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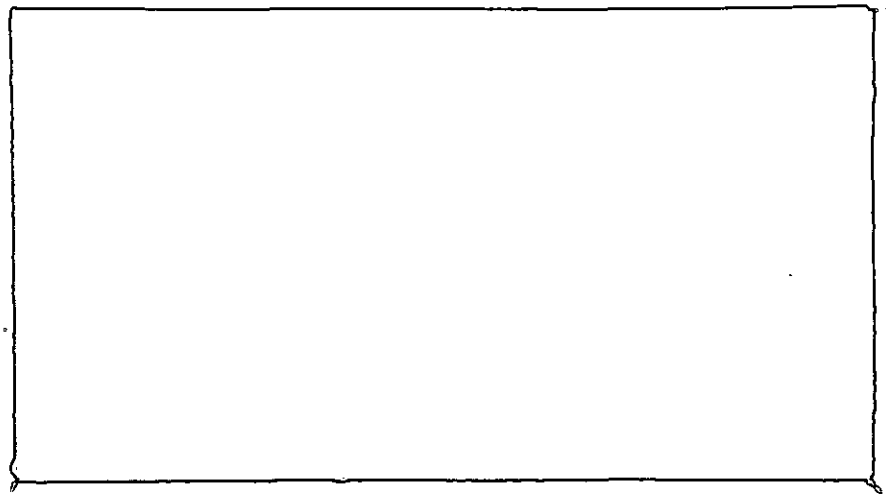
Environmental Engineering



Department of Civil Engineering
University of Massachusetts at Amherst

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Studies on the Acute Toxicity of Two-Cycle Outboard Motor Exhausts to Selected Benthic Invertebrates

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by

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Progress Report for the Division of Water Pollution Control

Massachusetts Water Resources Commission

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PREFACE

This progress report is the fourth in a series of detailed progress reports prepared for the Division of Water Pollution Control, Massachusetts Water Resources Commission, Contract Number 15-51451, "Effect of outboard motor exhausts on water quality and associated biota of small lakes."

This report presents information on the acute toxicity effects of exhausts from a 7.5 horsepower outboard engine on three species of freshwater benthic invertebrates; adult scuds, dragonfly nymphs, and damselfly nymphs. The authors are, respectively, assistant professor, and graduate research assistant, Department of Civil Engineering and Professor, Department of Zoology, University of Massachusetts at Amherst.

This report will be brought to the attention of various agencies, organizations, companies, industries, and individuals interested in the preservation of our natural resources.

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ABSTRACT

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The acute toxic effects of OMSE-water were evaluated on three species of benthic invertebrates and the variation in toxicity of OMSE-water with varying engine speeds to damselfly nymphs (Argia violacea) was also investigated.

The least sensitive invertebrate species to OMSE-water was found to be sprin collected dragonfly nymphs (Tetragoneuria cynosura) whose average 24, 48, and 96-hour TL_{50} values based on static bioassays were 0.186, 0.164, and 0.157 (540/1, 610/1, and 635/1) percent by volume of fuel consumed, respectively. The most sensitive test species were spring collected adult scuds (Crangonyx gracilis) whose average 24, 48, and 96-hour TL_{50} values based on static bioassays were 0.0300, 0.02945, and 0.02945 (3340/1, 3400/1, and 3400/1) percent by volume of fuel consumed, respectively.

OMSE-water generated at trolling speeds were found to be more toxic than OMSE-water generated at full throttle to summer collected dragonfly nymphs. The dramatic physical response exhibited by adult scuds when exposed to OMSE-water suggested the possible use of this species as a rapid response indicator of pollution levels of compounds similar to those found in OMSE-water.

INTRODUCTION

INTRODUCTION

The quantity of inland and estuarine waters capable of supporting the increasing sport of recreational boating is, in many parts of the country, quite limited. With this in mind, it can be seen how many of the heavily used recreational lakes and ponds could become subject to pollution from boaters. The problems of disposal of trash, disposal of sanitary wastes, increased coliform count from swimmers, maintenance of water safety, spillage of boating fuels (gasoline, lubricating oil, and associated products), development of nauseous fumes from subsurface exhausts of outboard motors, and effects upon aquatic biota from products derived from outboard motors all become a reality which must be dealt with if the preservation of the aquatic environment and surrounding watershed area are to be maintained at an optimal level of water quality. Problems such as these have already been reported to the Water Resources Commission of the Commonwealth of Massachusetts. Complaints have been received from home owners along the shores of Lake Arcadia and the Congamond Lakes (1,2)* both in Western Massachusetts. Among the items seemingly attributed to increased outboard motor operations on these lakes are an increase in dead fish, an increase of algae, shoreline erosion, disruption of small craft (such as small sailboats and rubber rafts) by high powered boats, presence of "mini oil slicks", and the retention of a fairly strong hydrocarbon odor. The accuracy of these claims may be subject to question and field investigation may have to be undertaken to verify their authenticity.

These problems have not been entirely overlooked by the scientific community. A number of papers have been presented on various aspects of the outboard motor pollution problem and other related investigations are currently in progress.

*Numbers in parentheses refer to equivalent referenced article.

The toxicity of compounds in water to aquatic biota has classicially been evaluated with the aid of acute and chronic biological assays (bioassays). Several species of fish have been used in acute toxicity bioassays to measure the effects of OMSE-products in the aquatic environment. Only one attempt has been made to investigate the effects of OMSE-water on other aquatic biota with the observations and results being inconclusive.

Fish possess the motivity to egress from areas that contain compounds that may interfere with their physiological activities. Therefore, when relating laboratory studies to natural conditions, fish may not remain in an area subject to the exhausting of outboard motor combustion products for a long enough period of time necessary for the development of adverse physiological effects. For this very reason, the use of fish mortality in a given area of water, under natural conditions,may serve only as a limited use indicator of OMSE-pollution. The aquatic inhabitants of the benthic community, for the most part, do not possess the ability to move large distances when faced with a stress condition such as those placed upon them by the compounds associated with OMSE-water. These stresses may be exerted by the compounds present in OMSE-water and/or the compounds that adsorb or settle onto the benthic deposits. Therefore, observation on membranes on the benthic community would serve to indicate long term conditions in a specified water area much better than observation of fish behavior or toxicity.

Benthic dwellers are also an important link in the aquatic food chain (3). Patrick (4) related that, "many invertebrates, such as worms, snails, and insects, feed directly on bacteria, fungi, and algae. They in turn are a source of food for the carnivorous species; thus, a closely integrated food chain is formed. Realizing the importance of the bio-dynamic cycle, the effect of a given waste is determined by using organisms representing three stages in the cycle. These organisms are as follows:

1. An alga that is important as a producer of oxygen, and as an organism that can convert inorganic substances into a direct source of food for many aquatic animals.

2. An invertebrate that serves as a direct food for fish. As representatives of this group, insects and snails have been used.

3. Fish, because of their recreational and economic importance."

Other investigators (5,6,7) also recommend the use of invertebrates in bioassays on toxic materials and relate the importance of these organisms in the natural aquatic food chain. A search of the literature has revealed that there is virtually little published information on the effects of outboard motors on aquatic invertebrates. For these reasons, it was decided to investigate the effects of OMSE-watr on fresh water invertebrates.

In general, lakes in Massachusetts are relatively small and shallow and therefore, have a limited quantity of dilution water. Possible environmental problems may arise from extensive outboard motor usage on lakes where dilution water is a limiting factor (ie. a high ratio of volume of fuel consumed to volume of dilution water). For this reason, invertebrates collected from a small lake were utilized to evaluate the toxicity of OMSE-water.

This particular experimentation consisted of the laboratory investigation of various factors involved with the outboard motor pollution problem. The foremost objectives were to establish the effect of these outboard motor derived compounds on specified aquatic biota. The specific scientific objectives of this investigation are:

1. The evaluation of the toxic effects of these outboard motor derived compounds on three species of benthic invertebrates: adult of scuds - Crangonyx gracilis, nymphs of dragonflies - Tetragoneuria cynosura, and nymphs of damselfiles - Argia violacea.

2. The evaluation of the outboard motor variable of speed of operation on the toxicity of OMSE-water to the nymphs of damselflies - Argia violacea.

LITERATURE REVIEW

LITERATURE REVIEW

Benthic invertebrates and other aquatic biota. Several investigators (8,9,10) have reported findings on the interaction of outboard motors and aquatic biota other than fish. The effects of other pollutants, similar to the components in OME-water, on invertebrates have been cited in the literature and a brief summary of only the pertinent papers will be discussed.

Lagler, et al (10) evaluated the effects of outboard motor usage on the production of free-swimming microorganisms (plankton) and benthic invertebrates. Plankton samples were collected from a bass control pond and a bass motor pond and then compared on a weight basis after concentration by centrifugation. The authors concluded that "although the plankton samples were few and had limitations because of the method of collection, it is evident that outboard motor use did not prohibit plankton development, and probably did not inhibit it in any way."

These same researchers (10) found that the numbers and volumes of bottom organisms present in an outboard's path in shallow water were substantially reduced by prolonged operation of the outboard motor. They noted that the organisms which presumably populated the bottom area corresponding to the path of travel of the outboard motor boat at the start of the experiments were not necessarily destroyed by the engine and may have been washed off to the sides or tossed up into the water and consumed by the bass present in the test pond. The bottom fauna populations in the control and motor use ponds are presented in Table 1. Differences in dominant species and species numbers between the control and motor ponds are possibly evident from the data but a statistical evaluation is not available. Differences in the volume of total organisms per square foot of sampling area between the control pond, motor pond, and motor path (2.48, 0.81, and 0.50 respectively) may indicate that the outboard motor had an effect on bottom fauna in the motor pond and motor path.

Table 1. Comparison of Bottom Fauna in Control and Motor Use Ponds - July, 1949 (10)

Item of comparison	Control Pond 19		Motor Pond 22 (exclusive of motor path)		Motor path in Pond 22	
Number of samples	20		20		6	
Area per sample, sq.ft.	.84		.84		.84	
Total area sampled, sq.ft.	16.80		16.80		5.04	
Organisms	Number	Vol. cc.	Number	Vol. cc.	Number	Vol. cc.
Oligochaeta	212	1.75	700	3.20	32	0.20
Hirudinea	83	9.90	2	0.10	--	--
Gastropoda	416	5.65	80	0.90	3	0.05
Pelecypoda	25	1.55	1	0.50	--	--
Amphipoda	478	1.30	3	Trace	--	--
Hydracarina	94	0.25	58	0.20	7	0.05
Ephemeroptera	703	2.50	158	2.60	9	0.20
Anisoptera	42	1.30	29	0.25	13	0.10
Zygoptera	8	1.50	4	0.90	1	0.05
Neuroptera	3	0.10	5	0.05	--	--
Trichoptera	111	0.90	58	0.60	9	0.10
Coleoptera	2	0.50	2	0.05	--	--
Corethra	146	0.35	250	0.80	14	0.05
Chironomidae	3,155	13.35	863	2.40	174	1.40
Other Diptera	69	1.15	4	1.50	37	0.30
TOTALS	5,547	41.60	2,260	13.60	309	2.55
Number per sq.ft.	330		135		61	
Volume per sq.ft.		2.48		.81		.50

In another aspect of their studies, Environmental Engineering, Incorporated researchers (8) secured grab samples of free floating phytoplankton and benthic invertebrates from a control lake and a motor lake to evaluate the effects of OME-water on these aquatic species. These investigators (8) made two statements about free floating phytoplankton and benthic communities which were subsequently apparently disregarded when conclusions on the effects of OME-water on the organisms were drawn:

1. "The free floating phytoplankton are not a good indicator of hydrocarbon pollution. The fauna found in the sediments are more indicative of the overall average conditions than the free floating algae."

2. "Unfortunately, the biological populations of sediments is a constantly changing one. It has been shown that populations vary with season (due to emergence of adult insects), depth, type of bottom, and other factors. Samples, therefore, should be obtained from a variety of locations during all the seasons of the year."

Based on one day's data from four locations on Lake X (the motor lake) and two locations on Cat Lake (control lake), these workers concluded that "phytoplankton and bottom organisms, which are necessary in a healthy ecological chain, were not affected by exhaust water hydrocarbons". Of these six grab samples, the one sample obtained from the area of heaviest outboard motor usage (old boat channel) was excluded from the conclusions due to a total absence of any bottom organisms (25 percent of Lake X data). This was presumed to be caused by the presence of hydrocarbons, which were apparently visible as slicks, upon the bottom sediments. In addition, the low numbers of benthic organisms found in the other five grab sample locations were noted as a possible suppression of the benthic community. This was attributed to the possibility of the recent emergence of adult insects (no mention made of the possibility that OME-water caused the suppression) and a seasonal sampling program was suggested. The benthic invertebrate data for the study is presented in Table 2.

Table 2. Benthic Organisms in Lake X and Cat Lake, Florida - April 21, 1969 (8).

Organism	Number of Organisms per Sample					
	Station					
	1	2	3	4	5	6
Annelids	4	4	1	0	1	3
Amphipods	8	0	0	0	0	0
Dipterans	1	1	5	0	0	2
Ephemeropterans	0	5	2	0	3	1
Odonata	0	0	1	0	0	0

Note: Stations 1-4 are located on Lake X (motor lake).

Stations 5 and 6 are located on Cat Lake (control lake).

Schenck, et al (9) introduced snails and daphnids into bioassay tanks containing a concentration of 1/2,560,000 of OME-water derived from a non-leaded gasoline and a concentration of 1/10,000 of OME-water with leaded gasoline as the fuel. No data for these species is presented in the article; however, the authors noted that the daphnids and snails appeared to live and reproduce equally well in the bioassay tanks, by visual inspection.

MATERIALS AND METHODS

MATERIALS AND METHODS

Acute toxicity studies employed static bioassays to measure the effects of OMSE-water on specific benthic invertebrates. All phases of this study utilized OMSE-water which was generated by a 7 1/2 horsepower outboard motor operating under specific conditions of engine revolutions per minute (rpm) burning a known volume of fuel, and exhausting the combustion products and crankcase drainage into a known volume of recipient dilution water.

OMSE-Water Preparation

Outboard motor. A 1970 model, 7 1/2 horsepower, Merc 75[1] outboard motor having an engine bore of 2 inches, a 1 3/4 inch stroke, and a total piston displacement of 11.0 cubic inches was used to generate the OMSE-water. This horsepower outboard motor was selected for two reasons: it is in the horsepower range os most in demand in Massachusetts (11) and is also within the horsepower range of outboard motors allowed on the Quabbin Reservoir (12). The speed of the engine operating within the test water was monitored with a tachometer[2] which was capable of measuring the outboard engine revolutions per minute (rpm) in two ranges; from 0 to 5,000 rpm, and 0 to 10,000 rpm. When not in use, the test engine was removed from the test tank and stored on an engine rack.

Test fuel. The test fuel was a commercial leaded gasoline[3], plus commercial outboard motor lubricating oil[4] recommended by the manufacturer.

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[1]Manufactured by Kiekhaefer Mercury, fond du Lac, Wisconsin.

[2]Model 67-100T-Tachometer manufactured by MERC-O-TRONIC Ins-ruments Corporation, Almont, Michigan.

[3]Gulf regular gasoline.

[4]Quicksilver-Formula 50 outboard motor oil manufactured by Kiekhaefer Mercury, Fond du Lac, Wisconsin.

Unleaded gasolines were not used in this study because of their reported harmful effects to outboard engines (13,14). A fuel to oil ratio of fifty to one was employed throughout the experiment as recommended by the outboard motor manufacturer (15). The test fuel mixture was blended in graduated cylinders to insure proper volume measurement as follows: a total of one gallon of fuel was prepared for each run by mixing 74 ml of lubricating oil with 3711 ml of gasoline to yield a ratio of 50/1 and a total volume of 3785 ml (one U.S. gallon). The prepared fuel mixture was then stored in a three gallon storage tank from which it was siphoned to the outboard engine for combustion. At no time did this storage period exceed two hours.

For acute toxicity evaluations, bioassay dilution water (and aquatic test species) was collected from Cranberry Pond, Sunderland, Massachusetts. This pond has no previous history of outboard motor usage and therefore, the biota present had never been subjected to the compounds associated with OMSE-water. This water was also used because it insured against excessive loss of aquatic test species during the acclimation period, since it was water native to their existence prior to capture for laboratory experimentation.

The composition of test water (Amherst tap water and Cranberry Pond water) prior to outboard motor operation must be known before any conclusion can be drawn on the contribution of various compounds by outboard motors. To assure that the water to be exhausted did not interfere in any manner with the toxicity exerted by OMSE exhausts and also with the measurement of hydrocarbon compounds it was characterized intermittently for the duration of the laboratory testing for the following quality parameters:

1. Turbidity
2. Total solids
3. Dissolved solids
4. Suspended solids
5. Color
6. pH
7. Alkalinity, phenolphthalein
8. Alkalinity, total
9. Hardness, total
10. Hardness, non-carbonate
11. Temperature
12. Copper
13. Organic material
14. Chlorine

These analyses were done using wet chemical techniques as described in Standard Methods (16). Copper was measured using an atomic absorption spectrophotometer[1] with the following instrumental parameters:

Light Source -- Cooper-Hollow Cathode Lamp
Wavelength -- 3247A°
Current -- 15 milliamperes
Oxidant -- Air at 30 psig
Fuel -- Acetylene
Sensitivity -- 0.10 µg/ml Copper for one percent absorption

Copper concentrations were monitored to insure that copper did not contribute to the death of test species used in the bioassays. The quantity of organic material present in the test waters was measured as total carbon with a total organic analyser[2].

Outboard motor testing area. The OMSE-water generating area consisted of two stainless steel test tanks, a raw water supply, an exhaust system for removal of gaseous products not retained within the test water, an engine stand, tank drainage system, gasoline storage area, organic extraction units, storage closet, etc.

[1]Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer manufactured by Perkin-Elmer Corporation, Norwalk, Connecticut.

[2]Beckman Model 915 Total Organic Carbon Analyser manufactured by Beckman Instruments, Incorporated, Fullterton, California.

Two test tanks, each 4 ft deep x 4 ft wide x 6 ft long, were fabricated from 26 gage-type 304 stainless steel sheets, reinforced with 3/4 in. plywood and braced by 2 in x 4 in wood supports. To prevent loss of test water due to splashing and allow for the operatio n of the test motors at high engine speeds (full throttle), 1 ft long stainless steel deflector shields were pop-riveted to the body of the stainless steel tank. The seams of the test tanks were double rolled and soldered with a 60/40 solder (60 percent lead and 40 percent tin) to assure water tightness. To prevent the test water from coming in contact with the solder and possibly altering the test water characteristics, the inside tank seams were caulked with General Electric Silicone Seal. Each tank was equipped with a set of two semi-steel rigid casters and four semi-steel swivel casters with plain bearings so that each tank could be readily moved. Motor mounts, which enabled the outboard motors to be raised and lowered into the test tanks, were permanently bolted to the wooden test tank structure.

The gases formed upon the combustion of the fuel mixture were vented below the water's surface where they were either absorbed or escaped into the atmosphere above the test tank. For safety reasons, an exhaust system was constructed to vent these gases outside the building which housed the test tanks. During operation, the test tank was covered with a plastic drop cloth sheet to form an exhaust hood and protruding below the plane of the plastic was a 10 in smoke pipe intake for an exhaust fan. Each test tank had an individual intake pipe which could be regulated with dampers so that they could be exhausted separately or together depending upon the testing schedule. Thedischarge piping was 8 in smoke pipe which discharged outside the testing building.

The Amherst tap water was directed into the test tanks through a rubber hose. Each test tank had a drain system made of 2 in polyvinyl chloride (PVC) pipe and was regulated by 2 in globe valves. The tank drainage network discharged into a floor drain. An observation platform was also constructed between the two tanks for ease in raising and lowering the test outboard motor.

Generation of OMSE-water. OMSE-water was generated by combusting one gallon of fuel mixture in a 7 1/2 horsepower outboard engine operating at 1700 ± 100 rpm and allowing the exhaust products to discharge into 400 gallons of Amherst tap water. This resulted in an OMSE-water which was termed a 'stock solution' and represented a 400/1 test mixture (400 gallons of recipient dilution water to one gallon of exhausted fuel). This stock solution served as a source of OMSE-water for both the hydrocarbon identification and bioassay portions of the investigation. for one series of bioassays a stock solution was prepared in the same manner as described above, except the outboard engine was operated at 3800 ± 100 rpm.

Amherst tap water was directed into a stainless steel tank to a depth corresponding to a volume of 400 gallons of water. This water was held in the test tank for varying periods of time to allow for temperature adjustment between the laboratory temperature and water temperature and for dissipation of any chlorine residual. the tap water was allowed to adjust near ambient so that a relatively uniform receiving water temperature could be maintained during the entire experimentation period. It was felt that this would prevent any shock due to water temperature differences to the test species and allow for a uniform gas absorption coefficient for all experiments. Only once during this entire testing period were any traces of chlorine found in the Amherst tap water and these traces were removed by allowing the tap water to stand for two days (no measurable total chlorine residual).

After this brief holding period, the outboard motor was started and allowed to run until it utilized one gallon of fuel. During this OMSE-water generation period, the exhaust gases were removed from the atmosphere just above the water's surface by an exhaust fan. The engine speed was checked at the beginning of the experiment and adjusted to 1700 $\pm$ 100 rpm for the duration of motor operation. Approximately three hours of motor operation at this engine speed were required to consume one gallon of fuel. Upon consumption of this quantity of fuel a portion of the OMSE-water was then removed for the experiments of choice.

Facilities. All test species acclimation and acute toxicity bioassays were conducted in a temperature controlled adequately equipped bioassay room maintained at $20^{o} \pm 2^{o}C$. A thermostatically controlled air conditioner[1] adequately maintained this temperature during the experimentation period.

Test species. To ascertain the effects of OMSE-water on invertebrates it was necessary to establish a reliable supply of test speciments that had not been exposed to any outboard motor emissions and, therefore, may have developed a kind of inbred immunity to OMSE-water. Specimens were collected from Cranberry Pond, located in Sunderland, Massachusetts. This pond was chosen because of its previous history of no outboard motor usage and also for its proximity to the Amherst campus of the University of Massachusetts.

The pond was first surveyed to determine the abundance of various invertebrates. Among the various orders collected and observed were Annelida, Amphipoda, Decapoda, Odonata, Hemiptera, Coleoptera, Diptera, Gastrapoda, and Pelecypoda. The most abundant Orders were those of Amphipoda and Odonata and because of the ease of collection and their ability to adapt to laboratory conditions, these

[1] 18,000 BTU/Hr Westinghouse Air Conditioner.

were chosen as the test orders. A listing of the species chosen as test specimens is presented in Table 3.

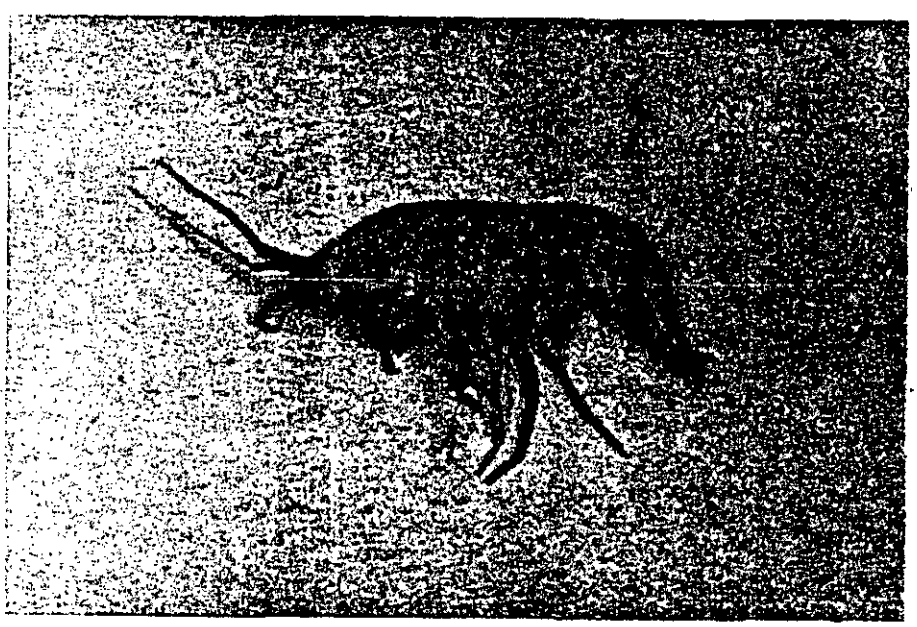
Table 3. Invertebrates Utilized in Acute Toxicity Bioassays

Common Name	Class	Order	Suborder	Genus & Species
Scuds (adult)	Crustacea	Amphiphoda	Haustoriidae	Crangonyx gracilis
Dragonfly(nymph)	Insecta	Odonata	Anisoptera	Tetragoneuria cynosura
Damselfly(nymph)	Insecta	Odonata	Zygoptera	Argia violacea

Photographs of the adult scud (Crangonyx gracilis), dragonfly nymph (Tetragoneuria cynosura), and damselfly nymph (Argia violacea) are presented in Figures 1, 2, and 3, respectively.

All test specimens were collected with a dip net and sorted in the field. Collection was restricted to the shoreline area of Cranberry Pond at depths of between 1 to 4 feet. Identification of the various test invertebrates was accomplished by stereomicroscopic observation at 20X magnification, coupled with taxonomic references (17,18) and communications with Entomologist W. J. Morse (19) and W. J. Nutting, Professor, Zoology Department, University of Massachusetts.

It is only the series of nymphal stages of Odonata which are aquatic. The adults are non-aquatic and for this reason only the nymphs of damselflies and dragonflies were used in the acute toxicity bioassays. The entire life cycle of sucs is completed in the aquatic environment and adult scuds were chosen as test species. To avoid problems of possible varying sensitivity to OMSE-derived compounds among the members of one test species due to differences in size, all test individuals in a particular species were approximately the same length throughout the testing



Lateral View (3.5 X)

Figure 1. Photograph of Adult Scud (Cragonyx gracilis)

Lateral-Ventral View (3.5 X)

Dorsal View (3.0 X)

Figure 2. Photograph of Dragonfly Nymph (Tetragoneuria cynosura)

Dorsal-Lateral View (2.5 X)

Figure 3. Photograph of Damselfly Nymph (Argia violacea)

period. The lengths of the various species tested were as follows: adult scud (Crangonyx gracilis), 1/2 to 5/8 inches; dragonfly nymph (Tetragoneuria cynosura), 3/4 to 7/8 inches; and damselfly nymph (Argia violacea), 1/2 to 5/8 inches.

Dilution water. In addition to the various test species of invertebrates, additional lake water from Cranberry Pond was collected which would serve as the dilution water for the bioassays. This was transported back to the laboratory in twenty gallon plastic barrels and held for future use. During the holding period, the lake water was aerated to hold any particulate matter in suspension and to aerate the dilution water. It is recognized that aeration of the dilution water may have improved its quality but it was necessitated to maintain a D.O. level suitable for aquatic life and to prevent the water from possibly becoming anaerobic.

Cranberry Pond water was not used for the generation of OMSE-water because of the large quantity required (400 gallons). Amherst tap water was expected to be of similar quality as that of Cranberry Pond water because of the similarity in geological location and availability as surface waters. For this reason and because of its ease of acquisition, Amherst tap water was chosen as a suitable substitute for Cranberry Pond water in the preparation of OMSE-water. As a check to verify the suitability of Amherst tap water as a substitute for Cranberry Pond water, the quality parameters listed earlier were used to compare both waters.

Procedural flow diagram. The actual measurement of toxic effects of OMSE-water on aquatic invertebrates followed several steps: generation of OMSE-water (described earlier), acclimation of test species,preparation of test solution, acute toxicity bioassay testing period, and analysis of results. A flow diagram of the stepwise procedure employed in the acute toxicity bioassays is presented in Figure 4.

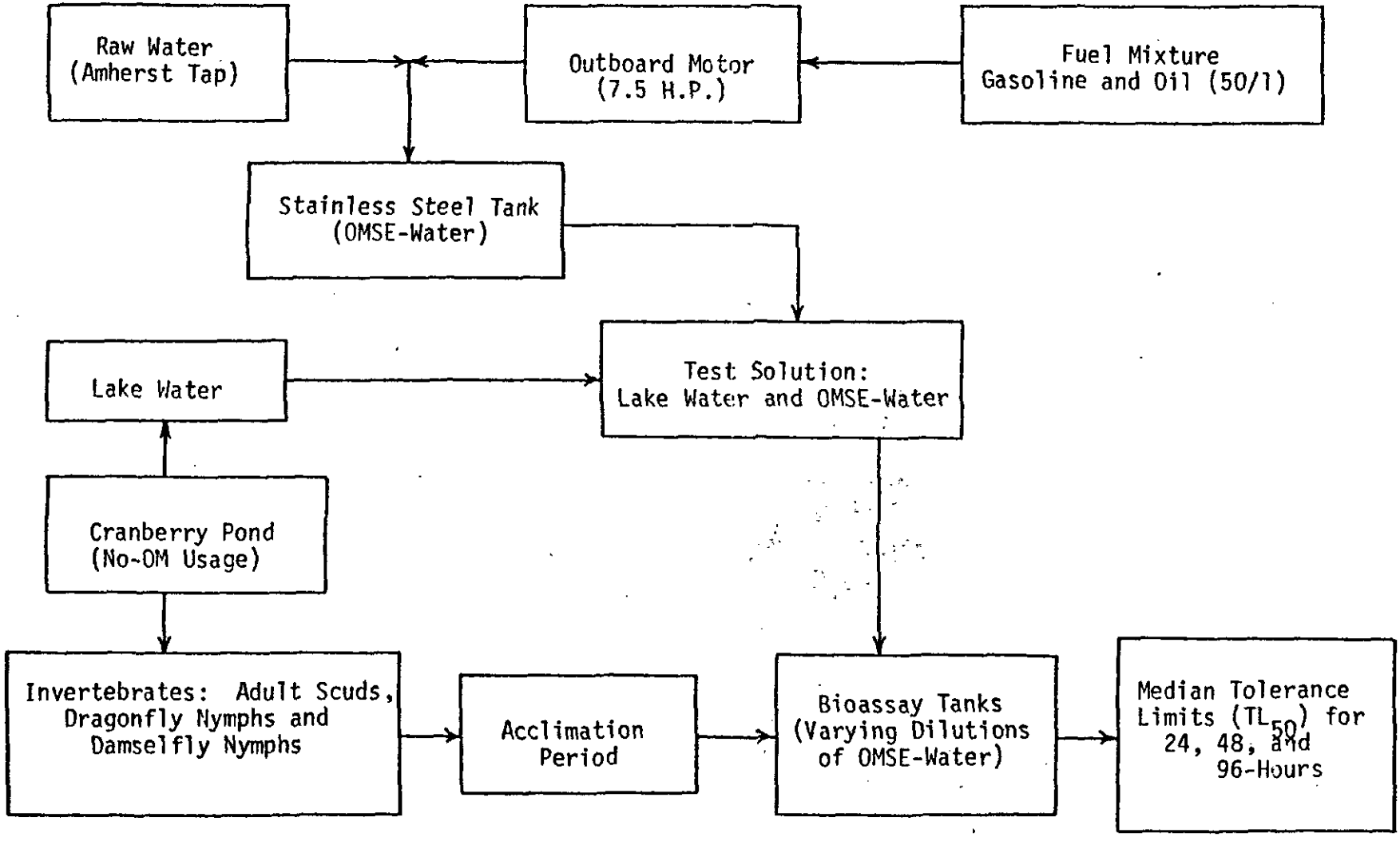


Figure 4. Flow Diagram of Benthic Invertebrate Acute Toxicity Bioassays.

Preparation of test aquaria. During the preliminary experimentation, it was observed that cannibalism occurred when the two different species of Odonata were maintained in the acclimation tank. In a time span of a week a significant reduction in damselfly populations was visually noted, while the dragonfly population remained fairly uniform. It was also observed that death due to fungal infestation began to occur after several weeks of acclimation. This occurred for both test species of Odonata, even when they were segregated to reduce the number of incidences of cannibalism.

To avoid the problems associated with long term acclimation, cannabilism, and infection, the test species were first field sorted and then held in five gallon plastic buckets containing Cranberry Pond water in the laboratory for a period of three to five days for observation of stress as recommended by W. J. Nutting, Professor, Zoology Department, University of Massachusetts. During this period and the four day acute toxicity bioassay period no feeding occurred. This was done to aid in avoiding possible growths of microorganisms and fungi which might cause disease or death among the test species.

After the observation period, the test species were placed in a series of one gallon test aquaria, each of which contained one liter of aerated lake water. Aeration of the dilution water and test species was accomplished with a commercial aquarium pump, glass tubing, and regulator valves at the rate of 30-180 bubbles per minute through a 3/32 inch orifice as recommended by Standard Methods (51). The species were then observed for an additional day to assure that no stress (in the form of noticeable injury) had occurred during transfer from the five gallon stock vessels to the one gallon test jars.

Immediately upon generation of the stock solution (one gallon of fuel exhausted into 400 gallons of Amherst tap water), approximately ten gallons of OMSE-water were transferred in glass containers to the temperature controlled bioassay laboratory. The test specimens were then carefully removed from the test chamber and then OMSE-water was added to the Cranberry Pond dilution water in varying quantities, which were measured in a graduated glass cylinder, to achieve the desired test concentrations. The contents were then stirred with a glass rod to insure that the toxicant was uniformly distributed throughout the test container, after which the test specimens were reintroduced into the test jars. The elapsed time between the generation of OMSE-water and the beginning of the acute toxicity bioassays was less than two hours for all bioassays.

Exploratory bioassays. The recommended method (16) for conducting acute toxicity bioassays was derived for fish, not invertebrates and therefore many of its recommendations may not hold for invertebrates. One such recommendation is that of a minimum volume of one liter of dilution water per gram of fish. Dragonfly nymphs weighed between 0.20 and 0.25 grams each and would allow for use of four to five creatures per liter of water. These values would even be higher for adult scuds and damselfly nymphs which weigh less than dragonfly nymphs. A knowledge of the allowable number of invertebrates per volume of dilution water would be valuable because for a fixed number of test jars, the more specimens tested would yield more valid results from a statistical standpoint. With this in mind, exploratory bioassays were set up to evaluate the allowable limit of invertebrates per volume of dilution water and to establish toxic ranges for OMSE-water to the various test invertebrate species.

Full-scale bioassays. The criterion for toxicity at various concednt of OMSE-water was death of the test specimens. Death was considered to have occurred when a test specimen showed no response to prodding with a dissecting needle.

For a majority of the bioassays it was found that between five to seven test concentrations plus a control series were sufficient to evaluate the toxicity of OMSE-water to aquatic biota. Generally, ten individuals per concentration (five test vessels with two individuals per vessel) served to evaluate the toxicity of OMSE-water at that concentration. However, if more test specimens were available, they were also included in the bioassays (yielding two to five creatures per liter of dilution water). The number of test members per concentration was kept constant for a particular series of bioassays except for summer bioassays where every available test member was used for testing purposes.

The environmental test parameters of pH, dissolved oxygen, and temperature were measured in the test jars, usually at the beginning of the bioassays and at the 24, 48, and 96th hours after the inception of testing. These time periods correspond to the hours during which the toxic effects (as death) of OMSE-water were evaluated on the test individuals. The dissolved oxygen concentration in each test container was maintained above 5.0 mg/l by very gentle aeration with flow regulated aquaria aerators, while the temperature was thermostatically maintained at $20^{o} \pm 2^{o}C$ (16). Once a test organism was considered dead it was immediately removed from the test jar to avoid fungal and microbial contamination of the test water and remaining live test specimens.

Derivation of median tolerance limit values. The procedure which was used for the calculation of the median tolerance limit (TL_{50}) was the one of graphical interpolation as presented in Standard Methods.

Safety factors may be applied to the TL_{50} values derived for the various test species at 24, 48 and 96-hours to obtain possible safe concentration levels of OMSE-water under natural conditions. Previous investigators (20,21,22) have used application factors ranging from 1/10 to 1/20 the 96-hour TL_{50} value obtained from acute toxicity bioassays.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Acute toxicity bioassays may give some indication of the extent of the stressed physiological function of invertebrates subject to OMSE-products. The results of acute toxicity bioassays on scuds (Crangonyx gracilis), nymphs of dragonflies (Tetragoneuria cynosura), and nymphs of damselflies (Argia violacea) and a discussion as to the significance of their reactions to OMSE-water will be presented in this section.

Dilution and test water characteristics. Amherst tap water was used as the recipient test water for the outboard motor subsurface exhausts because of its abundant availability. Cranberry Pond located in Sunderland, Massachusetts served as the source of the test invertebrates, dilution water for acute toxicity bioassays, and stock aquaria water for the laboratory acclimation of the invertebrate test species. Both of these waters were analyzed intermittently for selected physical and chemical characteristics during the investigation. The range of values obtained for three separate analyses of these water samples is summarized in Table 4.

Turbidity and color were determined by a precalibrated turbidimeter[1] and aqua tester[2], respectively. All samples analyzed were unfiltered and therefore, the Hellige aqua tester gave values of apparent color. Both the Amherst tap water and Cranberry Pond water were relatively clear, as indicated by the low range of color and turibidity values. The pH was measured by a pH meter[3] and all the pH values measured for samples from both water sources were near the neutral point.

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[1]Hellige turbidimeter manufactured by Hellige, Incorporated, Garden City, New York.

[2]Hellige aqua tester, manufactured by Hellige, Incorporated, Garden City, New York.

[3]Copenhagen pH Meter 28, Radiometer manufactured by Copenhagen Radiometer, Copenhagen, Denmark.

Since both waters are basically surface waters, some variation in temperature occurred over the testing period. As shown in Table 4, total chlorine residuals, as determined by the starch-iodide titration method, were immeasurable except fo one sample of Amherst tap water which showed a residual of 1.0 mg/l. This particular water sample was allowed to stand in the stainless steel test tank for two days after which time the chlorine residual was 0 mg/l. The chlorine having been dissipated, it was then used as test water for the generation of OMSE-water. Copper was undetectable in both waters with the atomic absorption technique used throughout this study. Based on the values obtained for copper and chlorine, both were considered to have negligible bearing on the toxic effect of OMSE-water to the test species.

The total hardness and alkalinity (mg/l as $CaCO_3$) for both waters were found to vary from 18-20 and 9-20 for Amherst tap and held constant at 24 and 13, respectively, for Cranberry Pond water. The range of values measured for hardness and alkalinity imply that soft waters were used throughout the investigation. The solids and total carbon analyses for both waters revealed that a high percentage of the solids were dissolved and that carbon was present in only very small quantities. The average value of triplicate analyses was reported for most analyses in Table 4. Based on the reported chemical and physical characteristics, Amherst tap water and Cranberry Pond water used in this investigation were found to be nearly identical.

Acute toxicity of OMSE-water to adults of scuds. Species mortality responses obtained in the bioassays conducted on Crangonyx gracilis exposed to OMSE-water are given in Appendix A, Tables A-1 to A-4, inclusive. Data in these tables also includes the measurement of dissolved oxygen, pH, temperature and number of test

Table 4. Dilution Water Characteristics for Acute Toxicity Bioassays of OMSE-Water to Scuds, Dragonflies, and Damselflies.

Analysis*	Water Sample	
	Amherst Tap	Cranberry Pond
Turbidity (JTU)	1.7-4.1	1.0-3.8
Color (Color units)	10-25	10
pH	6.0-6.9	6.7-7.3
Temperature (^{o}C)	20-24	15-20
Total chlorine (mg/l)	0-1.0	0
Copper (mg/l)	0	0
Alkalinity, total (mg/l as $CaCO_3$)	9-20	13
Alkalinity, phenolphthalein (mg/l as $CaCO_3$)	0	0
Hardness, total (mg/l as $CaCO_3$)	18-20	24
Hardness, non-carbonate (mg/l as $CaCO_3$)	6-11	11
Solids, total (mg/l)	33.5-115.5	50.0-97.7
Solids, dissolved (mg/l)	29.5-110.2	45.0-96.3
Solids, suspended (mg/l)	5.0-5.3	1.4-5.0
Total carbon (mg/l)	2.0-3.5	0.0-2.0

*Most values were based on the average of triplicate analyses.

survivors in the test and control bioassay chambers after exposure time periods of 0, 24, 48, and 96-hours. The scuds used as test species were collected from Cranberry Pond during the spring season of the year. Mention is made of this fact because a comparison of TL_{50} values was made between invertebrates collected in different seasons in a subsequent section.

It should be noted from the scud survival data in Tables A-1 and A-3, that the dissolved oxygen concentration in all bioassay jars was upwards of the 5.0 mg/l concentration recommended by Standard Methods (16) and Ellis (23) as a supply ample and favorable for fish life. The pH values which were measured at the time of preparation of the various OMSE-water concentrations ranged from 4.7 to 7.1. The lower pH values occurred in the higher concentrations of OMSE-water and these values gradually approached neutrality with the addition of more dilution water. It was noted that the pH values for all test concentrations increased during the four day bioassay period and reached values close to neutral.

These variations in pH with OMSE-water concentration and time can possibly be attributed to the carbon dioxide gas (rather than carbon monoxide because of the former's greater solubility in water) present in the exhausts of outboard motors. The reaction of carbon dioxide generated by the outboards with the Amherst tap dilution water is presumed to be identical to that of equation 1 as described by Sawyer (24). The increase in pH values for all concentrations with time indicates that equation 1 is no longer at equilibrium and that the rate of reaction favors the formation of carbon dioxide and water as the OMSE-water gives up carbon dioxide to maintain an equilibrium with that of the atmoshpere. These values indicate that death of invertebrates exposed to OMSE-water was not likely to have occurred due to a paucity of dissolved oxygen or an unfavorable pH. Observations of scuds in the control jars and stock tank revealed that no deaths

or visible adverse effects had occurred during the four-day bioassay period. This indicates that death of test speiees in the bioassay jars could be attributed to the addition of OMSE-products. The pH and dissolved oxygen values in all control jars were near neutrality and above 5.0 mg/l, respectively.

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+ \qquad \text{eq. 1}$$

A summary of the TL_{50} values for acute lethal toxicity of OMSE-water to scuds and the other invertebrates used in this study is presented for comparative purposes in Table 5.

The 24, 48, and 96-hour TL_{50} values obtained for spring (April-May) collected scuds in test No. 1 from the data in Table A-1 and A-2 are 0.0320, 0.0320, and 0.0320 percent by volume of fuel consumed, respectively. For convenience, in subsequent discussion, the corresponding values of parts of dilution water per parts of fuel consumed will be in parenthesis immediately following the percent by volume of consumed figures; as an example, 0.0320, 0.0320, and 0.0320 (3130/1, 3130/1, and 3130/1) percent of fuel consumed, respectively. The 24, 48, and 96-hour TL_{50} values for spring collected scuds in test No. 2 from the data in Tables A-3 and A-4 are 0.0280, 0.0269, and 0.0269 (3570/1, 3720/1, and 3720/1) percent by volume of fuel consumed, respectively. The average 24, 48, and 96-hour TL_{50} values for spring collectdd scuds from the combined data in Table 27 are 0.0300, 0.02945, and 0.02945 (3340/1, 3400/1, and 3400/1) percent by volume of fuel consumed, respectively.

The total population of scuds utilized in the derivation of TL_{50} values for test No. 1, test No. 2, and the average of test No. 1 and 2 are 80, 70, and 150 individual specimens, respectively. The average TL_{50} values for spring collected scuds from test No. 1 and 2 were obtained by taking an arithmetic average of TL_{50} values obtained in test No. 1 and test No. 2 for a specific exposure period.

Table 5. Summary of TL_{50} Values for Acute Lethal Toxicity of OMSE-Water to Benthic Invertebrates.

Test Number	Test Species	Season of Species Collection	Percent by Volume of Fuel Consumed			Parts of Dilution Water per Parts of Fuel Consumed		
			24-Hour	48-Hour	96-Hour	24-Hour	48-Hour	96-Hour
1	Scud	Spring	0.0320	0.0320	0.0320	3130/1	3130/1	3130/1
2	Scud	Spring	0.0280	0.0269	0.0269	3570/1	3720/1	3720/1
Average	Scud	Spring	0.0300	0.02945	0.02945	3340/1	3400/1	3400/1
3	Dragonfly	Spring	0.183	0.162	0.156	550/1	620/1	640/1
4	Dragonfly	Spring	0.181*	0.166	0.158	550/1*	600/1	630/1
Average	Dragonfly	Spring	0.186δ	0.164	0.157	540/1δ	610/1	635/1
5	Damselfly	Spring	0.158	0.150	0.132	630/1	670/1	760/1
6	Damselfly	Spring	0.167	0.160	0.145	600/1	625/1	690/1
Average	Damselfly	Spring	0.1625	0.155	0.1385	615/1	645/1	720/1
7	Damselfly**	Summer	0.183	0.176	0.168	550/1	570/1	590/1
8	Damselfly	Summer	0.114*	0.096	0.0931	880/1*	1040/1	1080/1
9	Dragonfly	Summer	0.128*	0.124	0.121	780/1*	810/1	830/1

*By extrapolation.

**Engine running at full throttle (3800 ± 100 rpm)

δCombined TL_{50} value.

A plot of the individual and average survivor curves based on TL_{50} values after varying exposure times for spring collected scuds appears in Figure 5. These survivor curves indicate that for the test No. 1 data no additional deaths (indicated by straight, horizontal TL_{50} line) occurred between the 24 and 96-hour observations periods and that all deaths to test scuds occurred prior to the observation at 24 hours. The survivor curve for test No. 2 and the average of test No. 1 and 2 represents a more classical bioassay response with a decreasing rate of death between consecutive observations after longer exposure times.

The differences between test No. 1 and test No. 2 TL_{50} results for spring collected scuds (0.0320 vs. 0.0280 for 24-hours, 0.0320 vs. 0.0269 for 48-hours, and 0.0320 vs. 0.0269 for 96-hours) are open to question, since all these concentrations contain an appreciable quantity of OMSE-products. Therefore, the average survivor curves and TL_{50} values for test No. 1 and 2 could be considered the best representation of the lethal effects of OMSE-water to spring collected scuds. All three survivor curves indicate that the lethal concentration for 50 percent survival[1] of spring collected scuds is achieved by the 48th hour of exposure to OMSE-water and this exposure period appears to be sufficient for occurrence of acute lethal toxicity to scuds. It is evident from these survivor curves that most, if not all of the scud deaths, occurred before the first observation period (24-hours) and this suggests that observation periods shorter than 24-hours be used in future scud bioassays to obtain a more meaningful bioassay response. This short response time to achieve a lethal concentration

[1] Lethal concentration for 50 percent test species survival is the concentration at infinite time derived by passing a straight line parallel to the time axis and asymptotic to the survival curve.

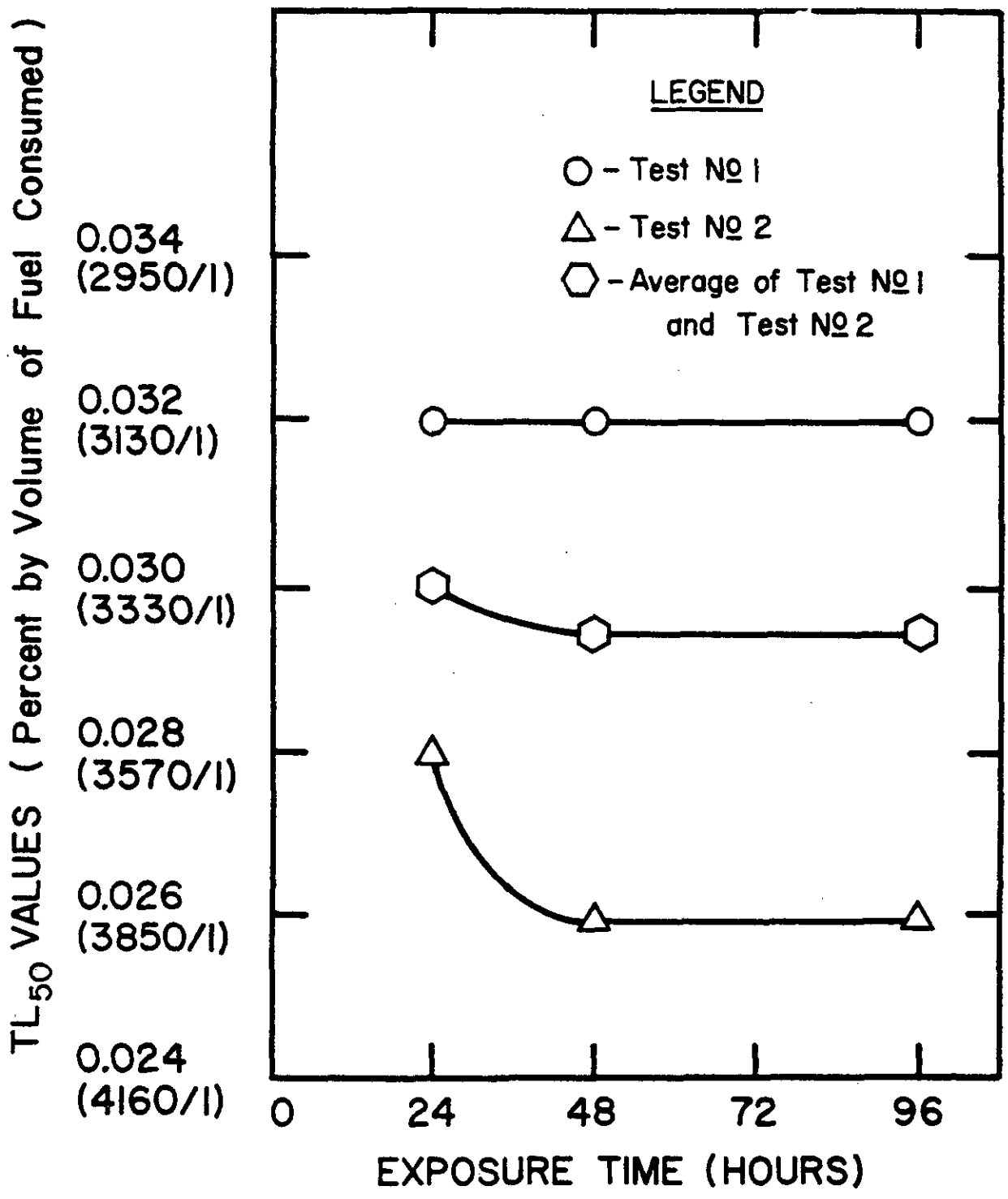

Figure 5. Plot of TL_{50} Values for Spring Collected Scuds versus Exposure Time to OSME-Water.

for 50 percent survival suggests that scuds may be of value as a biological indicator with rapid response to unfavorable conditions in their aqueous environment.

Acute toxicity of OMSE-water to nymphs of dragonflies. The results from the acute bioassays conducted on the nymphs of Tetragoneuria cynosura exposed to OMSE-water are listed in Appendix A, Tables A-5 to A-8, inclusive and Table A-15. Data in these tables include the measurement of dissolved oxygen, pH, temperature and number of survivors in the test and control bioassay chambers for exposure time periods of 0, 24, 48, and 96-hours. The dragonfly nymphs used in tests No. 3, No. 4, and No. 9 were all collected from Cranberry Pond; however, those used for tests No. 3 and 4 were obtained during the spring season (April-May) of the year whereas, those of test No. 9 were collected during the summer season (August-September).

The survivor data for spring collected dragonfly nymphs from Tables A-5 to A-8, inclusive, reveals that the D.O. concentrations in all bioassay chambers were upwards of 5.0 mg/l and that the supply was ample for fish life for both cold water and warm water species (5.0 mg/l D.O. and 4.0 mg/l D.O., minimum recommended value, respectively (16)). The authors question whether either of these D.O. values are valid for invertebrate bioassays since fish are vertebrates. No such recommended D.O. value for invertebrates could be found in the literature.

All invertebrates used for the bioassays were collected from relatively shallow shoreline areas which tend to be warmer waters. This was visually verified by noting the presence of only warm water fish (bass, bluegills, pumpkinseeds, etc.) while the cold water species known to be present in Cranberry Pond (trout) were absent from the collection area. This possibly suggests that the 4.0 mg/l lower limit for D.O. would be more adequate for fish or invertebrates collected from Cranberry Pond and used in this study.

The pH values incurred during tests No. 3 and No. 4 range from 4.9 to 7.0 and follow the same pattern as the pH for spring collected scuds (tests No. 1 and No. 2). These values of pH and D.O. suggest that neither were deleterious to the test dragonfly nymphs. Dragonfly nymphs in the control chambers or stock tank suffered no deaths or noticeable adverse effects during the four-day bioassay period. The pH and D.O. values in all control jars was near neutrality and above 5.0 mg/l, respectively. This indicates that all dragonfly nymph mortalities could be attributed to the addition of OMSE-water.

A summary of the TL_{50} values for acute lethal toxicity of OMSE-water to spring collected dragonfly nymphs is presented in Table 5.

The 24, 48, and 96-hour TL_{50} values obtained for spring collected dragonfly nymphs from the data for test No. 3 in Tables A-5 and A-6 are 0.183, 0.162, and 0.156 (550/1, 620/1, and 640/1) percent by volume of fuel consumed, respectively. Similar TL_{50} values derived from the data for test No. 4 in Tables A-7 and A-8 are 0.181, 0.166, and 0.158 (550/1, 600/1, and 630/1) percent, respectively. The 24-hour TL_{50} value of 0.181 percent by volume of fuel consumed was derived by linear extrapolation from the last two data points to a line drawn perpendicular to the 50 percent survival point on the arithmetic abscissa. Any significance in the <u>difference</u> between the 24-hour TL_{50} value of test No. 3 and the extrapolated 24-hour TL_{50} value of test No. 4 (0.183 vs. 0.181 or 550/1 vs. 550/1) is questionable. The 24-hour survivor data from test No. 3 and test No. 4 were combined and a new TL_{50} value derived which would eliminate any bias in averaging of an interpolated and extrapolated value. The combined 24-hour TL_{50} value and average 48- and 96-hour TL_{50} values for spring collected dragonfly nymphs from the combined data of tests No. 3 and 4 are 0.186, 0.164, and 0.157 (540/1, 610/1, and 635/1) percent by volume of fuel consumed, respectively.

A plot of the individual and average survivor curves based on TL_{50} values for spring collected dragonfly nymphs appears in Figure 6. These survivor curves indicate that the TL_{50} values for tests No. 3 and 4 and the combined test data are similar and any differences in TL_{50} values within the given 24, 48, and 96-hour observation periods for all three survivor curves are questionable. All three survivor curves indicate that the lethal concentration for 50 percent survival of spring collected dragonfly nymphs was not achieved after 96-hours of exposure to OMSE-water and that additional exposure time beyond 96-hours is needed to obtain this lethal concentration. Since this lethal concentration was not achieved within the standard four day acute toxicity bioassay period, the use of dragonfly nymphs to evaluate the acute toxic effects of OMSE-water is of uncertain suitability for this exposure period.

The survival data for summer (August-September) collected dragonfly nymphs (same size as spring collected; i.e. 3/4 in to 7/8 in length) from test No. 9 in Table A-15 indicate that the D.O. throughout the four-day bioassays period was greater than 5.0 mg/l. The pH values obtained for the test and control chambers were in the same range as the spring collected dragonfly nymphs and was therefore, considered a negligible factor in mortality of test species.

The 24, 48, and 96-hour TL_{50} values for exposure of summer collected dragonfly nymphs to OMSE-water were derived from these curves in a manner as previously described and are presented in Table 5. From the data of test No. 9 in Table A-15, the 24, 48, and 96-hour TL_{50} values obtained for summer collected dragonfly nymphs are 0.128, 0.124, and 0.121 (780/1, 810/1, and 830/1) percent by volume of fuel consumed. The 24-hour TL_{50} value of 0.128 is an extrapolated value, derived from the extension of a straight line from the 24-hour survivor values of 83 and 55.5 percent at concentrations of 0.115 and 0.125,

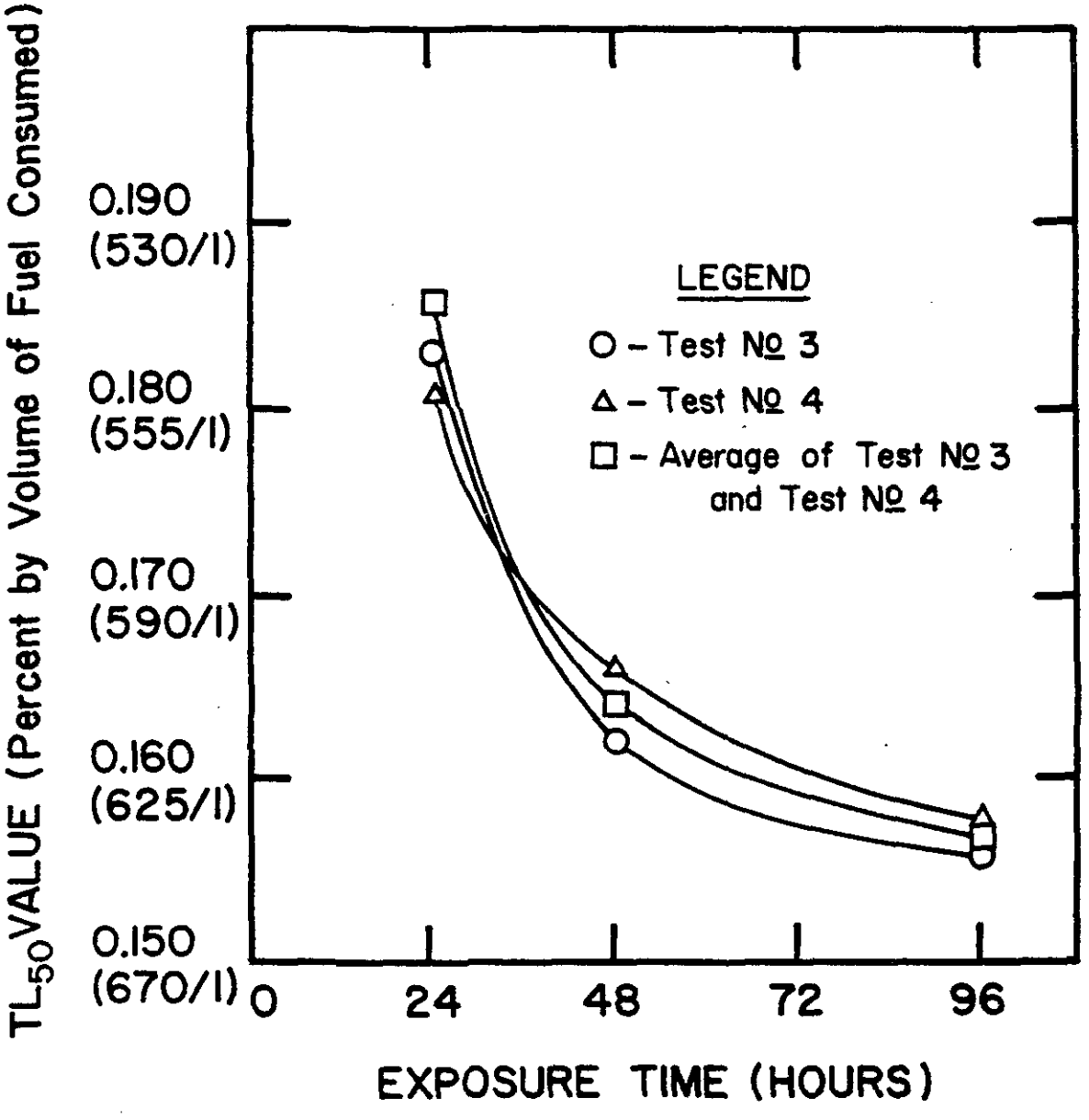

Figure 6. Plot of TL_{50} Values for Spring Collected Dragonflies *versus* Exposure Time to OSME-Water.

respectively. No partial kills below 50 percent were noted at any of the test concentrations for the 24-hour observation period; however, partial kills below 50 percent were noted during the 48- and 96-hour observation period. The 24-hour survivor data nearly parallels the 48-and 96-hour data and because a definite trend for the 24-hour data appear to be established it is felt by the authors that the 24-hour TL_{50} value (0.128 percent by volume of fuel consumed) represents a reliable TL_{50} value.

A plot of the average TL_{50} values for all (spring and summer collected) bioassays conducted on dragonfly nymphs as a function of exposure time is presented in Figure 7. The survivor curve for dragonfly nymphs collected in the summer reveals a typical bioassay response shape with the gradient of the curve leveling off with time. It appears that the summer collected dragonfly nymphs are more sensitive to the toxic effects of OMSE-water than those gathered in the spring. As shown in Figure 7, the TL_{50} values for the average of the spring collected species gradually decreased from 0.186 to 0.157 (540/1 to 635/1) percent concentration at 24 and 96 hours, respectively. By comparison, the curve obtained for the summer collected species indicates a gradual decrease in TL_{50} values from 0.128 to 0.121 (780/1 to 830/1) percent concentration at 24 and 96-hours, respectively The differences between 24, 48, and 96-hour TL_{50} values for spring and summer collected dragonfly nymphs suggests that fewer OMSE-products in receiving water are required during the summer to have an equivalent toxic effect on a similar spring collected species. This increased susceptibility during the summer of dragonfly nymphs to the toxic effects of OMSE-water may be due to the nymph passing from one nymphal stage to another as it approaches adulthood as a non-aquatic dragonfly. Pennak (17) relates the life cycle of Odonata as follows:

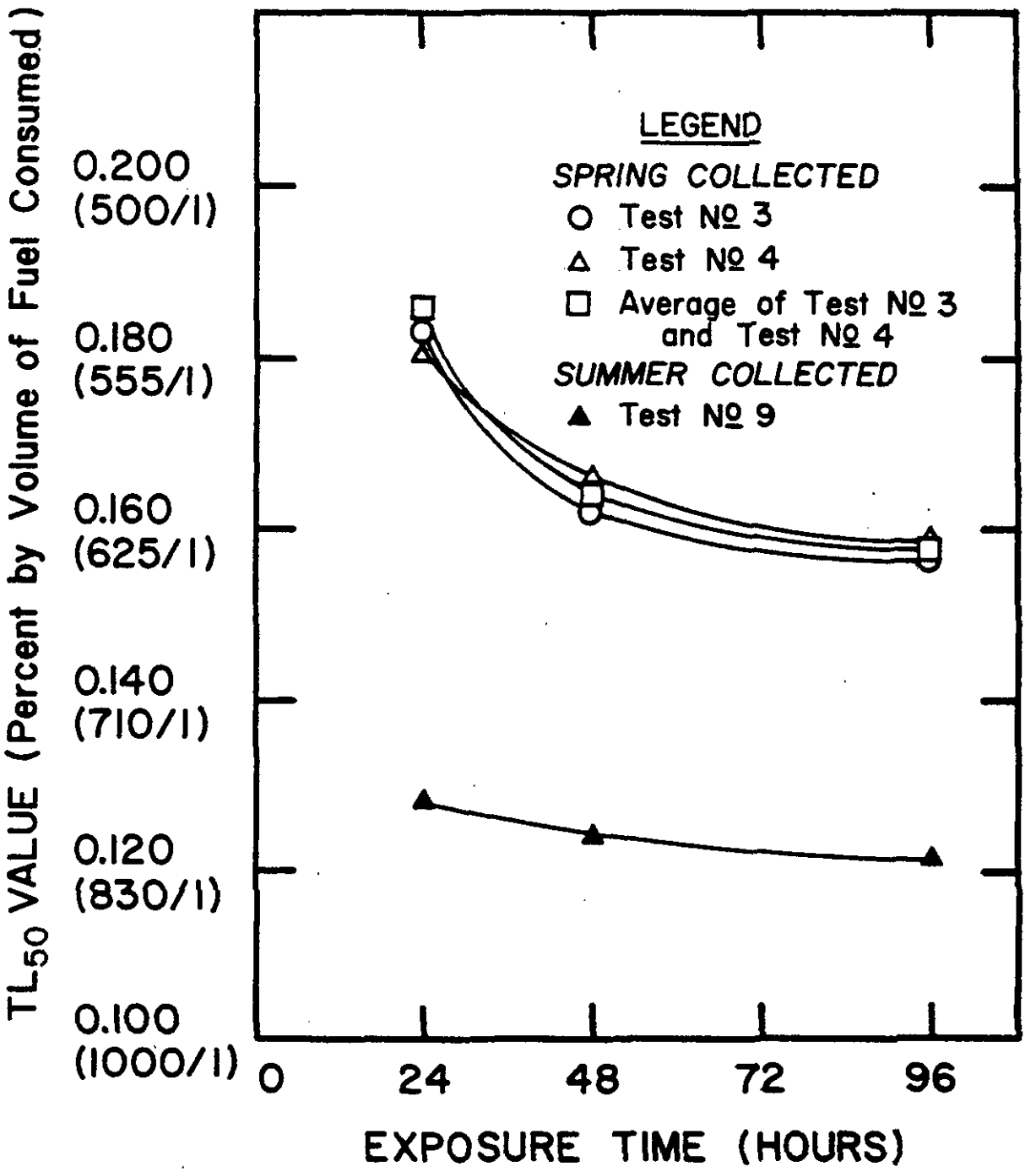

Figure 7. Plot of TL_{50} Values for Spring and Summer Collected Dragonflies *versus* Exposure Time to OSME-Water.

> "The complete life history of Odonata has been worked out for only a very few species, but it is probably that the great majority of species have from 11 to 14 nymphal instars. The length may last only three days; sometimes it may persist for as long as six months. It has been said that a one-year life cycle is the prevailing condition, but there are many exceptions."

Acute toxicity of OMSE-water to nymphs of damselflies. The results from the bioassays conducted on the nymphs of Argia violacea exposed to OMSE-water are given in ppendix A, Tables A-9 to A-15, inclusive. As with the bioassays conducted on the other invertebrate species data in thsse tables include the measurement of D.O., pH, temperature, and test species mortality response in the test and control bioassays chambers after exposure time periods of 0, 24, 48, and 96-hours. Damselfly nymphs used in tests No. 5 and 6 were collected during the spring season (April-May); whereas, those employed in tests No. 7 and 8 were gathered during the summer season (August-September).

Tests No. 7 and 8 also differ with respect to outboard motor operational speed: OMSE-water for test No. 7 was generated at 1700 $\pm$ 100 rpm and that for test No. 8 generated at a speed of 3800 $\pm$ 100 rpm.

Outboard motors have previously been reported (25,26,27) to operate more efficiently at higher engine speeds than at lower engine speeds. This suggests that fewer OMSE-products are emitted into receiving waters at higher engine speeds and may indicate that OMSE-water generated at higher speeds would be less toxic to aquatic biota than the OMSE-water generated at lower engine speeds. Tests No. 7 and 8 were designed to evaluate the differences in toxicity of OMSE-water generated at different engine speeds to summer collected damselfly nymphs.

The D. O. concentrations and pH values recorded in the control and test bioassays jars of test No. 5 to 8 inclusive suggest that neither were a limiting factor in damselfly survival and that all deaths could be attributed to the addition of the OMSE-water. No deaths occurred to the test species in the control

chambers or stock tanks during the bioassays exposure period.

The 24, 48, and 96-hour TL_{50} values for exposure of this test species to OMSE-water were derived in a manner as previously described and a summary of these acute lethal toxicity values is presented in Table 5.

The 24, 48, and 96-hour TL_{50} values obtained for the spring collected damselfly nymphs of test No. 5 in Tables A-9 and A-10 are 0.158, 0.150 and 0.132 (630/1, 670/1, and 760/1) percent by volume of fuel consumed, respectively. Similarly TL_{50} values accrued from the data of test No. 6 in Tables A-11 and A-12 are 0.167, 0.160, and 0.145 (600/1, 625/1, and 690/1) percent, respectively. The average 24, 48, and 96-hour TL_{50} values for the combined spring collected damselfly nymph bioassay data for tests No. 5 and 6 are 0.1625, 0.155, and 0.1385 (615/1, 645/1, and 720/1) percent by volume of fuel consumed, respectively (Table 5).

A plot of the survivor curves based on TL_{50} values versus time of exposure to OMSE-water for the spring collected damselfly nymphs of test No. 5, test No. 6, and the average of the two tests appears in Figure 8. These three survivor curves show that the lethal concentration for 50 percent survival of spring collected damselfly nymphs was not fulfilled after 96-hours of exposure to OMSE-water and this suggests that additional exposure time beyond 96-hours is required to obtain this lethal concentration. The differences in TL_{50} values among the three curves for any given exposure period may be questionable. Since the lethal concentration for spring collected damselfly nymphs was not achieved within the standard four day acute toxicity bioassays period, the use of damselfly nymphs to evaluate the acute toxic effect of OMSE-water is of uncertain suitability for this exposure period. The 24, 48, and 96-hour TL_{50} values for exposure of the summer collected damselfly nymphs to OMSE-water generated at 1700 $\pm$ 100 rpm (test No. 8) and at 3800 $\pm$ 100 rpm (test No. 7) were derived from these survivor curves in a manner as previously described and are recorded in Table 5. From the data of test No. 8,

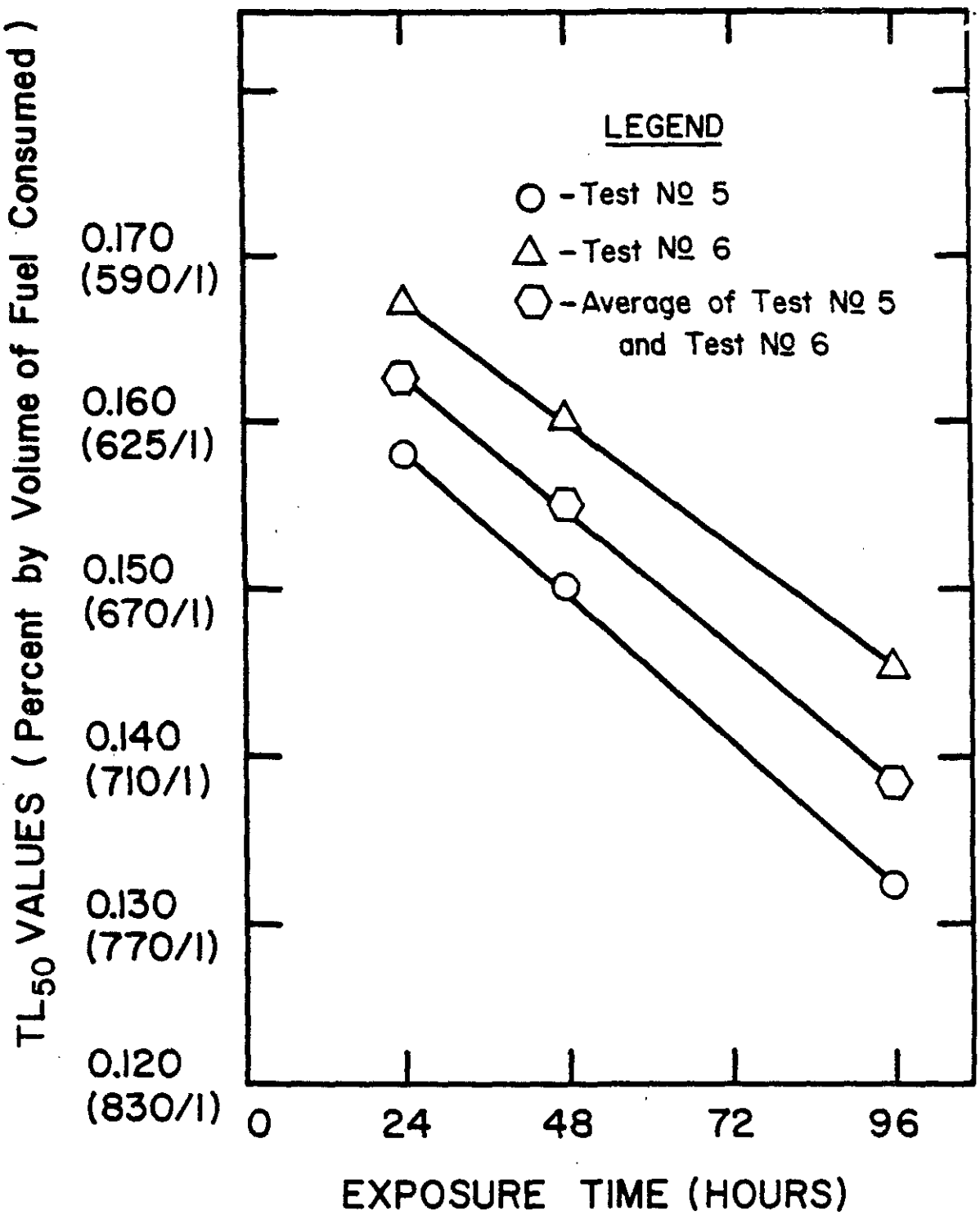

Figure 8. Plot of TL_{50} Values for Spring Collected Damselflies *versus* Exposure Time to OSME-Water.

the 24, 48, and 96-hour TL_{50} values obtained for the summer collected damselfly nymphs exposed to OMSE-water are 0.114, 0.0960, and 0.0931 (880/1, 1040/1, and 1080/1) percent by volume of fuel consumed, respectively. The 24-hour TL_{50} value of 0.114 is an extrapolated value; whereas, the 48 and 96-hour TL_{50} values of 0.114 are interpolated values. Although this 24-hour TL_{50} value is extrapolated, the 48 and 96-hour TL_{50} test mortality data indicate a definite toxic trend and the value of 0.114 percent of fuel consumed appears to be representative of the toxic trend exhibited by the damselfly nymphs. The 24, 48, and 96-hour TL_{50} values obtained from the exposure of summer collected damselfly nymphs to OMSE-water from an engine operating at full throttle (3800 $\pm$ 100 rpm) are 0.183, 0.176, and 0.168 (550/1, 570/1, and 590/1) percent, respectively.

A plot of the TL_{50} values for the average spring collected data, the summer collected data at 1700 $\pm$ 100 rpm, and the summer collected data at 3800 $\pm$ 100 rpm for damselfly nymphs is presented in Figure 9. All three survivor curves are indicative of typical bioassay plots where the gradient of each curve decreases with time but that of summer collected at low throttle is most classical. Several observations can be made from these survivor curves. In the first place, at trolling speeds (1700 $\pm$ 100 rpm) the damselfly nymphs collected during the summer exhibited a greater mortality than those similar test species collected in the spring season. In addition, outboard motor engines operating at low speeds (1700 $\pm$ 100 rpm) produced an exhaust composition that was more toxic to damselfly nymphs collected during the summer than was produced at full throttle engine speeds (3800 $\pm$ 100 rpm). These findings support the conclusions of various researchers (25, 26, 27) that outboards operate more efficiently at higher speeds of operation regarding percent of raw fuel wasted. However, this use of damselfly nymphs in acute toxicity bioassays is the first time any experimentation with

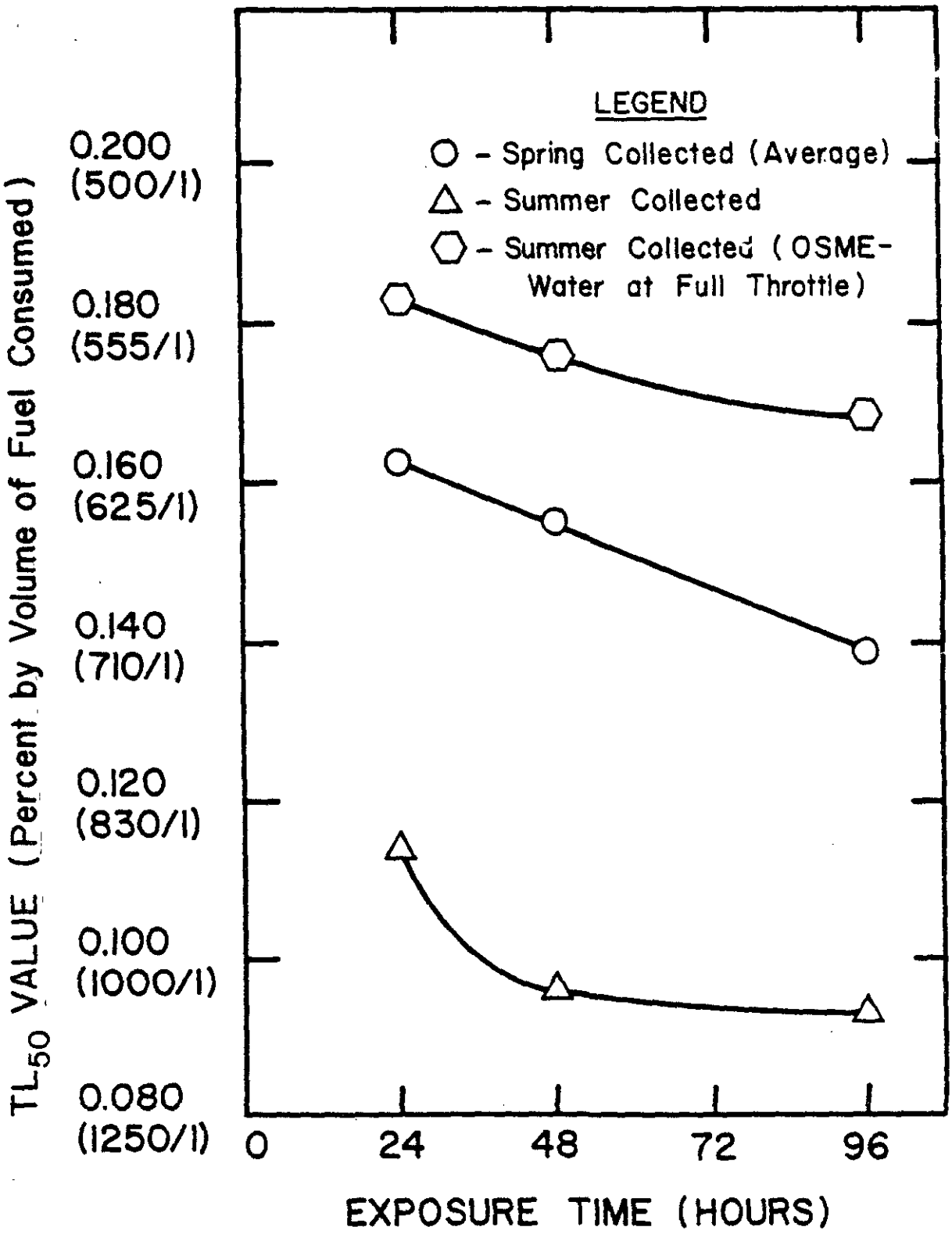

Figure 9. Plot of TL_{50} Values for Spring and Summer Collected Damselflies (Engine Speed at 1700 ±100 rpm) and Summer Collected Damselflies (Engine Speed at 3800 ± 100 rpm) *versus* Exposure Time to OSME-Water.

aquatic biota has been conducted to corroborate these earlier researchers' (25, 26, 27) findings.

Seasonal response of invertebrates to OMSE-water. Three different species of invertebrates each collected during the spring (April-May) season were exposed to varying concentrations of OMSE-water generated at trolling speeds (1700 ± 100 rpm) by a 1970 7 1/2 horsepower engine.

A plot of the combined survivor curves based on average TL_{50} values for spring collected scuds, dragonfly nymphs, and damselfly nymphs is presented in Figure 10. From these curves, it becomes apparent that spring collected dragonfly and damselfly nymphs exhibit approximately the same toxic response to OMSE-water after 24, 48, and 96-hours exposure. Neither of these two survivor curves show that the lethal concentration for 50 percent survival was achieved after the 96th hour of exposure and additional exposure time beyond 96-hours is suggested if this concentration is to be reached.

Scuds demonstrated the greatest sensitivity towards OMSE-products than any of the other spring collected invertebrates. The lethal concentration for 50 percent survival to spring collected scuds was accomplished by 24-hours and exposure times before the 24-hour observation period are suggested.

Two species of invertebrates (dragonfly and damselfly nymphs) were obtained from Cranberry Pond during the spring and summer seasons and exposed to varying concentrations of OMSE-water generated at trolling outboard motor speeds. A plot of the survivor curves based on average TL_{50} values for the spring and summer collected test species is presented in Figure 11. From these survivor curves it is evident that both summer collected invertebrate test species exhibited a higher mortality when exposed to OMSE-water than did the spring collected samples of

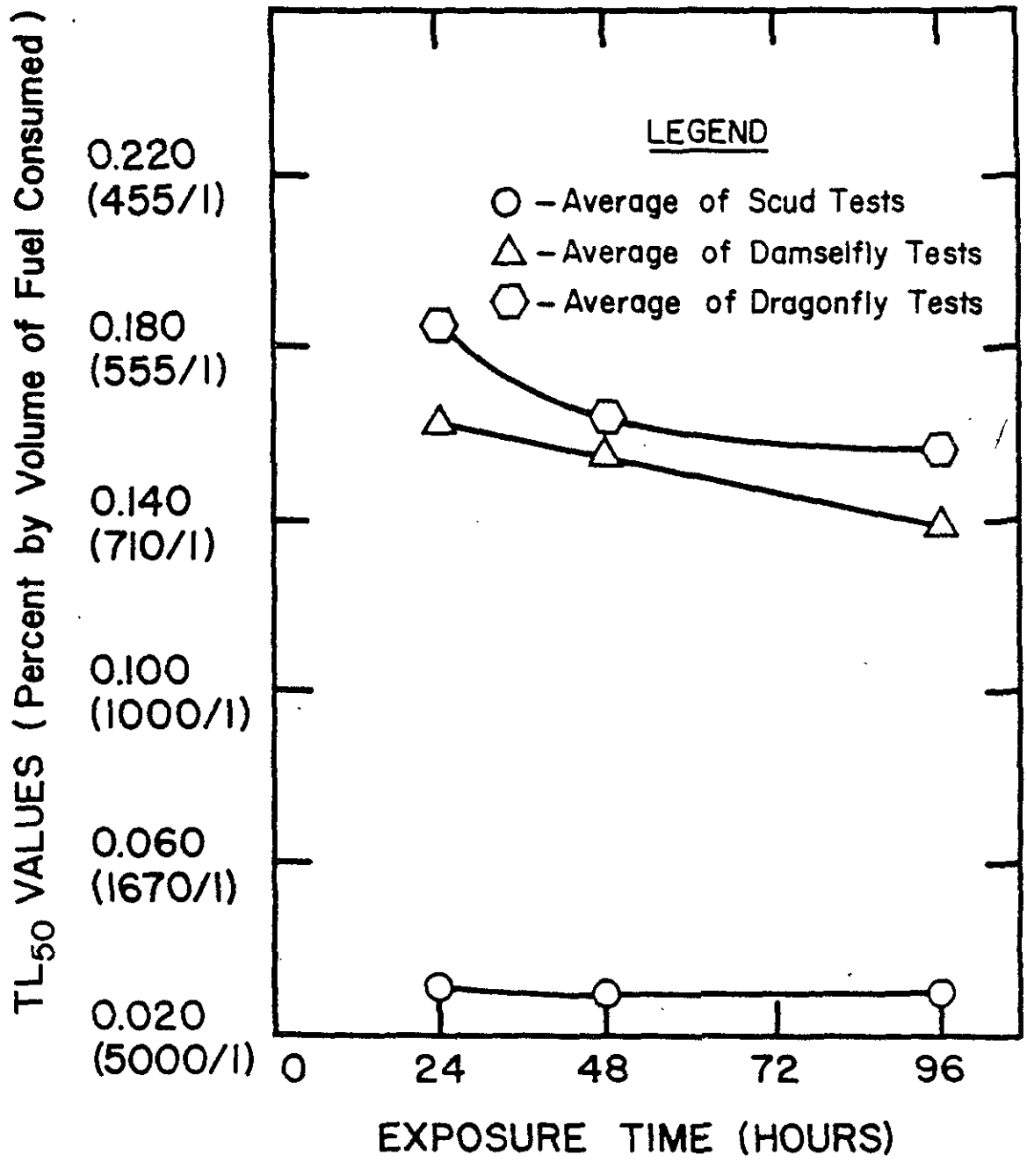

Figure 10. Plot of Average TL_{50} Values for Spring Collected Scuds, Dragonflies, and Damselflies *versus* Exposure Time to OSME-Water.

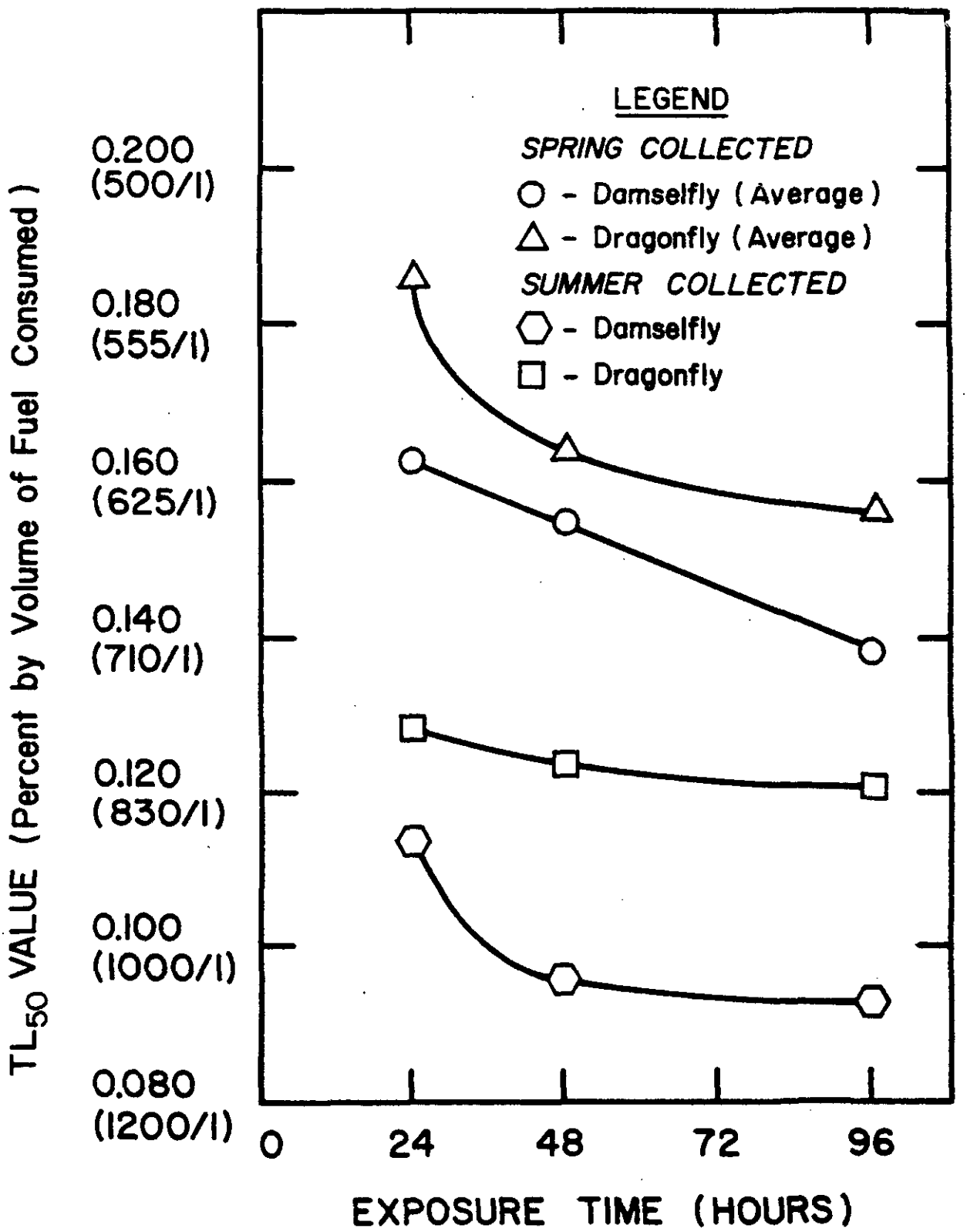

Figure 11. Plot of Average TL_{50} Values for Spring and Summer Collected Dragonflies and Damselflies *versus* Exposure Time to OSME-Water.

the same test species. Of the two test species, dragonfly nymphs showed a more pronounced mortality response between the two seasons of species collection than did the damselfly nymphs. After 96-hours of exposure, neither of the two test species showed that a lethal concentration for 50 percent survival had been accomplished in either of the two test seasons. An exposure period beyond 96-hours is suggested if this lethal concentration is to be reached.

Perhaps the increased toxicity of OMSE-water to both summer collected damselfly and dragonfly nymphs can be attributed to the particular stage in the life cycle of the invertebrate. Damselflies and dragonflies are of the family Odonata which in their preadult (nymphal) stage are strictly aquatic but, as adults, are terrestrial. During the spring the Odonata nymphs are in the less advanced nymphal stage; however, as time passes and the summer season approaches, these nymphal stages become more advanced and the creature nears the point of emergence (shedding of protective skin layer) as an adult. It is highly possible that during the later stages of nymphal development the creature's protective outer coating may become more permeable thus enabling the OMSE-products to exhibit a more toxic effect upon the test species.

Comparison of acute toxicity of OMSE-water to aquatic life. The experiments of English, et al (20) and Kuzminski, et al (21) included the exposure of aquatic vertebrate test species (fathead minnows and bluegills) to OMSE-water. From their studies they were able to derive 24, 48, and 96-hour TL_{50} values. Kuzminski, et al (21) noted that the dilution water characteristics of his study using Amherst tap water were similar to those of the study by English, et al (20). Since this study also utilized Amherst tap water and Cranberry Pond water (chemical and physical characteristics similar to those of Amherst tap water) it may be concluded

that all three studies used dilution water of similar chemical and physical composition and therefore, allowed for an unbiased comparison on the basis of chemical characteristics of dilution waters. A point of difference is that the bioassays conducted by English, et al (20) were performed at a water temperature of 25°C, while those of Kuzminski, et al (21) and this study were conducted at 20° ± 2°C. In addition, English, et al (20) used a fuel to oil ratio of 17/1; whereas, Kuzminski, et al (21) and this study used a ratio of 50/1.

The TL_{50} values for the three separate investigations are presented in Table 6 and were used to generate the plots of survivor curves in Figure 12. In order to facilitate comparison with the previous studies all 24, 48, and 96-hour TL_{50} values in Table 6 are based on average values for spring and summer collected and various sized test species. English, et al (20) obtained 24, 48, and 96-hour TL_{50} values for fathead minnows and bluegills of 0.0569, 0.0556, and 0.0556 (1760/1, 1800/1, and 1800/1) percent and 0.0700, 0.0513, and 0.0513 (1430/1, 1950/1, and 1950/1) percent by volume of fuel consumed, respectively. Kuzminski, et al (21) secured 24, 48, and 96-hour TL_{50} values for spring and summer collected fathead minnows (1 1/2" to 2" fork length) of 0.017, 0.017, and 0.014 (6000/1, 6000/1, and 7150/1) percent and 0.047, 0.038, and 0.032 (2150/1, 2640/1, and 3130/1) percent, respectively. The 24, 48, and 96-hour TL_{50} values obtained by Kuzminski, et al (21) for bluegills of 3/4" to 1" and 2" to 2 1/2" fork lengths were 0.032, 0.030, and 0.029 (3130/1, 3340/1, and 3450/1) percent and 0.044, 0.044, and 0.044 (2280/1, 2280/1, and 2280/1) percent by volume of fuel consumed, respectively. Corresponding average TL_{50} values obtained in this study for spring collected scuds, dragonfly nymphs, and damselfly nymphs are 0.030, 0.02945, and 0.02945 (3340/1, 3400/1, and 3400/1) percent, 0.186, 0.164, and 0.157 (540/1, 610/1, and 635/1) percent, and 0.1625, 0.155, and 0.1385 (615/1, 645/1, and 720/1)

Table 6. Summary of Average TL_{50} Values for Acute Lethal Toxicity of OMSE-Water to Various Aquatic Biota.

Species	Fork Length (inches)	Season Collected	TL_{50} Values: % By Vol. Fuel Consumed 24 Hr	48 Hr	96 Hr	Parts Dil. Water/ Parts of Fuel Consumed 24 Hr	48 Hr	96 Hr
OTHER STUDIES								
Fathead Minnow(78)	1 1/2-2	Spring	0.017	0.017	0.014	6000/1	6000/1	7150/1
Fathead Minnow (78)	1 1/2-2	Summer	0.047	0.038	0.032	2150/1	2640/1	3130/1
Bluegill (78)	3/4-1	Summer	0.032	0.030	0.029	3130/1	3340/1	3450/1
Bluegill (78)	2-2 1/2	Summer	0.044	0.044	0.044	2280/1	2280/1	2280/1
Fathead Minnow (45)	-	-	0.0569	0.0556	0.0556	1760/1	1800/1	1800/1
Bluegill (45)	-	-	0.0700	0.0513	0.0513	1430/1	1950/1	1950/1
THIS STUDY								
Scud	1/2-5/8	Spring	0.030	0.02945	0.02945	3340/1	3400/1	3400/1
Dragonfly nymph	3/4-7/8	Spring	0.186	0.164	0.157	540/1	610/1	635/1
Damselfly nymph	1/2-5/8	Spring	0.1625δ	0.155	0.1385	615/1δ	645/1	720/1
Dragonfly nymph	3/4-7/8	Summer	0.128*	0.124	0.121	780/1*	810/1	830/1
Damselfly nymph	1/2-5/8	Summer	0.114*	0.0960	0.0931	880/1*	1040/1	1080/1

*By Extrapolation

δCombined data

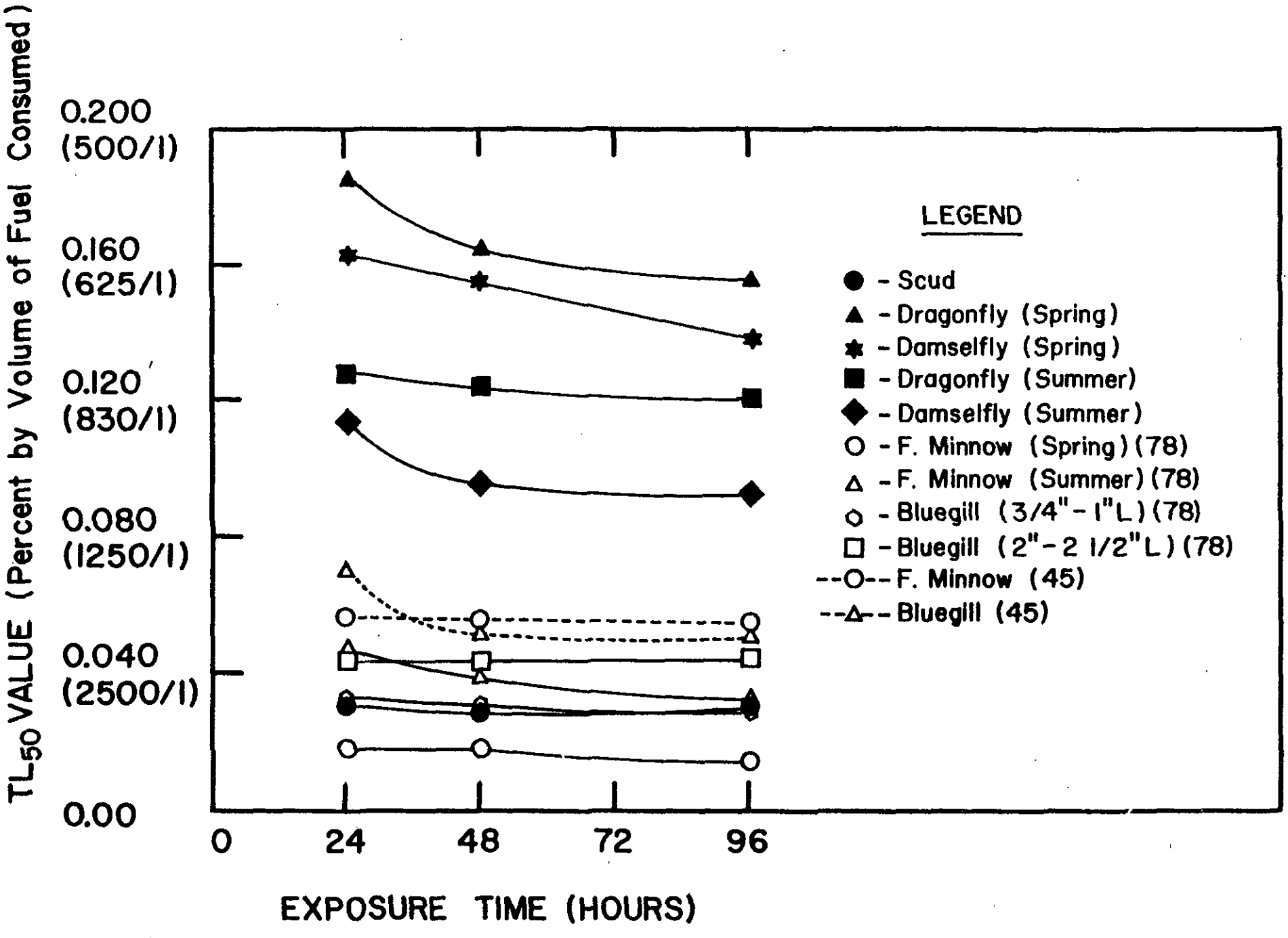

Figure 12. Plot of Average TL_{50} Values for Various Aquatic Biota (This Study and Others (45, 78)) *versus* Exposure Time to OSME-Water.

percent by volume of fuel consumed, respectively. Average 24, 48 and 96-hour TL_{50} values for summer collected dragonfly and damselfly nymphs for this study are 0.128, 0.124, and 0.121 (780/1, 810/1, and 830/1) percent, and 0.114, 0.0960, and 0.0931 (880/1, 1040/1, and 1080/1) percent, respectively.

It would appear, based on the survivor curves in Figure 12, that OMSE-water is more toxic to fish than it is to benthic invertebrates, except for spring collected scuds which appear to be as sensitive and even more sensitive to the toxic effects of OMSE-water than the various fish tested with the one exception of fathead minnows (1 1/2" to 2" fork length) collected during the spring. Dragonfly and damselfly nymphs collected in both the spring and summer appear to be far less sensitive to the toxic effects of OMSE-water than are bluegills, fathead minnows, and scuds. The shape of the survivor curves suggests that an exposure period of 96-hours is ample time to determine a lethal concentration for 50 percent survival for these latter three aquatic species. Perhaps the best test species for use as an indicator based on the plot of average TL_{50} values in Figure 12, of the acute toxic effects of OMSE-water would be spring collected fathead minnows with bluegills and scuds being additional aquatic test species for supplemental information. However, of these three aquatic biota, scuds were the easiest to collect and, with this in mind, they may be the species of choice for acute toxicity bioassays.

Several application factors have been suggested by various investigators (20, 21, 22) to establish a 'safe concentration' of toxicant for the protection of aquatic life based upon the 96-hour TL_{50} value obtained from acute lethal toxicity bioassays. These application factors include the 0.1 times the 96-hour TL_{50} as utilized by English, et al (20) from their fish toxicity studies, the 0.05 times the 96-hour TL_{50} value recommended by the Ohio River Valley Water

Sanitation Commission (22), and the 0.05 times the 96-hour TL_{50} values for the establishment by Kuzminski, et al (21) of possible 'safe concentrations' of OMSE-water to bluegills and fathead minnows. The application factor (0.05) of more recent studies (21,22) was applied to the results obtained for invertebrates. The average 96-hour TL_{50} for spring collected scuds, dragonfly nymphs, and damselfly nymphs are 0.02945, 0.157, and 0.1385 (3400/l, 635/l, and 720/l) percent by volume of fuel consumed, respecgively; whereas, the average 96-hour TL_{50} values for summer collected dragonfly and damselfly nymphs are 0.121 and 0.0931 (830/l and 1080/l) percent by volume of fuel consumed, respectively; whereas, the average 96-hour TL_{50} values for summer collected dragonfly and damselfly nymphs are 0.121 and 0.0931 (830/l and 1080/l) percent, respectively. Application of the safety factors to these 96-hour TL_{50} values give possible safe concentrations of OMSE-water of 0.00147, 0.00785, 0.00692 (68,000/l, 12,800/l, and 14,400/l) percent by volume of fuel consumed, respectively for spring collected scuds, dragonfly and damselfly nymphs. Possible safe concentrations of OMSE-water for summer collected dragonfly and damselfly nymphs are 0.00605 and 0.00466 (16,600/l and 21,600/l) percent, respectively. Application of these safety factors to yield possible safe concentrations is questionable since the safety factors are only hypothetical values and do not appear to be based upon any substantiating data.

Physical response of invertebrates to OMSE-water. During the course of experimentation the physical response of invertebrates when introduced into OMSE-water was noted. Dragonflies in both test and control chambers, demonstrate the least dramatic response by just settling to the bottom of the test chamber and exhibiting very little movement, when exposed to OMSE-water. Damselflies exhibited the same response as dragonflies when exposed to dilute test concentrations of OMSE-water; however, increases in physical movement and shedding of

tracheal gills for some test individuals occurred in the test chambers containing more OMSE-products. Actual measurement of the test concentration at which increased movement was noted was not recorded since this type of response was not the primary objective of the bioassays.

Perhaps the most dramatic responses of exposure to OMSE-water were exhibited by the test scuds. In all test concentrations, 0.006 to 0.200 (16,700/1 to 500/1) percent by volume of fuel consumed, increased physical movement was noted with respect to identical test specimens in the control and stock chambers when the test scuds were introduced into test chambers containing OMSE-products. The degree of physical movement increased (possibly indicating a sensory stress) as the concentration of OMSE-products increased in the test chambers.

Value as an indicator of the degree of pollution may possibly be made by noting the response of scuds when exposed to various suspect pollutants. This could then serve as a valuable rapid indicator organism to either supplement acute toxicity bioassay (TL_{50}) values or even replace toxicity testing if physical response can be justified as a measure of the degree of pollution. In any case, additional experimentation to ascertain the value of a rapid response test (with scuds as the test species) as a measure of pollution levels of various compounds, may reasonably be justified.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. The average 24, 48, and 96-hour TL_{50} values based on static bioassays for spring collected adult scuds were 0.0300, 0.02945, and 0.02945 (3340/1, 3400/1, and 3400/1) percent by volume of fuel consumed, respectively.
2. The average 24, 48, and 96-hour TL_{50} values based on static bioassays for spring and summer collected dragonfly nymphs were 0.186, 0.164, and, and 0.157 (540/1, 610/1, and 635/1) and 0.128, 0.124, and 0.121 (780/1, 810/1, and 830/1) percent by volume of fuel consumed, respectively.
3. The average 24, 48, and 96-hour TL_{50} values based on static bioassays for spring and summer collected damselfly nymphs were 0.1625, 0.155, and 0.1385 (615/1, 645/1, and 720/1) and 0.114, 0.0969, and 0.0931 (880/1, 1040/1, and 1080/1) percent concentration of OMSE-water, respectively. In addition, the 24, 48, and 96-hour TL_{50} values based on static bioassays for summer collected damselfly nymphs exposed to OMSE-water generated at an outboard motor engine speed of 3800 $\pm$ 100 rpm (full throttle) were 0.183, 0.176, and 0.168 (550/1, 570/1, and 590/1) percent by volume of fuel consumed, respectively.
4. Based on a dilutory application factor of 0.05 and the average 96-hour TL_{50} values, possible safe concentrations of OMSE-water for adult scuds, dragonfly nymphs, and damselfly nymphs are 0.00147 (68,000/1), 0.00465 (21,600/1), and 0.00605 (16,600/1) percent by volume of fuel consumed, respectively.
5. With respect to invertebrates collected during the spring season, adult scuds were the most sensitive and dragonfly nymphs the least sensitive to OMSE-water.
6. Summer collected dragonfly and damselfly nymphs were more sensitive to OMSE-water than similar spring collected species.

7. OMSE-water generated at an outboard motor engine speed of 1700 ± 100 rpm (trolling) was more toxic to summer collected dragonfly nymphs than was OMSE-water generated at outboard motor engine speeds of 3800 ± 100 rpm (full throttle).
8. The lethal concentration for 50 percent survival of spring collected adult scuds occurred for most cases during the first 24 hours of exposure to OMSE-water while acute lethality as measured by a 96-hour TL_{50} value for spring and summer collected dragonfly and damselfly nymphs did not occur during the full 96-hour exposure period to OMSE-water.
9. Spring collected dragonfly and damselfly nymphs were relatively equal in sensitivity to OMSE-water.
10. All test invertebrates exhibited a physical motor response at concentrations of OMSE-water less than the derived 24, 48, and 96-hour TL_{50} values for the test species. The most dramatic physical response when exposed to OMSE-water was demonstrated by the spring collected adult scuds.
11. Adult scuds because of their swift sensitivity and ease of collection could be used as a rapid response indicator of pollution levels of compounds similar to those found present in OMSE-water.
12. Based on average TL_{50} values, dragonfly and damselfly nymphs are less sensitive to OMSE-water than fathead minnows or bluegills based on similar previously published values for the fish species (20,21) ; however, spring collected adult scuds were more sensitive to OMSE-water (with the exception of spring collected fathead minnows)than were fathead minnows and bluegills.

RECOMMENDATIONS

1. That further tests be conducted on other invertebrate species to determine their relative motor responses and acute lethality reactions to OMSE-water at different periods of the year and different stages in life cycles.
2. That long term exposure studies using a continuous flow system be performed on invertebrates for sublethal responses such as reproduction, growth, respiration, and avoidance behavior.
3. That a study to establish a minimum safe dissolved oxygen level and weight of creature per liter of test water for invertebrates employed as test specimens in acute toxicity bioassays.
4. That the components of OMSE-water that are lethal to invertebrates perhaps by a chemical autopsy method be identified. Possible sites of susceptibility to OMSE-compounds could be those associated with oxygen transfer or nervous system enzymology.
5. That the bearing that the physical and chemical nature of receiving waters has upon the toxicity of OMSE-water to invertebrates be estimated.
6. That a rapid biological pollution indicatorusing the motor responses and acute lethality of adult scuds or other appropriate aquatic invertebrate species after exposure to the pollutant as the criterion for estimating pollution levels of compounds similar to those found present in OMSE-water be developed.

7. That field studies be conducted to verify the TL_{50} values and safe concentrations derived in this study and to assess the quantity of hydrocarbons present in OMSE-water which are adsorbed onto benthic deposits and its effects on other segments of the benthic community (Annelids, Poriferas, Pelecopods, etc.).

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LITERATURE CITED

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APPENDIX

APPENDIX A - Benthic Invertebrate Acute Toxicity Data

Table A-1. Survivor Data for Spring Collected Scuds (1/2" to 5/8"L) Exposed to OMSE-Water - Test No. 1.

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
11a	0.0125	8000/1	2	2	2	1	6.1	6.9	6.8	6.8	7.4	7.4	8.3	8.0
11b	"	"	2	2	2	2	6.1	6.8	6.8	6.8	7.5	7.5	8.4	8.0
11c	"	"	2	2	1	1	6.2	6.9	6.8	6.8	7.6	7.5	8.3	8.1
11d	"	"	2	2	2	2	6.1	6.8	6.8	6.7	7.6	7.5	8.2	8.0
11e	"	"	2	2	2	2	6.2	6.8	6.8	6.8	7.5	7.6	8.3	8.1
12a	0.020	5000/1	2	0	0	0	5.9	6.8	-	-	7.5	7.5	-	-
12b	"	"	2	2	2	2	6.0	6.7	6.9	6.8	7.5	7.5	8.2	8.0
12c	"	"	2	1	1	1	6.0	6.7	6.8	6.8	7.4	7.6	8.2	8.0
12d	"	"	2	2	2	1	5.9	6.7	6.8	6.8	7.5	7.6	8.1	8.0
12e	"	"	2	2	2	2	6.0	6.8	6.9	6.8	7.5	7.6	8.1	8.1
13a	0.032	3130/1	2	1	1	1	5.8	6.7	6.9	6.8	7.3	7.4	8.1	7.7
13b	"	"	2	1	1	1	5.8	6.7	6.8	6.7	7.4	7.5	8.0	8.0
13c	"	"	2	1	1	1	5.8	6.8	6.8	6.7	7.4	7.6	8.2	8.1
13d	"	"	2	0	0	0	5.8	6.7	-	-	7.5	7.6	-	-
13e	"	"	2	2	2	2	5.8	6.7	6.8	6.8	7.3	7.6	8.1	8.0
14a	0.050	2000/1	2	0	0	0	5.7	6.6	-	-	7.3	7.5	-	-
14b	"	"	2	0	0	0	5.8	6.7	-	-	7.4	7.5	-	-
14c	"	"	2	0	0	0	5.7	6.7	-	-	7.4	7.6	-	-
14d	"	"	2	0	0	0	5.7	6.7	-	-	7.4	7.4	-	-
14e	"	"	2	0	0	0	5.7	6.7	-	-	7.3	7.6	-	-

Table A-1. Survivor Data for Spring Collected Scuds, Continued

Jar	Concentration of OMSE-Water Percent	Dil.Fac.	No. Surviving Hours 0	24	48	96	pH Hours 0	24	48	96	D.O. (mg/l) Hours 0	24	48	96
15a	0.080	1250/1	2	0	0	0	5.2	6.6	-	-	7.0	7.5	-	-
15b	"	"	2	0	0	0	5.3	6.7	-	-	7.1	7.4	-	-
15c	"	"	2	0	0	0	5.3	6.6	-	-	7.0	7.5	-	-
15d	"	"	2	0	0	0	5.4	6.7	-	-	7.1	7.4	-	-
15e	"	"	2	0	0	0	5.2	6.5	-	-	7.1	7.4	-	-
16a	0.125	800/1	2	0	0	0	4.8	6.5	-	-	6.4	7.5	-	-
16b	"	"	2	0	0	0	4.9	6.4	-	-	6.5	7.6	-	-
16c	"	"	2	0	0	0	4.8	6.1	-	-	6.4	7.6	-	-
16d	"	"	2	0	0	0	4.9	6.2	-	-	6.5	7.4	-	-
16e	"	"	2	0	0	0	4.9	6.4	-	-	6.4	7.5	-	-
17a	0.200	500/1	2	0	0	0	4.7	5.6	-	-	6.1	7.5	-	-
17b	"	"	2	0	0	0	4.6	5.7	-	-	6.1	7.6	-	-
17c	"	"	2	0	0	0	4.7	5.7	-	-	6.1	7.5	-	-
17d	"	"	2	0	0	0	4.7	5.6	-	-	6.1	7.4	-	-
17e	"	"	2	0	0	0	4.7	5.7	-	-	6.0	7.6	-	-
1ca			2	2	2	2	6.6	6.7	6.8	6.9	7.7	7.6	8.3	8.0
1cb			2	2	2	2	6.7	6.8	6.7	6.7	7.6	7.6	8.2	7.9
1cc	Control		2	2	2	2	6.6	6.7	6.8	6.7	7.5	7.6	8.2	8.0
1cd			2	2	2	2	6.7	6.9	6.8	6.8	7.7	7.6	8.1	7.9
1ce			2	2	2	2	6.7	6.8	6.8	6.8	7.7	7.5	8.3	8.1

Table A-2. Composite Survivior Data for Spring Collected Scuds - Test No. 1

Dilution Factor	8000/1	5000/1	3130/1	2000/1	1250/1	800/1	500/1	Control
Percent of OMSE-Water	0.0125	0.020	0.032	0.050	0.080	0.125	0.200	-
Start								
D.O. (mg/l)	7.4-7.6	7.4-7.5	7.3-7.5	7.3-7.4	7.0-7.1	6.4-6.5	6.0-6.1	7.6-7.7
pH	6.1-6.2	5.9-6.1	5.8	5.7-5.8	5.2-5.4	4.8-4.9	4.6-4.7	6.6-6.7
Temperature (^{o}C)	20	20	20	20	20	20	20	20
24 Hours								
% Surviving	100	70	50	0	0	0	0	100
D.O. (mg/l)	7.4-7.6	7.5-7.6	7.4-7.6	7.4-7.6	7.4-7.5	7.4-7.6	7.4-7.6	7.5-7.6
pH	6.8-6.9	6.7-6.8	6.7-6.8	6.6-6.7	6.5-6.7	6.1-6.5	5.6-5.7	6.7-6.9
Temperature (^{o}C)	20	20	20	20	20	20	20	20
48 Hours								
% Surviving	90	70	50	0	0	0	0	100
D.O. (mg/l)	8.2-8.4	8.1-8.2	8.0-8.2	-	-	-	-	8.1-8.3
pH	6.8	6.8-6.9	6.8-6.9	-	-	-	-	6.7-6.8
Temperature (^{o}C)	20	20	20	-	-	-	-	20
96 Hours								
% Surviving	80	60	50	0	0	0	0	100
D.O. (mg/l)	8.1-8.2	8.0-8.1	7.7-8.1	-	-	-	-	7.9-8.1
pH	6.7-6.8	6.8	6.7-6.8	-	-	-	-	6.7-6.9
Temperature (^{o}C)	20	20	20	-	-	-	-	20

Table A-3. Survivor Data for Spring Collected Scuds (1/2" to 5/8" L) Exposed to OMSE-Water - Test No. 2

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
21a	0.006	16700/1	2	2	2	2	6.5	6.9	7.1	6.9	7.3	8.0	7.3	7.5
21b	"	"	3	3	3	3	6.6	7.0	7.0	7.0	7.7	7.5	7.5	7.7
21c	"	"	2	2	2	2	6.7	6.9	7.0	7.0	8.0	7.3	7.3	7.5
21d	"	"	3	3	3	3	6.6	6.7	7.0	7.0	8.0	7.3	7.5	7.5
22a	0.0095	10500/1	2	2	2	2	6.5	6.7	7.0	6.9	8.0	7.5	7.7	7.5
22b	"	"	3	3	3	3	6.6	6.7	7.0	7.0	8.0	7.7	7.7	7.5
22c	"	"	2	2	2	2	6.8	6.9	7.0	6.9	8.0	7.7	7.8	7.7
22d	"	"	3	3	3	3	6.7	6.8	7.0	6.9	7.8	7.8	7.3	7.7
23a	0.015	6670/1	2	2	2	2	6.2	6.7	7.0	7.0	8.0	7.7	7.7	7.8
23b	"	"	3	3	3	3	6.2	6.9	7.0	7.1	8.0	7.7	7.5	7.7
23c	"	"	2	2	2	2	6.3	6.8	7.0	7.0	8.0	7.7	7.7	7.5
23d	"	"	3	2	2	2	6.3	6.9	7.0	7.1	8.2	7.7	7.7	7.7
24a	0.024	4160/1	2	2	2	2	6.1	6.7	7.0	7.0	8.0	7.7	7.7	7.8
24b	"	"	3	1	1	1	6.3	6.7	6.9	6.9	8.0	7.8	7.8	7.5
24c	"	"	2	1	1	1	6.2	6.8	6.8	7.0	7.8	7.8	7.7	7.7
24d	"	"	3	2	2	2	6.3	6.7	7.0	7.1	8.0	7.8	7.5	7.7

Table A-3. Survivor Data for Spring Collected Scuds, Continued

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
25a	0.038	2640/1	2	1	1	1	6.2	6.5	6.9	6.8	7.8	7.8	7.8	7.7
25b	"	"	3	0	0	0	6.4	6.4	6.9	-	7.8	7.8	7.8	-
25c	"	"	2	1	0	0	6.0	6.4	7.0	-	7.7	7.8	7.8	-
25d	"	"	3	1	1	1	6.2	6.7	6.9	6.9	7.8	7.8	7.8	7.8
26a	0.060	1670/1	2	0	0		6.0	6.7	-	-	7.7	8.2	-	-
26b	"	"	3	0	0		5.9	6.7	-	-	7.8	8.2	-	-
26c	"	"	2	1	0	0	6.0	6.8	6.9	-	7.8	8.0	7.7	-
26d	"	"	3	1	0		5.8	6.5	6.9	-	7.7	7.7	7.7	-
2ca	Control		2	2	2	2	6.6	6.8	7.0	7.1	8.2	7.7	7.7	7.7
2cb			3	3	3	3	6.6	6.8	7.0	7.0	8.3	7.7	7.7	7.7
2cc			2	2	2	2	6.8	6.8	7.0	6.9	8.2	7.7	7.8	7.7
2cd			3	3	3	3	6.7	6.8	7.0	7.1	8.3	7.7	7.7	7.7

Table A-4. Composite Survivor Data for Spring Collected Scuds - Test No. 2

Dilution Factor	16700/1	10500/1	6670/1	4160/1	2640/1	1670/1	Control
Percent of OMSE-Water	0.006	0.0095	0.0150	0.024	0.038	0.060	-
Start							
D.O. (mg/l)	7.3-8.0	7.8-8.0	8.0-8.2	7.8-8.0	7.7-7.8	7.7-7.8	8.2-8.3
pH	6.5-6.7	6.5-6.8	6.2-6.3	6.1-6.3	6.0-6.2	5.8-6.0	6.6-6.8
Temperature (^{o}C)	20	20	20	20	20	20	20
24 Hours							
% Surviving	100	100	90	60	30	20	100
D.O. (mg/l)	7.3-8.0	7.5-7.8	7.7	7.7-7.8	7.8	7.7-8.2	7.7
pH	6.7-7.0	6.7-6.9	6.7-6.9	6.7-6.8	6.4-6.7	6.5-6.8	6.8
Temperature (^{o}C)	20	20	20	20	20	20	20
48 Hours							
% Surviving	100	100	90	60	20	0	100
D.O. (mg/l)	7.3-7.5	7.3-7.8	7.5-7.7	7.5-7.8	7.8	7.7	7.7-7.8
pH	7.0-7.1	7.0	7.0	6.8-7.0	6.9-7.0	6.9	7.0
Temperature (^{o}C)	20	20	20	20	20	20	20
96 Hours							
% Surviving	100	100	90	60	20	0	100
D.O. (mg/l)	7.5-7.7	7.5-7.7	7.5-7.8	7.5-7.8	7.7-7.8	-	7.7
pH	6.9-7.0	6.9-7.0	7.0-7.1	6.9-7.1	6.9	-	6.9-7.1
Temperature (^{o}C)	20	20	20	20	20	20	20

Table A-5. Survivor Data for Spring Collected Nymphs of Dragonflies (3/4" to 7/8" L) Exposed to OMSE-Water - Test No. 3

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
11a	0.020	5000/1	3	3	3	3	6.2	6.6	6.8	6.4	8.0	7.2	7.2	7.7
11b	"	"	3	3	3	3	6.1	6.6	6.8	6.9	8.0	7.2	7.0	7.3
11c	"	"	4	4	4	4	6.3	6.6	6.8	7.0	7.8	7.3	6.9	7.2
12a	0.0317	3150/1	3	3	3	3	5.8	6.5	6.8	6.9	8.0	7.3	7.2	7.5
12c	"	"	3	3	3	3	5.8	6.4	6.8	6.9	8.0	7.0	7.0	7.5
12b	"	"	4	4	4	4	6.0	6.4	6.8	7.0	8.0	7.2	7.3	7.3
13a	0.050	2000/1	3	3	3	2	5.9	6.6	6.8	6.8	7.8	7.7	7.3	7.5
13b	"	"	3	3	3	3	5.9	6.7	6.8	6.7	7.7	7.8	7.7	7.5
13c	"	"	4	4	4	4	5.8	6.5	6.7	6.8	7.7	7.5	7.3	7.7
14a	0.080	1250/1	3	3	3	3	5.5	6.3	6.7	6.8	7.3	7.3	7.3	7.3
14b	"	"	3	3	3	2	5.6	6.6	6.7	6.9	7.5	7.7	7.3	7.5
14c	"	"	4	4	4	4	5.5	6.6	6.6	6.9	7.3	7.8	7.7	7.3
15a	0.131	760/1	3	3	3	3	5.2	6.5	6.7	6.8	6.9	7.8	7.7	7.5
15b	"	"	3	2	2	2	5.3	6.5	6.7	6.9	7.0	8.0	7.7	7.7
15c	"	"	4	4	3	3	5.2	6.5	6.7	6.8	7.0	8.0	7.7	7.5

Table A-5. Survivor Data for Spring Collected Nymphs of Dragonflies, Continued

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
16a	0.200	500/1	3	1	0	0	4.9	6.3	6.6	6.7	6.7	8.0	7.8	7.7
16b	"	"	3	1	0	0	5.0	6.2	6.6	7.0	6.4	8.0	7.8	7.5
16c	"	"	4	2	2	1	4.9	6.1	6.6	6.8	6.4	8.0	8.0	7.8
1ca			3	3	3	3	6.7	6.6	6.9	6.9	8.0	7.7	7.7	7.7
1cb	Control		3	3	3	3	6.8	6.4	6.7	6.9	7.8	7.0	7.0	7.3
1cc			4	4	4	4	6.7	6.6	6.9	7.0	7.8	7.8	7.7	7.5

Table A-6. Composite Data for Spring Collected Nymphs of Dragonflies - Test No. 3

Dilution Factor	5000/1	3150/1	2000/1	1250/1	760/1	500/1	Control
Percent of OMSE-Water	0.020	0.0317	0.050	0.080	0.131	0.200	-
Start							
D.O. (mg/l)	7.8-8.0	8.0	7.7-7.8	7.3-7.5	6.9-7.0	6.4-6.7	7.8-8.0
pH	6.1-6.3	5.8-6.0	5.8-5.9	5.5-5.6	5.2-5.3	4.9-5.0	6.7-6.8
Temperature (^{o}C)	20	20	20	20	20	20	20
24 Hours							
% Surviving	100	100	100	100	90	40	100
D.O. (mg/l)	7.2-7.3	7.0-7.3	7.5-7.8	7.3-7.8	7.8-8.0	8.0	7.7-8.0
pH	6.6	6.4-6.5	6.5-6.7	6.3-6.6	6.5	6.1-6.3	6.4-6.6
Temperature (^{o}C)	20	20	20	20	20	20	20
48 Hours							
% Surviving	100	100	100	100	80	20	100
D.O. (mg/l)	6.9-7.2	7.0-7.3	7.3-7.7	7.3-7.7	7.7	7.8-8.0	7.0-7.7
pH	6.8	6.8	6.7-6.8	6.6-6.7	6.7	6.6	6.7-6.9
Temperature (^{o}C)	20	20	20	20	20	20	20
96 Hours							
% Surviving	100	100	90	90	80	10	100
D.O. (mg/l)	7.7-7.7	7.3-7.5	7.5-7.7	7.3-7.5	7.5-7.7	7.5-7.8	7.3-7.7
pH	6.9-7.0	6.8-6.9	6.7-6.8	6.8-6.9	6.8-6.9	6.7-7.0	6.9-7.0
Temperature (^{o}C)	20	20	20	20	20	20	20

Table A-7. Survivor Data for Spring Collected Nymphs of Dragonflies (3/4" to 7/8"L) Exposed to OMSE-Water - Test No. 4.

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
21a	0.063	1590/1	2	2	2	2	6.2	6.5	6.9	6.9	7.5	6.7	6.4	6.6
21b	"	"	2	2	2	2	6.2	6.5	6.8	6.9	7.5	6.4	6.1	6.6
21c	"	"	2	2	2	2	6.2	6.4	6.7	7.0	7.5	6.5	6.0	6.7
21d	"	"	2	2	2	2	6.2	6.6	6.9	7.0	7.5	6,9	6.7	7.0
21e	"	"	2	1	1	1	6.2	6.5	7.0	6.9	7.5	6.9	7.0	7.0
22a	0.100	1000/1	2	2	2	2	6.0	6.5	6.8	6.9	7.0	7.0	6.7	6.9
22b	"	"	2	2	2	2	6.0	6.6	6.9	7.0	7.0	6.9	6.7	6.6
22c	"	"	2	2	2	2	5.9	6.5	6.9	7.0	7.0	7.0	6.7	6.7
22d	"	"	2	2	2	2	6.0	6.5	6.9	6.9	7.0	6.9	6.9	6.9
22e	"	"	2	2	2	2	5.9	6.5	6.8	6.9	6.9	6.9	6.6	6.9
23a	0.115	870/1	2	2	2	1	5.8	6.5	6.9	6.8	6.7	7.0	6.7	6.7
23b	"	"	2	2	2	1	5.8	6.1	6.7	6.7	6.7	6.6	6.4	6.4
23c	"	"	2	2	1	2	5.9	6.4	6.8	6.8	6.9	6.7	6.4	6.7
23d	"	"	2	2	2	2	5.9	6.4	6.8	6.9	6.9	6.7	6.7	6.6
23e	"	"	2	2	2	1	5.9	6.4	6.9	6.9	6.9	6.9	6.7	6.7
24a	0.155	645/1	2	2	2	1	5.7	6.2	6.8	6.7	6.6	6.9	6.9	6.9
24b	"	"	2	2	2	1	5.7	6.3	6.8	6.8	6.7	6.9	6.7	6.6
24c	"	"	2	2	1	1	5.7	6.1	6.8	6.9	6.7	6.7	6.7	6.4
24d	"	"	2	2	2	1	5.7	6.1	6.8	6.8	6.7	6.7	6.7	6.7
24e	"	"	2	2	2	2	5.7	6.2	6.8	6.8	6.6	6.3	6.6	6.7

Table A-7. Survivor Data for Spring Collected Nymphs of Dragonflies, Continued

Jar	Conccentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
25a	0.175	570/1	2	2	0	0	5.5	6.0	6.8	6.7	6.4	6.9	6.9	6.9
25b	"	"	2	2	2	0	5.5	6.1	6.7	6.7	6.4	6.7	6.9	6.9
25c	"	"	2	0	0	0	5.5	5.8	6.7	6.7	6.4	6.2	7.0	6.9
25d	"	"	2	1	0	0	5.5	5.8	6.5	6.6	6.4	6.1	6.7	6.9
25e	"	"	2	1	0	0	5.5	5.8	6.6	6.7	6.4	6.4	6.9	6.7
2ca			2	2	2	2	6.8	6.3	6.9	7.0	7.7	7.0	6.7	7.0
2cb			2	2	2	2	6.8	6.3	6.9	7.0	7.7	6.7	6.7	7.2
2cc	Control		2	2	2	2	6.8	6.9	7.0	7.0	7.7	6.9	6.9	7.0
2cd			2	2	2	2	6.8	6.9	7.0	6.9	7.7	6.9	7.0	6.9
2ce			2	2	2	2	6.8	6.9	7.0	7.0	7.7	7.0	7.2	6.7

Table A-8. Composite Data for Spring Collected Nymphs of Dragonflies - Test No. 4

Dilution Factor	1590/1	1000/1	870/1	645/1	570/1	Control
Percent of OMSE-Water	0.063	0.100	0.115	0.155	0.175	-
Start						
D.O. (mg/l)	7.5	6.9-7.0	6.7-6.9	6.6-6.7	6.4	7.7
pH	6.2	5.9-6.0	5.8-5.9	5.7	5.5	6.8
Temperature (^{o}C)	20	20	20	20	20	20
24 Hours						
% Surviving	90	100	100	100	60	100
D.O. (mg/l)	6.4-6.9	6.9-7.0	6.6-7.0	6.7-6.9	6.1-6.9	6.7-7.0
pH	6.4-6.6	6.5-6.6	6.1-6.5	6.1-6.3	5.3-6.1	6.8-6.9
Temperature (^{o}C)	20	20	20	20	20	20
48 Hours						
% Surviving	90	100	90	90	20	100
D.O. (mg/l)	6.4-7.0	6.6-6.9	6.4-6.7	6.6-6.9	6.9-7.0	6.7-7.2
pH	6.7-7.0	6.8-6.9	6.7-6.9	6.8	6.5-6.8	6.9-7.0
Temperature (^{o}C)	20	20	20	20	20	20
96 Hours						
% Surviving	90	100	70	60	0	100
D.O. (mg/l)	6.6-7.0	6.6-6.9	6.4-6.7	6.4-6.9	6.7-6.9	6.7-7.2
pH	6.9-7.0	6.9-7.0	6.7-6.9	6.7-6.9	6.6-6.7	6.9-7.0
Temperature (^{o}C)	19	19	19	19	19	19

Table A-9. Survivor Data for Spring Collected Nymphs of Damselflies (1/2" to 5/8" L) Exposed to OMSE-Water - Test No. 5.

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
11a	0.050	2000/1	2	2	2	2	6.3	6.6	7.1	6.5	6.6	6.9	6.6	6.7
11b	"	"	2	2	2	2	6.3	6.6	7.1	6.4	6.4	6.9	6.6	6.7
11c	"	"	2	2	2	2	6.3	6.6	7.0	6.6	6.6	6.9	6.6	6.6
11d	"	"	2	2	2	1	6.3	6.6	7.1	6.6	6.6	7.0	6.6	6.7
11e	"	"	2	2	2	2	6.3	6.6	7.0	6.4	6.4	6.9	6.7	6.6
12a	0.067	1500/1	2	2	2	2	6.1	6.5	7.0	6.5	6.4	6.9	6.6	6.6
12b	"	"	2	2	2	2	6.1	6.3	7.0	6.5	6.6	6.7	6.6	6.4
12c	"	"	2	2	2	2	6.1	6.5	7.0	6.4	6.6	6.9	6.7	6.6
12d	"	"	2	2	2	2	6.2	6.5	7.0	6.5	6.7	6.7	6.7	6.6
12e	"	"	2	2	2	2	6.2	6.5	7.0	6.5	6.6	6.7	6.6	6.6
13a	0.087	1150/1	2	2	2	2	6.0	6.4	7.0	6.5	6.6	7.0	6.6	6.3
13b	"	"	2	2	2	2	6.0	6.4	7.0	6.4	6.7	7.0	6.7	6.4
13c	"	"	2	2	2	2	6.0	6.4	7.0	6.4	6.7	7.0	6.6	6.4
13d	"	"	2	2	2	2	6.0	6.4	7.0	6.5	6.7	7.0	6.6	6.4
13e	"	"	2	2	2	2	6.0	6.4	7.0	6.5	6.7	7.0	6.7	6.4
14a	0.115	870/1	2	2	2	2	5.8	6.2	6.9	6.4	6.6	7.2	6.7	6.3
14b	"	"	2	2	2	1	5.8	6.3	7.0	6.5	6.4	7.0	6.9	6.3
14c	"	"	2	2	1	1	5.8	6.3	7.0	6.5	6.4	7.0	6.7	6.4
14d	"	"	2	2	1	1	5.8	6.3	7.0	6.5	6.6	7.0	6.7	6.4
14e	"	"	2	2	1	1	5.8	6.3	7.0	6.5	6.4	7.0	6.7	6.4

Table A-9. Survivor Data for Spring Collected Nymphs of Damselflies, Continued

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
15a	0.150	670/1	2	2	1	1	5.6	6.0	6.9	6.4	6.3	6.9	6.7	6.1
15b	"	"	2	1	1	1	5.6	6.0	7.0	6.5	6.4	7.0	6.7	6.3
15c	"	"	2	1	1	0	5.6	5.9	7.0	6.5	6.3	7.0	6.7	6.3
15d	"	"	2	1	1	1	5.6	5.9	7.0	6.5	6.6	7.0	6.9	6.3
15e	"	"	2	1	1	1	5.6	6.0	7.1	6.5	6.3	6.9	6.9	6.3
16a	0.200	500/1	2	0	0	0	5.5	6.4	-	-	6.0	7.3	-	-
16b	"	"	2	0	0	0	5.5	6.1	-	-	5.9	7.3	-	-
16c	"	"	2	1	1	1	5.4	6.0	7.2	6.4	6.0	7.3	7.0	6.4
16d	"	"	2	0	0	0	5.5	6.2	-	-	6.1	7.3	-	-
16e	"	"	2	0	0	0	5.5	6.1	-	-	6.0	7.3	-	-
1ca			2	2	2	2	6.9	6.9	7.1	6.6	7.0	7.0	6.7	6.4
1cb			2	2	2	2	6.9	7.0	7.3	6.6	7.2	7.0	6.7	6.6
1cc	Control		2	2	2	2	7.0	7.0	7.3	6.6	7.0	7.2	6.7	6.6
1cd			2	2	2	2	6.9	7.0	7.3	6.6	7.0	7.0	6.7	6.6
1ce			2	2	2	2	6.9	7.0	7.3	6.6	7.0	7.2	6.7	6.6

Table A-10. Composite Data for Spring Collected Nymphs of Damselflies - Test No. 5

Dilution Factor	2000/1	1500/1	1150/1	870/1	670/1	500/1	Control
Percent of OMSE-Water	0.050	0.067	0.087	0.115	0.150	0.200	-
Start							
D.O. (mg/l)	6.4-6.6	6.4-6.7	6.6-6.7	6.4-6.6	6.3-6.6	5.9-6.1	7.0-7.2
pH	6.3	6.1-6.2	6.0	5.8	5.6	5.4-5.5	6.9-7.0
Temperature (^{o}C)	20	20	20	20	20	20	20
24 Hours							
% Surviving	100	100	100	100	60	10	100
D.O. (mg/l)	6.7-7.0	6.7-6.9	7.0	7.0-7.2	6.9-7.0	7.3	7.0-7.2
pH	6.5-6.6	6.3-6.5	6.4	6.2-6.3	5.9-6.0	6.0-6.4	6.9-7.0
Temperature (^{o}C)	20	20	20	20	20	20	20
48 Hours							
% Surviving	100	100	100	70	50	10	100
D.O. (mg/l)	6.6-6.7	6.6-6.7	6.6-6.7	6.7-6.9	6.7-6.9	7.0	6.7
pH	7.0-7.1	7.0	7.0	6.9-7.0	6.9-7.1	7.2	7.1-7.3
Temperature (^{o}C)	20	20	20	20	20	20	20
96 Hours							
% Surviving	90	100	100	60	40	10	100
D.O. (mg/l)	6.6-6.7	6.4-6.6	6.3-6.4	6.3-6.4	6.1-6.3	6.4	6.4-6.6
pH	6.4-6.5	6.4-6.5	6.4-6.5	6.4-6.5	6.4-6.5	6.4	6.6
Temperature (^{o}C)	20	20	20	20	20	20	20

Table A-11. Survivor Data for Spring Collected Nymphs of Damselflies (1/2" to 5/8" L) Exposed to OMSE-Water - Test No. 6.

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil. Fac.	0	24	48	96	0	24	48	96	0	24	48	96
21a	0.058	1720/1	2	2	2	2	6.1	6.8	6.9	6.8	7.5	7.2	7.6	8.6
21b	"	"	2	2	2	2	6.1	6.8	6.9	6.9	7.5	7.2	7.6	8.6
21c	"	"	2	2	2	2	6.1	6.8	6.9	6.8	7.5	7.2	7.5	8.6
21d	"	"	2	2	2	2	6.1	6.8	6.9	6.8	7.5	7.0	7.4	8.2
21e	"	"	2	2	2	2	6.1	6.9	6.9	6.8	7.5	7.0	7.5	8.2
22a	0.076	1320/1	2	2	2	2	5.9	6.8	6.9	6.9	7.5	7.2	7.6	8.5
22b	"	"	2	2	2	2	5.9	6.8	6.9	6.7	7.5	7.1	7.4	8.0
22c	"	"	2	2	2	2	5.9	6.8	6.9	6.9	7.5	7.2	7.6	8.2
22d	"	"	2	2	2	2	5.9	6.8	6.8	6.8	7.5	7.2	7.4	8.2
22e	"	"	2	2	2	2	5.9	6.8	6.8	6.8	7.5	7.2	7.5	8.3
23a	0.100	1000/1	2	2	2	2	5.9	6.6	6.8	6.8	7.4	7.2	7.5	8.6
23b	"	"	2	2	2	2	5.9	6.6	6.8	6.8	7.4	7.2	7.5	8.1
23c	"	"	2	2	2	2	5.9	6.6	6.8	6.8	7.4	7.2	7.4	8.1
23d	"	"	2	2	2	2	5.9	6.6	6.8	6.8	7.4	7.2	7.5	8.4
23e	"	"	2	2	2	2	5.9	6.6	6.8	6.8	7.4	7.2	7.5	8.3
24a	0.132	760/1	2	2	2	2	5.7	6.3	6.9	6.8	7.1	7.2	7.5	8.0
24b	"	"	2	2	2	1	5.7	6.3	6.8	6.8	7.1	7.2	7.5	8.3
24c	"	"	2	2	1	0	5.8	6.3	6.7	6.7	7.1	7.2	7.5	8.6
24d	"	"	2	2	2	2	5.8	6.5	6.8	6.7	7.1	7.2	7.5	8.2
24e	"	"	2	2	2	2	5.8	6.5	6.9	6.7	7.1	7.2	7.5	8.4

Table A-11. Survivor Data for Spring Collected Nymphs of Damselflies, Continued

Jar	Concentration of OMSE-Water		No. Surviving Hours				pH Hours				D.O. (mg/l) Hours			
	Percent	Dil.Fac.	0	24	48	96	0	24	48	96	0	24	48	96
25a	0.175	570/1	2	0	0	0	5.5	6.0	6.5	6.7	6.6	6.8	8.0	7.8
25b	"	"	2	1	0	0	5.5	6.0	6.5	6.7	6.6	7.0	7.8	8.4
25c	"	"	2	1	1	1	5.5	6.1	6.6	6.6	6.6	6.8	7.8	8.3
25d	"	"	2	1	1	0	5.5	6.1	6.6	6.6	6.6	7.0	7.7	8.3
25e	"	"	2	1	0	0	5.5	6.2	6.4	6.6	6.6	6.8	7.7	7.8
26a	0.225	445/1	2	0	0	0	5.1	5.6	-	-	6.1	7.0	-	-
26b	"	"	2	1	0	0	5.0	5.6	-	-	6.2	6.9	-	-
26c	"	"	2	0	0	0	5.1	5.8	-	-	6.2	7.0	-	-
26d	"	"	2	0	0	0	5.0	5.7	-	-	6.2	6.9	-	-
26e	"	"	2	0	0	0	4.9	5.6	-	-	6.1	6.9	-	-
2ca	Control		2	2	2	2	7.1	6.9	7.0	7.2	7.4	6.9	7.6	8.3
2cb			2	2	2	2	7.2	6.9	7.0	7.2	7.3	6.9	7.6	8.3
2cc			2	2	2	2	7.2	7.0	7.0	7.2	7.3	7.0	7.6	8.4
2cd			2	2	2	2	7.2	7.0	7.0	7.2	7.4	7.0	7.6	8.4
2ce			2	2	2	2	7.2	7.0	7.0	7.2	7.3	6.9	7.6	8.4

Table A-12. Composite Data for Spring Collected Nymphs of Damselflies - Test No. 6

Dilution Factor	1720/1	1302/1	1000/1	760/1	570/1	445/1	Control
Percent of OMSE-Water	0.058	0.076	0.100	0.132	0.175	0.225	-
Start							
D.O. (mg/l)	7.5	7.5	7.4	7.1	6.6	6.1-6.2	7.3-7.4
pH	6.1	5.9	5.9	5.7-5.8	5.5	4.9-5.1	7.1-7.2
Temperature (^{o}C)	19	19	19	19	19	19	19
24 Hours							
% Surviving	100	100	100	100	40	10	100
D.O. (mg/l)	7.0-7.2	7.1-7.2	7.2	7.2	6.8-7.0	6.9-7.0	6.9-7.0
pH	6.8-6.9	6.8	6.6	6.3-6.5	6.0-6.2	5.6-5.8	6.9-7.0
Temperature (^{o}C)	19	19	19	19	19	19	19
48 Hours							
% Surviving	100	100	100	90	20	0	100
D.O. (mg/l)	7.4-7.6	7.4-7.6	7.4-7.5	7.5	7.7-8.0	-	7.6
pH	6.9	6.8-6.9	6.8	6.7-6.9	6.4-6.6	-	6.9-7.0
Temperature (^{o}C)	21	21	21	21	21	21	21
96 Hours							
% Surviving	100	90	100	70	10	0	100
D.O. (mg/l)	8.2-8.6	8.0-8.5	8.1-8.6	8.0-8.6	7.8-8.4	-	8.3-8.4
pH	6.8-6.9	6.7-6.9	6.8	6.7-6.8	6.6-6.7	-	7.2
Temperature (^{o}C)	20	20	20	20	20	20	20

Table A-13. Survivor Data for Summer Collected Damselfly Nymphs (1/2" to 5/8"L) Exposed to OMSE-Water (Engine at 3800 RPM) - Test No. 7

Jar	Concentration of OMSE-Water Percent	Dil.Fac.	No. Surviving Hours 0	24	48	96	D.O. (mg/l) Initial	Final
11a	0.070	1430/1	5	5	5	5		
11b	"	"	5	5	5	3	7.0	7.1
11c	"	"	5	5	5	4		
11d	"	"	5	5	4	4		
12a	0.086	1160/1	5	5	5	2		
12b	"	"	5	5	5	4	6.9	6.8
12c	"	"	5	5	5	4		
12d	"	"	5	5	5	5		
13a	0.105	950/1	5	5	5	4		
13b	"	"	5	5	5	3	7.2	7.2
13c	"	"	5	5	5	5		
13d	"	"	5	5	5	5		
14a	0.125	800/1	5	5	5	5		
14b	"	"	5	5	5	5	7.0	7.2
14c	"	"	5	4	4	4		
14d	"	"	5	5	5	4		
15a	0.150	670/1	5	5	5	5		
15b	"	"	5	5	4	4	7.0	6.8
15c	"	"	5	5	5	4		
15d	"	"	5	5	5	5		
16a	0.180	560/1	5	3	3	2		
16b	"	"	5	2	1	0	6.8	6.9
16c	"	"	5	4	3	2		
16d	"	"	5	2	2	1		
17a			5	5	5	5		
17b	Control		5	5	5	5	7.4	7.8
17c			5	5	5	5		
17d			5	5	5	4		

Note: pH of stock OMSE-Water solution (400/1) was 5.3 at beginning of bioassays.

Table A-14. Composite Survivor Data for Summer Collected Damselfly Nymphs - Test No. 7

Dilution Factor	1430/1	1160/1	950/1	800/1	670/1	560/1	Control
Percent of OMSE-Water	0.070	0.086	0.105	0.125	0.150	0.180	-
Start							
D.O. (mg/l)	7.0	6.9	7.2	7.0	7.0	6.8	7.4
24 Hours							
% Surviving	100	100	100	95	100	55	100
48 Hours							
% Surviving	95	100	100	95	95	45	100
96 Hours							
% Surviving	80	75	85	90	90	25	95
D.O. (mg/l)	7.1	6.8	7.2	7.2	6.8	6.9	7.8

Table A-15. Summary of Survivor Data for Summer Collected Dragonfly and Damselfly Nymphs - Test No. 8 and Test No. 9.

Test No. and Species	Concentration of OMSE-Water Percent	Dil.Fac.	24 Hours Survivors Total Tested	24 Hours Percent Surviving	48 Hours Survivors Total Tested	48 Hours Percent Surviving	96 Hours Survivors Total Tested	96 Hours Percent Surviving
No. 8 Damselfly Nymphs	0.0303	3300/1	25/28	89	25/28	89	22/28	79
	0.0445	2250/1	21/22	95	20/22	91	18/22	82
	0.0644	1550/1	21/22	95	18/22	82	14/22	64
	0.080	1250/1	24/24	100	23/24	96	22/24	92
	0.100	1000/1	11/16	69	6/16	37.5	5/16	31
	Control	-	22/22	100	22/22	100	22/22	100
No. 9 Dragonfly Nymphs	0.054	1850/1	23/23	100	23/23	100	23/23	100
	0.085	1180/1	18/18	100	18/18	100	17/18	94
	0.092	1080/1	18/18	100	18/18	100	17/18	94
	0.105	950/1	15/18	83	14/18	77	14/18	77
	0.115	870/1	15/18	83	15/18	83	13/18	72
	0.125	800/1	10/18	55.5	8/18	44.5	6/18	33
	Control	-	17/17	100	17/17	100	17/17	100

Note: D.O. initial and D.O. final for all tests is greater than 5.0 mg/l and pH of stock OMSE-water solution (400/1) ranged from 5.1 to 5.3.