Creel Census Summary

Survey by boat of complete and incomplete fishing trips.

$$\text{Catch/Hr.} = \frac{\text{Catch of Any Species}}{\text{Total Hrs. Fished}}$$

2,959 fisherman checks (March 1–November 30, 1959)

ANNUAL AVERAGE CATCH PER HOUR

Bass	.058 Fish/Hr.
Crappie	.390 Fish/Hr.
Bream	.404 Fish/Hr.
Catfish	.355 Fish/Hr.
"Other"	.018 Fish/Hr.

HARVEST

Bass	.18 Fish/A.
Crappie	1.24 Fish/A.
Bream	1.28 Fish/A.
Catfish	1.14 Fish/A.
"Other"	.06 Fish/A.

AVERAGE WEIGHT

Crappie	6 Oz.
Bass	2.6 Lbs.
Fishing Pressure	3.19 M/H/A.

A STUDY OF THE COMPARATIVE USE OF DIFFERENT SPECIES OF FISH IN THE TOXICITY BIOASSAY OF PETROLEUM REFINERY EFFLUENT *

By NEIL H. DOUGLAS

Aquatic Biology Laboratory, Department of Zoology
Oklahoma State University
Stillwater, Oklahoma

ABSTRACT

Fish have been used as test animals in pollution abatement programs since the inception of bioassay research. Many kinds of fish have been used in the bioassay tests. The kinds used at times have been selected merely on availability factors and not necessarily on a basis of adaptation of the fish to bioassay tests. This paper presents a comparison of four different species of fish used as test animals in a series of toxicity bioassays of petroleum refinery effluents.

INTRODUCTION

Toxicity bioassays were made during 1958 to determine the differences in the resistance of four species of fish to pertoleum refinery effluents. The four species were chosen because they were easily obtained and they were used previously for bioassay in the Southwest by other workers.

To compare the resistance of one species to the other three it was necessary to use dilutions of effluents whose toxic strengths would neither kill all specimens nor permit all to live. Comparisons of the relative resistance of the four species to petroleum refinery effluents were made.

One of the purposes of the study was to determine if one of the species was more resistant or susceptible to refinery effluents than were the others. At no time were the effluents chemically tested to reveal the components. A determination of the toxicity of refinery effluents to biotic life was not an objective.

Another purpose was to compare the behaviors of the four species regarding their habitats, ease of capture, adjustment to laboratory confinement and reactions in test solutions.

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Bioassay methods for the determination of the toxicity of effluents, including petroleum refinery wastes, have become increasingly important in pollution abatement programs within recent years.

According to Tarzwell (1957b), bioassays to determine the toxicity of wastes to certain organisms, including fish, were first used in Europe about fifty years ago. Some early contributions to bioassay procedure were made in this country by Shelford (1918) and Belding (1927). Doudoroff *et al.* (1951) provided a standardized procedure for bioassay testing, entitled, "Bioassay Methods for the Evaluation of Acute Toxicity of Industrial Waste to Fish." Greenbank (1949) observed that it was only logical and proper that bioassay tests of harmful effects upon fish be made by the use of living fish.

There is, still, considerable variation within and misunderstanding about the requirements of a species of fish to be used. Turnbull, Demann, and Weston (1954) stated that the results obtained from any toxicity test will depend upon the size and kind of test animal that is used in the experiment. No test animal has been selected as a standard for several reasons, first, the locality of the test site should be considered in determining the animal used, and second, a test fish should be a representative of the fish fauna of the region of testing and in which the results are to be applied.

Tarzwell (1957b) reports that fry and other early life history stages of fish are generally more sensitive to industrial wastes than adult fish. Doudoroff *et al.* (1951) maintained that a test fish should be rather sensitive to adverse water conditions, should be common in unpolluted portions of the body of water that receives the toxic wastes, but be able to withstand captivity and testing procedure.

METHODS

Four species of fish were used in the toxicity bioassays of petroleum refinery effluents. The species were *Pimphales promelas* Rafinesque, the fathead minnow; *Hybognathus placita* Girard, the plains minnow; *Gambusia affinis* (Baird and Girard), the mosquito fish; and *Lebistes reticulatus* (Peters), the guppy.

All specimens used in the tests were collected with a fine mesh seine near Stillwater, Oklahoma with the exception of *L. reticulatus* which was reared in the laboratory. The native fish were removed from their natural waters and transported to the laboratory. Each species of fish was then placed into separate holding tanks, which had previously been filled with tap water and allowed to stand for not less than one week. The fish were kept in the holding tanks, fed, and observed for 10 days or longer which allowed them to become accustomed to the laboratory conditions and permitted the destruction of any that seemed unfit for testing.

Diseased and injured fish were separated from the healthy fish and were not used in the tests. If as many as 10 percent of the specimens of any species of fish were deemed unfit for testing, another collection of that species was made and the previous procedure was repeated before testing was begun (a procedure recommended by Doudoroff *et al.*, 1951).

All specimens were sorted into groups of approximately the same length and weight prior to testing. Sizing of the fish was important in maintaining the standard of not more than one fish of one or two grams weight for each liter of liquid in a test container (Doudoroff *et al.*, 1951). *Lebistes reticulatus* being a species of small fish did not present a problem of weight requirements. Fry, immature forms and exceptionally large specimens were not used.

Petroleum refinery effluents were collected in five-gallon polyethylene jugs from two pertoleum refineries (designated as X and Y) near Stillwater. The effluents were taken before they were diluted with stream water. Waste effluents were taken directly from a pipe leading from refinery X and from a stream leading from refinery Y. The effluents were placed into jugs, transported to the laboratory and allowed to adjust to the laboratory temperature (75° F.). Eight collections of effluents were made alternately, four from refinery X and four from refinery Y, for the first eight bioassay tests. Two collections of effluents for the ninth and tenth bioassay tests were made from refinery Y. The effluents were taken at different intervals during the year (1958) and at different times of the day. Each test was made with an effluent collected the previous day and no effluent was used in more than one test. At no time was it known whether a particular sample of effluent would be more or less toxic than the previously collected samples until an exploratory test was made.

Exploratory tests were made prior to the actual toxicity tests to make certain the dilutions of aerated tap water and petroleum refinery effluents which were selected would kill more than one-half of the test specimens. Exploratory tests were made in one-half gallon jars with one liter of effluent and tap water dilution per jar. Two fish of the same species were used in each of six jars, all at different dilutions. A control of one liter of tap water was used for each species of fish.

Bioassay test containers were polyethylene, retangular in shape, 11½ inches in length, 7½ inches in width, and 12 inches in depth. The containers were placed side by side in two rows on tables in the laboratory and each was filled with 10 liters of tap water which had been aerated for one week. Refinery effluents and the previously aerated tap water were mixed to form the dilutions for the bioassays after the approximate concentrations were determined from exploratory tests. Dilutions were duplicated using similar containers and the same number of specimens and species of fish. A total of 3,600 fish, 900 of each of four species, were used in 10 separate tests. Each test included 360 fish of all species. Ten specimens of a species were placed into each of a series of dilutions of effluents making a total of 20 test fish per dilution for each test. A control of 10 fish per species was maintained in 10 liters of previously aerated tap water for the duration of each of the 10 bioassay tests.

The effluents collected for the first eight bioassay tests were similar in toxic values and required the same dilutions. The testing dilutions used in the first eight tests were 32 percent, 18 percent, 10 percent and 6.5 percent. The strengths of the effluents collected for the ninth and tenth tests were similar to each other in toxicity but were more toxic than the first eight effluents. The dilutions used in the ninth and tenth tests were 18 percent, 10 percent, 4.2 percent and 1 percent.

The procedures of preparing duplicate containers and dilutions were repeated for each of the four species for each of the 10 tests.

After the tests commenced, results were recorded from observations made at 1 hour, 12 hours, 24 hours, 48 hours and 96 hours. The dead fish were removed and recorded when observed. Observations of the toxicity tests showing the numbers of fish per species that remained alive in each concentration at each observation were recorded.

Values expressed in TL_m (median tolerance limit-concentration which causes 50 percent mortality) were determined by plotting on semi-logarithmic graph-paper the data concerning the survival of each species of fish for each test at 24 hour and 48 hour observations.

Notes about the four species of fish concerning their behavior during capture, in the laboratory, and in the test solutions were also recorded.

OBSERVATIONS PRIOR TO TESTING

Critical observation and examination of fish to be used in bioassay testing is important from the time the fish are captured in natural waters until testing is completed. Death during bioassay testing must be directly traceable to toxic components in the test solution. Death from any other cause makes the results of tests unreliable. Poor care, such as, crowded conditions, extreme temperature, improper feeding method, rough treatment in capturing or confining, or the presence of disease among the fish will reduce the validity of the test.

Disease was a problem with *H. placita* and *P. promelas* until control measures were applied. Often in their natural habitat the fish appeared to be in good condition but some soon showed infection in the holding tanks. Either some of the specimens were diseased when captured or were exposed to disease organisms soon afterward and in confinement the disease spread rapidly. Some specimens of these species were found to have fin rot and anchor worms and were discarded.

Treatments with terramycin were especially successful in preventing outbreaks of fin rot. It was made a regular practice to treat water in the holding tanks with terramycin before the specimens were added.

OBSERVATIONS DURING TESTING

The reactions of the individual fish of each species were similar when they were introduced into a concentration that was sufficiently toxic to produce a

quick kill. All specimens swam rapidly and erratically, darting and jumping until exhausted, then they rose to the surface, swam on their sides and gulped convulsively. A few minutes later they died.

Most deaths occurred before the 24-hour observation period regardless of species. Among the fish which lived beyond the 24-hour observation period, the death rate declined sharply except for *L. reticulatus.* Specimens of *L. reticulatus* succumbed during the entire times of each test and some died as late as the 96-hour period.

In weaker dilutions of effluents the percentages of fish survival were established for each species. The strengths of the effluents and the percentages of specimens of each species of fish surviving for each test were plotted on semi-logarithmic graph-paper and the TL_m values were determined by employing straight-line graphical interpolations (Henderson, 1956).

A trend seemed to exist throughout the ten bioassay tests in which resistance of one species was greater than any of the other three species. In tests 1-9, *L. reticulatus* was clearly the most resistant species, however, in test 10, *G. affinis* was the most resistant. *Pimephales promelas* and *H. placita,* varied in resistance throughout the 10 tests and both were much less resistant that *L. reticulatus* and *G. affinis.*

All specimens of the four species in the control solutions survived the entire period of each test.

An examination of the median tolerance limits for each of the four species in the 10 tests reveals that the four formed an arrangement of a definite order of resistance to petroleum refinery effluent. In Plate I graphs are presented in which the TL_m values for the 10 tests for each species were combined and show the comparative resistance. *Hybognathus placita* was the least resistant, *P. promelas* was second, *G. affinis* was third and *L. reticulatus* was the most resistant.

It was interesting that the observations prior to testing show to some extent the resistant effect of each species to petroleum refinery effluents. Of the four species, *H. placita,* the least resistant to the effluents, was the most excitable, difficult to capture and difficult to keep. *Lebistes reticulatus,* the more resistant of the species tested, was the least excitable and was readily available.

Statistical analyses of the 24-hour TL_m values for each species of fish in each test (Tables I and II) indicate that the difference between TL_m values are significant and not a result of chance. A five percent multiple range test (Table II, Number 2) was made by combining the TL_m values of each of the four species in each of the 10 tests thus resulting in 40 TL_m values (Table I). The multiple range test produced results which were expected, showing the TL_m values for *L. reticulatus* to be significantly different than those for *G. affinis, P. promelas,* and *H. placita.* The TL_m values for *G. affinis* were significantly different than those for *H. placita,* however, there was not a significant difference existing between the values for *P. promelas* and *G. affinis,* and those for *P. promelas* and *H. placita.*

TABLE I

TOTALS OF THE 24-HOUR TL_M VALUES

	Species 1	*Species 2*	*Species 3*	*Species 4*	*Total*
Test #1	23.00	21.00	21.25	24.00†	89.25*
Test #2	23.50	24.00	20.00	27.50	95.00
Test #3	12.75	13.00	13.50	18.00	57.25*
Test #4	21.50	22.00	16.25	47.00‡	106.75
Test #5	13.00	21.50	12.75	37.00¶	84.25*
Test #6	12.50	20.00	12.50	32.00	77.00
Test #7	12.25	13.00	10.00	16.50	51.75*
Test #8	7.65	13.00	7.30	14.00	41.90
Test #9	6.50	13.25	3.30	13.00	36.05
Test #10	2.20	17.00	2.30	11.00	32.50
TOTAL	134.80	177.75	119.15	240.00	671.70

† Average of total tests. ‡ and ¶ Extrapolations.

* Effluents from refinery X, other effluents from refinery Y.

Bioassay Test Animal:
Species 1 *P. promelas.* Species 2 *G. affinis.* Species 3 *H. placita.* Species 4 *L. reticulatus.*

PLATE I

Total 24 and 48 Hour TL_m Values for Each Species in 10 Bioassay Tests

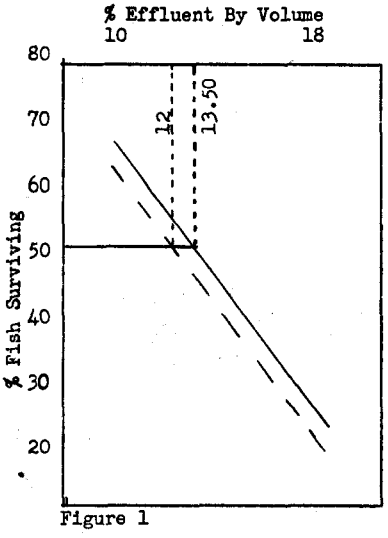

Figure 1

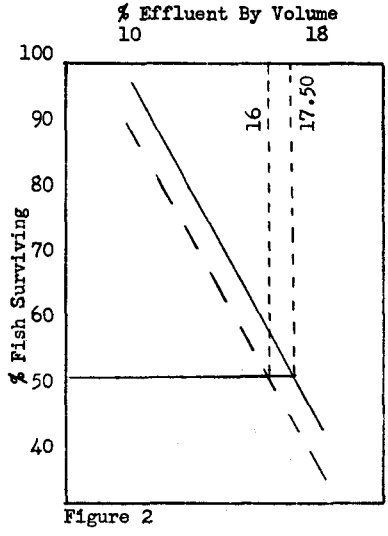

Figure 2

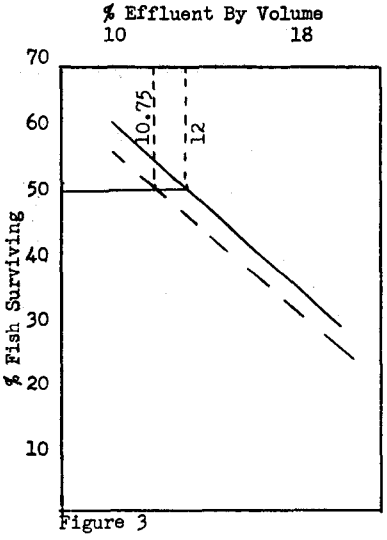

Figure 3

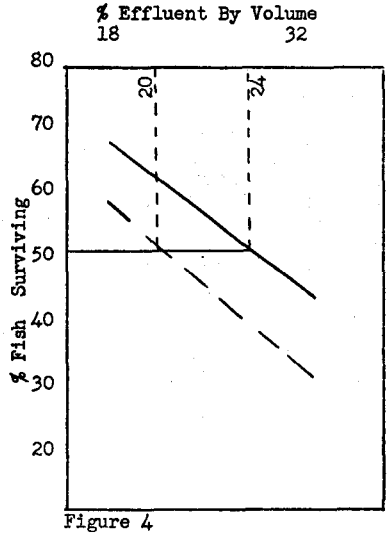

Figure 4

Figure 1. Species 1 *P. promelas*—24 Hour TL_m 13.50; 48 Hour TL_m 12.
Figure 2. Species 2 *G. affinis*—24 Hour TL_m 17.50; 48 Hour TL_m 16.
Figure 3. Species 3 *H. placita*—24 Hour TL_m 12; 48 Hour TL_m 10.75.
Figure 4. Species 4 *L. reticulatus*—24 Hour TL_m 24; 48 Hour TL_m 20.
Legend—24 Hour TL_m ————; 48 Hour TL_m — — — —

TABLE II

STATISTICAL ANALYSIS OF THE 24-HOUR TL_M VALUES

1. Analysis of Variance

Source	df	ss	ms	f
Total	39	3,144.1728	.....	....
Tests	9	1,590.5465	176.7273	....
Fish	3	876.7603	292.2534	11.66
Error	27	676.8660	25.0691	....

2. 5% Multiple Range Test

P		2	3	4
Rp		4.602	4.844	4.971
ID	*H. placita* (Species 3)	*P. promelas* (Species 1)	*G. affinis* (Species 2)	*L. reticulatus* (Species 4)
Mean	11.92	13.48	17.76	24.00

3. Results

Species 4 mean is significantly different than the means of species 1, 2, and 3.
Species 2 mean is significantly different than the mean of species 3.
Species 1 and 2 exhibit no significant difference between means.
Species 1 and 3 exhibit no significant difference between means.

DISCUSSION

A knowledge of the life history of a fish seems important in determining its value as a test animal. Such factors as the breeding habits, rate of growth, life span and distribution may determine if that particular species is a suitable and an advantageous fish for use in bioassay testing.

Some species of fish die soon after spawning. Such a species should not be used during the spawning season because of the inability to determine the cause of death during testing. Markus (1934) in his studies of the life history of *P. promelas* found that the death rate of the adult minnow was very high after the spring spawning period. Through one summer, 85 percent of an adult population died after spawning. Their off-spring which matured and spawned later that summer or the following spring had 80 percent mortality during the summer. It may be that the individuals that survived did not take part in the spawning and this enabled them to survive.

Pimephales promelas has a wide distribution, ranging throughout the Great Plains region of the United States eastward and southward through the Ohio and Cumberland systems to the Tennessee River Basin. It is not found on the Atlantic slope and the Gulf states east of the Mississippi River (Moore, 1957).

Gambusia affinis was distributed originally in central United States from southern Illinois to Alabama and southern Texas and on the Atlantic Coast from New Jersey to Florida. It is now more widely distributed by planting (Moore, 1957). It breeds during the spring and summer months but there is no indication of death following reproduction. The species is easily introduced into different localities and has a great appetite for its own young (Axelrod and Schultz, 1955).

Hybognathus placita normally ranges from Wyoming and South Dakota to Texas and on the Gulf Coast to Alabama (Moore, 1957). Bailey (1954) reports the species abounds in moderate to large rivers, backwaters, and bayous and ascends creeks infrequently except in the Great Plains. This fact certainly is not encouraging to one seeking a consistently obtainable species. There is little known about the life history of the species. As a test animal, it was found to have more undesirable factors than the other test species. The specimens proved to be far more difficult to collect, were very excitable, and had a higher mortality rate prior to testing than those of the other species. The species seems to be the least desirable of four species studied.

Lebistes reticulatus have broods about every four weeks, with the brood size averaging about 45 individuals (Axelrod and Schultz, 1955). The distribution of *L. reticulatus* is not a problem since it can be reared in the laboratory. Some pregnant females failed to survive the 96-hour durations of the weaker dilutions. Perhaps, for reliable test results, a separation of sexes is advisable especially with fish that bear their young alive. *Lebistes reticulatus* was the most convenient species used because specimens were small, of uniform size, free from

disease, and available in the laboratory in large quantities. The use of *L. reticulatus* in test solutions compared favorably with the other species tested.

A good test fish should adjust to laboratory conditions, by accepting conditions, calmly, feeding readily, and remaining healthy and vigorous. A fish which can be captured with ease and adjusts quickly to indoor confinement is more desirable for testing. Perhaps the best test fish would be one that can be raised in the laboratory in plentiful numbers, grows to maturity quickly, is resistant to common diseases and still is similar to native fish in resistance to waste effluents.

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A REPORT ON THE OPERATION OF A FISHWAY ON LAKE BISTINEAU, LA.

By DONALD W. GEAGAN and HARRY E. SCHAFER, JR.
Louisiana Wild Life and Fisheries Commission

Lake Bistineau is a 17,200 acre impoundment located in northwest Louisiana, in portions of Bossier, Webster, and Bienville Parishes. In 1954 the construction of a fishway on the spillway was completed. This fishway consisted of a four foot wide cement chute, 58 feet long with a one on five slope. Within the chute are 17, four feet high, wooden baffles located four feet 2.5 inches apart. Those baffles at the lower end of the fishway contain one rectangular opening in the top center two feet deep and 12.5 inches wide. The size of the opening gradually deminishes in width with each succeeding baffle to 8.5 inches for the top baffles. At the lower end of the chute are three openings 8.5 inches wide allowing fish to enter the structure. At the upper end is a well containing a basket or trap which when lowered retains all fish using the fishway and when raised allows unobstructed entrance into the lake proper.

The fishway was opened on March 1st in 1954 and in 1955. However, the actual periods during which fish were regularly found using the structure were April 10, 1954 through July 26, 1954 and May 4, 1955 through July 14, 1955. During these periods the basket trap was raised three times daily at 6 a. m., 12 noon, and 6 p. m.; all fish caught were identified and counted. All trash and commercial species were disposed of. Game species of available size which included Largemouth bass, *Micropterus salmoides* (Lacepede) and Spotted bass, *M. punctulatus* (Rafinesque) over ten inches; Black crappie, *Pomoxis nigromaculatus* (LeSueur) over 7 inches; Bluegill, *Lepomis macrochirus* Rafinesque; Redear sunfish, *L. Microlophus* (Gunther) and Warmouth, *Chaenobryttus gulosus* (Cuvier) over 5 inches were measured, weighed, and tagged in the operculum with a monel strap tag. Game fish under available size were counted. All game fish were released into the lake proper.

The fishway was in operation 1.8 times more days in 1954 than in 1955, however, the number of fish using the structure was 403 fish, approximately 9% more in 1955. Tables 1 and 2 give a breakdown on the species and number using the fishway for each year. The species using the fishway most frequently both years was the Freshwater drum, comprising 54% in 1954 and 31% in 1955. The Catostomidae comprised 17% of 1954 and 30% in 1955. From the figures it became apparent that the fishway is a good source of stocking the impoundment with commercial species. In 1954 only 26.7% of the total number of fish were game species and in 1955, 24.9% were game species, Table 3. Of the game fish using the structure in 1955, approximately 39% were available size.

The trap was raised three times daily to ascertain if any species migrate and utilize the fishway at a particular time. It was found, Table 4 that more