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

By

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DIFFERENT SPECIES OF FISH IN

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REFINERY EFFLUENT

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PREFACE

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 several different species of fish used as test animals in a series of bioassay tests.

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INTRODUCTION

Bioassay tests were made to determine the differences in the resistance of four species of fish to petroleum refinery effluents. The four species of fish were chosen because they were easily obtained and they were used previously for bioassay in this locality by other workers.

To compare the resistance of one species to the other three species it was necessary to use 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. Several different dilutions of the effluents with tap water were used for each test. 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.

The tests were made during the spring and fall semesters of 1958, in the Oklahoma State University fisheries laboratory in Stillwater, Oklahoma.

REVIEW OF THE LITERATURE

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.

Even though bioassay procedures have become standardized, there is 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. They also said that 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.

There has been some differences in opinions concerning the requirements of a test fish. In Report Number Six of the Waste Control

Laboratory of the Atlantic Refining Company (1939) it was reported that goldfish, Carassius auratus (Linnaeus) were used as test animals for the determination of toxicity of waste instead of native fish because they were adjusted to laboratory surroundings and confinement. The results of the tests with these fish were said to be more reliable than the results obtained when using native fish because the native fish were too nervous in captivity. It was also stated that the test results were comparable to wild fish that had been kept in laboratories and had become accustomed to the surroundings. Authors differ in their opinions of the values of goldfish as test animals. According to Hart, Doudoroff, and Greenbank (1945) goldfish are not ideal test animals because they are relatively hardy fish which were introduced into this country after being domesticated for countless generations.

Results using other species have been more satisfactory. Turnbull, Demann, and Weston (1954) report that the Atlantic Refinery Company used bluegill sunfish, Lepomis macrochirus Refinesque, obtained from fish hatcheries for test animals. Several other species of fish have been used in recent years by Doudoroff and Katz (1950) and the results published in Sewage and Industrial Wastes, Volume 22.

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.

MATERIALS AND METHODS

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

All specimens used in the toxicity 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 in testing.

Petroleum refinery effluents were collected in five-gallon polyethylene jugs from two petroleum 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 dumping 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, $11\frac{1}{2}$ inches in length, $7\frac{1}{2}$ 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 previously aerated tap water were mixed to form the dilutions for the bioassay tests after the approximate concentrations were determined from the exploratory tests. Necessary volumes of tap water to make the desired dilutions were removed from the containers and replaced with effluents. Dilutions were duplicated (indicated by letters A and B in tables 1-10 of the appendix) using similar containers and the same number of specimens and species of fish. A total of 3600 fish, 900 of each of four species, were used in 10 separate tests. Each test included 360 fish of each 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.

Specimens of H. placita were difficult to capture and transport to the laboratory. They were easily injured during capture and died unless oxygenating apparatus was used during transportation. Individuals were excitable and perhaps the shock of removing the fish from seines to holding tanks was a cause of death for some specimens.

Specimens of P. promelas were less difficult to capture and transport to the laboratory. The specimens were not particularly susceptible to injury during capture and oxygenating apparatus was not necessary for survival of the specimens during transportation. They were excitable, but calmed somewhat after several days of confinement.

Individuals of G. affinis were easily captured and were transported with ease when weather conditions were not extreme. They showed no harmful effects from capture and adapted readily to the laboratory conditions.

Members of L. reticulatus were the most convenient of the species used because no problems existed concerning capture or transportation since they were reared in the laboratory.

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 time 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 the 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 resistance than *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 1 and 2) indicate that the differences between TL_m values are significant and not a result of chance. A five percent multiple range test (Table 2, 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 1). 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.

The species which statistical analyses reveal to have no significant difference in TL_m values have other equally important characteristics, already described, which influences their use as test animals (Table 3).

TABLE 1. 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 (1)	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 (2)	106.75
Test #5	13.00	21.50	12.75	37.00 (3)	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	7.60	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

(1) Average of total tests, (2) and (3) Interpolations

* 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

TABLE 2. STATISTICAL ANALYSES 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	<u>H. placita</u> (Species 3)	<u>P. promelas</u> (Species 1)	<u>G. affinis</u> (Species 2)	<u>L. reticulatus</u> (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 offspring 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 licalities 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 the 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.

TABLE 3. OBSERVATIONS PRIOR TO TESTING AND RELATIVE RESISTANCE OF FISH SPECIES DURING TESTING

	Results of Observations Prior to Testing				Relative Resistance to Effluents
	Ease of Capture	Ease of Transportation	Nervous Reaction in Confinement	Resistance to Fin Rot	
Species #1	Fairly Easy	Fairly Easy	Nervous	Poor Resistance	Third Least Resistant
Species #2	Easy	Easy	Calm	Fair Resistance	Second Least Resistant
Species #3	Difficult	Difficult	Very Nervous	Poor Resistance	Least Resistant
Species #4	Easy	Very Easy	Calm	Good Resistance	Most Resistant

Species #1---*P. promelas*
Species #2---*G. affinis*
Species #3---*H. placita*
Species #4---*L. reticulatus*

SUMMARY

1. Bioassay tests were made to determine the differences in the resistance of four species of fish to petroleum refinery effluent.

2. Studies of the behaviors of the four species regarding their habitats, ease of capture, adjustment to laboratory confinement and reactions in test solutions were made.

3. Results of ten bioassay tests are presented.

4. A TL_m value was determined for each species of fish for each bioassay test and the values were combined per species to reveal a comparison of the relative resistance of the four species to petroleum refinery effluents. The results of this comparison are presented.

5. The 24 hour TL_m values for the four species of fish were tested statistically and the results are considered.

6. Life history characteristics of the species that may influence test results are discussed.

7. *Lebistes reticulatus* seems to be the most desirable of the species tested because it can be raised in the laboratory in large numbers and its resistance to common diseases is high.

8. A definite order of resistance to refinery effluents was established for the four species. *Hybognathus placita* was the least resistant, *P. promelas* was second, *G. affinis* was third and *L. reticulatus* was the most resistant.

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APPENDIX

TABLE 1. BIOASSAY TEST 1, JAN. 25, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

		32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
		1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1	A	10	0	-	-	-	10	10	7	6	4	10	10	10	10	9	10	10	10	10	10
	B	10	0	-	-	-	10	10	10	10	8	10	10	10	10	10	10	10	10	10	10
#2	A	10	0	-	-	-	10	10	8	7	6	10	10	10	7	6	10	10	10	8	6
	B	10	0	-	-	-	10	10	6	4	3	10	10	10	10	8	10	10	9	6	5
#3	A	10	0	-	-	-	10	10	7	4	2	10	9	7	5	4	10	10	10	9	7
	B	10	0	-	-	-	10	10	9	9	4	10	10	10	9	4	10	10	10	10	8
#4	A	10	10	10	9	6	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	B	10	10	10	9	8	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery X

TABLE 2. BIOASSAY TEST 2, MAR. 6, 1958

Bioassay Test Animal
#1 P. *promelas* (Fathead Minnow)
#2 G. *affinis* (Mosquito Fish)
#3 H. *placita* (Plains Minnow)
#4 L. *reticulatus* (Guppy)

Number of Test Animals Surviving

		32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
		1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1	A	9	0	-	-	-	10	10	9	9	8	10	10	10	10	10	10	10	10	10	10
	B	8	0	-	-	-	10	9	9	8	7	10	10	10	10	10	10	10	10	10	10
#2	A	10	0	-	-	-	10	10	10	10	8	10	10	10	9	8	10	10	10	10	10
	B	10	0	-	-	-	10	10	10	9	8	10	10	10	10	8	10	10	10	10	8
#3	A	7	0	-	-	-	10	6	6	3	0	9	7	7	5	5	10	10	10	9	8
	B	4	0	-	-	-	9	8	6	4	3	10	10	10	10	10	10	10	10	10	7
#4	A	10	8	4	1	1	10	10	10	8	7	10	10	10	10	10	10	10	10	10	10
	B	10	7	3	3	3	10	10	10	9	9	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

TABLE 3. BIOASSAY TEST 3, APR. 1, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

		32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
		1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1	A	0	-	-	-	-	8	0	-	-	-	10	10	10	10	10	10	10	10	10	10
	B	0	-	-	-	-	8	0	-	-	-	10	10	7	7	7	10	10	10	10	10
#2	A	7	0	-	-	-	9	0	-	-	-	10	10	10	10	6	10	10	10	10	9
	B	5	0	-	-	-	10	0	-	-	-	10	9	8	8	2	10	10	10	10	8
#3	A	0	-	-	-	-	7	1	0	-	-	10	10	10	10	8	10	10	10	10	9
	B	0	-	-	-	-	8	2	0	-	-	10	10	10	10	10	10	10	10	10	8
#4	A	10	0	-	-	-	10	10	4	4	4	10	10	10	10	10	10	10	10	10	10
	B	10	0	-	-	-	10	9	6	6	6	10	10	10	9	9	10	10	10	10	10

Effluent from Refinery X

TABLE 4. BIOASSAY TEST 4, APR. 9, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

	32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1 A	3	0	-	-	-	10	9	7	7	7	10	10	10	10	10	10	10	10	10	10
B	4	0	-	-	-	10	10	8	6	6	10	10	10	10	10	10	10	10	10	10
#2 A	4	0	-	-	-	10	8	8	4	2	10	10	10	9	9	10	10	10	8	8
B	5	0	-	-	-	10	7	7	3	2	10	10	10	10	8	10	10	10	10	9
#3 A	0	-	-	-	-	9	7	4	4	4	10	10	10	9	9	10	10	10	9	9
B	0	-	-	-	-	8	8	4	4	4	10	10	10	10	9	10	10	10	10	10
#4 A	10	9	9	7	4	10	10	10	9	8	10	10	10	10	9	10	10	10	10	10
B	10	5	5	3	1	10	10	10	10	7	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

TABLE 5. BIOASSAY TEST 5, APR. 16, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

		32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
		1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1	A	0	-	-	-	-	2	0	-	-	-	9	8	8	8	8	10	10	10	10	10
	B	0	-	-	-	-	4	0	-	-	-	10	10	10	10	10	10	10	10	10	10
#2	A	5	0	-	-	-	10	10	10	10	10	10	10	10	10	9	10	9	9	9	9
	B	6	0	-	-	-	10	5	5	5	5	10	10	10	10	10	10	10	10	10	9
#3	A	0	-	-	-	-	0	-	-	-	-	10	10	9	9	9	10	10	10	10	10
	B	0	-	-	-	-	0	-	-	-	-	8	8	8	8	8	10	10	10	10	10
#4	A	10	10	6	3	2	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	B	10	7	6	4	4	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery X

TABLE 6. BIOASSAY TEST 6, APR. 25, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

	32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1 A	0	-	-	-	-	3	0	-	-	-	9	8	7	7	7	10	10	10	10	10
B	0	-	-	-	-	5	0	-	-	-	10	10	9	9	9	10	10	9	9	9
#2 A	4	0	-	-	-	10	8	8	8	7	10	10	10	10	9	10	10	10	10	10
B	3	0	-	-	-	10	4	4	4	4	10	10	10	10	10	10	10	10	10	10
#3 A	0	-	-	-	-	0	-	-	-	-	10	10	9	9	9	10	10	10	9	9
B	0	-	-	-	-	0	-	-	-	-	8	8	7	7	7	10	10	9	9	9
#4 A	10	7	5	4	4	10	10	9	9	9	10	10	10	10	10	10	10	10	10	10
B	10	6	5	2	2	10	10	8	7	7	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

TABLE 7. BIOASSAY TEST 7, SEPT. 29, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

		32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
		1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1	A	0	-	-	-	-	10	2	0	-	-	10	10	8	5	5	10	10	9	9	8
	B	0	-	-	-	-	10	1	0	-	-	10	10	7	6	6	10	10	7	6	6
#2	A	10	0	-	-	-	10	5	0	-	-	10	10	10	8	7	10	10	10	10	10
	B	10	0	-	-	-	10	3	0	-	-	10	10	10	9	9	10	10	10	10	10
#3	A	0	-	-	-	-	0	-	-	-	-	10	5	4	4	4	10	8	8	7	7
	B	0	-	-	-	-	0	-	-	-	-	10	6	6	6	6	10	7	7	7	7
#4	A	10	0	-	-	-	10	10	6	6	6	10	10	10	10	10	10	10	10	10	10
	B	10	0	-	-	-	10	9	2	2	2	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery X

TABLE 8. BIOASSAY TEST 8, OCT. 14, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

	32% Dilution					18% Dilution					10% Dilution					6.5% Dilution				
	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1 A	0	-	-	-	-	10	0	-	-	-	10	8	0	-	-	10	10	9	9	9
B	0	-	-	-	-	10	0	-	-	-	10	7	0	-	-	10	10	7	7	7
#2 A	5	0	-	-	-	10	0	-	-	-	10	10	9	9	9	10	10	10	10	10
B	4	0	-	-	-	10	0	-	-	-	10	10	10	10	10	10	10	10	10	10
#3 A	0	-	-	-	-	0	-	-	-	-	10	5	0	-	-	10	8	8	8	8
B	0	-	-	-	-	0	-	-	-	-	10	6	0	-	-	10	6	6	6	6
#4 A	10	0	-	-	-	10	4	1	1	1	10	10	10	10	10	10	10	10	10	10
B	10	0	-	-	-	10	4	1	1	1	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

TABLE 9. BIOASSAY TEST 9, OCT. 23, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

	18% Dilution					10% Dilution					4.2% Dilution					1% Dilution				
	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1 A	0	-	-	-	-	5	0	-	-	-	10	10	10	10	10	10	10	10	10	10
B	0	-	-	-	-	6	0	-	-	-	10	10	10	10	10	10	10	10	10	10
#2 A	10	0	-	-	-	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
B	10	0	-	-	-	10	9	9	9	9	10	10	10	10	10	10	10	10	10	10
#3 A	0	-	-	-	-	0	-	-	-	-	10	8	6	6	6	10	10	10	10	10
B	0	-	-	-	-	0	-	-	-	-	10	2	2	2	2	10	10	10	10	10
#4 A	10	0	-	-	-	10	9	9	9	9	10	10	10	10	10	10	10	10	10	10
B	10	0	-	-	-	10	9	9	9	9	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

TABLE 10. BIOASSAY TEST 10, OCT. 28, 1958

Bioassay Test Animal
#1 P. promelas (Fathead Minnow)
#2 G. affinis (Mosquito Fish)
#3 H. placita (Plains Minnow)
#4 L. reticulatus (Guppy)

Number of Test Animals Surviving

	18% Dilution					10% Dilution					4.2% Dilution					1% Dilution				
	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	1 hr.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
#1 A	0	-	-	-	-	0	-	-	-	-	10	5	0	-	-	10	10	10	10	10
B	0	-	-	-	-	0	-	-	-	-	10	4	2	2	2	10	10	10	10	10
#2 A	10	4	4	4	4	10	9	9	7	7	10	10	10	10	10	10	10	10	9	9
B	10	8	5	5	5	10	8	8	7	7	10	10	10	10	10	10	10	10	9	9
#3 A	0	-	-	-	-	0	-	-	-	-	4	2	1	1	1	10	10	10	10	10
B	0	-	-	-	-	0	-	-	-	-	6	2	2	2	2	10	10	10	9	9
#4 A	10	0	-	-	-	10	6	6	6	6	10	10	10	10	10	10	10	10	10	10
B	10	0	-	-	-	10	7	6	5	5	10	10	10	10	10	10	10	10	10	10

Effluent from Refinery Y

PLATE I

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

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	— — —

PLATE I

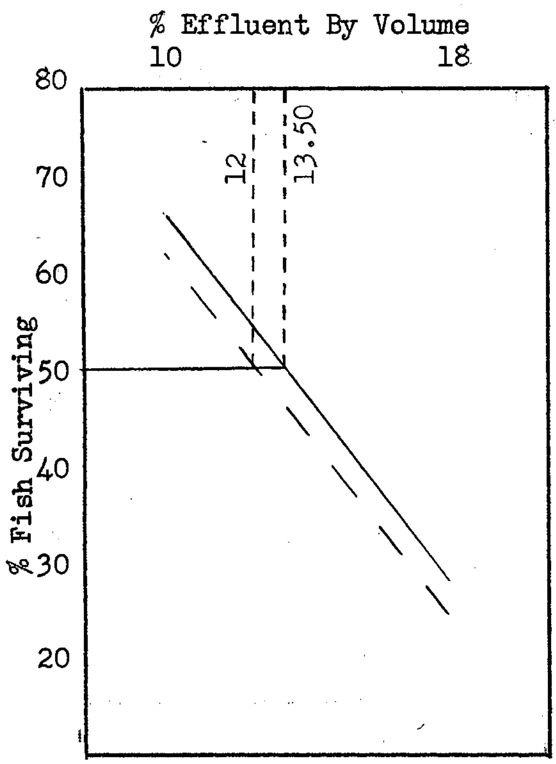

Figure 1

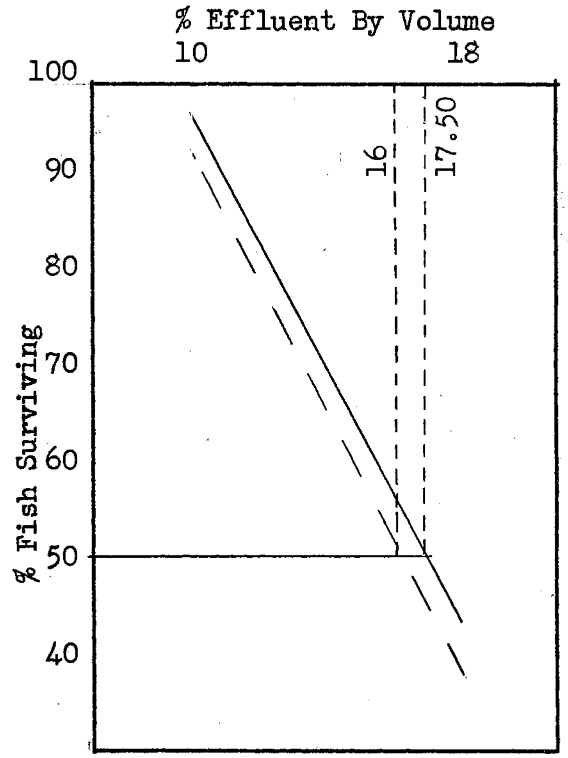

Figure 2

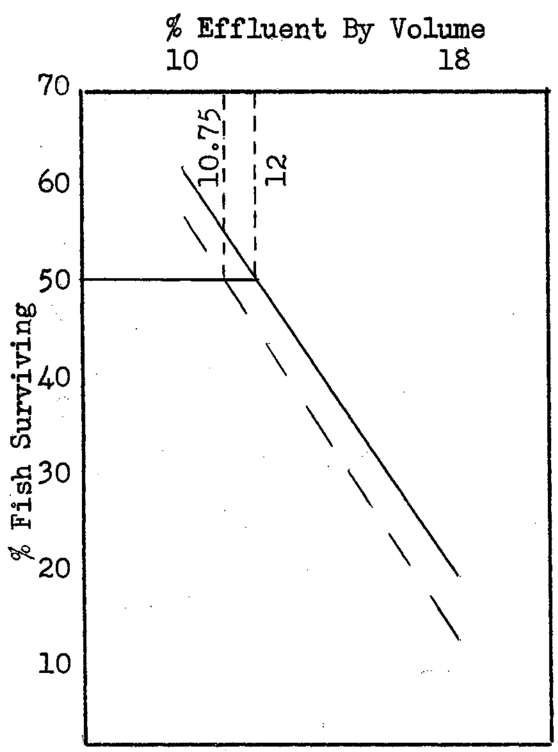

Figure 3

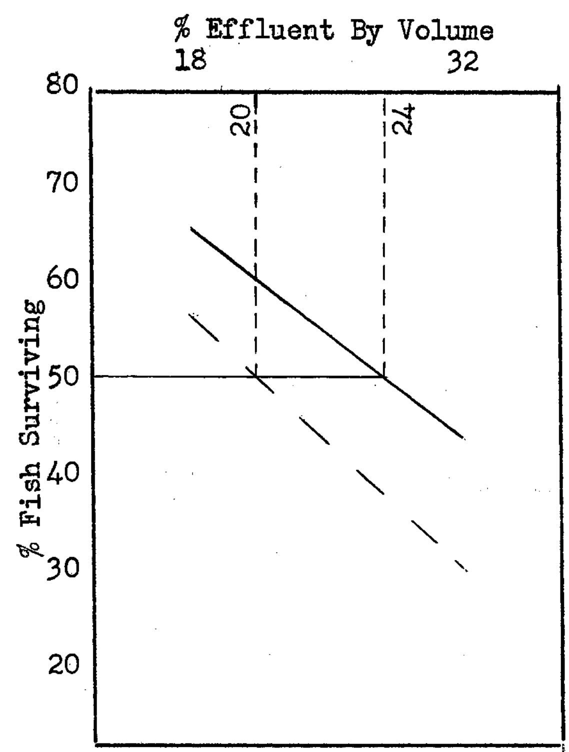

Figure 4

VITA

Neil Harrison Douglas

Candidate for the Degree of

Master of Science

Thesis: THE COMPARATIVE USE OF DIFFERENT SPECIES OF FISH IN THE BIOASSAY OF PETROLEUM REFINERY EFFLUENT

Major Field: Zoology

Biographical:

Personal data: Born at Moorefield, West Virginia, February 17, 1932.

Education: Graduated from Oklahoma State University with a major in Zoology in January, 1955; completed the requirements for the Master of Science degree from Oklahoma State University with a major in Zoology in May, 1959.

Experience: Employed by the United States Fish and Wildlife Service as a fisheries biologist assistant in Alaska during the summer of 1953; served two years (1955-1957) in the United States Army in Germany; employed by the Oklahoma Refiners Waste Control Council doing research during the summer of 1957; graduate teaching assistant in Zoology, Oklahoma State University, 1957-1959.

Organizations: Oklahoma Academy of Science, Phi Sigma.