THE SUITABILITIES AND RELATIVE RESISTANCES

OF TWELVE SPECIES OF FISH AS BIOASSAY

ANIMALS FOR OIL-REFINERY

EFFLUENTS

By

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PREFACE

The toxicity bioassay has become a widely used tool in the evaluation of industrial effluents. The results of bioassays are published extensively. The results of some studies are not comparable to others even though the same method was used because different species of test fish were used.

The purpose of the study was twofold. The first aim was to provide information concerning the suitability of each of the 12 species as a bioassay animal. The information was obtained from observations made during the study and from the literature. The second aim was to rank the 12 species of fish by their relative resistances to oil-refinery effluents. The study would provide information to aid in the choice of test species and would allow a comparison of oil-refinery studies in which the species herein treated have been or might be used.

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INTRODUCTION

The Zoology Department, Oklahoma State University, has been engaged in a study of oil-refinery effluents since June, 1957. A grant from the National Institutes of Health was awarded to Dr. W. H. Irwin and Dr. 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 in this paper was done under the supplemental grant.

The thesis provides information concerning 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. The collecting and testing of species were begun in May, 1961, and completed by January, 1962.

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 (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

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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 which is 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). Henderson and Tarzwell (1957) have reported that 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) have recommended the use of the guppy.

Investigators have studied the resistances of fishes to a multitude of substances and situations including coal-tar wastes (Shelford, 1917), lead salts (Carpenter, 1930), brine water from oil wells (Clemens and Jones, 1954; Clemens and Finnell, 1955; Chipman, 1959), oxygen levels (Whitworth, 1961; Moss and Scott, 1961), ferro- and ferricyanide solutions (Tarzwell and Henderson, 1957), and endrin (Katz and Chadwick, 1961). Ellis (1937) in a study of the lethal limits of 114 substances reported information about the relative resistances of fishes. Doudoroff and Katz (1950) have reviewed the literature on the toxicity of alkalies, acids, and inorganic gases. The metals as salts have been reviewed (Doudoroff and Katz, 1953). Algicides, insecticides, and weedicides

have been reviewed and summarized (Ingram and Tarzwell, 1954). Henderson, Pickering, and Tarzwell (1959) reported the toxicity of ten chlorinated hydrocarbon insecticides to four species of fish.

Turnbull et al. (1954), Clemens and Summers (1954), and Gould and Dorris (1961) have used fishes as test organisms in bioassays of oil-refinery effluents but gave no relative resistance rankings. Clemens and Finnell (1956) made a field study from which they reported finding different species of fish at different distances downstream from an oil-refinery effluent outfall. They interpreted the findings as a measure of the relative resistances of fishes involved.

Douglas (1959) ranked four species by their relative resistances to petroleum-refinery effluents. On the basis of average results he ranked Lebistes reticulatus the most resistant. It was followed by Gambusia affinis, Pimephales promelas, and Hybognathus placita in order of decreasing resistance. The mean tolerance limit of L. reticulatus was significantly different from that of H. placita. No other significant differences were found.

Ward and Irwin (1961) ranked 13 species by their resistivity to oil-refinery effluents. By mean values they found the guppy to be the most resistant. 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 followed in order of decreasing resistance. The guppy and the black bullhead were statistically different from each other and from all the other species. The golden shiner was found to differ in resistance from P. notatus and all other more sensitive species. Notropis lutrensis was calculated to be significantly different from C. erythrogaster, I. punctatus, and

$\underline{M}$. $\underline{salmoides}$. $\underline{Lepomis}$ $\underline{microlophus}$ proved to be statistically different from $\underline{M}$. $\underline{salmoides}$. No other significant differences were found.

Douglas (1962) ranked 16 species by their resistance to oil-refinery waste waters. Four were species of fish he had ranked in 1959. He found that on the basis of means the guppy ranked as the most resistant fish. It was followed by $\underline{C}$. $\underline{auratus}$, $\underline{G}$. $\underline{affinis}$, $\underline{C}$. $\underline{carpio}$, $\underline{P}$. $\underline{promelas}$, $\underline{E}$. $\underline{inconstans}$, $\underline{L}$. $\underline{macrochirus}$, $\underline{L}$. $\underline{sicculus}$, $\underline{N}$. $\underline{percobromus}$, $\underline{S}$. $\underline{atromaculatus}$, $\underline{M}$. $\underline{dolomieui}$, $\underline{H}$. $\underline{placitus}$, $\underline{C}$. $\underline{commersoni}$, $\underline{S}$. $\underline{gairdneri}$, $\underline{N}$. $\underline{zonatus}$, and $\underline{E}$. $\underline{spectabile}$ in order of descending resistance. $\underline{Labistes}$ $\underline{reticulatus}$ was found to differ significantly in its resistance from all the species listed after $\underline{G}$. $\underline{affinis}$ in the preceding sentence. $\underline{Carassius}$ $\underline{auratus}$ and $\underline{G}$. $\underline{affinis}$ were found to differ significantly in resistance from $\underline{N}$. $\underline{zonatus}$ and $\underline{E}$. $\underline{spectabile}$. $\underline{Cyprinus}$ $\underline{carpio}$ was found to differ statistically from $\underline{E}$. $\underline{spectabile}$. No other significant differences between species were found.

MATERIALS AND METHODS

Materials

The oil-refinery effluents used contained 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 mentioned period as determined by the chemists of the
refinery.

TABLE I

CHEMICAL CHARACTERISTICS OF OIL-REFINERY EFFLUENTS

	pH	NH_3 ppm	Phenol ppm	Sulfide ppm	C.O.D. ppm	Alk.[1] ppm	Alk.[2] 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
[1]Phenolphthalein Alkalinity
[2]Methyl Orange Alkalinity

Dilution water was tap water which originated in Lake Carl Blackwell.
Dechlorination was achieved by at least 24 hours of aeration prior to
use. Tests for residual chlorine showed that it was reduced to 0.018
ppm in 12 hours. The chemical composition of the dilution water had

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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.

TABLE II

CHEMICAL CHARACTERISTICS OF DILUTION WATER

Iron	0.0	Silicon	3.6	Magnesium	12.0
HCO_3	130.0	Calcium	37.0	Potassium	25.0
CO_3	0.0	Clorides	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.

There was evidence that 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.

Methods

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 solu-
tions. 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 not withstanding the rating of other factors.

Guppies, Lebistes reticulatus (Peters), which were used in all the tests were raised in the Aquatic Biology Laboratory. 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 ways; (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 test 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 the Aquatic Biology Laboratory in 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 to be tested in the full-scale bioassay was subjected to the exploratory test. An independent exploratory test was made for each species, including the reference species. An exploratory test consisted of several concentrations of effluent and dilution water with two fish in each concentration. The exploratory test provided information which was used to determine the range of concentrations for the full-scale bioassay. Any effluent remaining in a partially filled container from the exploratory test was discarded. The exploratory test 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 of effluent. 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 during the test 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.

Initial dissolved-oxygen and hydrogen-ion concentration tests 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 checked 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.

Oxygen requirements

Workers have reported different lethal oxygen thresholds for the same species of fish. Wiebe, McGavock, Fuller, and Marcus (1934) reported that the lethal threshold for Notropis whipplei (Girard) was approximately 1.0 ppm oxygen. Wilding (1939) reported that oxygen concentrations below 2.25 ppm were lethal to Notropis whipplei. Moore (1942) reported the lethal threshold for Lepomis humilis (Girard) to be 1.4 ppm of dissolved oxygen. Wiebe et al. (1934) reported 0.5 ppm

dissolved oxygen to be the lethal threshold for Lepomis humilis. Moyle and Clothier (1959) have stated that Esox lucius Linnaeus survived dissolved-oxygen concentrations of between 0.9 to 2.7 ppm. Moore (1942) found that 2.3 ppm oxygen killed Esox lucius. Cooper and Washburn (1949) found the toleration level for Esox lucius to be 0.4 to 0.3 ppm of dissolved oxygen. All of the fishes mentioned are considered in this paper.

The contradictions in the reported lethal oxygen levels for the same species of fish were in part attributable to differences in the methods of investigation (field versus laboratory studies), hydrogen-ion concentrations, carbon dioxide concentrations, temperatures, sizes of fish, individuals within a species, the activity of the specimens, and other factors (Whitworth, 1958).

None of the reported factors were directly applicable to the stated test conditions of the study; therefore, none of the reported dissolved-oxygen concentrations were used as a basis for minimum oxygen levels in the study. 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 data on mortality of more than 50 percent and less than 50 percent on semi-logarithmic graph paper. 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 was applied to adjust 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.

ECOLOGICAL SUITABILITIES

Species Suitabilities

Chaenobryttus gulosus (Cuvier)

Source and size.--The warmouth, *Chaenobryttus gulosus* (Cuvier),
was seined from a pond near Boomer Lake, Payne County, Oklahoma, during
August and October, 1961. Boomer Lake pond (R. 2E., T. 19N, S.E. 1/4
Sec. 2) was shallow, mud bottomed, and had a profusion of *Najas*
guadalupensis (Spring) Morong. 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 had an average
length of approximately 5.1 cms and a wet weight of approximately 1.9
grams.

Transport and laboratory observations.--The fish were transported
to the laboratory in water held in covered tanks. Oxygen was applied
to the water through breaker stones. The short trip did not adversely
affect the fish. *Chaenobryttus* adjusted well to laboratory-holding con-
ditions. Initially they tended to aggregate in a corner of a holding tank
or hide under a thermometer which had been placed in the tank. After
a few days they distributed themselves more or less 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.

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The fish again showed a tendency to aggregate closely upon introduction into test solutions. After a short time they redistributed themselves more evenly throughout the test solution. The specimens tended to hover near the surface of the lethal solutions and near the bottom of the non-lethal solutions. Chaenobryttus withstood oxygen concentrations of 1.9 ppm without fatality. Oxygen was not applied to the test solutions since it was not needed. The test concentrations had a pH range of from 8.5 to 7.4.

Distribution and life history.--Chaenobryttus gulosus is found throughout the central and eastern United States to the Gulf Coast. The species has been found from Kansas and Iowa to the Mississippi River drainage in southern Wisconsin, the southern two-thirds of the Lower Peninsula of Michigan, Lake Erie, and the Allegheny River tributaries of Pennsylvania. Chaenobryttus gulosus is distributed 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 principally found in ponds and lakes although it can be found in the backwaters of rivers. It is found in association with dense weed beds, brush, roots, old tree stumps, and soft bottoms. The young are found 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 object taken. Chaenobryttus hybridizes with a great number of sunfishes but seldom produces large numbers of hybrids in natural populations. (Larimore, 1957).

The fish is a desirable animal for laboratory experimentation because it is easy to transport from the field, and easy to keep alive in the laboratory. The species has a quiet disposition, quickly becomes adapted to laboratory conditions, readily feeds on a wide variety of foods, and is tolerant to low dissolved-oxygen conditions. (Larimore, 1957).

Esox lucius Linnaeus

Source and size.--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 slightly under 1.4 grams in weight and 6.4 cms in total length.

Transport and laboratory observations.--Esox lucius was transported to Stillwater, Oklahoma, in plastic bags with oxygen atmospheres. Transport time was one day. Approximately 30 of the 400 died in transit. A few specimens remained after the tests were completed. At the end of a six week holding period they were still easily excited. The pike tended to dart into 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 distrubed.

Small specimens of <u>Gambusia</u> <u>affinis</u> (Baird and Girard) were fed to the northern pike during the holding period. The pike ate by slowly moving to 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.

<u>Esox</u> <u>lucius</u> tended to lie 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 their test containers.

Some fish were observed to come to the surface of the test solution as the dissoved-oxygen content decreased. It was necessary to bubble oxygen through the test containers and controls to keep the dissolved-oxygen content above the necessary minimum. It was noted, however, that there was no mortality in a container that had only 1.4 ppm dissolved oxygen. Test concentrations had a pH range of from 8.5 to 7.2.

Distribution and life history.--<u>Esox</u> <u>lucius</u> is found in Canada, northern United States, and northern Eurasia. The species occurs in northern New England through the Great Lakes region to eastern Nebraska and south to Missouri in the United States. (Moore, 1957).

The species prefers clean 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 some-
what 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

Source and size.--The plains killifish, Fundulus kansae Garman,
was seined from a tributary of the Cimarron River at a location south
and west of Lacy, Oklahoma (R. 9W., T. 19N, S.E. 1/4 Sec. 20). Collec-
tions were made on June 13, June 27, and July 11, 1961. Specimens used
weighed slightly over 1.7 grams each and averaged 5.8 cms in length.

Transport and laboratory observations.--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 sur-
vived transport well. Some tail-rot was noted after only a few days of
holding. Water in holding tanks had to be 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 added oxygenation. Test solutions had a pH range of 8.7 to 7.2.

Distribution and life history.--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 the 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

Source and size.--The brassy minnow, Hybognathus hankinsoni Hubbs,

was seined from a Brady, Nebraska, commercial minnow dealer's pond on June 3, 1961, and again on November 12, 1961. A random sample of ten fish had an average wet weight of 1.2 grams and a total length of 5.2 cms.

Transport and laboratory observations.--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 shrunken. 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.

Distribution and life history.--*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)

Source and size.--The bigmouth buffalo, Ictiobus cyprinellus (Valenciennes), was obtained on September 30, 1961, from a state hatchery at Lonoke, Arkansas. The specimens averaged 5.9 cms. in total length and 2.2 grams in wet weight.

Transport and laboratory observations.--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 conditions appeared to be due to the reactions of the food, fish secretions, and excretions. A few dead fish were found each day in the holding tanks. Close examination revealed anchor worms (Lernaca sp.) were present on many specimens. The parasitized fish were carefully separated from the non-parasitized, and no parasitized specimens were knowlingly 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 from 8.3 to 7.2

Distribution and life history.--<u>Ictiobus cyprinellus</u> 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). It's 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).

<u>Lepomis humilis</u> (Girard)

Source and size.--The orangespotted sunfish, <u>Lepomis humilis</u> (Girard), was obtained from two locations. In August of 1961, fish for

two tests were seined from a pond near Boomer Lake (R. 2E., T 19N., S.E. 1/4 Sec. 2), 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, (R. 2E., T. 23N., S.W. 1/4 of Sec. 3). The fish used averaged 5.0 cms in total length and 1.7 grams in weight.

Transport and laboratory observations.--The fish were transported in covered water tanks to which oxygen was applied through a breaker stone. In the laboratory L. humilis held well. 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 which they took voraciously by short darting movements.

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 tended to stay 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.

Distribution and life history.--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 more commonly found in a sluggish stream or pond in which there is a scarcity of vegetation. Muddy water is not disadvantageous to 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 crsutaceans, 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 indicate that the species can attain a size of 1.8 cms to a little larger than 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 size of more than four inches (Ward, 1959).

Menidia audens Hay

Source and size.--The Mississippi silversides, Menidia audens Hay, was seined from Lake Texoma, Oklahoma, (R. 4E., T. 7S., N.W. 1/4 Sec. 15) on August 18, 1961, and on August 22, 1961. The average fish had a wet weight of nearly 0.8 grams and a total length of approximately 6.4 cms.

Transport and laboratory observations.--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 tended to remain motionless in test solutions. They exhibited no jumping while in lethal test solutions. Specimens tended to become dark before death. They were capable of surviving in test solutions without oxygenation. Test solutions had a pH range of from 8.4 to 7.5.

Distribution and life history.--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 deep 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 from his observations that 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

Source and size.--The Arkansas River shiner, Notropis girardi Hubbs and Ortenburger, was seined from the Cimarron River near Lacey,

Oklahoma (R. 9W., T. 19N., S.W. 1/4 Sec. 16). On June 3, 1961, 700 to 800 specimens were taken in one foot deep water moving swiftly 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. A series of ten fish taken at random had an average weight of 0.6 grams, and were approximately four cms in length.

Transport and laboratory observations.--The fish were transported to Stillwater, 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.

Distribution and life history.--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)

Source and size.--The sand shiner, Notropis stramineus (Cope), was seined from Rock Creek near Dougherty, Murray County, Oklahoma, (R. 3E., T. 2S., S.W. 1/4 Sec. 7) on August 17, 1961. A random sample showed them to weigh 1.4 grams each and have an average total length of 5.4 cms.

Transportation and laboratory observations.--The sand shiners were transported the day they were seined, in two water tanks over which a cover and a net were placed to minimize the loss of fish and water. Oxygen was applied to the water 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 for approximately three weeks.

Notropis stramineus tended to cluster in the corners near the bottom of the test containers having more dilute concentrations. In

higher 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 higher test concentrations to insure the retention of leaping fish.

Ten specimens lived and showed no oxygen 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 from 8.4 to 7.4.

Distribution and life history.--<u>Notropis</u> <u>stramineus</u> 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 (Moore, 1957).

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)

Source and size.--The steelcolor shiner, <u>Notropis</u> <u>whipplei</u> (Girard), was seined from the Mountain Fork River at Beavers Bend State Park on the apron of a dam (R. 25E., T. 5S., N.W. 1/4 Sec. 14). The water, which was one half to two feet deep, flowed swiftly with some white water. The steelcolor shiner averaged approximately 6.7 cms in total length and 1.8 grams in wet weight.

Transport and laboratory observations.--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 hitting 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 of the water and 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 of test containers with the more dilute concentrations. Nets were used over the higher test 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.

Distribution and life history.--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)

Source and size.--The bullhead minnow, Pimephales vigilax (Baird and Girard), was seined from Lake Texoma at the mouth of Brier Creek (R. 4E., T. 7S., N.W. 1/4 Sec. 15) on July 20, 1961, and again on

August 17, 1961. The fish weighed approximately 1.4 grams apiece, and each was a little over 5.0 cms in total length.

Transport and laboratory observations.--Pimephales vigilax was transported in covered water containers to which oxygen was applied. Little death occurred during the trip. In the laboratory the bullhead minnow took a 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 insure the retention of 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 10 test fish. Test solutions had an initial high pH of 8.8 and a final low of 7.6.

Distribution and life history.--Pimephales vigilax is found in the Mississippi River system from Minnesota south to Texas, Louisiana, and Mississippi. It extends 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° at the collection time. The species eats algae, water mites, rotifers, crustaceans, annelids, insects, and molluscs (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 reaches three inches at maturity.

Tilapia nilotica Linnaeus

Source and size.--The Nile tilapia, Tilapia nilotica Linnaeus, was obtained from the U. S. Fish Culture Station, Marion, Alabama. The fish were generally about 5.5 cms in total length and were just under or over two grams apiece.

Transport and laboratory observations.--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 <u>L</u>. <u>humilis</u>, or <u>H</u>. <u>hankinsoni</u>. Ten fish in a test con-
tainer survived an oxygen content of 1.2 ppm for 96 hours. Test solu-
tions had a pH range of from 8.9 to 7.4.

In 1960 Ben-Tuvia quoted Trewavas as stating that <u>T</u>. <u>nilotica</u> 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 <u>Tilapia</u> <u>nilotica</u> utilizes both plant and animal food. Smaller
Nile tilapia feed primarily on entomostricans and chironomids while
larger sizes may utilize algae to a greater extent. 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

<u>Esox</u> <u>lucius</u> 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. It was necessary to provide the species with a suitably sized
fish as food which proved to be time consuming. Several <u>E</u>. <u>lucius</u> 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 in the laboratory. Holding tank water "fouled" quickly. The fish held poorly in the laboratory, even for periods of moderate duration. Nets were required over toxic test solutions to ensure the retention of leaping 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 in the laboratory. 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 laboratory 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. It was necessary to use nets because some specimens attempted to leap from toxic test 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 test 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.

Chaenobyttus gulosus proved to be a good bioassay animal. In ponds and lakes the species is associated with vegetation, which may make it somewhat 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 good as a test species. The species spawns over a prolonged period, thus 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, seinable water. Specimens do not outgrow a desirable test size. The species transported well and survived well in the laboratory. It ate both a dry food and a live food. Specimens remained quiet in test solutions. The respiration of specimens did not require test solutions to be oxygenated.

Notropis _girardi_ proved to be an excellent test species. The species does not outgrow a usable test size. The species sustained transport without undue mortality and adapted to holding conditions well. Specimens ate a dry food almost immediately upon introduction. 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 in the laboratory. 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 (Appendix Table I). 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

	A.O.V.				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F.	P.
Total	139	7570.078			
Tests (unadj.)	22	5101.893	231.904		
Fish Species (adj. for tests)	12	2124.420	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 <0.005 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 pre-
sented 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.

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 Adjusted for Treatment	Populations 1 2 3 4 5 6
Esox lucius	6.3003753	|
Notropis girardi	11.9431691	|
Notropis whipplei	12.2599563	|
Menidia audens	12.3742204	|
Hybognathus hankinsoni	13.9306705	| |
Pimephales vigilax	15.6642834	| |
Chaenobryttus gulosus	16.5482376	| |
Notropis stramineus	17.1867204	| |
Lepomis humilis	17.1893568	|
Ictiobus cyprinellus	20.0961375	|
Tilapia nilotica	20.1596482	|
Fundulus kansae	22.2008517	|
Lebistes reticulatus (reference species)	22.4593841	|

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 assumed to be not 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.

Lebistes reticulatus and P. kansae were the most resistant species tested. Their means were significantly different from those of Lepomis humilis and all less resistant species. Tilapia nilotica and I. cyprinellus proved to be significantly more resistant than C. gulosus and all less resistant species. Lepomis humilis and N. stramineus were significantly different in resistance than H. hankinsoni or any less resistant species. Chaenobryttus gulosus and P. vigilax were significantly different from M. Audens and all less resistant species. 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 mean 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, _L_. _reticulatus_.

DISCUSSION

The fishes tested in the study were rated on the basis of their ecological suitabilities for bioassay. Each species was ranked on a scale which ranged through poor, fair, good, and excellent. The resistance of each species was 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 was a poor test species. It was found to be just over six-tenths as resistant as the guppy. Ictiobus cyprinellus was a poor test animal which 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 was a good test animal. It was slightly less than three-fourths as resistant as the reference species. Lepomis humilis was rated good as a test animal. It was slightly more than three-fourths as resistant as the guppy. Menidia audens was an excellent bioassay animal. It was slightly over one-half as resistant as the guppy. Notropis girardi was found to be an excellent test animal with approximately one-half the resistance of the reference

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species. Notropis stramineous was an excellent bioassay animal with slightly over three-fourths the resistance of L. reticulatus.

It is recognized that other investigators may rank the fishes treated in the study differently because of different emphasis, uses, and experiences with them. Certainly 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 were downgraded if they required live food, particularly if the food consisted of a fish of a definite size. It is possible that providing fish for food would present no problem to some investigators and thus would result in a different evaluation of the species. Some species appear to be inherently less adaptable to holding conditions than others. Perhaps somewhat modified holding containers would allow the presently rated less adaptable species to become more adaptable.

Hybognathus hankinsoni was taken from waters which had extremely high total dissolved solids and was held in water with much less total dissolved solids. Fundulus kansae was taken from a stream with a high salt content and was held in water with much less salts. Neither of the species held acceptably in the laboratory. The differences in the waters of their sources and holding water may have caused unacceptable

holding conditions which could have been eliminated if holding water were like the water of the natural habitat of the fish.

Restraining devices were needed over test solutions in which some species were used. Species that demonstrated jumping might not do so in the bioassay of other kinds of effluents.

Different species apparently have different metabolic rates. It appears that species which have low respiration rates or those that can withstand low oxygen tensions would be more advantageously used in laboratories or field situations where oxygenation facilities are not available.

The pH of all test solutions in the study 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 feeding and demonstrated a high respiration rate were found to be in poor condition at the end of the 96 hour test period, even in controls. It is suggested that the species not be used for extended tests or that the bioassay procedure be modified to provide for the feeding during tests. It has been found that 48 hour tests with oil-refinery effluents produced TL_m^{48} 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 more or less

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). Their 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 (1) the use of local fishes, by Henderson and Tarzwell (1957), and (2) non-native species by Warren and Doudoroff (1958). In both cases it was suggested that relative resistances comparisons be made.

Several of the species included in the present study have been ranked against other substances, but the sensitivity of different species to different chemicals varies greatly (Henderson and Tarzwell, 1957). The study gives information on 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.

SUMMARY

1. A study was made to determine the ecological suitabilities and relative resistances of 12 species of fish as bioassay animals for oil-refinery effluents.

2. Ecological observations were made on the locality occupied, capture, size, ease of transport, holding capabilities, and test reactions of each species.

3. The literature was searched for distribution and life-history information that could influence an investigator's future choice of test species.

4. On the basis of ecological information, a general acceptability statement was made for each species.

5. Esox lucius, Fundulus kansae, Hybognathus hankinsoni, and Ictiobus cyprinellus proved to be poor bioassay species.

6. Notropis whipplei, Pimephales vigilax, and Tilapia nilotica were rated fair as test species.

7. Chaenobryttus gulosus and Lepomis humilis are judged to be good bioassay species.

8. Menidia audens, Notropis girardi, and Notropis stramineus were determined to be excellent bioassay animals.

9. Bioassay tests were performed, and median tolerance limits for each species were determined.

10. The median tolerance limits were statistically treated.

11. The relative resistance of each species studied was determined in relation to that of the reference species.

12. *Esox lucius* was almost one-fourth as resistant as the reference species.

13. *Menidia audens*, *Notropis girardi*, and *Notropis whipplei* were approximately one-half as resistant as the guppy.

14. *Hybognathus hankinsoni* was about six-tenths as resistant as the guppy.

15. *Pimephales vigilax* was nearly seven-tenths as resistant as the reference species.

16. *Chaenobryttus gulosus*, *Lepomis humilis*, and *Notropis stramineus* were about three-fourths as resistant as the guppy.

17. *Tilapia nilotica*, and *Ictiobus cyprinellus* were nearly nine-tenths as resistant as the reference species.

18. *Fundulus kansae* was nearly as resistant as the guppy, *Lebistes reticulatus*.

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APPENDIX

TABLE I

NINETY SIX-HOUR MEDIAN TOLERANCE LIMITS PLACED IN THE STATISTICAL ANALYSIS DESIGN

Test Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Species																								
L. reticulatus	A*	25.2	25.0	27.5	29.5	37.0	26.0	17.3	13.2	25.0	25.5	21.0	17.3	14.3	16.3	13.7	15.5	12.2	12.5	11.8	23.0	12.8	25.0	25.5
	B	25.2	24.0	28.3	33.5	37.0	21.3	17.3	12.4	24.8	23.8	21.0	16.8	15.5	17.8	17.8	15.5	12.1	12.5	12.5	23.0	12.8	28.0	25.7
N. girardi	A	13.5	15.0	20.0	14.5																			
	B	13.5	15.3	18.8	16.5																			
E. lucius	A	7.7	11.8	6.2																				
	B	8.5	10.0	11.0																				
H. hankinsoni	A	11.0																		7.2	13.6	8.5		
	B	11.0																		7.5	14.4	7.7		
F. kansae	A		25.0		29.5	39.5	21.1																	
	B		22.5		32.0	38.8	20.0																	
P. vigilax	A							12.5	10.0	12.4	17.3													
	B							12.2	8.5	12.4	16.3													
N. whipplei	A									12.2	12.4	11.4	7.5											
	B									12.4	12.4	11.8	7.8											
N. stramineus	A										16.8	17.8	12.5	7.5										
	B										17.3	18.0	12.5	7.5										
M. audens	A										12.4	12.8	6.2	4.2										
	B										12.4	12.8	7.7	2.9										
L. humilis	A												12.5	7.5							15.5	12.2		
	B												12.5	7.5							14.4	11.8		
C. gulosus	A												11.3	7.5		10.0		8.0						
	B												8.5	7.5		9.1		10.0						
I. cyprinellus	A														12.5	12.5	12.5		12.1					
	B														16.3	12.5	12.5		12.1					
T. nilotica	A																				21.0	12.5	24.3	24.3
	B																				18.3	12.5	24.3	24.3

* A and B designate replications

TL$_m^{96}$ figures represent the calculated concentrations (percent of effluent by volume) that should kill 50 percent of test animals.

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