vacation, the mortality rate in all lots increased sharply (Fig. 1). Both Loren G. Hill and the project leader were out of town for a little over a week, and the care of the threadfin shad was intrusted to Joe E. Coward. The reason for this great mortality is unknown.

Data obtained in the performance of these experiments indicate that a slow drop in temperature to 5.0° C. will eliminate threadfin shad from a lake. A sudden drop in temperature would be even more deadly because the threadfin shad would not have time to become acclimated to colder temperatures. Deaths of threadfin shad at 12.2 to 14.2° C. in the Colorado River at Austin, Texas (Hubbs, 1951), were probably caused by a sudden drop in temperature. For example, threadfin shad, acclimated at 15.0° C., live less than a day when suddenly put at 6.0° C. Some threadfin shad could survive limited periods at lake temperatures as low as 6.0 to 7.0° C. A breeding stock of threadfin shad will survive the winter in a lake that does not go below 9.0° C. provided the drop in temperature is slow enough for them to become acclimated to cold temperatures. However, they are sluggish at low temperatures and it is possible that predators would eliminate them from a lake because of their reduced swimming speed.

Lake Fort Smith usually becomes too cold in winter for threadfin shad to survive. During 23 winters (Table 1), the minimum water temperature fell below 5.0° C. (41.0° F.) during 16 and remained above 5.0° C. during 3. No evidence of overwintering by threadfin shad, stocked in Lake Fort Smith in the summers of 1959, 1960 and 1961, was obtained and a temperature kill of threadfin shad was observed during the late winter of 1959-1960 when temperature fell below 5.0° C.

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THE RELATIVE RESISTANCES OF SEVENTEEN SPECIES OF FISH TO PETROLEUM REFINERY EFFLUENTS AND A COMPARISON OF SOME POSSIBLE METHODS OF RANKING RESISTANCES [1, 2]

DEWEY L. BUNTING II AND W. H. IRWIN[3]
Oklahoma State University

ABSTRACT

Eighteen species of fish including a reference species, were subjected to toxicity bioassay using petroleum refinery effluent as a toxicant. Twenty-four-hour and 96-hour median tolerance limits were calculated using a straight-line graphical interpolation based on ten specimens per concentration with a replication. Collection, laboratory, and bioassay histories were recorded for each test species and a general suitability statement made for each.

Twenty-four-hour and 96-hour adjusted resistances obtained by the "Preadjusted-Abbreviated Doolittle" method were subjected to analysis of variance and to a modification of Duncan's new five percent multiple range test. Six methods were employed to adjust the relative resistance for differences in tests. The tests were ranked according to results obtained by each adjustment from most to least resistant. The "Interval" method was preferred over the other adjusted procedures on the

[1] Contribution 379 Zoology Department, Oklahoma State University.

[2] The project was supported by the National Institute of Health Research Grant WP-67(C2).

[3] W. H. Irwin, currently associated with U. S. Department of Health, Education, and Welfare, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

bases of computational ease, ready addition of new data, compatible rankings with the "Preadjusted-Abbreviated Doolittle" method, and a lack of a "number effect."

INTRODUCTION

The sensitivity of fish to specific chemical compounds that occur in petroleum refinery effluents has been extensively studied (Carpenter, 1930; Ellis, 1937; Shelford, 1917; Turnbill, *et al.*, 1954; and Wallen, *et al.*, 1957). Little attention has been given to comparative aspects of sensitivity, and even less to the final effluent that contains the by-products of the refining procedure. Douglas (1960), Douglas and Irwin (1962), Gould and Irwin (1962), and Ward and Irwin (1962) made comparative studies of the relative resistances of 40 species of fish to petroleum refinery effluents, and provided information concerning the suitabilities of species as toxicity bioassay test animals. Gould and Dorris (1961) studied the effects of storage on the toxicity of oil refinery effluents.

The purposes of the study were to provide comparative measures of relative resistances and toxicity bioassay suitabilities of 17 species of fish to petroleum refinery effluent, and to compare several methods of adjusting resistance data for test differences. The species studied were: *Cottus carolinae* (Gill), banded sculpin; *Cyprinus carpio* Linnaeus, carp; *Dionda nubila* (Forbes), ozark minnow; *Dorosoma cepedianum* (LeSueur), gizzard shad; *Fundulus olivaceus* (Storer), blackspotted topminnow; *Hybopsis amblops* (Rafinesque), bigeye chub; *Ictalurus nebulosus* (LeSueur), brown bullhead; *Lepomis auritus* (Linnaeus), redbrest sunfish; *Notropis camurus* (Jordan and Meek), bluntface shiner; *Notropis cornutus* (Mitchill), common shiner; *Notropis dorsalis* (Agassiz), bigmouth shiner; *Notropis rubellus* (Agassiz), rosyface shiner; *Notropis spilopterus* (Cope), spotfin shiner; *Notropis venustus* (Girard), blacktail shiner; *Noturus exilis* Nelson, slender madtom; *Salmo trutta* (Linnaeus), brown trout, and *Tilapia nilotica* (Linnaeus), Nile tilapia. The bioassays were conducted in the Aquatic Biology Laboratory of Oklahoma State University from October 1961 to December 1962.

MATERIALS AND METHODS

Collection, Transport, and Maintenance of Test Fish

The majority of fish used in toxicity bioassay were collected in the wild. Some species were obtained from state and federal hatcheries. Fish were sorted in the field whenever possible to facilitate hauling a single species to a container. Guppies were reared in the laboratory, and specimens used in bioassay were the descendants of a common brood stock.

Specific identification of each species was corroborated using the keys and descriptions found in Moore (1957), Trautman (1957), and Hubbs and Lagler (1949). The nomenclature used follows that recommended by the American Fisheries Society (1960).

Fish species collected in Oklahoma were transported to the laboratory in tanks supplied with oxygen. Hauling tanks were covered with nylon netting to minimize fish loss due to water splashing.

Upon arrival at the laboratory, fish were transferred to porcelainized holding tanks. The tanks were large enough to accommodate 100 to 200 fish each depending upon the size of individual specimens. Holding water was tap water that had previously been aerated for at least 24 hours to remove chlorine.

Fish were fed daily upon dry meal (poultry mash and powdered egg) and/or live food consisting of either *Daphnia* or chironomid larvae, or both. Suggestions of Doudoroff, *et al.* (1951) concerning the care and feeding of test animals were followed where applicable. The length of time a species was held in the laboratory prior to testing was determined by its behavior, size, food, and condition.

The guppy was employed as a reference species in all bioassays. They were reared in the laboratory at about 80 degrees F. and fed daily a mixture of dry meal and powdered egg. Ward (1962) presented a detailed account of the rearing method employed. Specimens were

graded prior to testing and only those approximately 0.6 to 0.7 inches in length were used.

COLLECTION AND CHEMICAL NATURE OF THE EFFLUENT

Petroleum refinery effluent was collected from a retention pond from which it was normally pumped into a nearby watercourse. Five-gallon polyethelene carboys were filled with the effluent and capped securely. A collection, usually 30 to 50 gallons, was transported to the laboratory and emptied into a 30-gallon polyethelene container to cool. Tests were begun within 24 hours of the collection time. No effluent sample was used twice. All effluents were from the same refinery, and all were toxic to the species tested.

Table I shows extremes and averages of some chemical characteristics of effluents used between June 7, 1962 and December 17, 1962. Values given are those obtained by chemists at the refinery.

TABLE I. SOME CHEMICAL CHARACTERISTICS OF PETROLEUM REFINERY EFFLUENTS [1]

Chemical Characteristics	Range		Average
	low	high	
pH	8.8	10.4	9.70
Ammonia as NH_3	67.0	93.0	79.30
Phenol	5.2	34.0	13.00
Sulfide	0.0	1.9	0.33
Phenophthalein Alkalinity	140.0	300.0	170.00
Methyl Orange Alkalinity	180.0	420.0	311.10
Chemical Oxygen Demand	275.0	480.0	344.00

[1] All chemical values in ppm.

SOURCE AND CHEMICAL NATURE OF THE DILUTION WATER

Dilution water was tap water treated the same as the fish holding water. Gould and Irwin (1962) state that tests for residual chlorine showed a reduction to 0.018 ppm after 12 hours aeration. Table II contains the results of a chemical analysis performed January 8, 1963, by Curtis E. Moutrey & Associates, Inc., Tulsa, Oklahoma, on a sample of the dilution water.

TABLE II
SOME CHEMICAL CHARACTERISTICS (PPM) OF THE DILUTION WATER

Calcium	45	Fluoride	1
Magnesium	14	Hydroxide	0
Manganese	0	Carbonate	0
Iron	0.01	Bicarbonates	176
Aluminum	0	Carbon Dioxide	6
Sulfate	24	Silica as SiO_2	2
Chloride	54	Total Solids	348
Sodium and Potassium as Na	35	$CaCO_3$ as Saturation	+1
HCO_3 Alkalinity as $CaCO_3$	144	Turbidity as SiO_2	0
Stability Alkalinity as $CaCO_3$	143	Specific Conductance [1]	405

[1] Micromhos

EXPLORATORY BIOASSAY

Preceding each bioassay, an exploratory test was performed with each species including the guppy. The exploratory test helped in the establishment of the range of concentrations needed. Exploratory testing substantially reduced the number of concentrations and the number of fish required. Two specimens were used in each concentration and the tests concluded after approximately 12 hours.

BIOASSAY PROCEDURE

The bioassay procedure as outlined by Doudoroff, *et al.* (1951) was followed. Based on information gained in exploratory tests, concentrations to bracket the median tolerance limit were selected from a

progressive bisection of intervals on a logarithmic scale. The median tolerance limit, TL_m, is defined as the concentration at which 50 percent of the test animals survive for a specified exposure time (Doudoroff, *et al.*, 1951). Three to five concentrations per test were used. Tests with concentrations no more than five percent apart seemed most accurate. Each test solution was ten liters in volume, contained ten fish, and had two replications. Because of the large size of *Dorosoma cepedianum* specimens, four replications per concentration were used with five specimens in each replication.

Bioassays were inspected at 1, 6, 12, 24, 48, and 96 hours to record the number of surviving fish and to remove dead specimens. Dissolved oxygen concentration was determined at intervals throughout each test. If the oxygen level dropped below 2 ppm the test solution was aerated with oxygen at the rate of one bubble per second. Henderson and Tarzwell (1957) reported that rates of 180 bubbles per minute will not greatly affect the toxicity of solutions containing volatile compounds. Tests were terminated at the end of 96 hours. Initial and final pH values were determined colormetrically using a B & L Spectronic 20. Dissolved oxygen concentrations were determined by the Alsterburg modification of the Winkler method. Averages of dilution water temperature are given in Table III. All testing was performed in a constant temperature room.

TABLE III
DILUTION WATER TEMPERATURE (DEGREES F.)

Month, 1962	Range	Average
July	77.0 - 77.5	77.2
August	73.0 - 77.5	75.6
September	74.5 - 75.5	75.0
October	73.0 - 77.0	74.4
November	68.0 - 72.5	70.0
December	68.7 - 72.0	69.4

CALCULATION OF RELATIVE RESISTANCE

The methods of Finney (1962), Reed and Muench (1938), and Berkson (1953) for determining median tolerance limits were considered. They were not used because each method requires several concentrations that exhibit survival values between 0 percent and 100 percent. The method adopted was the straight-line graphical interpolation as suggested by Douboroff, *et al.* (1951) and Henderson and Trazwell (1957). The two closest concentrations exhibiting percent survival figures above and below 50 percent were plotted on semi-logarithmic paper, percent survival being on the arithmetic scale and percent concentration of effluent on the logarithmic scale. A straight line was drawn between the two points. A concentration determined by the intersection of the above line and the percent survival line was the TL_m. Actual survival of 50 percent of the test animals in a concentration never was used to represent the TL_m except where the median survival occurred in the highest concentration. Twenty-four-hour (TL_m^{24}) and ninety-six-hour (TL_m^{96}) TL_m values were determined for each species in each replication of every test. The first two and last two replications in each concentration involving *Dorosoma cepedianum* were combined to form two final replications per concentration.

The TL_m^{24} values were also determined for each species of fish by pooling replications and basing percent survival on 20 fish rather than 10 percent concentration.

RANKING OF RELATIVE RESISTANCE

Because of variability in toxicity of the effluent samples it seemed inadvisable to compare and rank resistances of test species without first adjusting TL_m values statistically. The "Preadjusted-Abbreviated Doolittle" method was used by Douglas and Irwin (1962), Gould and Irwin (1962), and Ward and Irwin (1962). Since the method was complex and data for additional species could not be added without

complete recomputation, several shorter and simpler techniques were also applied. Species were ranked according to the results obtained by each adjustment procedure, and the rankings compared.

"Preadjusted-Abbreviated Doolittle" Method. The TL_m^{24} and TL_m^{96} values were analyzed in separate two-way classifications with unequal numbers in subclasses. The data were nonorthogonal, necessitating the use of special methods of analyses to obtain a sum of squares for any one effect free from other effects. To obtain a sum of squares for species effects, the reduction in the total sum of squares attributable to all sources of variation, including species, was computed. From the result was subtracted the reduction in the total sum of squares attributable to all sources of variation other than species effects. The difference in the two reductions gives a sum of squares for species effects adjusted for other effects. Graybill (1961) presents the well-known theory of two-way classifications with unequal numbers in subclasses.

The general computational procedures for obtaining species effects (adjusted resistance values) follows.

1. The method of least-squares was applied to the data and resulted in a set of linear equations involving both test effects and species effects.
2. Test effects were solved in terms of species effects, and substituted in the equations in procedure 1 to obtain a reduced set of equations involving species effects.
3. The Abbreviated Doolittle Method, for solving simultaneous linear equations, was applied to the set of reduced equations from procedure 2 to obtain species effects. The combination of procedures 2 and 3 will be referred to as the "Preadjusted-Abbreviated Doolittle." Adjusted data were subjected to Analysis of Variance and to a modification of Duncan's new five percent multiple range test (Kramer, 1956).

"Interval" Method. The length of the interval between the TL_m of a test species and the TL_m of the reference fish in the same test is considered a relative measure of difference in resistance between the two species. Using TL_m^{24} values based on 20 fish per concentration, interval lengths were calculated, averaged, and average values subtracted from 100 for each species within all tests in which a species appeared. The species were ranked by intervals from least resistance (low interval) to most resistant (high interval), the guppy being the most resistant.

"Proportion" Methods. Four different proportional methods for test adjustment were employed. The first, third and fourth methods contained TL_m values based on 20 test animals per concentration. The second method contained TL_m values based on 10 animals per concentration and a replication. The species were ranked from most resistant to least resistant according to results obtained from each method.

1. An "Individual Proportion," TF/G, was obtained by dividing the TL_m for the guppy (G) from each test into the TL_m for the test species (TF) from the same test. The resulting proportions for each species were averaged.
2. A "Replicate Proportion," TF/G,' was calculated in the following manner. All available TL_m values for each test species (TF) were averaged, as were the TL_m values of the guppy (G) for the same tests. The average for the guppy was then divided into the average for the test species.
3. An "Individual Proportion," G/TF, was obtained by dividing each TL_m for a test species (TF) into the TL_m for the guppy (G) from the same test. The results were then averaged for each species.
4. The "Average Proportion," G/TF,' involved averaging all available TL_m values for each test species (TF), and all TL_m values for the guppy (G) from the same tests. The resulting test fish average was then divided into the corresponding average for the guppy.

FIELD, LABORATORY, AND BIOASSAY HISTORIES AND SUITABILITIES OF THE TEST SPECIES

Cottus carolinae

Specimens of *Cottus carolinae* were collected in Tyner Creek, Adair Co., Oklahoma, June 5 and June 15, 1962. All specimens were taken at

night from about six inches of rapidly flowing water. Collecting during the daylight hours proved ineffective. The fish were transported to the laboratory with no casualties the day following each collection. During a laboratory holding period of 20 days four small specimens from the June 5 collection succumbed. The fish ate *Daphnia* well, though not avidly, and occasionally ate dry meal. They became noticeably thinner toward the end of the holding period and were never observed to eat for an hour or more after the introduction of food. Chironomid larvae were offered as food on three occasions without success.

The first specimens were used in bioassay after a holding period of five days. All specimens rested on the bottom of the test containers and exhibited little or no distress in highly toxic solutions. Because the fish congregated at the bottom of the test containers, all samples for the determination dissolved oxygen and pH were siphoned from the lower third of the test solutions. Differences of 2 ppm dissolved oxygen were noted between the surface and lower third of some solutions. On no occasion was the level low enough to warrant oxygenation.

Cottus carolinae was rated a poor test animal. Specimens did not eat standard foods well, and their behavior in the test containers made special care necessary to obtain water samples for chemical analysis.

Cyprinus carpio

Six hundred specimens of *Cyprinus carpio* were obtained from the U. S. Fish and Wildlife Service, Stuttgart, Arkansas, June 28 and July 30, 1962. Both collections were transported in molded plastic containers with oxygen atmospheres. Only a few specimens of the July 30 collection died enroute. When the fish were placed in holding tanks they became excitable, but after a few moments quieted. Within 24 hours the specimens ate dry meal and appeared in a healthy and hardy condition during the entire holding period of over two weeks.

The specimens were not excited when placed in the test solutions, and reacted slowly to highly toxic concentrations. Oxygenation was not necessary except in the higher concentrations of the second test.

Cyprinus carpio was rated an excellent test species. It was easily transported, ate standard laboratory foods, and was not excitable in test containers.

Dicnda nubila

Approximately 200 specimens of *Dionda nubila* were collected in Barren Fork Creek, Adair Co., Oklahoma, November 8, 1962, and were immediately transported to the laboratory. A few casualties occurred among some injured specimens. The fish were placed in two holding tanks and treated with terramycin. They were fed dry meal, *Daphnia*, and chironomid larvae, but were never observed to eat actively. The fish were used as test animals five and 12 days after arrival at the laboratory. Approximately 20 specimens were held for a two-week period after testing was completed. These were noted to become progressively thinner and to lose their color completely. One or two fish died each day during the last week of the holding period.

Dionda nubila was considered a fair test animal. Specimens did not eat standard laboratory foods well, and were not easily obtained in the field.

Dorosoma cepedianum

Approximately 400 specimens of *Dorosoma cepedianum* were collected in Fourteen Mile Creek, Cherokee Co., Oklahoma, November 28 and December 6, 1962. The fish were herded into shallow water and captured with the bare hands. Contact with the herding seine was kept at a minimum to avoid scaling. Both collections were transported in good condition with a small mortality occurring in the December 6 collection. Upon arrival at the laboratory the tanks were moved indoors, the water aerated, treated with terramycin and acriflavine, and allowed to equilibrate to room temperature. Equilibration time was about 36 hours for each collection. The November 28 collection held very well, no casualties occurred during the entire period of ten days. Some specimens were held 45 days. The December 6 collection exhibited a

mortality of one to two fish every two days over the entire holding period. Specimens were used as bioassay animals within three days after arrival.

Test specimens from the November 28 collection reacted slowly to toxic concentrations. Fish in acute distress swam on their sides at the surface of the solutions. All deaths occurred during the first 48 hours of the testing period.

Fish that died during the first 48 hours in tests made from the December 6 collection exhibited the same behavior as those from the November collection. In these tests deaths were recorded until the 96-hour period. Specimens that died between 48 and 96 hours showed signs of hemorrhaging around the snout and at the bases of the fins. All concentrations were oxygenated and dissolved oxygen levels were maintained above 3 ppm.

Dorosoma cepedianum was rated a poor test species. They were very susceptible to injury and disease, one of the two collections did not hold well, and they did not eat the laboratory foods.

Fundulus olivaceus

Approximately 400 specimens of *Fundulus olivaceus* were collected in an isolated pool of Terrapin Creek, Cherokee Co., Oklahoma, July 30, 1962. The fish were collected by seining in shallow waters near the edges of the pool. Specimens were transported to the laboratory with a loss of two fish. During the ten-day holding period there were no casualties. The fish ate dry meal reluctantly and *Daphnia* readily. The dry meal was their usual diet due to a lack of an adequate supply of *Daphnia*.

The fish were excited in test solutions of intermediate and high toxicity. All test containers were covered to keep the specimens from jumping out. No violent reactions were observed in highly toxic test solutions. The fish floated to the surface and twitched occasionally before dying. Oxygenation of the test solutions was never required.

Fundulus olivaceus was rated a fair test animal. It ate well and was easy to hold. Specimens were difficult to obtain, and were initially excitable in the test containers.

Hybopsis amblops

Approximately 200 specimens of *Hybopsis amblops* were collected in Barren Fork Creek, Adair Co., Oklahoma, December 15, 1962. The fish were transported without loss. They were fed dry meal twice daily after arrival at the laboratory. The fish were held for a period exceeding 14 days. The first specimens were used as bioassay test animals after a period of two days. Test specimens reacted slowly and nonviolently to toxic test solutions. Oxygenation was not required during either testing period.

Hybopsis amblops was considered a fair test animal. Specimens were easily transported and ate well, but were difficult to obtain in large numbers.

Ictalurus nebulosus

Approximately 1,000 specimens of *Ictalurus nebulosus* were obtained from the Hamilton Fish Hatchery, Hamilton, Arkansas, July 8, 1960, and were transported with a loss of two specimens. The fish were first used as test animals after a holding period of ten days and others at intervals through 25 days. All bioassay procedures for the species were performed by Claud M. Ward.

Lepomis auritus

More than 500 specimens of *Lepomis auritus* were obtained from the Tennessee State Fish Hatchery at Humboldt, Tennessee, June 27, 1962. Fish were placed in plastic containers with oxygen atmospheres, the containers placed in styrofoam ice-chests to retard temperature changes and hauled to Stillwater, Oklahoma. Fish remained in the containers for approximately 36 hours. Upon arrival 100 specimens were dead. The remaining fish were transferred to holding tanks and treated with terramycin and acriflavine. Because the specimens proved to be excitable,

a portion of each tank was covered with wooden planks. When disturbed the fish aggregated in the shadows. They ate dry meal well the third day after arrival and by the seventh day came to the surface in anticipation of food. The first specimens were used as bioassay test animals after a holding period of seven days and others at intervals through 20 days.

Lepomis auritus was rated a good test animal. Specimens did not transport well, but acclimated to laboratory conditions quickly and were easily fed and cared for.

Notropis camurus

Specimens of *Notropis camurus* were collected in Barren Fork Creek and Fourteen-Mile Creek, Cherokee Co., Oklahoma, June 20, 1960, November 8, 1962, and November 27, 1962. All collections were transported without casualty. The fish were easily excited and never became entirely acclimated to laboratory conditions. All holding containers were covered with netting to prevent loss of specimens. *Notropis camurus* proved to be a voracious eater, requiring three daily feedings of dry meal and *Daphnia*.

Specimens placed in highly toxic concentrations reacted instantaneously and violently by attempting to jump from the test containers. In intermediately toxic solutions, deaths were preceded by a long period of floating with occasional muscular twitching. Oxygenation and cover netting were required for all test concentrations and the controls.

Notropis camurus was rated a good test species. It transported easily, held and ate well in the laboratory, but was extremely excitable in both the holding tanks and test containers.

Notropis cornutus

Approximately 100 specimens of *Notropis cornutus* were obtained from the Department of Zoology, Southern Illinois University, Carbondale, Illinois, October 13, 1961. They were transported without casualty and appeared to be in good condition upon arrival. After transfer to holding containers, the fish were treated with terramycin and acriflavine. They began to eat almost immediately, taking dry meal and *Daphnia* readily. Holding tanks were covered with netting.

Specimens were used as bioassay animals after a holding period of two weeks. Because of the large size and excitability of the fish, test containers were covered with nylon netting. All test solutions including the controls were oxygenated during the entire test period.

Notropis cornutus was considered a fair test species. It was transported with ease, held well, ate well in the laboratory, but was fairly excitable and difficult to obtain in large numbers.

Notropis dorsalis

Approximately 500 specimens of *Notropis dorsalis* were collected in the Des Moines River near Madrid, Iowa, September 29, 1962 and separated from other species before preparation for transportation. Upon arrival at the laboratory the fish were treated with terramycin and acriflavine because they showed evidence of tail-rot. A few days after treatment all evidence of the fungus had disappeared. The fish were fed dry meal twice daily and ate voraciously. Specimens were used as test animals after a holding period of six days. A few fish held for four months were still in a healthy condition.

Test specimens reacted violently in highly toxic concentrations, several jumped from the solutions, and all test containers were covered with netting. Oxygenation of test solutions was never necessary.

Notropis dorsalis was rated a poor test species. It was difficult to sort from other species without excessive handling, was susceptible to disease, and was excitable in test containers.

Notropis rubellus

Specimens of *Notropis rubellus* were collected in Barren Fork Creek, Cherokee Co., Oklahoma, November 8, and December 15, 1962. No loss was experienced in transporting the November collection to the laboratory, but several small specimens of the December collection died enroute.

Upon arrival the transporting tanks were moved indoors, the water aerated, treated with terramycin, and allowed to equilibrate to room temperature before the fish were transferred to holding tanks. No casualties occurred during the equilibration time.

Specimens belonging to the November collection were used as test animals after a holding period of five days and others at intervals through the twenty-second day. Specimens used after the twenty-second day died during the last 21 hours. By the twenty-third day the fish in the holding tanks had also succumbed.

Specimens collected on December 15 were used as test animals after holding periods of two and three days. Some specimens were held in the laboratory for three weeks without a casualty.

Notropis rubellus was rated a fair test species. It was difficult to hold specimens in the laboratory, and they were difficult to collect in the field.

Notropis spilopterus

Specimens of *Notropis spilopterus* were collected in the Des Moines River near Madrid, Iowa, September 29, 1962, sorted from other species and transported in plastic containers with oxygen atmospheres. The fish were treated with terramycin upon arrival at the laboratory to deter infection. Eight specimens died during the two-day transportation period and four during the six-day holding period before testing was begun. Every two or three days a few additional fish died during the remainder of a 25-day holding period. The fish seemed to eat dry meal adequately but became noticeably thinner after the first week.

Test specimens were excited when placed in the test containers though covers were unnecessary. They reacted slowly and nonviolently to toxic test solutions. Oxygenation was required only in the highest concentrations during the last 24 hours of a test period.

Notropis spilopterus was rated a poor test animal. It was difficult to identify in the field, ate poorly, and was excitable in test containers.

Notropis venustus

Specimens of *Notropis venustus* were collected in Comanche Lake, Stephens Co., Oklahoma, June 27 and August 27, 1962, and transported to the laboratory without casualties. They ate dry meal voraciously and were fed at least four times daily. Heavy casualties in the June collection occurred 13 days after arrival. Over-feeding resulted in fouled water and by morning only a few remained alive.

Test specimens were excitable and all test containers had to be covered with netting. The fish reacted quickly to toxic solutions and in a violent manner. All concentrations required oxygenation after the first 24 hours.

Notropis venustus was rated a fair test animal. It required constant feeding, and was excitable in test containers.

Noturus exilis

More than 600 specimens of *Noturus exilis* were collected at night in Tyner Creek, Adair Co., Oklahoma, June 5 and June 15, 1962. Seining during the daylight hours proved ineffective because the fish were located on the bottom among rocks and debris. At night they moved to open water, presumably to eat, and were easy to obtain. Transportation was accomplished without loss. During a holding period of 20 days approximately 25 fish belonging to the June 5 collection died. Death followed a power failure which stopped aeration pumps for 24 hours. When aeration was reestablished, surviving specimens were in poor condition; but recovered, within a few hours. The fish ate *Daphnia* sparingly and occasionally partook dry meal. Holding tanks were covered with wooden planks to provide a darkened area and subsequently reduce excitation.

Fish remained near the bottom of the test containers but would move about vigorously when disturbed. Specimens exhibited little reaction to the test solutions other than initial and brief distress in highly toxic concentrations. Dissolved oxygen concentration in the test solutions was never depleted enough to warrant oxygenation.

Noturus exilis was rated a poor test species. It required constant attention in the laboratory, and did not eat standard laboratory foods.

Salmo trutta

Specimens of *Salmo trutta* were obtained from the Nebraska Game, Forestation, and Parks Commission Hatchery at Benkleman, Nebraska, March 19, 1962. They were transported to the laboratory without casualties. The fish were fed dry meal and *Daphnia* twice daily, but were seldom observed eating. Specimens were used as test animals after a holding period of two days and others at intervals through the fourth day.

Test specimens reacted quickly and nonviolently to toxic solutions. Many dead specimens taken from the test containers showed signs of cannibalism. Oxygenation was required in all test containers and controls.

Salmo trutta was rated a poor test animal. It did not eat adequately in the laboratory and needed feeding during the test period.

Tilapia nilotica

Approximately 300 specimens of *Tilapia nilotica* were obtained from the U. S. Fish and Wildlife Service at Stuttgart, Arkansas, July 30, 1962. Specimens were transported in plastic containers in styrofoam ice-chests. Transportation was accomplished without loss of specimens. Fish immediately ate dry meal and *Daphnia* and after several days came to the surface in anticipation of food. Specimens were used as test animals after holding periods of four, five, eight, and nine days.

Test fish reacted quickly to toxic solutions and exhibited a narrow sensitivity range. Only two concentrations were needed to bracket the median tolerance limit. Oxygenation of the test concentrations was never required.

Tilapia nilotica was rated a good test species. Though it was not easily obtained, it transported well, held well in the laboratory, ate well, and had a narrow sensitivity range.

RESULTS

Analyses of Variance, on the basis of unadjusted TL_m values and information obtained from the "Preadjusted-Abbreviated Doolittle" method, were computed for the 24-hour and 96-hour data (Tables IV and V). The Total, Tests (unadjusted), and Sampling error sums of squares were calculated from unadjusted values. Sums of squares for Species (adjusted for tests) were calculated from adjusted values. Experimental error sums of squares were obtained by subtraction. In each analysis the hypothesis that there was no difference in the resistance of the test species was rejected at the five per cent level.

TABLE IV
STATISTICAL ANALYSIS OF TWENTY-FOUR-HOUR TL_m VALUES

Analysis of Variance				
Source	d.f.	S.S.	M.S.	F
Total	191	41,906.84		
Tests (unadjusted)	33	27,539.55		
Species (adjusted)	17	13,573.28	798.42	52.08
Experimental error	45	690.05	15.33	
Sampling error	96	103.96	1.08	

d.f. = degrees freedom, S.S. = sums of squares, M.S. = mean square, F = variance ratio.

A modification of Duncan's new five per cent multiple range test was applied to both sets of adjusted values to investigate the significances indicated by the Analyses of Variance. The results of the application are given in Table VI.

The vertical lines appearing in Table VI designate populations of values. Any value appearing to the left of a vertical line is a member of that population. All values within a population are assumed to be statistically equal. Values to the left of different lines are assumed to be statistically different. A value directly to the left of two lines is not statistically different from either population.

Table VII contains the results of ranking the 24-hour adjusted values on the basis of six adjustment procedures. Species are ranked by number from 1 (most resistant) to 18 (least resistant) by each procedure. The same number appearing in the same row in two or more columns shows an identical ranking for that species. The "Pre-adjusted-Abbreviated Doolittle" and "Interval" methods are based upon the absolute differences between the guppy and the test species. The basis for the other methods is the relative, or proportional, difference.

COMPARISON OF RELATIVE RESISTANCES

Importance and Use of the Reference Species

A major problem encountered in determining relative resistances of test species to complex industrial wastes is the inability to duplicate experimental treatments. The relation of resistance values for different species run in different tests or the same species in several tests can-

TABLE V

STATISTICAL ANALYSIS OF NINETY-SIX-HOUR TL_m VALUES

Analysis of Variance				
Source	d.f.	S.S.	M.S.	F
Total	179	39,328.11		
Tests (unadjusted)	33	26,858.25		
Species (adjusted)	17	11,580.17	681.18	38.22
Experimental error	39	695.33	17.82	
Sampling error	90	104.36	1.15	

d.f. = degrees freedom, S.S. = sums of squares, M.S. = mean square, F = variance ratio.

TABLE VI

RESULTS OBTAINED BY APPLYING DUNCAN'S NEW FIVE PER CENT MULTIPLE RANGE TEST TO ADJUSTED RESISTANCE VALUES

Species	24-Hour Values	24-Hour Populations	Species	96-Hour Values	96-Hour Populations
L. reticulatus	100.00	|	*L. reticulatus*	100.00	|
N. cornutus	96.75	|	*N. cornutus*	95.20	|
T. nilotica	94.58	|	*T. nilotica*	94.64	|
C. carpio	93.97	|	*C. carpio*	94.16	|
F. olivaceus	92.96	|	*F. olivaceus*	93.01	|
N. spilopterus	90.89	|	*N. spilopterus*	90.89	|
S. trutta	90.19	|	*N. rubellus*	90.20	|
N. dorsalis	90.11	|	*D. nubila*	90.17	|
L. auritus	89.72	|	*N. dorsalis*	90.11	|
D. nubila	89.59	|	*N. camurus*	90.08	|
N. camurus	89.24	|	*L. auritus*	89.66	|
H. amblops	88.17	|	*H. amblops*	89.66	|
N. venustus	87.90	|	*S. trutta*	87.90	|
N. rubellus	86.58	|	*D. cepedianum*	87.53	|
D. cepedianum	84.06	|	*N. venustus*	86.91	|
I. nebulosus	82.56	|	*I. nebulosus*	84.08	|
N. exilis	68.75	|	*N. exilis*	68.94	|
C. carolinae	63.80	|	*C. carolinae*	64.36	|

TABLE VII

A COMPARISON OF SIX METHODS USED TO RANK THE RESISTANCES OF THE TEST SPECIES[1]

Species	Rank	Preadjusted-Abbreviated Doolittle	Rank	Interval	Rank	Proportion Individual, TF/G	Rank	Proportion Replicate, TF/G'	Rank	Proportion Individual, G/TF	Rank	Proportion Average, G/TF'
L. reticulatus	1	100.00	1	100.00	1	1.000	1	1.000	1	1.00	1	1.00
N. cornutus	2	96.75	2	97.00	2	0.760	4	0.734	2	1.32	3	1.32
T. nilotica	3	94.58	3	93.90	4	0.746	3	0.742	4	1.38	4	1.34
C. carpio	4	93.97	4	93.50	3	0.753	2	0.753	3	1.34	2	1.29
F. olivaceus	5	92.96	5	91.90	6	0.655	6	0.674	6	1.58	6	1.51
N. spilopterus	6	92.96	6	91.10	10	0.436	10	0.425	14	3.75	11	2.30
S. trutta	7	90.19	13	87.10	16	0.283	16	0.273	18	4.89	16	3.61
N. dorsalis	8	90.11	9	90.30	12	0.392	12	0.373	12	3.41	12	2.58
L. auritus	9	89.72	10	90.00	7	0.649	7	0.627	5	1.56	7	1.60
D. nubila	10	89.59	7	91.10	14	0.316	15	0.338	13	3.66	15	2.97
N. camurus	11	89.24	8	90.60	11	0.420	11	0.418	10	2.65	10	2.26
H. amblops	12	88.17	11	87.50	9	0.551	9	0.572	9	1.82	9	1.82
N. venustus	13	87.90	12	87.50	8	0.588	8	0.597	7	1.72	8	1.69
N. rubellus	14	86.58	14	87.00	15	0.311	14	0.352	15	3.93	13	2.70
D. cepedianum	15	84.06	15	83.40	18	0.216	18	0.209	17	4.87	18	4.35
I. nebulosus	16	82.56	16	82.50	5	0.694	5	0.675	8	1.78	5	1.48
N. exilis	17	68.75	17	68.40	13	0.374	13	0.363	11	2.77	14	2.77
C. carolinae	18	63.80	18	63.10	17	0.248	17	0.262	16	4.45	17	3.95

[1] Data based on 24-hour values.

not be determined easily without some method of cross-referencing. Comparisons may be more easily accomplished by providing a point of reference to which the resistance values may be compared. The simplest procedure appears to be the use of a reference species in every test. The reference species provides a resistance value to which the resistances of the tested species may be compared and adjusted.

Qualities possessed by a reference species of necessity, must be more stringent than those of most test species. The reference organism should possess all the qualities of a good test species. Probably the most stringent quality should be genetic homogeneity. Mather (1946) states that a general method of achieving genetic uniformity is afforded by inbreeding, though a reduction in vigor may occur in specimens of an inbred population. Loss of vigor varies from species to species (Mather, 1946) and should be considered in the selection of a reference organism. A species easily reared in the laboratory with no loss of vigor under constant environmental conditions should satisfy genetic requirements. The laboratory reared fish can be graded for size and maturity, and thus the variability resulting from age, size, and sex can be minimized. Variability in response to a toxic material may be further minimized by testing an adequate sample. Deichmann and LeBlanc (1943) have indicated that as few as six animals gave LD_{50} values compatible with those obtained using 60 to 90 animals. Proper sample size is important in resistance studies employing tolerance limits calculated by the use of straight-line graphical interpolation, because confidence limits cannot be determined without a fitted line.

A major difficulty encountered in all types of bioassay is the incompatibility of estimates between laboratories. Even under supposedly controlled conditions relative sensitivities vary significantly (Allmark, 1946; Miller, 1954). Allmark (1946) has concluded that with the use of a standard reference a more reliable index to toxicity may be obtained, even though the error contributed by the investigator may be large. Excessively high variability in test results is automatically blamed on the test animal, "but never on *Homo sapiens* who performed the experiment" (Dews and Berkson, 1954).

COMPARISON AND EVALUATION OF RANKING METHODS

Relative resistances were adjusted and ranked by the use of six procedures. Resistance values ranked by the four "Proportional" methods varied appreciably when compared to the "Preadjusted-Abbreviated Doolittle" and "Interval" rankings, though they were generally agreeable. Variability in "Proportion" rankings may be understood by examining resistance values themselves. The magnitude of a "Proportional" resistance was directly affected by the magnitude of the original tolerance limits from which it was derived. For example, consider the reference species in two tests to have tolerance limits of 4.0 and 24.0, and the test species in the same tests to have limits of 2.0 and 22.0. The "Preadjusted-Abbreviated Doolittle" and "Interval" resistance values would both be essentially 2.0 for each test (see below), while the values for the TF/G "Proportion" would be 0.5 and 0.9, respectively. The G/TF "Proportion" would exhibit the same "number effect" with adjusted resistance values of 2.00 and 1.09.

The use of "Proportional" methods to obtain adjusted resistance values is questioned. They should prove useful in helping to analyze relative toxicity changes of an effluent, since changes in the magnitude of resistance values reflect changes in effluent toxicity. An increase in the size of resistance values would be accompanied by an increase in proportion even though the relative resistance remained approximately the same.

Resistance values ranked by use of the "Interval" method were in close agreement with those obtained by use of the "Preadjusted-Abbreviated Doolittle" method. Three major differences in rank occurred, but only one of them was of sufficient magnitude to be considered an important deviation. *Salmo trutta* was ranked seventh by the "Preadjusted-Abbreviated Doolittle" and thirteenth by the "Interval" method. The difference between the two ranked values was 3.01. A close examination of both methods failed to reveal the source of deviation. Dif-

ferences between resistance values of the two methods, except for *S. trutta*, were less than 1.7 per species.

The "Interval" method may be considered a less rigorous statistical treatment than the "Preadjusted-Abbreviated Doolittle" method, but is easier to compute, new resistance values may be added without complete recomputation, and results are compatible enough with the "Preadjusted-Abbreviated Doolittle" to warrant its preferential use in obtaining adjusted resistances.

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THE CORPS OF ENGINEER ACTIVITIES ON POLLUTION AND WATER QUALITY CONTROL[1]

FRED J. DICKSON[2]

Mr. Chairman and Members of the Society:

The majority of you know that I was a State employee for a number of years. It is felt that I understand your various problems. Since the first of this year I have been employed as a biologist by the South Atlantic Division, Corps of Engineers. Since then I have had the opportunity to observe the activities and efforts of the Corps in providing the fullest utilization and benefits of its projects to the public. I now have a much better perspective of the development, management and conservation of the water resources—with particular reference to fish and wildlife phases. I feel that many of you may have some misunderstandings, as I did, about the Corps' activities, therefore, with your indulgence I would like to tell you as simply and plainly as I can, just what I have found to be the views and objectives of the Corps in this important work and how we can all move together to do a better job in preserving and enhancing our heritage.

Let me sincerely say the Corps of Engineers has a strong desire to cooperate with local, State, and Federal organizations. Many consultations are held each year with the responsible fish and wildlife agencies.

We have all been interested in the effects pollution and water quality have on our fish and wildlife, and until the most recent legislation, very little could be done by those most concerned.

It is realized that cooperation in water quality control is largely dependent on the free exchange of information among the engineers, scientists, and administrators from various levels and agencies of government, from industry, and from universities to explore research needs in the field of streamflow regulation for quality control. All planning must be carried out with multiple purpose needs and possibilities in mind.

Agencies which have authority to review, accept or reject a plan of development must rely on something more tangible and objective than human judgment as a means of fitting these demands into a plan of development and as a means of evaluating it.

This is the reason why we are required to show economic justification, generally as a benefit—cost ratio based on dollar evaluations of the costs of proposals versus the benefits they will produce over the life of the project.

[1] Presented at the Southern Division, American Fisheries Society, 29 September to 2 October, 1963, Arlington Hotel, Hot Springs, Arkansas.

[2] Biologist, Master Planning Branch, Engineering Division, U. S. Army Engineer Division, South Atlantic, Corps of Engineers.