna*. Both foods were eaten, but *Daphnia* was taken more readily. Specimens were held in the laboratory for three weeks.

The fish remained motionless in test solutions. They exhibited no jumping while in lethal test solutions. Specimens became dark before death. They were capable of surviving in test solutions without oxygenation. Test solutions had a pH range of 8.4 to 7.5.

*Menidia audens* may be found in the lower Mississippi Valley as far north as southern Arkansas and Oklahoma. In Texas it is found in the Red River drainage (Moore, 1957).

There is no published life history work of *M. audens* (Saunders, 1959). Saunders (1959) found that the species usually frequented shallow water of six feet or less although it could be seen near the surface of deeper water.

Food studies showed that *M. audens* eats plankton, algae, crustaceans, insects, arachnids, and fry of fish. Apparently it is not a bottom feeder. It seldom exceeds four inches (Saunders, 1959).

Saunders (1959) concluded *M. audens* possessed the ability to survive drastic temperature changes. The species has become well established in Red River impoundments to the exclusion of *Labidesthes sicculus* (Cope) (Saunders, 1959).

#### *Notropis girardi* Hubbs and Ortenburger

The Arkansas River shiner, *Notropis girardi* Hubbs and Ortenburger, was seined from the Cimarron River near Lacey, Oklahoma. On June 3, 1961, 700 to 800 specimens were taken in one-foot deep water over a sandy bottom. Water temperature was 76° F. and air temperature, 84° F. The specimens were taken in association with *H. placitus*, *H. aestivalis*, and *N. percobromus*. The females of the Arkansas River shiners were gravid. Fish averaged 0.6 gm. in weight and 4.0 cms. in length.

The fish were transported to Sillwater, Oklahoma, in an open water container to which oxygen was supplied. Few deaths occurred in transit and in the initial portion of the holding period. The fish accepted dry food almost immediately. Moore (1944) fed specimens a commercial dog food, which was readily eaten. Specimens ate mostly at the surface. Individuals were startled by a sudden sound close to the tank but otherwise were quiet. *Notropis girardi* moved around slowly in the holding containers. The sight of a person approaching did not excite them. Toward the end of the holding period the compressed



air in one tank failed with a resulting kill of the fish. Otherwise the species held extremely well. Specimens were held for more than three weeks.

In test solutions *N. girardi* moved slowly, maintained a loose school formation, and did not become excited at visual stimuli. The oxygen levels of test solutions were not sufficiently reduced to require oxygenation. Whitworth (1961) stated that *N. girardi* tolerated 1.0 ppm dissolved oxygen without death. Test solutions had a pH range of 8.7 to 7.5.

The Arkansas River shiner is found in the Arkansas River and its large silty tributaries in Kansas, Oklahoma, and Arkansas (Moore, 1957). The specimens are usually found in the main channels of larger rivers although occasionally they are found in backwater pools (Moore, 1944).

Hubbs and Ortenburger (1922) suggested that spawning time was in early July. In 1940 on July 12, eggs were obtained by stripping a female. Eggs were taken from the Cimarron River on July 24, 1940, and again July 11, 1942, and both times the air temperature was approximately 80° F. (Moore, 1944).

Young attained a length of 24.5 mm. in 40 days. The species may have a life span of three years. Adults of the species are small (Moore, 1944).

#### *Notropis stramineus* (Cope)

The sand shiner, *Notropis stramineus* (Cope), was seined from Rock Creek near Dougherty, Murray County, Oklahoma, on August 17, 1961. Specimens averaged 1.4 gms. in weight and 5.4 cms. in length. The sand shiners were transported, the day they were seined, in two net covered, water tanks supplied with oxygen through breaker stones. The species withstood transport well.

The fish tended to aggregate in the corners of the holding containers. They ate a dry food readily. Specimens were kept in healthy condition in the laboratory three weeks.

*Notropis stramineus* tended to cluster in the corners near the bottom of the test containers having dilute concentrations. In high concentrations the specimens tended to move about slowly near the top of the test solution but occasionally became frantic and would leap from the solution. Nets were used over the high concentrations to insure the retention of leaping fish.

Ten specimens lived and showed no distress in test solutions to which no oxygen was applied. An oxygen level of 1.4 ppm produced no death. Test solutions had a pH range of 8.4 to 7.4.

*Notropis stramineus* is found from the Rocky to the Appalachian Mountains and from the Great Lakes to Mexico, with the exception of the Gulf Coast east of Mississippi. The species appears to be common in sandy streams of small to moderate size (Moore, 1957). Trautman (1957) found the largest populations on sand- and gravel-bottomed riffles and in pools of large- and moderate-sized streams with at least a moderate current of clear water. It is rarely found in low gradient streams or among rooted-aquatic vegetation (Trautman, 1957). The species was surprisingly tolerant of inorganic pollutants which did not cover sand or gravel (Trautman, 1957).

#### *Notropis whipplei* (Girard)

The steelcolor shiner, *Notropis whipplei* (Girard), was seined from the Mountain Fork River at Beavers Bend State Park on the apron of a dam. The water, one-half to two feet deep, flowed swiftly with some white water. The steelcolor shiner averaged 1.8 gms. in weight and 6.7 cms. in length.

The fish were transported in covered tanks of water to which oxygen was applied through breaker stones. The fish held extremely well in spite of the fact that the water temperature reached 92° F. Specimens demonstrated breeding colors as described by Hubbs and Cooper (1936).

The fish were quite active the first ten days in holding tanks and could be heard striking the sides of the holding containers when startled. Several specimens received injured snouts from repeated collisions against sides of the holding containers. Sound appeared to disturb them more than sight stimuli. Covers placed over the holding tanks reduced reaction to the stimuli and kept the fish confined. The fish had adjusted sufficiently to holding conditions by the end of two weeks to allow the removal of the holding tank covers. Some specimens were held for 25 days. Few deaths occurred in the holding tanks during the period.

*Notropis whipplei* ate a dry food on the surface and while falling through the water. *Daphnia magna* was used as food once and was accepted well. The fish ate several times a day enthusiastically.

The fish were inclined to lie quietly near the bottom in the dilute concentrations. Nets were used over the high concentrations to keep the steelcolor shiners confined. Oxygenation of all test solutions and controls was necessary. Death occurred within 48 hours to several of ten fish in a test container which held dilution water and had no oxygenation. The pH of test solutions ranged from 8.4 to 7.5.

The steelcolor shiner can be found in the Mississippi Valley from Indiana, Ohio, and New York, south to Alabama and west to Oklahoma (Moore, 1957).

*Notropis whipplei* is usually found in streams but is not uncommon in some lakes. It seems to prefer swift running water over a clean bottom where there is little vegetation (Hubbs and Cooper, 1936). In Illinois dense populations were found in a lake and in a sluggish stream (Lewis and Gunning, 1959).

The species spawns in Michigan in the latter part of May and early June. Elsewhere specimens may spawn in July and August (Hubbs and Cooper, 1936). Hankinson (1930) called the species a late breeder because he took only comparatively small young-of-the-year in collections made late in the season. Lewis and Gunning (1959) found that one-year-olds spawn in late August and early September, and two-year-olds spawn in July. The young develop rapidly in the late summer or early fall of their first year. In winter the spent adults suffer heavy mortality (Lewis and Gunning, 1959).

*Notropis whipplei* is chiefly insectivorous (Hubbs and Cooper, 1936; Lewis and Gunning, 1959), but also eats water mites, zooplankton, algae, some other plant material, and small fishes (Hubbs and Cooper, 1936). Two inches was found to be the maximum size attained in the Illinois populations although larger individuals have been reported (Lewis and Gunning, 1959). The species is resistant to hot-weather handling and transport (Lewis and Gunning, 1959).

#### *Pimephales vigilax* (Baird and Girard)

The bullhead minnow, *Pimephales vigilax* (Baird and Girard), was seined from Lake Texoma at the mouth of Brier Creek, July 20, 1961, and again, August 17, 1961. The fish averaged 1.4 gms. in weight and 5.0 cms. in length.

*Pimephales vigilax* was transported in covered water containers to which oxygen was applied. Few deaths occurred during the trip. The bullhead minnow ate dry food well. Specimens were held satisfactorily for a period of three weeks. *Pimephales vigilax* was an extremely nervous test animal in test concentrations. Nets were required over test containers to retain the fish. Fish in the higher concentrations stayed near the surface of test solutions. The fish in the controls continued to mill at a slow pace near the bottom of the dilution water.

Oxygenation of some test concentrations was needed; however, *P. vigilax* survived in test solutions without oxygenation. A two ppm level of dissolved oxygen caused no death to fish. Test solutions had an initial high pH of 8.8 and a final low of 7.6.

*Pimephales vigilax* is found in the Mississippi River system from Minnesota south to Texas, Louisiana, and Mississippi, east from Minnesota to Pennsylvania and south from Pennsylvania to Georgia and Alabama (Moore, 1957).

*Pimephales vigilax* has a preference for large streams (Harlan and Speaker, 1956). The fish inhabits sluggish pools of larger inland streams of the Ohio River system and their connecting backwaters and bayous where the bottom is gravel, silt, or deep mud (Trautman, 1957). Harlan and Speaker (1956) stated the species was rare to absent in lakes but Parker (1962) stated that specimens could be found in lakes and impoundments. The species is highly tolerant of turbidity (Trautman, 1957).

The bullhead minnow spawns in early spring through midsummer (Harlan and Speaker, 1956). Parker (1962) found several females with eggs in September of 1961. The water temperature was 78° F. at the collection time. The species eats algae, water mites, rotifers, crustaceans, annelids, insects, and mollusks (Parker, 1962).

In Ohio the young-of-the-year reach 0.8 to 2.0 inches. One-year-olds were found to range from 1.2 to 2.5 inches. Adults attain a length of 1.5 to 3.0 inches (Trautman, 1957). Harlan and Speaker (1956) found the species reached three inches at maturity.

### *Tilapia nilotica* Linnaeus

The Nile tilapia, *Tilapia nilotica* Linnaeus, was obtained from the U. S. Fish Culture Station, Marion, Alabama. The fish averaged 2.0 gms. in weight and 5.5 cms. in length.

*Tilapia nilotica* arrived in Stillwater, Oklahoma, on November 16, 1961, after several days in transit by air-freight. Of the approximately 500 shipped, only about seven died during shipment. They were shipped in plastic bags with oxygen atmospheres. *Tilapia nilotica* adapted to laboratory conditions extremely well. Specimens have been held for over five months. A dry food and *Daphnia magna* were fed to the fish. Both foods were eaten, but *Daphnia* was taken with more zeal.

The species showed a jumping, surfacing reaction to lethal test solutions. Specimens often apparently lost their equilibrium and displayed quick, spiraling movements before falling to the bottom of the test container. It was difficult to tell dead from live fish because live fish remained near the bottom of the test container, motionless on their sides in much the same manner as dead fish.

The Nile tilapia reduced the oxygen content of test solutions at a greater rate than *L. humilis*, or *H. hankinsoni*. Ten fish in a test container survived an oxygen content of 1.2 ppm for 96 hours. Test solutions had a pH range of 8.9 to 7.4.

In 1960 Ben-Tuvia quoted Trewavas as stating that *T. nilotica* is distributed over most of the African continent. It is also found in almost every lake and river of Israel according to Ben-Tuvia (1960). The fish was introduced into the Southeastern United States in 1957 (McBay, 1961).

Spawning in ponds occurs when water temperature reaches 70° F. In aquaria *Tilapia nilotica* utilizes both plant and animal food. Smaller Nile tilapia feed primarily on entomostriacans and chironomids while larger sizes may utilize algae. Fish have been kept in the laboratory on dry food pellets (McBay, 1961). In ponds which were fertilized, fry were raised to a length of 2-3 inches in one and one-half to two months (Swingle, 1960). Fish of less than six inches may be killed in ponds when the temperature drops to 48° F. (McBay, 1961).

## RANKING BY SUITABILITIES

*Esox lucius* was rated a poor test animal. The species appears to be available in a suitable size for testing only a short part of its life. The species required fish as a food. Providing suitably sized food specimens was time consuming. Several *E. lucius* were found strangled after they had attempted to eat too large a fish. The species reacted violently to sight or sound stimuli throughout the entire six-week holding period. Because of the high respiration rate of the species, oxygenation of all test concentrations and controls was required to insure the maintenance of oxygen levels above the minimum. Individuals tended to leap in toxic test solutions, making necessary the use of nets over the containers.

*Fundulus kansae* was judged to be a poor test species. The species required much attention. Holding tank water "fouled" quickly. The fish held poorly, even for periods of moderate duration. Nets were required over toxic test solutions to retain the specimens. Oxygenation was required for all concentrations, both tests and controls.

*Hybognathus hankinsoni* was ranked a poor test animal. The species was difficult to seine. The animals did not hold well. They became thin and shrunken. The species could not survive on a laboratory preparation, ground alfalfa pellets, or a milk supplement diet.

*Ictiobus cyprinellus* was rated a poor test species. Frequent changing of water in the holding tanks was required because of "fouling." The fish appear susceptible to parasites. Specimens were easily disturbed by stimuli throughout the holding period. Nets were required to retain specimens in the more concentrated test solutions. Oxygenation was needed to maintain satisfactory oxygen levels in the test solutions. It appears that the species would be available in a suitable size for testing only a part of its first growing season.

*Notropis whipplei* was ranked a fair test animal. The species did not initially adapt well to holding conditions. The fish received snout injuries by striking the sides of the holding tanks throughout their first two weeks in the laboratory. Holding-tank covers were necessary because sight or sound stimuli excited the fish. After two weeks specimens adjusted sufficiently to allow the removal of

tank covers. The species required frequent feeding but a dry food maintained it satisfactorily. Nets were necessary while testing because some specimens attempted to leap from toxic solutions. Oxygenation was required for all test solutions and controls.

*Pimephales vigilax* was rated a fair test animal. The species transported without undue mortality, ate a dry food, and held well for three weeks. Deaths increased appreciably after that period. Specimens were undesirably nervous in test solutions. Nets were required to retain specimens in toxic concentrations. Oxygenation of some test solutions was required.

*Tilapia nilotica* was judged to be a fair test species. The species probably cannot be raised outdoors in areas in which the temperature drops below 48° F. Specimens transported and held well. They were maintained in the laboratory using a dry food. Toxic solutions caused the fish to leap, a situation which required the use of cover nets. Dead and live fish appeared similar in position in test solutions; therefore, a disturbance of the concentration for survival counts became necessary. The species withstood low-oxygen tensions.

*Chaenobryttus gulosus* proved to be a good bioassay animal. In ponds and lakes the species is associated with vegetation, which may make it difficult to seine. Because of a prolonged spawning period suitably sized specimens may be available throughout the year although fish older than one year would become too large for bioassay. Specimens transported well and held well for over one month. The species required a live food, *Daphnia magna*. The species withstood low-oxygen tensions without mortality.

*Lepomis humilis* was rated a good test species. The species spawns over a prolonged period, providing a continuous supply of young. Seldom do specimens outgrow a suitable testing size. The species transported and held well. A live food was required. Specimens remained quiet, and no leaping occurred in toxic solutions. Low-oxygen levels produced no mortality.

*Menidia audens* was judged an excellent test species. Apparently it frequents shallow, seizable water. Specimens do not outgrow a desirable test size. The species transported and survived well and ate both a dry food and a live food. Specimens remained quiet in test solutions and did not require oxygenation.

*Notropis girardi* proved to be an excellent test species. The species does not outgrow a usable size. The species sustained transport without undue mortality and adapted to holding conditions well. Specimens ate a dry food almost immediately. Specimens did not respire at a sufficient rate to require oxygenation of test solutions.

*Notropis stramineus* was judged an excellent bioassay species. The species does not outgrow a suitable testing size. Specimens transported well and held well. A dry food maintained them over a long period of time. Jumping did occur in the more concentrated test solutions. The species survived low oxygen tensions without death.

### RELATIVE RESISTANCES

Median tolerance limits were calculated for each species in each test. The Doolittle technique was applied. The analysis of variance is presented in Table III.

TABLE III  
RESULTS OF THE APPLICATION OF THE DOOLITTLE TECHNIQUE TO MEDIAN TOLERANCE LIMITS OF FISH TESTED

Source	Degrees of Freedom	Sum of Squares	A.O.V. Mean Square	F.	P.
Total	139	7,570.078			
Tests (unadj.)	22	5,101.893	231.904		
Fish Species (adj. for tests)	12	2,124.420	177.035	40.678	<0.0005
Within	45	82.610	1.835		
Error	60	261.155	4.352		

A.O.V.—Analysis of Variance, F.—Variance Ratio, P.—Probability.

A hypothesis, there were no differences in the resistances of the fishes to the toxicity of oil-refinery effluents, was tested. The probability value of <0.0005

showed there were fewer than five chances in 10,000 of the species possessing the same resistances. Therefore, the hypothesis was not accepted and additional treatment was applied to solve for species differences.

The results of the matrix inversion and the application of a modification of Duncan's new five percent multiple range test are presented in Table IV. The species are listed in ascending order of resistance. The mean  $TL_m^{96}$  adjusted for treatments, is given for each species. The  $TL_m^{96}$  mean differences can be determined from the  $TL_m^{96}$  means.

Vertical lines in Table IV are used to designate statistically determined populations of means. A  $TL_m^{96}$  mean directly to the left of a line is considered to be a member within the population designated by the line. Any two  $TL_m^{96}$  means directly to the left of the same line are not determined to be statistically different. Any two  $TL_m^{96}$  means not directly to the left of the same line are determined to be statistically different. A  $TL_m^{96}$  mean directly to the left of two population lines cannot be determined statistically different from either population.

TABLE IV  
RESULTS OF THE APPLICATION OF A MODIFICATION OF DUNCAN'S NEW  
FIVE PERCENT MULTIPLE RANGE TEST TO MEAN  $TL_m^{96}$  VALUES  
ADJUSTED FOR TREATMENT

Species	$TL_m^{96}$ Means					
	Adj. for Treatment	1	2	3	4	5 6
<i>Esox lucius</i> .....	6.3003753					
<i>Notropis girardi</i> .....	11.9431691					
<i>Notropis whipplei</i> .....	12.2599563					
<i>Menidia audens</i> .....	12.3742204					
<i>Hybognathus hankinsoni</i> .....	13.9306705					
<i>Pimephales vigilax</i> .....	15.6642834					
<i>Chaenobryttus gulosus</i> .....	16.5482376					
<i>Notropis stramineus</i> .....	17.1867204					
<i>Lepomis humilis</i> .....	17.1893568					
<i>Ictiobus cyprinellus</i> .....	20.0961375					
<i>Tilapia nilotica</i> .....	20.1596482					
<i>Fundulus kansae</i> .....	22.2008517					
<i>Lebistes reticulatus</i> .....	22.4593841					
(reference species)						

*Lebistes reticulatus* and *F. kansae* were the most resistant species tested. Their means were significantly different from those of *Lepomis humilis* and all species less resistant. *Tilapia nilotica* and *I. cyprinellus* proved to be significantly more resistant than *C. gulosus* and all species less resistant. *Lepomis humilis* and *N. stramineus* were significantly different in resistance than *H. hankinsoni* or any species less resistant. *Chaenobryttus gulosus* and *P. vigilax* were significantly different from *M. audens* and all species less resistant. *Hybognathus hankinsoni*, *M. audens*, *N. whipplei*, and *N. girardi* were all significantly different in resistance from *E. lucius*. *Esox lucius* was significantly different from all other fish tested. No other significant differences were found.

#### RANKINGS BY RESISTANCES

*Esox lucius* was nearly one-fourth as resistant as the reference species. *Menidia audens*, *N. girardi*, and *N. whipplei* were approximately one-half as resistant as the guppy. *Hybognathus hankinsoni* was about six-tenths as resistant as the guppy. *Pimephales vigilax* was nearly seven-tenths as resistant as the guppy. *Chaenobryttus gulosus*, *L. humilis*, and *N. stramineus* were about three-fourths as resistant as the guppy. *Tilapia nilotica*, and *Ictiobus cyprinellus* were nearly nine-tenths as resistant as the reference species. *Fundulus kansae* was nearly as resistant as the guppy.

#### DISCUSSION

The fishes tested were rated on their ecological suitabilities for bioassay. Each species was ranked on a scale which ranged through poor, fair, good, and excellent. The resistances were evaluated and compared with that of the reference species and with each of the other eleven species.

*Esox lucius* proved to be a poor test animal. The northern pike was only about one-fourth as resistant as *L. reticulatus*. *Fundulus kansae* was a poor bioassay animal and showed approximately the same resistance as the reference species. *Hybognathus hankinsoni*, a poor test species, was found to be just over six-tenths as resistant as the guppy. *Ictiobus cyprinellus*, a poor test animal, had about nine-tenths the resistance of the reference species. *Notropis whipplei*, a fair test animal, was a little less than one-half as resistant as the reference species. *Pimephales vigilax* was a fair test animal with nearly seven-tenths the resistance of the guppy. *Tilapia nilotica* was a fair bioassay animal with nearly nine-tenths the resistance of the reference species. *Chaenobryttus gulosus*, a good test animal, was slightly less than three-fourths as resistant as the reference species. *Lepomis humilis*, rated good as a test animal, was slightly more than three-fourths as resistant as the guppy. *Menidia audens* was an excellent bioassay animal with slightly over one-half the resistance of the guppy. *Notropis girardi* was found to be an excellent test animal with approximately one-half the resistance of the reference species. *Notropis stramineus* was an excellent bioassay animal with slightly over three-fourths the resistance of *L. reticulatus*.

Other investigators may rank the fishes treated in the study differently because of different emphasis, uses, and experiences with them. Investigators need to re-evaluate the species because of availability of suitably sized specimens for a particular locality. The fishes treated in the study were ranked as though they were equally available by distribution.

Problems encountered in the long distance transportation may be of no importance when transporting short distances. Some methods of transportation are not so effective as others for a particular species. *Menidia audens* did not respond well to transportation in plastic bags with oxygen atmospheres but transported excellently in water tanks to which oxygen was applied through breaker stones. Species requiring live food, particularly a definite sized fish as food were problematic. Providing fish for food would present no problem to some investigators and would result in different evaluation of some species. Some species were less adaptable to holding conditions than others. Modified holding containers might meet the requirements of additional species.

*Hybognathus hankinsoni* was taken from waters with high total dissolved solids and held in water with much less total dissolved solids. *Fundulus kansae* was taken from a stream with a high salt content and held in water with much less salts. Neither of the species held acceptably. The differences in the waters of their sources and the holding water may have caused unacceptable conditions. Holding water like the natural habitat might eliminate the difficulty.

Restraining devices were needed over some test solutions. Species that jumped might not do so in other kinds of effluents. Different species have different metabolic rates. Species with low respiration rates or those that can withstand low oxygen tensions would be more advantageous in laboratory or field situations where oxygenation facilities are not available.

The pH of all test solutions initially were alkaline, because of the alkalinity of the dilution water and the even greater alkalinity of the effluent. During the course of the test all pH values turned toward neutrality. The change was thought to be due to the accumulation of food wastes, carbon dioxide from respiration, and the volatility of some effluent compounds. Some fishes tended to reduce the pH further and at a faster rate than others. Investigators planning to do work within a narrow pH range should consider this factor.

The species which required frequent feedings and demonstrated high respiration rates were found to be in poor condition at the end of the 96 hour test period, even in controls. Such species should not be used for extended tests or the bioassay procedure should be modified to provide for the feeding during tests. Forty-eight hour tests with oil-refinery effluents produced  $TL_{m48}$  values similar to those of 96 hour tests.

The most resistant species was nearly four times as resistant as the most sensitive species. Within the range there was a normal distribution of species with a few resistant, a few sensitive, and a majority intermediate.

#### APPLICATION OF THE STUDY

The suitability of a test species is an important factor to consider in selecting an animal for bioassay. Factors that should be considered in the selection of a test species were reviewed by Henderson and Tarzwell (1957), Warren and

Doudoroff (1958), and Doudoroff *et al.* (1951). Recommendations made are based on experiences with various species. The study reported herein presents information on the suitability of additional species. The information can be used by investigators in evaluating perspective bioassay fishes.

Suggestions have been made for the use of local fishes, by Henderson and Tarzwell (1957), and non-native species by Warren and Doudoroff (1958). Relative resistances comparisons have been suggested in both cases.

Several of the species included in the present study have been ranked according to sensitivities to other substances. Species vary in their sensitivities to different chemicals (Henderson and Tarzwell, 1957). The study gives information concerning the relative resistances of the 12 species tested to oil refinery effluents only.

A review of the literature on almost any chemical will show a wide range of lethal or harmful values obtained by different investigators because the values reflected in part the use of different test species (Henderson and Tarzwell, 1957). It is hoped the present study will provide useful information for comparing existing or future reports of bioassays on oil-refinery effluents.

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## **GROWTH OF CHANNEL CATFISH, *Ictalurus punctatus*, AND BLUE CATFISH, *Ictalurus furcatus*, IN THE KENTUCKY LAKE PORTION OF THE TENNESSEE RIVER IN TENNESSEE**

By JOHN R. CONDER and RAY HOFFARTH  
*Tennessee Game and Fish Commission*

### ABSTRACT

The age and rate of growth of channel and blue catfish were determined by the pectoral spine section aging technique. At the end of their first year's growth, channel catfish weighed 0.10 pound and were 4.3 inches in length (total length), and blue catfish weighed 0.12 pound and were 5.3 inches in length. At the end of ten years, channel catfish weighed 9.2 pounds and were 25.2 inches long, and blue catfish weighed 24.26 and were 33.3 inches long.

### INTRODUCTION

The channel and blue catfish data in this paper have been collected and compiled because of the need for more complete knowledge of the age and rate of growth of these fish in Kentucky Reservoir. Also, there is considerable concern on the part of Kentucky Reservoir fishermen who are catching predominately small catfish and accurate scientific information is needed to combat the many and varied opinions held by the fishermen.

Kentucky Lake is the largest of the Tennessee Valley Authority's reservoirs and is managed primarily for flood control and navigation. It contains approximately 150,409 surface acres, of which 110,898 are within Tennessee. Its average water level normally fluctuates from two to five feet and occasionally during flood stage, it will fluctuate ten plus feet.