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HAZARD ASSESSMENT OF RICE HERBICIDES MOLINATE AND THIOBENCARB TO LARVAL AND JUVENILE STRIPED BASS

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Hazard Assessment of Rice Herbicides Molinate and Thiobencarb to Larval and Juvenile Striped Bass[1,2/]

by

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SUMMARY

Acute toxicity tests with rice herbicides molinate (Ordram®), thiobencarb (Bolero®), and molinate-thiobencarb mixtures on larval and juvenile striped bass Morone saxatilis produced median lethal concentrations (96-h LC50 values) indicating that thiobencarb (0.35 to 0.67 mg/L) was 7 to 21 times more toxic than molinate (2.1 to 14 mg/L), and molinate-thiobencarb mixtures at 1:1 LC50 value ratios had additive toxic effects. Prolarval and postlarval stages were approximately twice as sensitive to the herbicides as juvenile striped bass.

The herbicides are discharged from May through June into agricultural drains and subsequently, the Sacramento River and Sacramento-San Joaquin Estuary, California. Although no chronic eggs-to-fry tests were completed, an assessment based on worst-case analyses using safety factors comparing measured and expected environmental concentrations of molinate and thiobencarb with estimated chronic eggs-to-fry no-observed-effect concentration values indicated that minimal toxicological hazard exists to young striped bass from exposure to rice herbicides in the Sacramento River and downstream estuary. This assessment is based on safety factors of at least 6 fold for molinate and at least 2 fold for thiobencarb; compliance with public health action levels in the Sacramento River will result in safety factors of at least 7 fold for molinate and at least 11 fold for thiobencarb. A similar assessment, based on data from other researchers, indicated that a potentially excessive hazard exists to opossum shrimp Neomysis mercedis in the Sacramento-San Joaquin Estuary during years with minimal dilution flows in Sacramento River. This invertebrate is a primary food item of young bass. The hazard to opossum shrimp can be minimized by lowering the maximum herbicide concentrations in agricultural drains to 54 ug/L molinate and 12 ug/L thiobencarb.

The lack of operational techniques prevented the successful completion of striped bass eggs-to-fry tests. Research and development on successful laboratory culturing and testing techniques progressed over the duration of the study. Several factors conducive to striped bass culture are identified and discussed.

1/ Environmental Services Division Administration Report No. 82-7; final report of the Rice Herbicide Study.

2/ Study funded by California Department of Fish and Game Striped Bass Stamp Fund, State Water Resources Control Board (Interagency Agreement No. 1-161-420-2), and Rice Research Board (Standard Agreements No. C-1053 and C-1519).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CONCLUSIONS

1. Acute (96 and 144-h) toxicity tests indicated that 6, 13, and 24-day old larvae (mean 96-h LC50 values of 3.3 mg/L molinate and 0.27 mg/L thiobencarb) were approximately twice as sensitive to molinate-thiobencarb mixtures than 45 and 90-day old juveniles (mean 96-h LC50 values of 7.7 mg/L molinate and 0.58 mg/L thiobencarb). Molinate-thiobencarb mixtures had additive acute toxic effects on striped bass.
2. The sensitivity of 6, 13, and 24-day old larvae to molinate was variable but they were more sensitive than 28-day old larvae and 90-day old juveniles. The sensitivity of 6-day old larvae to thiobencarb was greater than the sensitivity of 13, 24, and 28-day old larvae and 45 and 90-day old juveniles.
3. Thiobencarb (mean 96-h LC50 values of 0.59 mg/L) was on the average 14 times more toxic than molinate (mean 96-h LC50 value of 8.3 mg/L).
4. Two similar static tests with thiobencarb on 13-day old larvae by different laboratories produced similar 96-h LC50 values indicating the test procedures yield reproducible results.
5. An assessment based on worst-case analyses using safety factors comparing measured and expected environmental concentrations of molinate and thiobencarb with estimated chronic no-observed-effect concentration values indicated that minimal toxicological hazard exists to young striped bass from exposure to the rice herbicides in the Sacramento River and downstream estuary. This assessment is

based on safety factors of at least 6 fold for molinate and 2 fold for thiobencarb; compliance with public health action levels in the Sacramento River will result in safety factors of at least 7 fold for molinate and 11 fold for thiobencarb.

6. An assessment, based on data from other researchers, indicated that potentially excessive hazard exists to the opossum shrimp from exposure to the rice herbicides in the Sacramento-San Joaquin Delta during years with minimal dilution flows in Sacramento River. This assessment is based on safety factors of 0.6 to 2.6 for molinate and 0.5 to 6.0 for thiobencarb. The hazard can be minimized by lowering maximum herbicide concentrations in the agricultural drains from the present 90 to 54 ug/L molinate and from the present 24 to 12 ug/L thiobencarb.

7. Conditions which were identified as conducive to striped bass survival during culture and testing include the following: i) water with hardness and alkalinity of $\geq$200 mg/L $CaCO_3$; ii) feeding prolarvae and early postlarvae brine shrimp nauplii supplemented with unicellular algae; iii) the use of round glass test compartments with deep ($\geq$15 cm) water columns; and iv) the use of flow-through techniques for culturing to prevent the build-up of toxic nitrogenous wastes.

RECOMMENDATIONS

1. It is desirable to collect additional information on the chronic effects of rice herbicides on striped bass eggs-to-fry and opossum shrimp to complement and confirm the safety factors from the current hazard assessment.

2. The DFG should recommend to the Department of Food and Agriculture guidelines of 54 ug/L molinate and 12 ug/L thiobencarb for protection of aquatic organisms in the agricultural drains and downstream Sacramento River and Sacramento-San Joaquin Delta. It is anticipated that current Department of Food and Agriculture pesticide use practices (best management practices) will result in molinate and thiobencarb concentrations at or below these recommended guidelines.

3. Future culturing procedures for striped bass should include the following: i) water of sufficient hardness and alkalinity ($\geq$200 mg/L $CaCO_3$); ii) supplementing the diet of prolarvae and early postlarvae with unicellular algae; and iii) flow-through techniques.

4. Future testing procedures for striped bass larvae should include round glass test compartments with a minimum water depth of 15 cm.

INTRODUCTION

Identification of Problem

Molinate (Ordram®) and thiobencarb (Bolero®) are herbicides used to control weeds which infest inundated rice fields in the Sacramento River Valley, California (Figure 1). Seasonal (May through June) discharges of these herbicides into agricultural drains and the Sacramento River have been monitored since 1981 (Finlayson et al. 1982; Finlayson and Lew 1983a; 1983b; 1984; 1985; 1986). The California Department of Fish and Game (CDFG) became concerned about discharges of rice pesticides because of coincident fish losses in the agricultural drains. Carp Cyprinus carpio has been the primary species killed in the Colusa Basin Drain with estimated annual losses of 7,000 to 30,000 from 1980 to 1983; no fish losses were observed in 1984 through 1986 with the implementation of holding times following herbicide application required by the California Department of Food and Agriculture (Finlayson and Lew 1984; 1985; 1986). Previous studies have defined the acute and chronic toxicities of molinate and thiobencarb to juvenile resident and anadromous fishes (Finlayson and Faggella 1986).

Rice herbicides and other toxics may be responsible for the decline of striped bass in the Sacramento-San Joaquin Estuary (State Water Resources Control Board 1981; Stevens et al. 1985). The 90-km section of the Sacramento River extending from Colusa downstream to Sacramento receives up to 33% of total flow from agricultural drain water originating from rice fields; the Sacramento River downstream of Sacramento receives up

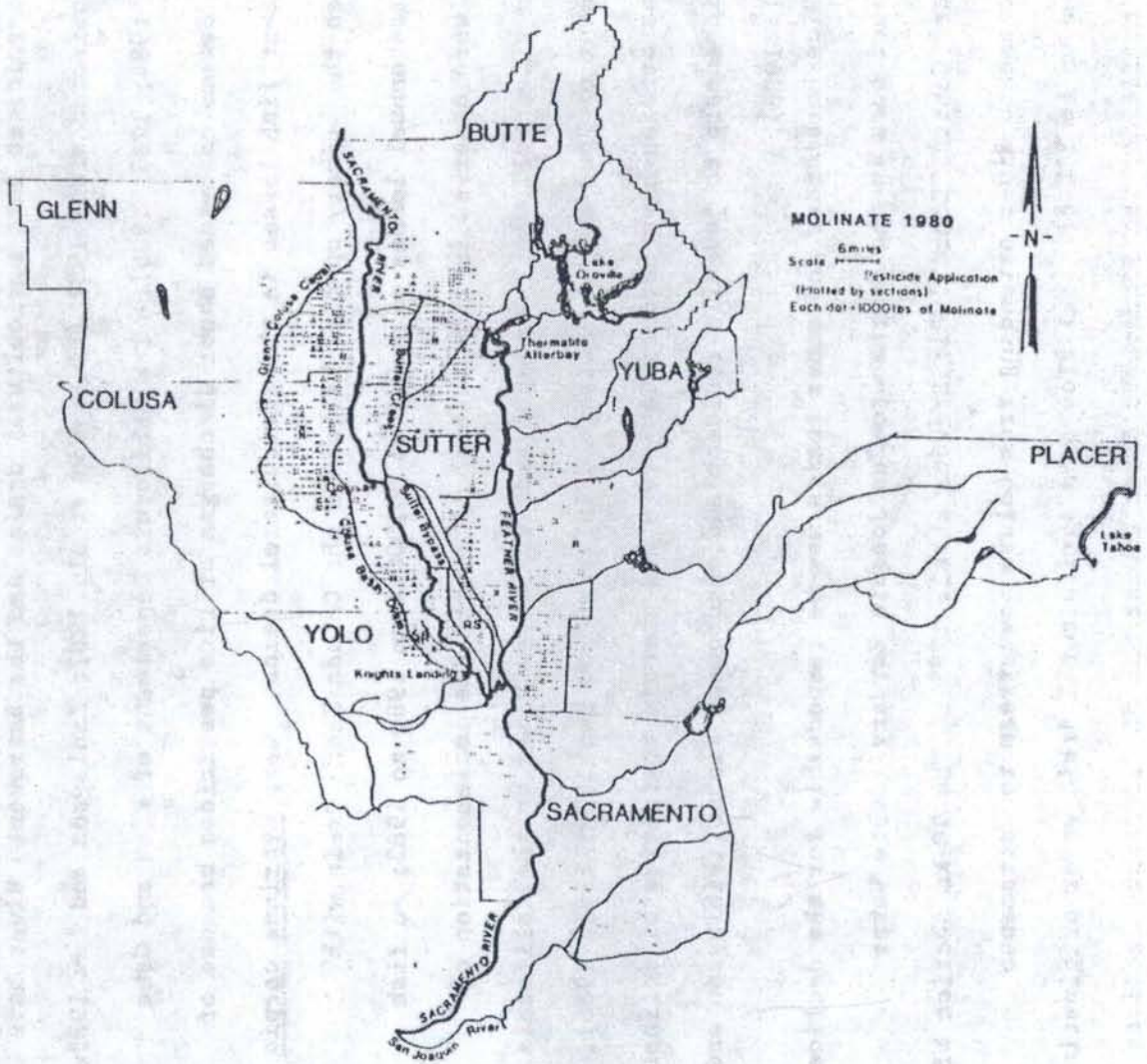

FIGURE 1. RICE ACREAGE IN THE SACRAMENTO VALLEY FOR 1980 BASED ON MOLINATE USEAGE.

to 24% of total flow from agricultural drain water (Cornacchia et al. 1984). This section and downstream estuary serve as spawning and nursery habitats from May through June for anadromous striped bass Morone saxatilis, American shad Alosa sapidissima, and white sturgeon Acipenser transmontanus (Farley 1966; Turner 1976). Chinook salmon Oncorhynchus tshawytscha and steelhead trout Salmo gairdneri downstream migrants, also occupy this area. The effects of these herbicides on survival and development of striped bass larvae and juveniles were unknown until the study reported here was completed. Results of investigations completed in 1984 and 1985 have been previously reported (Finlayson and Faggella 1984; Faggella and Finlayson 1985). Here we report a summary of all work completed through 1986 and provide a hazard assessment of rice herbicides on larval and juvenile striped bass. Additionally, we evaluated toxicity data for the herbicides on opossum shrimp Neomysis mercedis collected for the State Water Resources Control Board (1985).

Study Goal and Objectives

The goal of this study was to collect data necessary for an assessment of possible toxic effects caused by molinate and thiobencarb on larval and juvenile striped bass in the Sacramento River and Sacramento-San Joaquin Estuary. In obtaining this goal, several objectives were accomplished as follows:

1) Estimating environmental concentrations of molinate and thiobencarb in the Sacramento River and associated agricultural drains;

2) Researching, developing, and implementing operational techniques for the successful culturing and testing of larval and juvenile striped bass;

3) Estimating the acute and chronic toxicities of the herbicides on embryonic, larval, and juvenile striped bass;

4) Integrating monitoring and toxicity information into worst-case hazard assessment models for judging toxicological impacts of agricultural return water containing rice herbicides on striped bass; and

5) Recommending appropriate guidelines to regulatory agencies which will protect striped bass from toxic effects of rice herbicides.

Study Schedule

Tasks Completed in 1984 - Between October 1983 and December 1984, the following tasks were completed relative to the study objectives:

1) Monitored herbicide concentrations in the Sacramento River and associated agricultural drains during 1984 rice growing season and prepared a report on the findings (Finlayson and Lew 1984);

2) Developed prototype testing and culturing protocols and facilities for early-life-history-stages (ELHS) of striped bass;

3) Conducted 24 acute and chronic toxicity tests with the herbicides on striped bass embryos, larvae, and juveniles and prepared a progress report on the findings (Finlayson and Faggella 1984);

4) Collected and preserved striped bass specimens from the culture system for a histological record of ELHS development. Specimens have been processed by California State University under contract to CDFG and are currently being analyzed; and

5) Collected and preserved striped bass larvae which had been exposed to molinate and thiobencarb in 144-h tests for evaluation of possible histopathological effects of molinate and thiobencarb. Specimens have been processed by California State University under contract to DFG and are currently being analyzed.

Tasks Completed in 1985 - Between January and December 1985 the following tasks were completed relative to the study objectives:

1) Constructed a glass and solenoid valve type diluter to replace the flow meter type diluter used in 1984 tests for better toxicant delivery and stability;

2) Installed airlifts in the test compartments for aeration and water circulation and exchange;

3) Constructed 36 new glass and polypropylene screen test compartments for increased testing capacity;

4) Installed an oil-less air compressor, two-stage regulator, and PVC pipe delivery system for the test chamber airlifts;

5) Installed a water chiller in the recirculating water bath system for better temperature control;

6) Modified the saltwater injection system for better control of dilution water salinity;

7) Installed ventilation fans to remove volatilized molinate from the bioassay room for less cross-contamination of test aquaria;

8) Modified the striped bass rearing system to allow for using either circular or up-welling currents, and increased the holding capacity to allow for holding up to three families concurrently;

9) Enlarged brine shrimp rearing capacity for increased food supply of larval striped bass;

10) Conducted 27 acute and chronic toxicity tests with the herbicides on striped bass embryos, larvae, and juveniles and prepared a progress report on the findings (Faggella and Finlayson 1985); and

11) Monitored rice herbicide concentrations in the Sacramento River and associated agricultrual drains during 1985 rice growing season and prepared a report on the findings (Finlayson and Lew 1985).

Tasks Completed in 1986 - Between January and December 1986 the following tasks were completed relative to the study objectives:

1) Constructed a gas stripping tower for removal of supersaturated gas from the American River water;

2) Installed pressurizing pump and filters for consistent delivery of sediment-free dilution water;

3) Installed pressurizing pump in salt water system for consistent injection of salt water into the dilution water;

4) Installed stirrers in stock solution containers for maintenance of homogeneous toxicant solutions;

5) Installed plastic covers over the banks of test aquaria to reduce cross-contamination of molinate caused by volatilization;

6) Darkened sides of test compartments to reduce positive phototaxis behavior and corresponding stress of larval striped bass;

7) Installed a copper tube heat exchanger coil in the recirculating water bath system for more stable and efficient temperature control;

8) Conducted 21 acute and chronic continuous flow toxicity tests with the herbicides on striped bass embryos, larvae and juveniles;

9) Conducted one static acute toxicity test with thiobencarb on striped bass larvae and contracted with the U.S. Environmental Protection Agency for a similar quality assurance test; and

10) Monitored rice herbicide concentrations in the Sacramento River and associated agricultural drains during 1986 rice growing season and prepared a report on the findings (Finlayson and Lew 1986).

METHODS AND MATERIALS

Testing Schedule

From May 1984 through December 1986, 21 acute 96 or 144-h toxicity tests were completed on prolarval, postlarval, and juvenile striped bass (Table 1). Other acute and chronic tests were initiated but not completed during this period primarily because of less than satisfactory survival (<75%) of the control groups (Appendix A).

Culture Techniques

Embryos and Larvae - Striped bass embryos (12 to 6 h prehatch) were obtained from the CDFG Central Valley Fish Hatchery located near Elk Grove, California. The embryos were transported to the Nimbus facility in air-tight plastic bags containing oxygen inside styrofoam ice chests. Transport time was approximately 30 minutes. Some of the embryos were exposed to the herbicides in tests. Those embryos not used in the toxicity tests were hatched in modified plexiglass MacDonald hatching jars described by Finlayson and Faggella (1984).

The hatching jars were placed in culture tanks with one striped bass family per tank. The culture system consisted of three, 400-L circular fiberglass tanks with the water volumes adjusted to approximately 200 L. Initial loading of striped bass prolarvae did not exceed 50/L in the tanks. The tanks were interconnected to biological filters made of two, 114-L plastic Nalgene® tanks (Figure 2). Aquarium gravel (30 to 35 cm deep) was used as substrate for bacterial growth in the filters. Water flowed by

Table 1. Schedule of acute tests completed with molinate, thiobencarb, and molinate-thiobencarb mixtures in 1984 through 1986.

Test series	Begin date	Bass family	Age (developmental stage)	Herbicide	Test duration (h)
1984-5	5-11-84	84-2	6-day (prolarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	144
1984-7	5-29-84	84-2	24-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	144
1985-4	5-23-85	85-3	13-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	144
1985-5	5-31-85	85-4	6-day (prolarvae)	Molinate	144
1985-6	6-7-85	85-4	13-day (postlarvae)	Molinate-Thiobencarb Mixture	144
1985-7	6-7-85	85-3	28-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	144
1985-8	6-21-85	85-3	45-day (juvenile)	Thiobencarb Molinate-Thiobencarb Mixture	144

Table 1. (Continued)

Test series	Begin date	Bass family	Age (developmental stage)	Herbicide	Test duration (h)
1986-5	7-7-86	86-4	90-day (juvenile)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	96
1986-8A [a/]	6-7-86	86-3	13-day (postlarvae)	Thiobencarb	96
1986-8B [a/]	6-13-86	86-5	13-day (postlarvae)	Thiobencarb	96

[a/] 96-h static test.

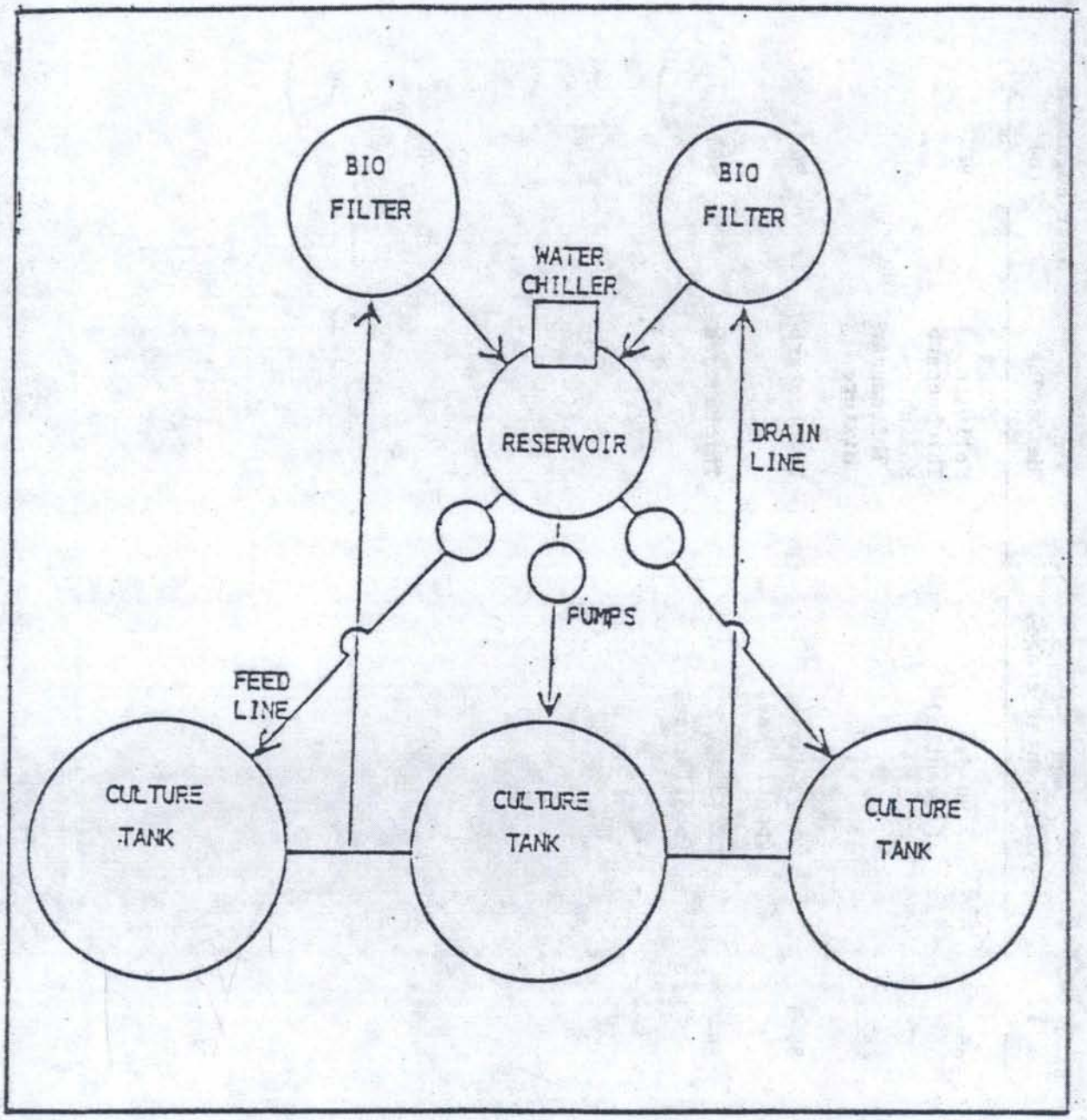

FIGURE 2. SCHEMATIC DIAGRAM OF THE RECIRCULATING REARING SYSTEM.

gravity out of each culture tank through 500-um mesh polypropylene screen into PVC pipe drains, through the biological filters, and then into the chilling reservoir. The water in the reservoir was maintained at 17 to 19° C using a Frigid Unit® model D1-100 1/2-ton refrigeration unit. The cooled water was then recirculated to the circular rearing tanks with March® model AC3CMD impellor pumps. Water flowed into the tanks as upwelling (to 13 days after hatch) and circular (thereafter) currents by adjusting the height and position of PVC discharge pipes.

Water temperature, salinity, dissolved oxygen, and ammonia in the culture system were measured daily. Water temperature was measured with a glass thermometer, dissolved oxygen with a YSI® model 57 dissolved oxygen meter calibrated daily, salinity with a YSI® model 33 conductivity meter, and ammonia with an Orion® model 407A specific-ion meter and an Orion® ammonia probe calibrated daily. The biological filters were backflushed and approximately 10% of the water was replaced daily. Salinity of the water was maintained at 2 to 5 o/oo using Marine Environment® artificial sea salts.

The striped bass prolarvae were fed brine shrimp nauplii beginning at 4 days after hatch. Automatic feeders (Figure 3) suspended over the culture tanks dispensed live brine shrimp nauplii through 24 VDC 316 stainless steel solenoid valves controlled by repeat-cycle timers. Each feeder was calibrated to dispense nauplii at approximately 400/L of tank volume, eight times (every 3 h) per day. Larvae were

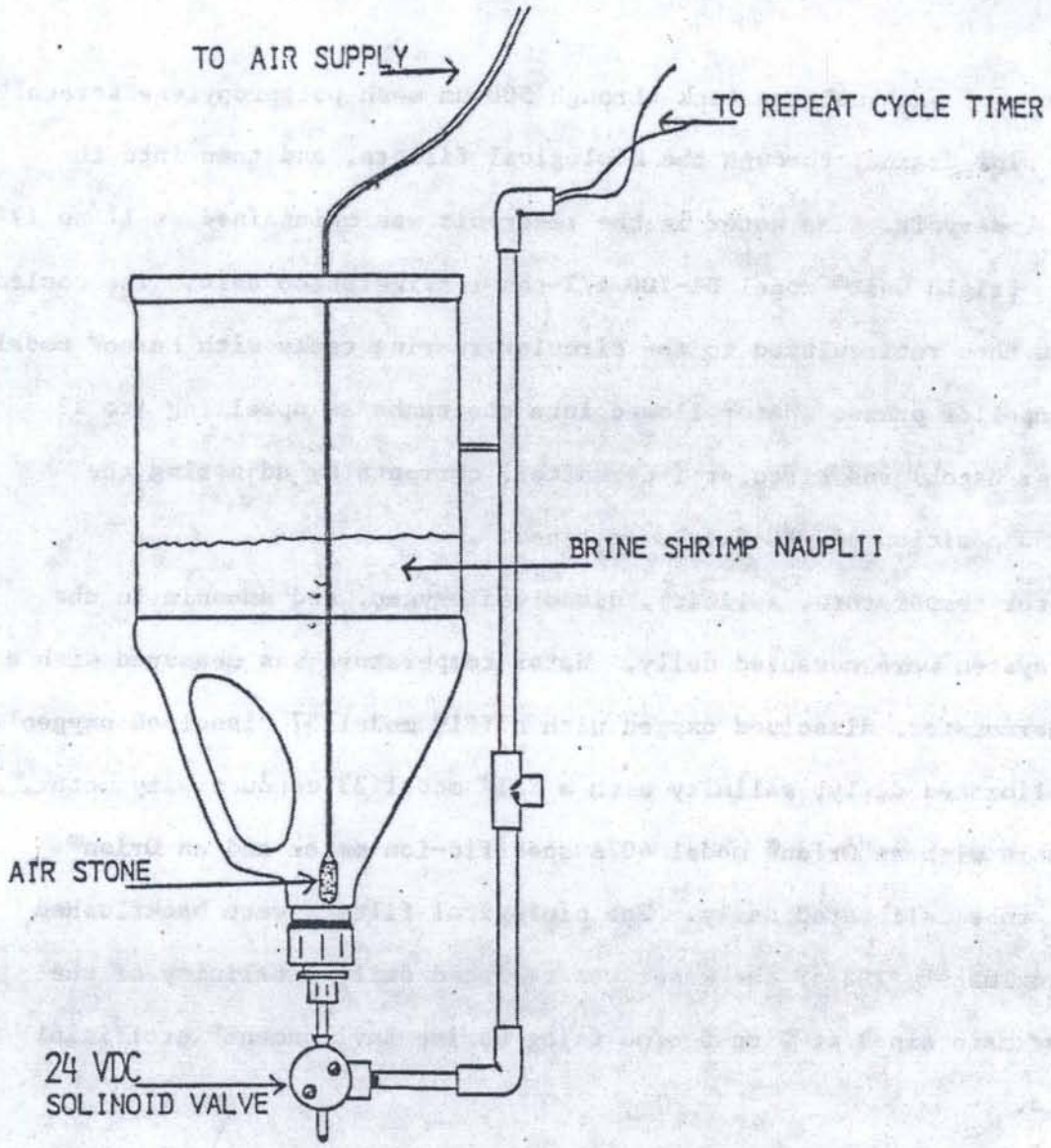

FIGURE 3. AUTOMATIC FEEDER FOR REARING SYSTEM.

occasionally removed from the system and measured using an ocular micrometer to the nearest 0.01 mm to establish growth rates.

The brine shrimp nauplii were cultured in bottomless inverted one-gallon plastic bottles. Salinity of the culture media was adjusted to 35 o/oo with sodium chloride, and 30 ml of San Francisco Bay Brand® artemia cysts were added per gallon of culture media. The nauplii were separated from the shells and unhatched cysts using a device (Figure 4) similar to that described by Lewis and Heidinger (1981). The resulting density was 500,000 nauplii/L.

For the static test conducted by the U.S. Environmental Protection Agency, striped bass embryos (12 h prehatch) were obtained from CDFG Central Valley Hatchery and shipped to Oregon via air freight using the procedures listed above. The larvae were raised using the culture procedures listed above, except as noted in Appendix B.

Juveniles - Juvenile striped bass at 80 days of age were obtained from the CDFG Central Valley Hatchery at Elk Grove, California and transported to the Nimbus facility using techniques described above. The juveniles were kept for 14 days in a 400-L circular tank with adequate supply (12 L/min) of flowing freshwater prior to testing; water quality was temperature of 17° C, pH of 7.0 to 7.1, hardness of 20 to 21 mg/L $CaCO_3$, and dissolved oxygen >90% of saturation. The juvenile bass were fed Rainbrook® fish food twice daily at 2.5% of their body weight (5.0% total). The fish were not fed for 4 days prior to or during the 4 day tests.

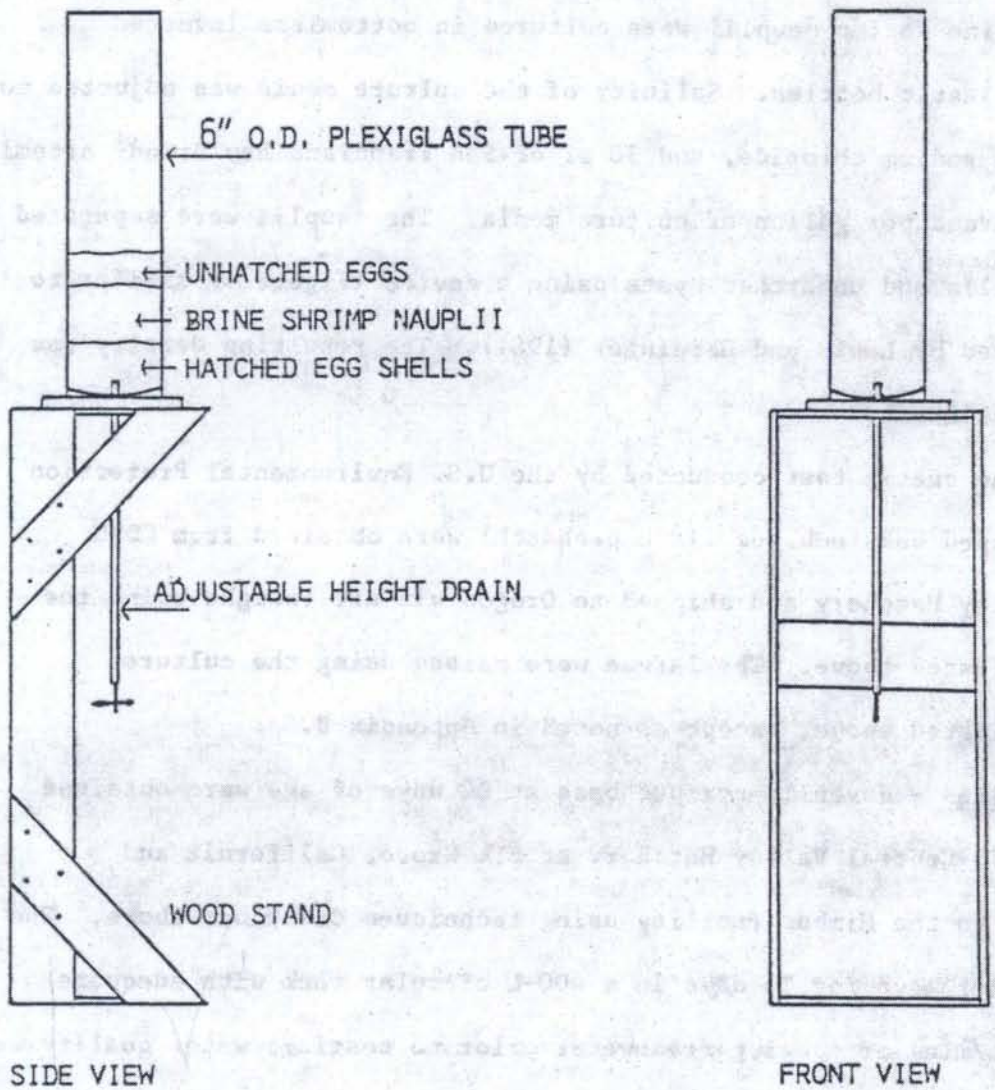

FIGURE 4. BRINE SHRIMP SEPARATOR.

Testing Techniques

Continuous Flow - The 96 and 144-h continuous flow toxicity tests were conducted in the laboratory trailer (Figure 5) previously described by Finlayson and Faggella (1984) using procedures outlined by American Society for Testing and Materials (1980) and Finlayson and Faggella (1986). Tests were conducted with three herbicide solutions concurrently; molinate, thiobencarb, and molinate-thiobencarb mixture at a 1:1 LC50 value ratio. Fish were exposed using solenoid valve proportional diluters of plexiglass, glass, and stainless steel described by Faggella and Finlayson (1985). Each test consisted of a control group in replicate and a geometric series (dilution factor of 0.6) of five herbicide concentrations in replicate bracketing the LC50 value. The flow rate through the 15-L aquaria was 4 L/h. A photoperiod of 14-h light (0500 to 1900 h) and 10-h dark was provided by automatically controlled overhead fluorescent lights. Two overhead ventilation fans with a total discharge capacity of 2,000 cfm kept molinate and thiobencarb vapors in the 1,200-cf bioassay room to a minimum.

Dilution water used for the tests came from the American River. Native quality of the American River water during the tests was pH of 7.1, alkalinity of 20 mg/L $CaCO_3$, hardness of 18 mg/L $CaCO_3$, temperature of 14 to 17° C, conductivity of 50 uS, and dissolved oxygen of $\geq$90% of saturation. Supersaturated gas from the American River water was brought into equilibrium with air using a gravity fed gas stripping column (Figure 5). The degassed water was stored in a 300-L Nalgene® tank and pressurized to 18 psi with a March® model TW-7R-MD 3/4 hp pump. Water used for

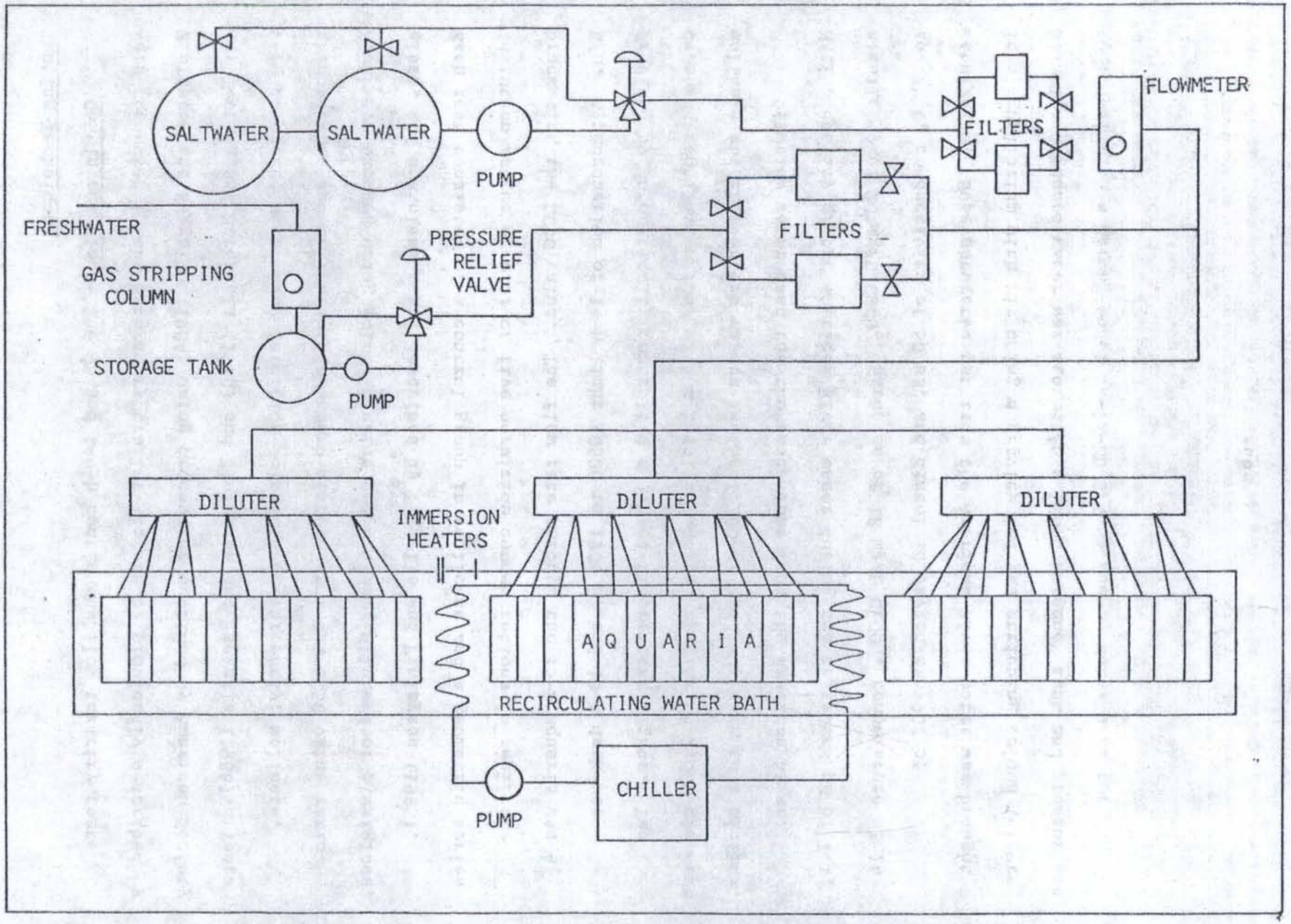

FIGURE 5. SCHEMATIC DIAGRAM OF DILUTION WATER DELIVERY SYSTEM FOR THE LABORATORY TRAILER.

dilution in the tests on embryonic and larval striped bass was adjusted to 2 ± 1 o/oo salinity by injecting artificial sea water (35 o/oo) at 22 psi into the degassed native water with a March® model TE-7R-MD 3/4 hp pump (Figure 5). The artificial sea water was made by mixing Marine Environment® artificial sea salts with native American River water for 4 h using an electrical 316 stainless steel mixer. The artificial sea water was aged and aerated for 20 h prior to use. Native American River water was used for dilution in the tests on juvenile bass. Dilution water was filtered through 10-um porosity cellulose filters. Temperature control in the test aquaria was provided by a recirculating water bath. The temperature of the recirculating water bath was maintained at 17 to 19° C by either heating with Cole-Parmer® quartz immersion heaters or cooling with an Edwards® model CC1 one-ton water chiller. Water temperatures in the water bath and aquaria were continually monitored with a two channel Cole Parmer® temperature recorder.

The embryonic and larval striped bass were exposed in 600-ml glass beakers (Figure 6) and one-liter (13 x 13 x 13-cm) plate glass and 500-um mesh polypropylene screen test compartments (Figure 7), respectively, inside 15-L glass aquaria. For the tests with embryonic striped bass, the toxicant delivery tubes from the diluters were placed in the bottom of the beakers to provide an upwelling current. The newly hatched prolarvae flowed out of the beakers into the test compartments. Each test compartment had an airstone which provided for an exchange of test water with the aquaria and helped keep the larvae suspended. A Dayton® model

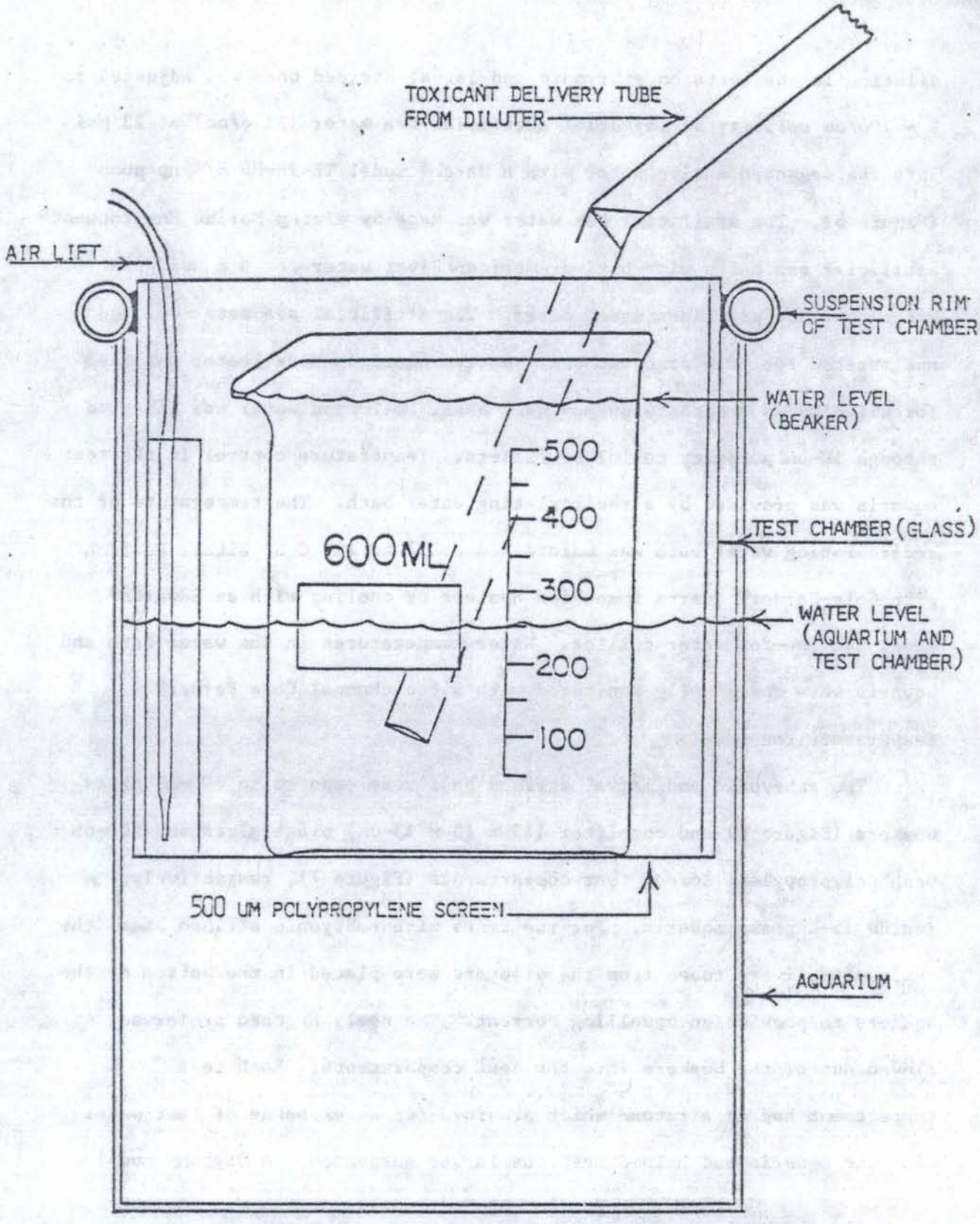

FIGURE 6. EMBRYONIC AND LARVAL TEST VESSEL.

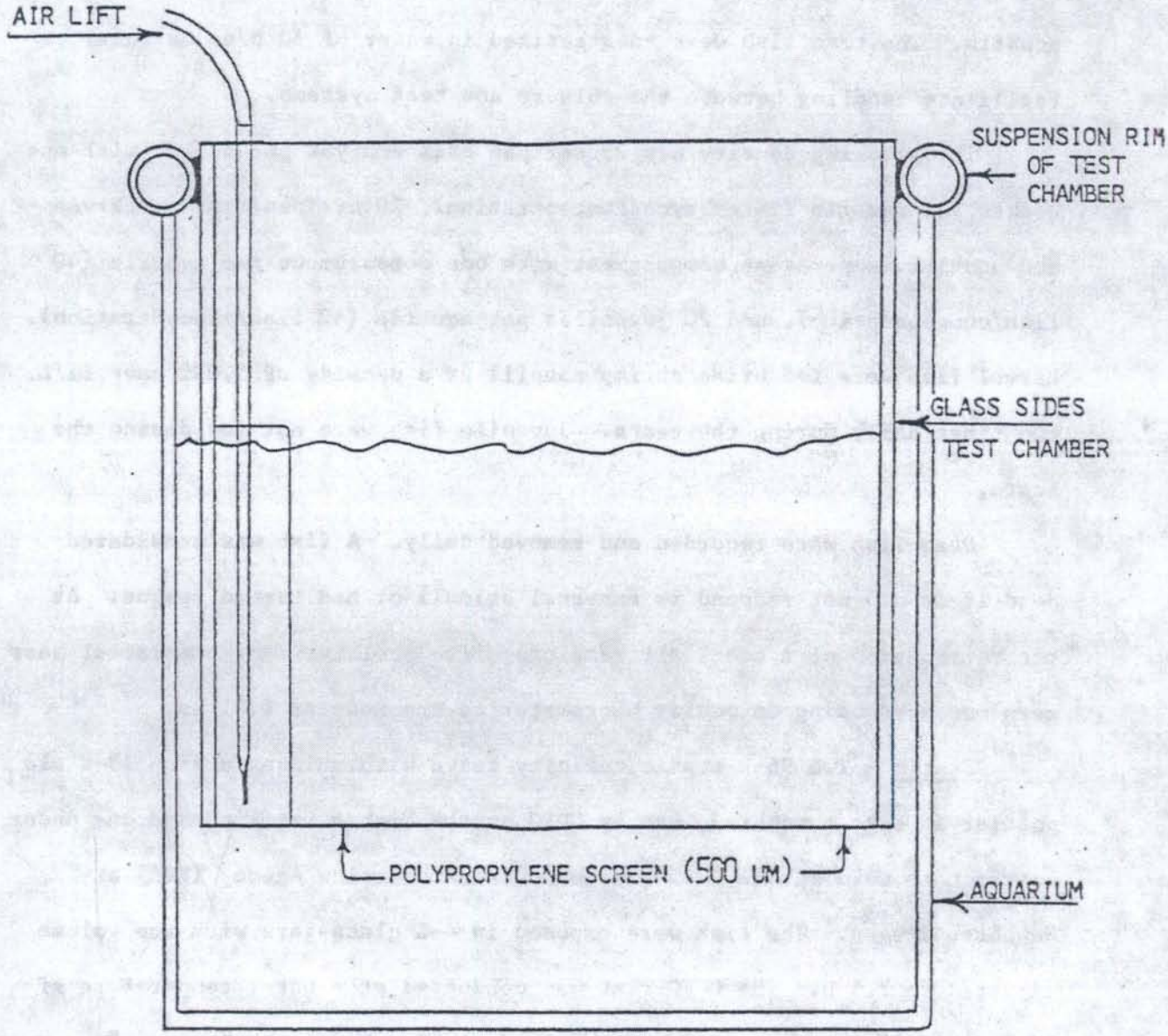

FIGURE 7. GLASS AND POLYPROPYLENE TEST VESSEL.

42706 3/4 hp oil-less air compressor with a 76-L storage tank supplied pressurized air to the test compartments through two-stage regulators, PVC manifolds, and valves. The juvenile bass were exposed in 15-L glass aquaria. The test fish were anesthetized in water of 10 o/oo salinity to facilitate handling between the culture and test systems.

The stocking density was 25 striped bass embryos per beaker with one beaker per aquaria (50 embryos/concentration), 20 striped bass prolarvae and postlarvae per test compartment with one compartment per aquaria (40 fish/concentration), and 20 juveniles per aquaria (40 fish/concentration). Larval fish were fed brine shrimp nauplii at a density of 5,000 nauplii/L, two times daily during the tests. Juvenile fish were not fed during the tests.

Dead fish were recorded and removed daily. A fish was considered dead if it did not respond to external stimuli or had turned opaque. At the termination of a test, all remaining live prolarval and postlarval bass were measured using an ocular micrometer to the nearest 0.01 mm.

Static - Two 96-h static toxicity tests with thiobencarb on 13-d old postlarvae were conducted, one by CDFG at the Nimbus facility and one under contract to CDFG by the U.S. Environmental Protection Agency (EPA) at Newport, Oregon. The fish were exposed in 4-L glass jars with the volume adjusted to 3.5 L. The CDFG test was conducted at a water temperature of 17.5 to 18.5° C and a salinity of 2.5 o/oo using Marine Environment® artificial sea salts (Appendix C). The EPA test was conducted at a temperature of 17.0° C and a salinity of 4 o/oo using Marine Environment®

artificial sea salts (Appendix B). The dilution water for the EPA test was made from distilled-deionized tap water and that for the CDFG test was made from native American River water. The stocking density in the tests was 25 larvae per jar (50 larvae/concentration).

Chemistry

Water temperatures in the continuous flow tests were continuously measured with thermister probes and monitored on a Cole-Parmer® two-channel script chart recorder. Conductivity and pH of dilution water were automatically measured hourly using a Montedoro-Whitney® model WQM-1 water quality monitor.

Molinate and thiobencarb stock solutions used in continuous flow tests were prepared from Ordram 8EC® and Bolero 8EC® commercial herbicide formulations (containing 90.3 and 85.4% active ingredient, respectively) by sonicating in deionized water. A Heat System's Ultrasonics® model W-370 Cell Disrupter was used for sonication. The solutions were made up in 12-L quantities and stored in 20-L glass jars with lids. The stock solutions were checked analytically at the beginning and end of a test. The solutions were delivered to the proportional diluters through silicone tubing using Gilson® model HP1 Minipuls peristaltic pumps.

For the static tests, thiobencarb test solutions were prepared by dilution of a stock solution (213 mg/L thiobencarb). The stock solution was prepared in 0.2% Nanograde® acetone to increase solubility of thiobencarb. The maximum concentration of acetone was 0.001% or 0.01 mg/L

in the highest herbicide test concentration; solvent controls were not tested.

Water samples for confirmation of molinate and thiobencarb test concentrations were collected at 24 h and 72 h during the 96-h tests and additionally at 120 h during the 144-h tests. Water samples from the continuous flow tests were collected by dipping chemically clean 250-ml amber glass bottles into the aquaria. Water samples from static tests were collected in chemically clean glass beakers and transferred to 250-ml amber glass bottles. The bottles were filled to the top and sealed with teflon-lined caps. The EPA test samples were shipped packed in ice in styrofoam chests from Oregon to California via ground transportation. All samples were stored at the Nimbus facility in a refrigerator at 4° C for up to five days until analyzed. Repeated analyses of water samples stored in this manner for two weeks indicated insignificant losses of herbicides.

Water samples were extracted with Baker®-10 Solid Phase Extraction C-8 disposable columns. The Baker®-10 columns were pre-conditioned with 2 ml of methanol followed by 2 ml of deionized water. Water samples were passed through the columns at a rate of 5 ml per minute. The herbicides were eluted from the columns with two 2-ml aliquots of ethyl acetate. The extracts were transferred to a 10-ml graduated tube and adjusted to a desirable volume for analysis by gas chromatography. The instrumental conditions of the Varian-Aerograph® model 3700 gas chromatograph used for the analysis were as follows:

Column: DC-200, Length: 183 cm, I.D.: 2 mm

Detector Temperature: 290° C

Injector Temperature: 210° C

Column Temperature: 192° C for thiobencarb

180° C for molinate

Carrier Flow: 30 ml/min

Carrier Gas: N_2

Detector: TSD

Detection Limits: 1 ug/L for both molinate and thiobencarb

In 1984, 1985, and 1986, water samples were split and analyzed for molinate concentrations by Stauffer Chemical Company (168 samples) and for thiobencarb concentrations by Chevron Chemical Company (71 samples); our molinate concentrations were 89% (r = 0.97) of those reported by Stauffer Chemical Company, and our thiobencarb concentrations were 88% (r = 0.93) of those reported by Chevron Chemical Company (Finlayson and Lew 1984, 1985, 1986).

Data Analysis

Generally, median lethal concentration (LC50) values were not calculated if mortality of the control group was >25%. Abbot's correction factor was used to adjust for control group mortality of >10% and ≤25%

$$Mx(a) = 1 - Sx/Sc$$

where Mx(a) is adjusted mortality in concentration x, Sx is survival in concentration x, and Sc is survival in controls (Finley 1971). The Mx(a) was used to calculate the adjusted number dead in concentration x

$$Dx(a) = Mx(a) * Ex$$

where Dx(a) is the adjusted number dead in concentration x and Ex is the number exposed in concentration x.

The LC50 values were determined using either moving average method when $\geq$2 toxicant concentrations had partial mortality (between 0 and 100% mortality) or nonlinear interpolation method when $\leq$1 toxicant concentration had partial mortality (Peltier and Weber 1985). Confidence intervals (95%) surrounding LC50 values were calculated using Fiellers Theorem (moving average), or conservative (>95%) confidence intervals were calculated using binomial probabilities (nonlinear interpolation).

In herbicide mixture tests, molinate and thiobencarb concentrations at the LC50 level were interpolated from least squares regression (solution concentration versus herbicide concentration). To determine the type of toxic interaction of molinate and thiobencarb in mixtures, toxicities of the herbicides in mixtures were expressed as toxic units and summed (Lloyd 1961; Brown 1968):

$$(Mm/Mi) + (Tm/Ti) = S$$

where M is molinate and T is thiobencarb, i is LC50 of individual herbicide tested separately, and m is the concentration of the individual herbicide at the mixture LC50 value. Additive indexes (AI) were calculated by adjusting for asymmetry of S and substituting 95% confidence intervals for the 96-h LC50 values and establishing a test range (Marking 1977) as follows:

If $S \geq 1.0$ then $AI = -S + 1.0$;

If $S \leq 1.0$ then $AI = 1/S - 1.0$.

This index is symmetrical about AI = 0 (additivity); positive values indicate synergism, negative values antagonism. The theoretical range of completely (simple) additive toxicity (AI = 0) for the herbicide mixture tests were estimated by adjusting the herbicide mixture LC50 values to S = 1.0, substituting the adjusted 95% confidence intervals into the two AI equations and establishing an additive range (Finlayson and Verrue 1982). To determine the type of interaction, the two ranges were compared as follows: i) additive toxicity if the test range broadly (>50%) overlapped the additive range; ii) antagonistic toxicity if most of the test range (>50%) was below the additive range; and iii) synergistic toxicity if most of the test range (>50%) was above the additive range.

Significant differences in survival and growth between control and treatment groups were used to establish no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) values. Survival data of striped bass from control and treatment groups in the acute tests were compared by the binomial chi-square test for data arranged in two groups (Cochran and Cox 1968). Significant differences in length measurements of larvae from control and treatment groups were determined by the Kruskal-Wallis test with Dunn's multiple comparison procedure (Daniel 1978).

RESULTS

Culture System

There were varying degrees of success in maintaining larval and juvenile bass in the culture system. Generally, the survival of control groups in the testing system parallelled the survival of that family remaining in the culture system. Poor survival in the culture system was associated with several factors including: i) failure of a recirculating pump and subsequent loss of filtration and aeration; ii) high density of fish coupled with failure of the biological filter in removing ammonia wastes; iii) stress caused by low water hardness and alkalinity; iv) poor inflation of gas bladders; v) lack of feeding activity; vi) parasitic gill protozoan and secondary fungal infections; and vii) inadequate space and environmental control systems.

The growth of larval striped bass from families spawned in 1984 and 1985 were similar and comparable to that reported by others (Figure 8).

Testing System

From 1984 through 1986, 24 tests were begun with embryonic bass, three with 4-day old bass, nine with 6 to 7-day old bass, eight with 13-day old bass, nine with 24 to 28-day old bass, three with 45-day old bass, and nine with 90-day old bass (Table 2). Generally, the percentage of the tests which were successfully completed (<25% mortality in controls) increased with increased age of bass.

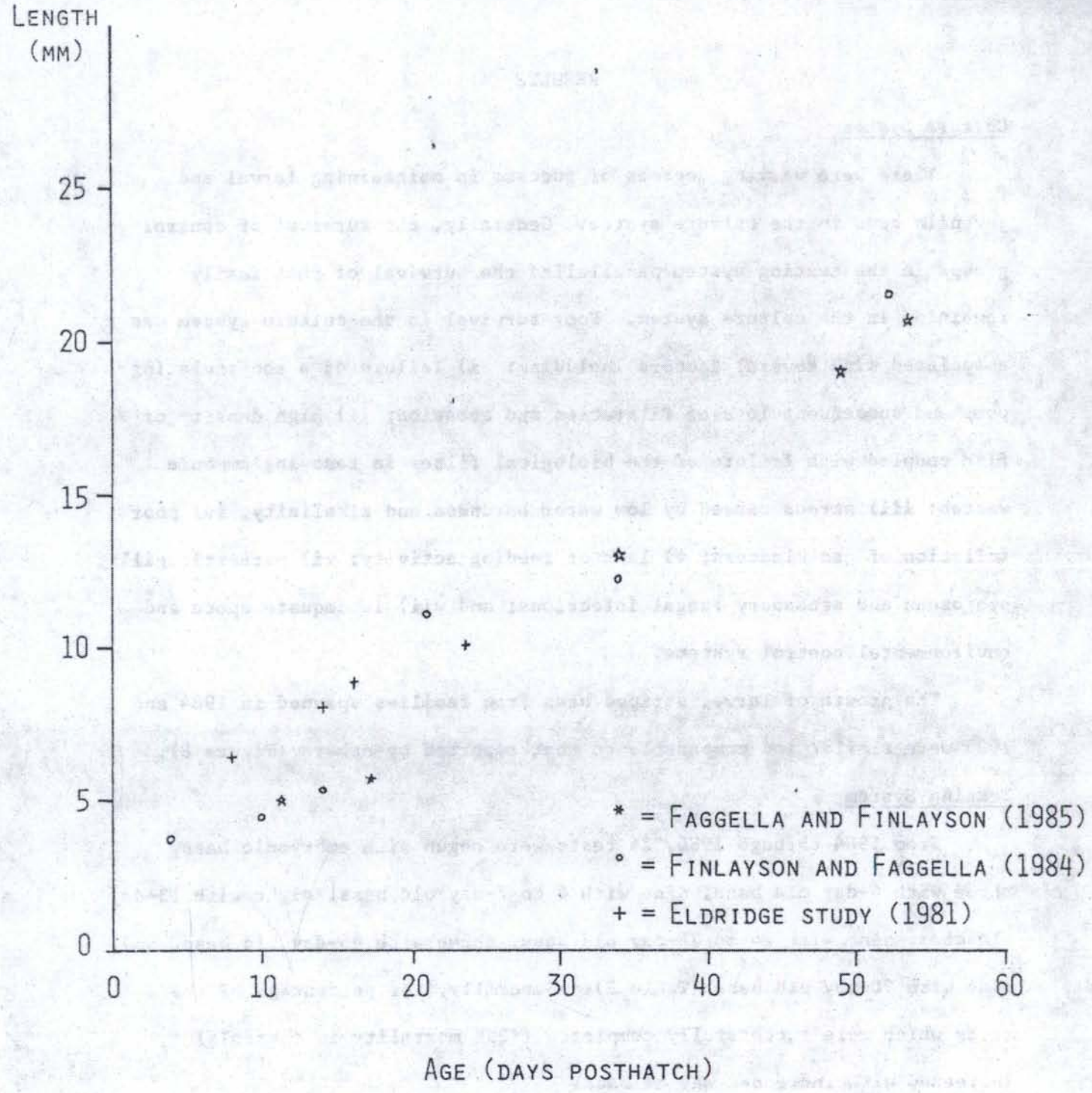

FIGURE 8. LARVAL GROWTH REPORTED BY FAGGELLA AND FINLAYSON (1985), FINLAYSON AND FAGGELLA (1984), AND ELDRIDGE ET AL. (1981).

Table 2. Number of tests which were successful and unsuccessful (≥25% mortality in control group) in 1984, 1985, and 1986.

Age	Successful	Unsuccessful
Prenatch		24
4-day		3
6 to 7-day	4	5
13-day	4	4
24 to 28-day	6	3
45-day	1	2
90-day	6	3

Acute Toxicity Tests

Tests on prolarvae and postlarvae were conducted at temperatures of 17.4 to 20.0° C and salinities of 1.6 to 4.0 o/oo (Table 3). Tests on juvenile bass were conducted in freshwater at a temperature of 17.4° C. Dissolved oxygen concentrations for all the tests ranged from 80 to 100% of saturation and total ammonia concentrations were <0.1 mg/L.

Molinate concentrations averaged 90, 81, and 86% and thiobencarb concentrations averaged 93, 53, and 86% of expected based on known stock solution concentrations and dilutions for years 1984, 1985, and 1986, respectively.

Survival of fish in control groups in the acceptable 96-h and 144-h continuous flow tests varied from 75 to 96% for 6-day old, 77 to 92% for 13-day old, 82 to 98% for 24-day old, 91 to 93% for 28-day old, 75 to 85% for 45-day old, and was 100% for 90-day old striped bass. Survival of 13-day old bass from control groups in the 96-h static tests were 62 and 90% for EPA and CDFG conducted tests, respectively. Molinate and thiobencarb concentrations caused dose related responses in survival of 6-day old (Tables 4 and 5), 13-day old (Tables 6, 7, and 8), 24-day old (Table 9), 28-day old (Table 10), 45-day old (Table 11), and 90-day old bass (Table 12). Thiobencarb (mean 96-h LC50 = 0.59 mg/L) was on the average 14 times more toxic than molinate (mean 96-h LC50 = 8.3 mg/L). Generally, the sensitivity of striped bass to the herbicides decreased with increased age (Table 13). The sensitivity of 6, 13, and 24-day old larvae to molinate was variable but they were more sensitive than 28-day old

Table 3. Mean (± S.D.) characteristics of dilution water during toxicity tests.

Test series	Salinity (o/oo)	Temperature (°C)	pH	Dissolved oxygen (mg/L)
1984-5	2.0±0.2	17.4±0.9	8.9±0.3	9.8±0.2
1984-7	2.1±0.3	17.6±0.8	8.9±0.4	9.9±0.5
1985-5	1.6±0.9	18.9±0.9	7.0±0.3	8.7±0.3
1985-6	1.6±0.9	18.9±0.9	7.1±0.4	8.9±0.3
1985-8	2.5±0.7	20.0±1.5	7.1±0.2	9.0±0.3
1986-5	0.0	17.4±0.3	7.2±0.1	8.5±0.2
1986-8A	4.0	17.0±0.5	7.8±0.1	9.0±0.3
1986-8B	2.5±0.0	18.0±0.3	7.9±0.1	9.4±0.2

Table 4. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on survival of 6-day old striped bass larvae (mean values with S.D. in parentheses) during Test Series 1984-5.

				Molinate-Thiobencarb Mixture	
Molinate concentration	Mortality (%)	Thiobencarb concentration	Mortality (%)	Molinate and Thiobencarb concentrations	Mortality (%)
Control <0.31(0.23)	6	Control <0.1(0)	12	Control <0.16(0.08) <0.10(0)	4
2.6(0.55)	23 [a/]	0.12(0.01)	15	0.79(0.21) 0.07(0.03)	7
4.2(0.54)	20 [a/]	0.19(0.02)	31 [a/]	2.0(0.76) 0.16(0.05)	19 [a/]
6.6(0.52)	54 [a/]	0.37(0.04)	44 [a/]	3.1(1.0) 0.24(0.08)	46 [a/]
13.0(1.3)	100 [a/]	0.72(0.10)	100 [a/]	6.0(2.3) 0.49(0.15)	100 [a/]
20.0(1.8)	100 [a/]	1.2(0.05)	100 [a/]	8.6(2.9) 0.83(0.19)	100 [a/]

[a/] Significantly greater than control (x^2, 0.05).

Table 5. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on growth and survival (mean values with S.E. in parentheses) of 6-day old striped bass larvae during Test Series 1985-5.

				Molinate-Thiobencarb Mixture			
Molinate concentration	Mortality (%)	Fish length (mm)	N [a]	Molinate and Thiobencarb concentrations	Mortality (%)	Fish length (mm)	N
Control				Control			
0.02(0.02)	16	5.55(0.12)	36	0.03(0.01) 0.001(0.00)	25	4.52(0.13)	37
1.5(0.1)	37 [b]	4.70(0.39) [c]	6	0.7(0.3) 0.05(0.02)	30	3.56(0.03) [c]	28
0.9(0.5)	52 [b]	4.71(0.24) [c]	10	1.0(0.3) 0.06(0.03)	27	4.23(0.18)	20
4.6(0.6)	100 [b]	--	--	1.7(0.3) 0.09(0.06)	28	4.88(0.03)	31
8.4(3.5)	100 [b]	--	--	2.7(0.7) 0.17(0.06)	63 [b]	4.27(0.17)	19
13.3(2.1)	100 [b]	--	--	4.4(0.9) 0.26(0.10)	68 [b]	5.03(0.07)	21

[a] N = Number of fish measured.

[b] Significantly greater than control (x^2, 0.05).

[c] Significantly greater than control (z, 0.05).

Table 6. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on growth and survival (mean values with S.E. in parentheses) of 13-day old striped bass larvae during Test Series 1985-4 and 1985-6.

Molinate concentration	Mortality (%)	Fish length (mm)	N [a]	Thiobencarb concentration	Mortality (%)	Fish length (mm)	N	Molinate-Thiobencarb Mixture: Molinate and Thiobencarb concentration	Mortality (%)	Fish length (mm)	N
Control				Control				Control			
0.21(0.24)	18	5.62(0.05)	59	0.001(0.00)	23	5.80(0.09)	20	0.04(0.02) <0.001(0.00)	8	5.50(0.06)	39
2.0(0.3)	17	5.72(0.09)	23	0.07(0.04)	13	5.52(0.07)	23	0.88(0.2) 0.05(0.05)	23 [b]	5.39(0.08)	28
3.1(0.7)	21	5.55(0.10)	27	0.11(0.08)	57 [b]	5.12(0.14) [c]	13	1.3(0.3) 0.09(0.04)	28 [b]	5.13(0.10) [c]	20
4.9(1.0)	23	5.34(0.08) [c]	23	0.22(0.14)	37	4.96(0.12) [c]	14	2.0(0.5) 0.15(0.06)	45 [b]	5.16(0.09) [c]	22
8.0(1.7)	49 [b]	5.06(0.10) [c]	17	0.44(0.06)	82 [b]	4.80(0.03) [c]	6	3.2(0.5) 0.27(0.5)	53 [b]	4.87(0.09) [c]	25
15.5(3.7)	100 [b]	--	--	0.86(0.23)	100 [b]	--	--	5.9(0.1) 0.48(0.06)	100 [b]	4.67(0.08) [c]	17

[a] N = Number of fish measured.

[b] Significantly greater than control (x^2, 0.05).

[c] Significantly greater than control (z, 0.05).

Table 7. Acute effects (96-h) of thiobencarb concentrations (mg/L) on survival (mean values with S.D. in parentheses) of 13-day old striped bass larvae during Test Series 1986-8B conducted by DFG.

Thiobencarb concentration	% Mortality	Fish length (mm)	N [b/]
Control			
<0.002	10	5.4±0.3 [a/]	24
0.09(0.05)	0	--	--
0.17(0.11)	4.2	--	--
0.27(0.15)	10	--	--
0.47(0.27)	21.3 [c/]	--	--
0.8(0.5)	60 [c/]	--	--

[a/] Mean ± S.E.

[b/] Number of fish measured.

[c/] Significantly greater than control (x^2, 0.05).

Table 8. Acute effects (96-h) of thiobencarb concentrations (mg/L) on survival (mean values with S.D. in parentheses) of 13-day old striped bass larvae during Test Series 1986-8A conducted by U.S. Environmental Protection Agency.

Thiobencarb concentration	Mortality (%)	Fish length (mm)	N [b]
Control (<0.01)	38	5.1 [a]	23
0.16(0.01)	34	5.0	33
0.31(0.01)	42	5.0	27
0.45(0.00)	42	5.0	30
0.80(0.03)	94 [c]	5.0	15
1.45(0.03)	100 [c]	4.9	1

[a] Mean length.

[b] Number of fish measured.

[c] Significantly greater than control (x^2, 0.05)..

Table 9. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on growth and survival (mean values with S.D. in parentheses) of 24-day old striped bass larvae during Test Series 1984-7.

Molinate				Thiobencarb				Molinate–Thiobencarb Mixture			
Molinate concentration	Mortality (%)	Fish length (mm)	N [a]	Thiobencarb concentration	Mortality (%)	Fish length (mm)	N	Molinate and Thiobencarb concentration	Mortality (%)	Fish length (mm)	N
Control <0.29(0.19)	2	8.02(0.71)	25	Control <0.1	17	8.33(0.75)	25	Control <0.15(0.06) 0.10(0)	4	7.82(0.53)	25
3.6(1.3)	60 [b]	8.18(1.11)	10	0.20(0.04)	33	8.48(0.31)	5	0.49(0.18) 0.1(0)	10	7.42(0.40) [c]	25
5.4(1.7)	52 [b]	8.30(0.33)	18	0.27(0.05)	20	8.24(0.65)	25	2.6(1.1) 0.19(0.06)	87 [b]	8.28(0.29)	3
8.9(2.5)	34 [b]	7.82(0.31)	20	0.45(0.05)	57 [b]	7.52(0.70)	25	3.8(0.58) 0.30(0.03)	92 [b]	7.40(0.95)	2
13.0(1.3)	100 [b]	--	--	0.75(0.09)	92 [b]	7.93(0.07)	4	8.0(0.19) 0.60(0.05)	100 [b]	--	--
20.0(1.4)	100 [b]	--	--	1.3(0.12)	100 [b]	--	--	14.0(0.5) 1.1(0.05)	100 [b]	--	--

[a] N = Number of fish measured.

[b] Significantly greater than control (x^2, 0.05).

[c] Significantly smaller than control (z, 0.05).

Table 10. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on growth and survival (mean values with S.E. in parentheses) of 28-day old striped bass larvae during Test Series 1985-7.

Molinate concentration	Mortality (%)	Fish length (mm)	N [a]	Thiobencarb concentration	Mortality (%)	Fish length (mm)	N	Molinate-Thiobencarb Mixture: Molinate and Thiobencarb concentration	Mortality (%)	Fish length (mm)	N
Control				Control				Control			
0.03(0.02)	7	12.5(0.3)	23	<0.01(0.00)	8	12.6(0.5)	14	0.04(0.02) <0.01(0.01)	9	12.9(0.3)	10
1.9(0.2)	15	12.1(0.2)	24	0.04(0.02)	14	12.8(0.3)	20	0.9(0.2) 0.05(0.05)	0	12.4(0.3)	19
0.02(0.02)	8	12.0(0.3)	20	0.10(0.06)	6	12.0(0.3)	15	1.3(0.3) 0.09(0.04)	4	12.2(0.4)	12
5.0(0.6)	10	11.8(0.3)	17	0.18(0.11)	0	12.5(0.2)	25	2.0(0.5) 0.15(0.06)	8	11.9(0.2)	18
7.0(1.8)	17	12.1(0.2)	14	0.35(0.17)	33 [b]	12.2(0.3)	10	3.2(0.5) 0.27(0.15)	0.5	11.8(0.2)	23
15.5(3.0)	55 [b]	11.8(0.2)	6	0.44(0.26)	41 [b]	10.9(0.4) [c]	10	5.9(0.1) 0.48(0.06)	39 [c]	10.8(0.2) [c]	7

[a] N = Number of fish measured.

[b] Significantly different than control (x^2, 0.05).

[c] Significantly different than control (z, 0.05).

Table 11. Acute effects (144-h) of molinate and thiobencarb concentrations (mg/L) on growth and survival (mean values with S.E. in parentheses) of 45-day old striped bass fry during Test Series 1985-8.

Thiobencarb concentration	Mortality (%)	Fish length (mm)	N[a/]	Molinate-Thiobencarb Mixture: Molinate and Thiobencarb concentrations	Mortality (%)	Fish length (mm)	N
Control				Control			
<0.01(0.00)	25	20.0(0.3)	20	0.02(0.02) <0.01(0.00)	15	18.9(0.3)	25
0.20(0.18)	5	24.4(0.6)	13	3.5(1.2) 0.35(0.11)	3	18.8(0.3)	28
0.27(0.08)	0	24.0(0.4)	15	4.9(1.4) 0.49(0.16)	33[b/]	18.2(0.3)	13
0.40(0.25)	37	23.5(0.4)	6	7.7(2.1) 0.74(0.25)	69[b/]	18.1(0.5)	7
0.77(0.23)	90[b/]	19.5(3.5)	2	13.1(4.0) 1.1(0.4)	100[b/]	--	--
1.3(0.35)	--	--	--	20.8(4.9) 1.7(0.7)	100[b/]	--	--

[a/] N = Number of fish measured.

[b/] Significantly different than control (x^2, 0.05).

Table 12. Acute effects (96-h) of molinate and thiobencarb concentrations (mg/L) on survival (mean values with S.D. in parentheses) of 90-day old striped bass juveniles during Test Series 1986-5.

				Molinate-Thiobencarb Mixture	
Molinate concentration	Mortality (%)	Thiobencarb concentration	Mortality (%)	Molinate and Thiobencarb concentration	Mortality (%)
Control 0.008(0.003)	0	Control <0.005(0.000)	0	Control 0.012(0.005) <0.005(0.000)	0
1.6(0.05)	0	0.24(0.02)	0	1.4(0.1) 0.10(0.01)	0
3.4(0.04)	0	0.47(0.01)	0	2.7(0.2) 0.19(0.02)	0
6.2(0.0)	2.5	0.89(0.03)	97.5 [a/]	4.2(0.4) 0.30(0.02)	0
10(0)	0	1.5(0.0)	97.5 [a/]	7.0(0.6) 0.50(0.04)	18 [a/]
19(0)	97.5 [a/]	2.6(0.1)	100 [a/]	12(2.0) 0.75(0.01)	100 [a/]

[a/] Significantly greater than control (x^2, 0.05).

Table 13. Median lethal (LC50 values) and 95% confidence interval (in parentheses) concentrations (mg/L) for molinate and thiobencarb on striped bass.

Fish age	Solution	Molinate		Thiobencarb	
		96-h	144-h	96-h	144-h
6	Molinate	6.6(6.1-7.1)	5.4(4.9-5.9)		
	Molinate	2.1(1.8-2.4)	2.1(1.7-2.4)		
	Thiobencarb			0.35(0.30-0.39)	0.32(0.29-0.35)
	Molinate-Thiobencarb Mixture	3.8(3.5-4.2)	2.7(2.4-2.9)	0.33(0.30-0.37)	0.22(0.19-0.24)
	Molinate-Thiobencarb Mixture	>4.4	>4.4	>0.26	>0.26
13	Molinate	11(8.0-16)	8.8(8.0-16)		
	Thiobencarb			0.51(0.44-0.86)	0.24(0.21-0.27)
	Thiobencarb [a/]			0.62(0.57-0.68)	
	Thiobencarb [b/]			0.70(0.61-0.87)	
	Molinate-Thiobencarb Mixture	3.0(2.7-3.3)	2.0(1.7-2.4)	0.24(0.21-0.26)	0.16(0.13-0.19)
24	Molinate	7.9(7.1-8.7)			
	Thiobencarb			0.67(0.61-0.74)	0.46(0.41-0.52)
	Molinate-Thiobencarb Mixture	3.1(2.6-3.6)	1.4(1.2-1.7)	0.24(0.20-0.28)	0.11(0.09-0.13)
28	Molinate	>16	>16		
	Thiobencarb			>0.44	>0.44
	Molinate-Thiobencarb Mixture	>5.9	>5.9	>0.48	>0.48
45	Thiobencarb				0.57(0.40-0.77)
	Molinate-Thiobencarb Mixture	7.5(7.0-8.3)	6.6(4.7-7.5)	0.65(0.60-0.72)	0.58(0.43-0.66)
90	Molinate	14(13-15)			
	Thiobencarb			0.67(0.60-0.75)	
	Molinate-Thiobencarb Mixture	7.9(7.4-8.6)		0.52(0.49-0.56)	

[a/] U.S. Environmental Protection Agency 96-h static test.

[b/] DFG 96-h static test.

larvae and 90-day old juveniles. The sensitivity of 6-day old larvae to thiobencarb was greater than the sensitivity of 13, 24, and 28-day old larvae and 90-day old juveniles. The molinate-thiobencarb mixtures produced AI ranges suggesting simple additive toxicity to 13-day old, slightly less than additive toxicity to 6 and 90-day old, and slightly greater than additive toxicity to 24-day old striped bass (Figure 9). Collectively the four tests indicate simple additive toxicity of molinate and thiobencarb to striped bass larvae and juveniles. The mean LC50 values for the molinate-thiobencarb mixtures were 5.1 mg/L molinate and 0.40 mg/L thiobencarb (Table 13).

Generally, the no-observed-effect concentration (NOEC) values for the 144-h acute tests increased with age of larval striped bass (Table 14). Molinate NOEC values ranged from <1.5 to 7.0 mg/L for 6 to 28-day old bass, and thiobencarb NOEC values ranged from 0.07 to 0.40 mg/L for 13 to 45-day old bass, respectively. The herbicide mixture NOEC values ranged from 0.79 mg/L molinate and 0.07 mg/L thiobencarb to 3.5 mg/L molinate and 0.35 mg/L thiobencarb for 6 and 45-day old bass, respectively. The lowest-observed-effect concentration (LOEC) values also increased with age of fish. Survival was as sensitive as growth as an indicator of acute toxic effects from molinate and thiobencarb on striped bass larvae (Tables 5, 6, 9, 10, 11, and 14).

Table 14. No-observed-effect concentrations (NOEC) and lowest-observed-effect concentrations (LOEC) of molinate and thiobencarb (mg/L) during 6-day tests on striped bass larvae.

Age		NOEC		LOEC	
		Molinate	Thiobencarb	Molinate	Thiobencarb
6-day	Molinate [a,b]	<1.5		1.5	
	Thiobencarb [b]		0.12		0.19
	Molinate-Thiobencarb Mixture [b]	0.79	0.07	2.0	0.16
13-day	Molinate [a]	3.1		4.9	
	Thiobencarb [a,b]		0.07		0.11
	Molinate-Thiobencarb Mixture [b]	<0.88	<0.05	0.88	0.05
24-day	Molinate [b]	3.6		3.6	
	Thiobencarb [b]		0.27		0.45
	Molinate-Thiobencarb Mixture [b]	0.49	0.08	2.6	0.19
28-day	Molinate [b]	7.0		16	
	Thiobencarb [b]		0.18		0.35
	Molinate-Thiobencarb Mixture [b]	3.2	0.27	5.9	0.48
45-day	Thiobencarb [b]		0.40		0.77
	Molinate-Thiobencarb Mixture [b]	3.5	0.35	4.9	0.49

[a] Based on significantly reduced growth compared to control ($P < 0.05$).

[b] Based on significantly reduced survival compared to control ($P < 0.05$).

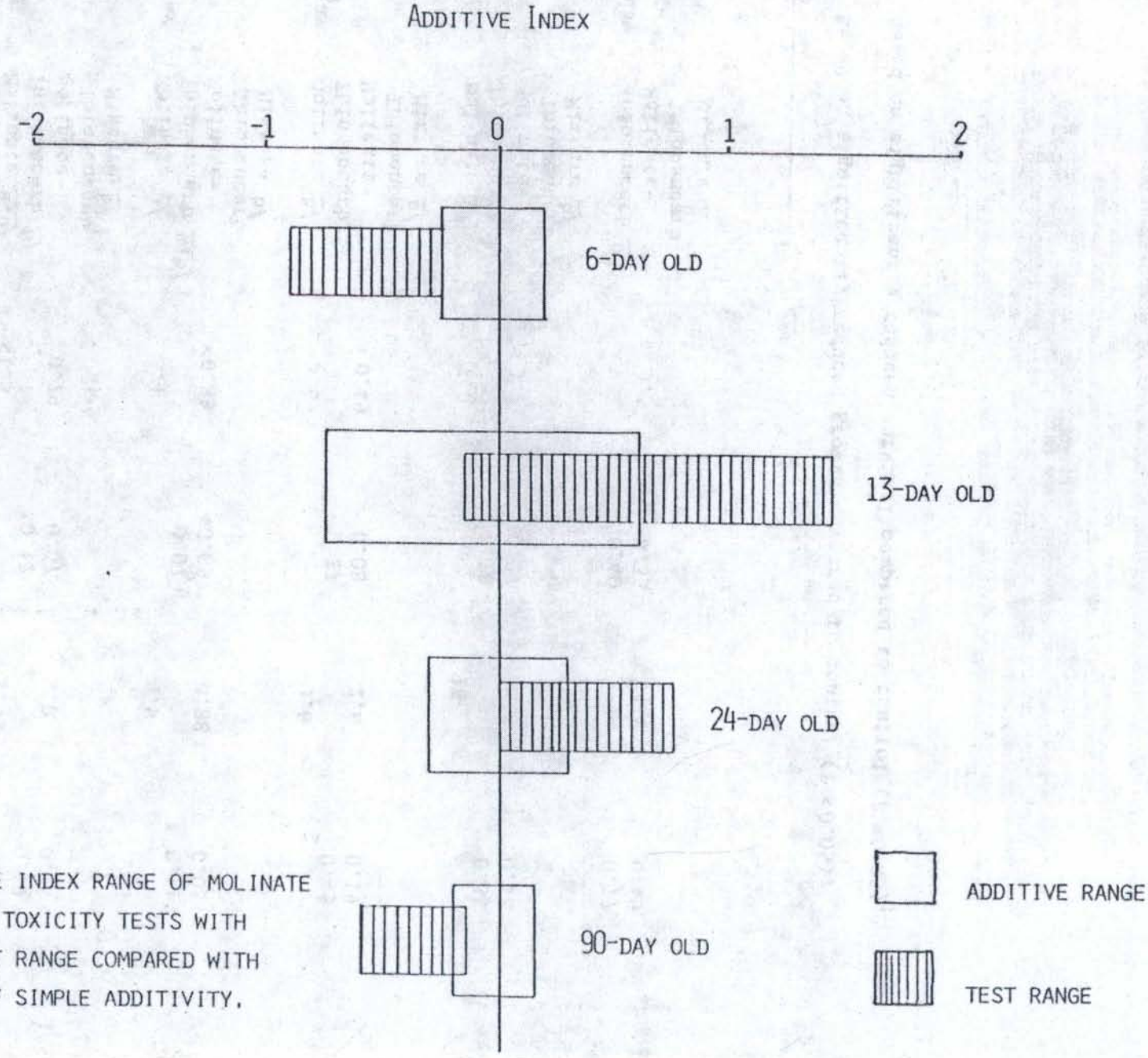

FIGURE 9. ADDITIVE INDEX RANGE OF MOLINATE AND THIOBENCARB IN TOXICITY TESTS WITH STRIPED BASS. TEST RANGE COMPARED WITH CALCULATED RANGE OF SIMPLE ADDITIVITY.

DISCUSSION

Culturing and Testing Techniques

Varying degrees of success in culturing and testing striped bass occurred during the 3-year study for a variety of reasons. Sufficient water hardness and alkalinity (>200 mg/L $CaCO_3$) in culture and test water are important factors for the health of striped bass (Stickney 1986). The water hardness and alkalinity used for culturing and testing striped bass in our study varied from 20 to 100 mg/L $CaCO_3$. Stress caused by low water hardness could lead to poor feeding, increased occurrence of disease, and weaker test organisms.

There is no evidence to indicate that brine shrimp nauplii are a nutritionally complete diet even through they are recommended food for larval striped bass (American Fisheries Society 1976; Lewis and Heidinger 1981; Rogers et al. 1982). To the contrary, nauplii have been shown to lack essential fatty acids and some elements required by marine fish. Cowgill et al. (MS 1986) has suggested correcting deficiency diseases in larval fish by supplementing brine shrimp with algae grown in nutrient enriched media. Survival of prolarvae and early postlarvae may be improved by offering them a combination of brine shrimp nauplii and unicellular algae. Additionally, algae are smaller than nauplii and not mobile thus, more available than nauplii as a food item to young bass.

Nitrogenous wastes, particularly ammonia, are highly toxic to striped bass larvae (Stickney 1986). Although water recirculation systems for culturing striped bass have been used successfully, our experience

indicates that the systems are difficult to maintain and are easily disrupted resulting in toxic concentrations of nitrogenous wastes. Flow through or partial recirculating systems are much easier to operate and keep nitrogenous wastes below toxic levels.

Larval striped bass control groups had better survival in circular clear glass test chambers with deep (>15 cm) water columns under well-lighted conditions. Light may have improved the food catching ability of the striped bass. A small water current provided by weak aeration helped keep the prolarvae suspended. Test chambers with netting should be avoided because larval striped bass often become impinged in the netting.

Acute Toxicity of Rice Herbicides

The 96-h and 144-h continuous flow LC50 values indicated that the sensitivity of striped bass to the rice herbicides decreased with increased age. The LC50 values of molinate and molinate-thiobencarb mixtures for larvae 24-days old and younger were generally lower than those for larvae and juveniles 28-days old and older. The LC50 values of thiobencarb for larvae 6-days old were lower than those for larvae and juveniles 13-days old and older. The 96-h LC50 values of molinate and thiobencarb for 90-day old bass agree with those for yearling striped bass of 8.1 to 12 mg/L molinate and 0.76 mg/L thiobencarb reported by Finlayson and Faggella (1986).

The herbicide mixtures produced AI ranges indicating additive toxicity of molinate and thiobencarb to larval and juvenile striped bass. These data indicate that both herbicides were twice as toxic when present

together at 1:1 LC50 value ratios than they were individually. Finlayson and Faggella (1986) reported additive toxicity from molinate-thiobencarb mixtures to juvenile channel catfish, chinook salmon, and steelhead trout.

Survival was as sensitive as growth as an indicator of toxic effects on striped bass larvae from molinate and thiobencarb. Woltering (1984) also found survival to be as sensitive an indicator of toxic effects in chronic and early-life-history-stage toxicity tests with a variety of toxicants and fish species.

Hazard Assessment of Rice Herbicides to Striped Bass and Opossum Shrimp

The hazard assessment procedure compares estimated or measured environmental concentrations with toxic adverse effects likely to result from those exposures. Three possible measurements of risk are possible from an assessment: i) minimal hazard; ii) potentially excessive hazard; and iii) uncertain hazard. Risk should not be confused with safety which is a value judgement, largely social in origin, on the perceived acceptability of the risk.

The Standard Guide (E1023-84) for assessing the hazard of materials to aquatic life used here was developed by the American Society for Testing and Materials (ASTM 1984). Our assessment evaluates the toxicological hazard to striped bass and opossum shrimp Neomysis mercedis resulting from exposure to rice herbicides in the Sacramento River and downstream estuary. Opossum shrimp are a primary food item of young bass in the Sacramento-San Joaquin Delta (Orsi and Knutson 1979). It is based on the measured and estimated acute and chronic toxicity of molinate-thiobencarb mixtures to

young striped bass and opossum shrimp and the measured and expected environmental concentrations of the herbicides.

The procedure is based on reasonable worst-case analyses that are consistent with scientific validity. Judgements in the measurements of risk are done using safety factors, the quotient of a toxicologically significant concentration divided by an appropriate environmental concentration. If these safety factors are large then the risk is judged minimal, and if the factors approach or are less than one then the risk is judged unacceptable. If the risk cannot be judged minimal or unacceptable then it is judged uncertain, requiring additional information. The value of the safety factor for minimal risk will vary with the quantity, quality, and kind of data available concerning the environmental concentrations and toxic effects, and the degree of confidence in the validity of any assumptions and extrapolations that were used.

Environmental Concentrations - Extensive and accurate data are available on the measured environmental concentrations of rice herbicides in the Sacramento River and Sacramento-San Joaquin Delta. The CDFG has been monitoring molinate and thiobencarb concentrations in the Sacramento River since 1982 (Finlayson and Lew 1983a; 1983b; 1984; 1985; 1986), and the Regional Water Quality Control Board-Central Valley Region has been monitoring rice herbicide concentrations in the Sacramento-San Joaquin Delta since 1983 (unpublished data). Expected worst-case environmental concentrations of rice herbicides were estimated from the State Water

Resources Control Board dilution flow model for rice herbicide concentrations in the Sacramento River (Cornacchia et al. 1984).

Restrictions on the use of these herbicides influence environmental concentrations in the agricultural drains and ultimately, the Sacramento River. The Department of Food and Agriculture began mandatory use restrictions during the 1984 rice season which have since been modified yearly (California Department of Food and Agriculture MS 1984; 1985). Rather than conduct individual assessments for years 1984, 1985, and 1986, we chose 1985 as the worst-case (highest levels of herbicides) year for measured rice herbicide concentrations in the Sacramento River (Finlayson and Lew 1985; Regional Water Quality Control Board-Central Valley Region 1985, unpublished data). Two worst-case hypothetical environmental concentration scenarios were also assessed: i) minimum compliance with the Department of Health Services action levels (20 ug/L molinate and 1 ug/L thiobencarb) for Sacramento River drinking water; and ii) minimum compliance with the DFG guidelines (90 ug/L molinate and 24 ug/L thiobencarb) for protection of aquatic life in agricultural drains and expected minimal dilution (24%) in the Sacramento River below Sacramento using the State Water Resources Control Board model (Cornacchia et al. 1984). Both maximum and estimated daily weighted mean (one-half maximum) concentrations were used in the assessments (Table 15).

Toxicologically Significant Concentrations - A review of acutely lethal levels for striped bass (Tables 13 and 14) indicated that 13-day old postlarvae were the most sensitive and 90-day old juveniles were the least

Table 15. Measured and estimated worst-case concentrations (ug/L) of molinate and thiobencarb in Sacramento River and Sacramento-San Joaquin Delta.

Location and scenario	Molinate	Thiobencarb
I. Sacramento River @ Sacramento		
A. Measured concentration (1985 data)[a,b/]		
1. Maximum	16	4.1
2. Average	8.0	2.0
B. Estimated concentration (DHS action levels)[c/]		
1. Maximum	20	1.0
2. Average	10	0.5
C. Estimated concentration (DFG guidelines)[d/]		
1. Maximum	22	5.8
2. Average	11	2.9
II. Sacramento-San Joaquin Delta @ Rio Vista		
A. Measured concentration (1985 data)[e/]		
1. Maximum	10	2.3
2. Average	5.0	1.2

a/ Concentrations of molinate ($\geq$1 ug/L) in 1985 lasted for 38 days in Sacramento River at Sacramento; average molinate concentration is weighted numeric mean for 38-day period.

b/ Concentrations of thiobencarb ($\geq$0.5 ug/L) in 1985 lasted for 26 days in Sacramento River at Sacramento; average thiobencarb concentration is weighted numeric mean for 26-day period.

c/ Maximum concentrations represent minimum compliance with DHS action levels and average concentrations estimated as weighted numeric means for 38-day and 26-day exposure periods for molinate and thiobencarb, respectively.

d/ Maximum concentrations represent minimal compliance with DFG guidelines in all agricultural drains and worst-case dilution (24%) in Sacramento River; average concentrations estimated as weighted numeric means for 38-day and 26-day exposure periods for molinate and thiobencarb, respectively.

e/ Maximum concentrations from Regional Water Quality Control Board - Central Valley Region 1985 data; average concentrations estimated as weighted numeric means for 38-day and 26-day exposure periods for molinate and thiobencarb, respectively.

sensitive to molinate, thiobencarb, and molinate-thiobencarb mixtures. Sensitivities to molinate-thiobencarb mixtures were used in the hazard assessment because of the additive toxic effects from the two herbicides on larval striped bass (Figure 9) and young chinook salmon (Table 16; Finlayson and Faggella 1986). The 96-h LC50 values for 13-day and 90-day old striped bass life-history-stages were used in estimating safety factors for acutely lethal levels (Table 17). The acute NOEC values from the test on 6-day old prolarvae were also used because these data were more definitive than and approximated those for 13-day old prolarvae (Table 14).

Chronic striped bass eggs-to-fry NOEC values for molinate and thiobencarb were estimated from tests on 90-day old juvenile striped bass by dividing those 96-h LC50 values by corresponding acute-chronic ratios. The NOEC is the lower level of the MATC (maximum allowable toxicant concentration) range and is a conservative estimate of an unacceptable chronic level; the upper level of the MATC is the LOEC. The acute-chronic ratios are quotients of the juvenile 96-h LC50 values divided by the chronic eggs-to-fry NOEC values for chinook salmon exposed to molinate, thiobencarb, and a molinate-thiobencarb mixture (Table 16). It appears that chinook salmon (Table 16) are a suitable model for predicting effects of the herbicides on striped bass (Table 17) given the similarity of 96-h LC50 values of molinate and thiobencarb alone and molinate-thiobencarb mixtures for juvenile fish.

Acute (7 and 14-day tests) LC50 values from tests with molinate and thiobencarb alone and a molinate-thiobencarb mixture on opossum shrimp

Table 16. Toxicologically significant concentrations (mg/L) of molinate and thiobencarb on chinook salmon juveniles and eggs-to-fry.

Solution	Juvenile 96-h LC50 [a]		Eggs-to-fry 90-day MATC [b]		Acute-chronic ratios (LC50/NOEC)-(LC50/LOEC)	
	Molinate	Thiobencarb	Molinate	Thiobencarb	Molinate	Thiobencarb
Molinate alone	14		0.42-0.73		33-19	
Thiobencarb alone		0.76		0.028-0.049		27-16
Molinate-Thiobencarb Mixture	9.3	0.43	0.16-0.23	0.009-0.013	58-40	48-33

[a] From Finlayson and Faggella (1986).

[b] Unpublished data; MATC represented by NOEC-LOEC range; California Department of Fish and Game, Pesticide Investigations Unit, Rancho Cordova, California 95670 (1987).

Table 17. Toxicologically significant concentrations (mg/L) of molinate and thiobencarb on striped bass.

Solution	6-day old 144-h NOEL		13-day old 96-h LC50		90-day old 96-h LC50		Eggs-to-fry MATC [a]	
	Molinate	Thiobencarb	Molinate	Thiobencarb	Molinate	Thiobencarb	Molinate	Thiobencarb
Molinate alone	1.5	--	11	--	14	--	0.42-0.71 [b]	
Thiobencarb alone	--	0.12	--	0.54	--	0.67		0.025-0.043 [c]
Molinate-Thiobencarb Mixture	0.79	0.09	3.0	0.24	7.9	0.52	0.14-0.20 [d]	0.011-0.016 [d]

[a] MATC represented by NOEC-LOEC range.

[b] Estimated using acute-chronic ratios of 33 and 19 from chinook salmon juvenile and eggs-to-fry tests.

[c] Estimated using acute-chronic ratios of 27 and 16 from chinook salmon juvenile and eggs-to-fry tests.

[d] Estimated using acute-chronic ratios of 58 and 40 for molinate and 48 and 33 for thiobencarb from chinook salmon juvenile and eggs-to-fry tests.

(State Water Resources Control Board 1985) indicated simple additivity for the two herbicides (Table 18). Chronic 42-day tests on molinate and thiobencarb alone produced NOEC values for growth of 0.026 and 0.007 mg/L, respectively. A chronic 42-day test was conducted with a molinate-thiobencarb mixture for the State Water Resources Control Board (1985) but the results were invalid because of poor survival in the control group and bad laboratory practices. However, we estimated chronic MATC values for the molinate-thiobencarb mixture as one-half of those values for the individual herbicides, assuming additivity in the mixture during the 42-day test. Additivity was demonstrated in the chronic 90-day test on chinook salmon (Table 17) and in the 7 and 14-day acute tests on opossum shrimp.

Hazard Assessment - The acute safety factors indicate no hazard to striped bass from a short-term (4 to 6 day) exposure to the combined rice herbicides; safety factors ranged from 36 to 1,580 for molinate and 16 to 1,040 for thiobencarb (Table 19). However, exposure in the Sacramento River and downstream estuary lasts from 30 to 40 days and coincides with the eggs-to-fry stage of striped bass. The safety factors (Table 19) indicate minimal hazard to striped bass eggs-to-fry because the estimated chronic NOEC values (Table 17) are greater (at least 2 fold) than the worst-case maximum environmental concentrations (Table 15). For average herbicide concentrations, the safety factors using estimated chronic NOEC values ranged from 13 to 28 for molinate and from 3.9 to 22 for thiobencarb. It is anticipated that future herbicide use restrictions will result in compliance or better with the DHS action levels of 20 ug/L

Table 18. Toxicologically significant concentrations (mg/L) of molinate and thiobencarb on opossum shrimp Neomysis mercedis in acute and chronic tests. Data from State Water Resources Control Board (1985).

	LC50 Values		LC50 Values	
	7-day		14-day	
Solution	Molinate	Thiobencarb	Molinate	Thiobencarb
Molinate alone	2.5		0.82	
Thiobencarb alone		0.21		0.091
Molinate-Thiobencarb Mixture	2.1	0.071	0.77	0.024

	MATC Values [a]	
	42-day	
Solution	Molinate	Thiobencarb
Molinate alone	0.026-0.045	
Thiobencarb alone		0.006-0.013
Molinate-Thiobencarb [b] Mixture	0.013-0.023	0.003-0.006

[a] MATC represented by NOEC-LOEC range.

[b] NOEC and LOEC values for mixture estimated as one-half of values for herbicides alone.

Table 19. Estimated safety factors (toxicologically significant concentration/environmental concentration) for 6-day old prolarval, 13-day old postlarval, 90-day old juveniles, and eggs-to-fry striped bass in Sacramento River and Sacramento-San Joaquin Delta. Safety factors based on combined herbicides.

	Safety Factors							
	Molinate				Thiobencarb			
	Acute			Chronic	Acute			Chronic
Location and scenario	6-day old NOEC	13-day old LC50	90-day old LC50	Eggs-to-fry NOEC	6-day old	13-day old LC50	90-day old LC50	Eggs-to-fry NOEC
I. Sacramento River @ Sacramento								
A. Measured concentration (1985 data)								
1. Maximum	49	190	490	8.9	22	58	130	2.7
2. Average	99	380	990	17	45	120	260	5.7
B. Estimated concentration (DHS action levels)								
1. Maximum	40	150	400	7.0	90	240	520	11
2. Average	79	300	390	14	180	480	1,040	22
C. Estimated concentration (DFG guidelines)								
1. Maximum	36	140	360	6.4	16	41	90	1.9
2. Average	72	270	720	13	31	83	180	3.9
II. Sacramento-San Joaquin Delta @ Rio Vista								
A. Measured concentration (1985 data)								
1. Maximum	79	300	790	14	40	100	230	4.6
2. Average	150	600	1,600	28	79	200	430	9.2

molinate and 1 ug/L thiobencarb in the Sacramento River at Sacramento; minimal compliance and better will result in safety factors of at least 7 for molinate and 11 for thiobencarb during the 30 to 40 days of exposure in May and June.

The acute safety factors indicate no hazard to opossum shrimp from a short term (7 to 14 day) exposure to the combined rice herbicides; safety factors ranged from 38 to 410 for molinate and from 4.1 to 142 for thiobencarb (Table 20). However, exposure in the Sacramento River and downstream estuary lasts from 30 to 40 days. The safety factors (Table 20) indicate potentially excessive hazard to opossum shrimp because estimated chronic NOEC values (Table 18) are 0.6 times the worst-case maximum environmental concentrations (Table 15). Safety factors ranged from 0.6 to 1.3 for molinate and from 0.5 to 3.0 for thiobencarb. For average herbicide concentrations, the safety factors using estimated chronic NOEC values ranged from 1.2 to 2.6 for molinate and from 1.0 to 6.0 for thiobencarb. Compliance with the DHS action level for thiobencarb will result in acceptable environmental concentrations, but the DHS action level for molinate will not protect against toxic conditions for the opossum shrimp. Likewise, compliance with the present DFG guidelines (90 ug/L molinate and 24 ug/L thiobencarb) for agricultural drains will not protect the opossum shrimp under minimal dilution (24% of total flow) conditions below Sacramento. Lowering the DFG guidelines to 54 ug/L molinate and 12 ug/L thiobencarb will result in a mean safety factor of 1.0 below

Table 20. Estimated factors (toxicologically significant concentration/environmental concentration) for opossum shrimp Neomysis mercedis in Sacramento River and Sacramento-San Joaquin Delta. Safety factors based on combined herbicides.

	Safety Factors					
	Molinate			Thiobencarb		
	Acute		Chronic	Acute		Chronic
Location and scenario	7-day LC50	14-day LC50	42-day NOEC	7-day LC50	14-day LC50	42-day NOEC
I. Sacramento River @ Sacramento						
A. Measured concentration (1985 data)						
1. Maximum	130	48	0.8	17	5.8	0.6
2. Average	260	96	1.6	35	12	1.3
B. Estimated concentration (DHS action levels)						
1. Maximum	100	38	0.6	71	24	3.0
2. Average	210	76	1.3	142	48	6.0
C. Estiamted concentration (DFG guidelines)						
1. Maximum	95	35	0.6	12	4.1	0.5
2. Average	190	70	1.2	24	8.2	1.0
II. Sacramento-San Joaquin Delta @ Rio Vista						
A. Measured concentration (1985 data)						
1. Maximum	210	77	1.3	31	10	1.3
2. Average	410	150	2.6	62	21	2.5

Sacramento and should protect the opossum shrimp during worst-case dilution years.

It would be desirable in the hazard assessment to have empirical information on the chronic effects of molinate and thiobencarb on striped bass eggs-to-fry and opossum shrimp to confirm the estimated acute-chronic ratios and safety factors. The present assessment indicates minimal toxicological hazard to young striped bass from exposure to rice herbicides in the Sacramento River and Sacramento-San Joaquin Delta. We presently have no viable method for testing striped bass <6-days old. There is no reason to believe that striped bass have a different acute-chronic ratio to rice herbicides than chinook salmon. Cornacchia et al. (1984) lists an acute-chronic ratio for channel catfish Ictalurus punctatus exposed to molinate alone at 36 and for mysid shrimp Mysidopsis bahia exposed to thiobencarb alone at 17. Our acute-chronic ratios for chinook salmon exposed to the herbicides alone (Table 16) approximate these values for these species suggesting that our acute-chronic ratios for the herbicide mixture are reasonable.

The present assessment indicates potentially excessive hazard to opossum shrimp from exposure to rice herbicides in the Sacramento River and Sacramento-San Joaquin Delta during worst-case dilution years. The potentially excessive hazard to opossum shrimp can be corrected by lowering the DFG guidelines for herbicide concentrations in agricultural drains to 54 ug/L molinate and 12 ug/L thiobencarb which will result in a mean

chronic safety factor of 1 below Sacramento. Because molinate and thiobencarb are additive, the guidelines for molinate range from 0 to 90 ug/L and those for thiobencarb range from 0 to 24 ug/L (Table 21).

The worst-case safety factors used here are reasonable and conservative for protection of striped bass and opossum shrimp and allow for a margin of error. This margin of error is created by the estimations and assumptions inherent to this hazard assessment. The safety factors use chronic NOEC values rather than chronic LOEC values and sublethal effects are expected to occur at concentrations somewhere between the NOEC and LOEC values. The safety factors use maximum concentrations rather than average (one-half maximum) concentrations although the latter typically controls the degree of sublethal effects during chronic exposure. Finally, neither organism would be expected to be exposed to the worst-case environmental concentrations of the herbicides for more than a few days because of additional dilution in the Sacramento-San Joaquin Delta created by San Joaquin River flows and San Francisco Bay tidal action. This additional dilution is seen in the measured concentrations at Sacramento and Rio Vista in 1985 (Table 15) and the safety factors calculated for the two locations (Tables 19 and 20). The minimal 4 fold conservative margin of error is needed, however, because not all aquatic species have been tested under all conditions.

Table 21. Maximum concentrations (ug/L) of molinate and thiobencarb in agricultural drain water needed for protection of striped bass and opossum shrimp in the Sacramento River and Sacramento-San Joaquin Delta. Calculations assume simple additivity of molinate and thiobencarb and minimal dilution from Sacramento River.

Molinate	Thiobencarb
90	0
80	3
70	7
60	10
54	12
50	13
40	15
30	17
20	20
10	22
0	24

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Appendix A. Schedule of uncompleted acute and chronic tests with molinate, thiobencarb, and molinate-thiobencarb mixtures in 1984 through 1986.

Test series	Begin date	Age (developmental stage)	Herbicide	Comments
1984-1	5-1-84	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	100% mortality in all treatments
1984-2	5-4-84	6-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	100% mortality in all treatments
1984-3	5-5-84	4-day (prolarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	100% mortality in all treatments
1984-4	5-11-84	13-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	100% mortality in all treatments
1984-6	5-18-84	7-day (prolarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	20% mortality in controls
1984-8	6-8-84	28-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	20% mortality in controls
1985-1	4-27-85	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	50% mortality in controls
1985-2	5-9-85	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	50% mortality in controls

Appendix A. (Continued)

Test series	Begin date	Age (developmental stage)	Herbicide	Comments
1985-3	5-10-85	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	50% mortality in controls
1985-5	5-31-85	6-day (prolarvae)	Thiobencarb Molinate-Thiobencarb Mixture	75% mortality in highest treatment
1986-1	5-10-86	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	50% mortality in controls
1986-1	5-15-86	12-h prehatch (embryonic)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	50% mortality in controls
1986-3	6-12-86	11-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	75% mortality in controls
1986-4	6-14-86	13-day (postlarvae)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	75% mortality in controls
1986-6	8-6-86	90-day (juvenile)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	0% mortality in all treatments
1986-7	8-12-86	90-day (juvenile)	Molinate Thiobencarb Molinate-Thiobencarb Mixture	25% mortality in controls

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Appendix B

RESULTS OF A PROTOCOL TEST FOR CONDUCTING ACUTE TOXICITY EVALUATIONS WITH EARLY LIFE STAGES OF STRIPED BASS

Submitted to
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by
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Standard Agreement C-1518

RESULTS OF A PROTOCOL TEST FOR CONDUCTING ACUTE TOXICITY EVALUATIONS WITH EARLY LIFE STAGES OF STRIPED BASS

Introduction

The U.S. Environmental Protection Agency's research group at Newport, Oregon is involved in a program to develop and test the robustness of toxicity evaluation procedures for marine organisms, with the immediate goal of providing a suite of tests for use in regulating effluents discharged into the marine environment. A related State of California project is the development of culture and testing procedures for several species of estuarine organisms of special interest in San Francisco Bay and Delta region. As part of the latter investigation, the EPA laboratory in Newport conducted a parallel rearing and toxicty test evaluation in conjunction with California Fish and Game. Support for the EPA effort was made available by California Fish and Game (CF&G) through a cooperative agreement already in place between EPA and Oregon State University. Coordination of the details of the rearing and test procedures was provided by Brian Finlayson of California Fish and Games Pesticide Investigation Unit to Gary Chapman of EPA's Newport facility.

Methods

Details of the method were stipulated by CF&G. In summary, fish were reared and tested in reconstituted sea water made up with distilled-deionized tap water from the EPA laboratory and sea salts provided by CF&G. Brine shrimp (Artemia) cysts were supplied by CF&G and reared in 10 ppthousand seawater. Eggs and prolarvae were held nominally at 17 C and 4 ppt reconstituted seawater. Fish were fed Artemia nauplii at about 90,000 per feeding. Approximately 10 percent of the 325 liter recirculating seawater volume was replaced each day.

The striped bass eggs were provided by CF&G and were picked up at the Eugene, Oregon airport shortly after midnight on May 24, 1986. The package was immediately transported to Newport, arriving at the laboratory at 0300 hrs. The temperature of the egg transport water was 21 C. At 0319 hrs the eggs were placed into a McDonald hatching jar at a temperature of 17 C. The water flow through the hatching jar was 525 ml/min with the remaining 1175 ml/min of water flow providing a circular flow of water in the rearing tank in which the hatching jar was placed.

At 0735 the 520ml/min overflow from the hatching jar was at 17 C. but all of the eggs had hatched and only a few prolarvae were still in the jar. Observation of the hatched prolarvae through a dissecting scope led to the following notation in the notebook: "As a rough estimate I'd say about 1/2 of the larvae are active."

On May 27 the fish were "well eyed." Three-a-day feedings started this morning, about 75 hours after hatch. By the morning of May 30 "There are still lots of fish around the edge of the tank, but not as many as previously. The numbers appear to be fewer."

On May 31 the water temperature climbed to 19 C by 1515 hrs with the room air temperature in the low 80s (F). The custodial staff readjusted the thermostat; the room temperature thereafter remained in the mid-60s.

On June 1 the fish appeared to possess a swim-bladder and on June 2 the beating of the heart was discernible. A June 2 notebook notation read: "I'm concerned that the number of fish surviving until time for the test will either be insufficient for the test or make minimal stress capture difficult."

On June 4 the parallel group of fish being reared by CF&G were reported to have been killed as a result of a pump failure.

On June 5 twelve one-gallon jars were filled with 4 ppt reconstituted seawater and placed in a 17 C water bath. Assignment of concentration of test chemical to the jars was done by random number. The test chemical was the carbamate herbicide Thiobencarb and was supplied as an aqueous stock solution by CF&G. The stock solution was received at Newport on June 4 and was stored at room temperature. Stock solution was added to the test jars at 1410 hrs on June 6. Samples of the test solutions were taken within the hour and were stored in a refrigerator.

Because of an impending shipment of striped bass eggs due to arrive in Eugene on the evening of June 6, start up of the toxicity test was put off until June 7. Many larvae were collected from the rearing tank on the afternoon of June 6. Because of low numbers of fish in the tank the following method of capture was used: small numbers of fish were "siphoned" into a length of 8 mm i.d. glass tubing by covering one end of the tube with an index finger and inserting the other end several inches below the water surface and in the vicinity of the bass larvae; removing the finger caused a flow of water (and entrained bass larvae) into the tube; the upper end was re-covered and the contents transferred to a beaker; finally, the beaker was emptied into a bucket of 12 ppt aerated seawater and the

fish provided with Artemia. The fish were held in this manner until their transfer to the toxicity test chambers on the afternoon of June 7 (a holding period of about 24 hrs.) About 300 fish were transferred at this first capture; very few dead larvae were noted the next day and the fish appeared to be in good condition.

Beginning at 1345 hrs on June 7 groups of 4 to 7 larvae were removed from the holding bucket using the glass tube "siphon" procedure described above. The larvae were deposited into a white, glass spoon, excess water pipetted off, and the larvae placed into a test jar beginning with jar no. 1 and proceding in order through jar no. 12. This procedure was repeated for a second, third, fourth and fifth group per jar, until each jar contained 25 larvae. Distribution of test organisms was completed at 1510 hrs.

Test jars were placed in the head box for the recirculation system supplying the rearing tank. Each jar received gentle aeration. Water temperature was checked daily in from one to four jars and never deviated from 17.0 C. Jars were removed from the water bath early each afternoon after the aeration was removed and the water gently stirred for ten revolutions to concentrate dead and weak larvae in the center of the jar. All possibly dead fish were removed and placed in a small petri dish for observation under a dissecting scope. Fish showing sign of life, even if limited to weak ventilatory or heart activity, were placed back into the test jars. Fish showing no sign of life were removed and discarded. After 96 hours all fish that could be found were removed, observed, and enumerated in the following classifications: dead; live but immobile; weakly mobile; and normal. All but the dead fish were placed into sample vials and preserved in 10% formalin.

Discussion with Brian Finlayson indicated that those fish that were live but immobile would be termed dead by the criteria used by CF&G. Because the count of prolarvae into the test jars was believed to be more accurate than the final count of live and dead fish, all jars were assumed to have received 25 fish at the start of the test (except for jar no. 10 which was known to have received 26 and jar no. 7 which yielded 26 dead larvae.) Therefore, the final number of dead fish was taken to be the sum of the number of observed deaths plus the number of immobile fish at 96 hours plus the number of missing fish.

Following termination of the test, water samples were taken and shipped on ice to CF&G for thiobencarb analysis. In addition, samples were removed for pH analysis and Winkler dissolved oxygen determination. Temperatures were measured in each jar after they were removed from the water bath, but before fish were removed. The last four jars (9-12)

warmed appreciably prior to temperature measurement and do not represent test temperatures.

Results.

Between the receipt of the striped bass eggs and the start of the toxicity test with thiobencarb, the temperature ranged between 16.2 and 19.0 C; salinity varied between extreme values of 3.5 and 4.7 ppt (Table 1).

Mortality in the thiobencarb toxicity test showed an obvious dose related progression at the two highest concentrations of 0.67 and 1.20 mg/L (Table 2). Considerable intermediate mortality was seen at 0.21 and 0.38 mg/L and in one control jar. Minor mortality was seen at 0.12 mg/L and in the other control jar.

The 96-hr observations for condition of the larvae are given in Table 3. Normal larvae predominated in controls and the two lowest concentrations (0.12 and 0.21 mg/L). Most surviving larvae at 0.38 mg/L were weak and those at 0.67 were immobile. The 96-hr LC50 appeared to be between 0.38 and 0.67 mg/L.

No effects of thiobencarb were seen on the final lengths of the larvae from the 96-hr toxicity test. The lengths appear to be less than those for larvae from tests conducted by CF&G in previous years. Reported in arbitrary scaled units, the conversion to millimters indicated that all treatments had fish with a mean length between 4.94 and 5.18 mm (Table 5). Individual lengths ranged from 4.75 to 5.58 mm.

Several weeks following the termination of the test, analytical results from CF&G indicated that the thiobencarb concentrations were about 30 percent greater that nominal due to a similarly greater concentration in the stock solution. Based on the analyzed stock solution concentration, measured concentrations in the test jars averaged about 0.02 mg/L higher than nominal (Table 6) and were very stable over the period of the test.

Discussion.

Success with the first group of striped bass eggs indicated that the procedures used in collection, shipping, and receiving of the eggs were acceptable. A subsequent failure with a second group of eggs was apparently caused by a combination of less viable eggs and a problem in transit from Sacramento to Eugene that led to a 12 hr delay in receipt of the eggs at Eugene.

The toxicity test results provided an apparent 96-hr LC50 that was in reasonable agreement with those obtained in earlier tests by CF&G. A combination of factors, primarily the 17 C rearing temperature, resulted in the larvae being tested at a relatively small size (5 mm) and before many larvae had begun actiye feeding. I believe that a warmer temperature would have resulted in a more typically sized larvae at two weeks post hatch and more feeding larvae being represented in the population. However, this may also have resulted in a poorer survival in the stock prior to the test, a survival that was probably only about 5-10 percent under the existing conditions. Using more developed larvae in the test would presumably have allowed a somewhat better control survival.

Past success with rearing striped bass larvae notwithstanding, I believe that a smaller first food would provide a better and more natural prey for striped bass than the Artemia nauplii currently prescribed. Although available egg numbers may preclude broader experimentation at our laboratory, I would like to try rearing larvae on a diet that included a smaller prey organism (perhaps a rotifer) in addition to Artemia.

Success in rearing many organisms has been achieved with artificial media composed of deionized water and sea salts or off-the-shelf chemicals. Such media provide a standard matrix for the conduct of toxicity tests. and this allows for better comparison of data between tests, between effluents, and between laboratories. Often this success is achieved in spite of, rather than because of, the charactisitics of the medium. It would be interesting to look at the rearing of striped bass in a medium blending natural surface freshwater and natural seawater, preferably in a minimally acceptable flow-through mode.

Measurement of dissolved oxygen, pH, and ammonia were hampered by lack of meters for these measurements caused by a delay in funding of the EPA-Oregon State University cooperative agreement to which the striped bass test was to be attached. The agreement was funded effective June 6, 1986, and the equipment will be available for the next round of striped bass testing.

A significant loss of thiobencarb from similar test solutions in CF&G tests suggested a difference between the results from the present study and those conducted by CF&G. Discussion with Brian Finlayson elucidated a probable cause for this discrepency between the two studies. Our tests were conducted in freshly prepared 4 ppt seawater, while the CF&G tests were composed in water from the striped bass rearing system. This suggests that. bacterial decomposition of the thiobencarb was the mode of detoxication in the CF&G tests.

Table 1. Summary of temperature and salinity values in the rearing apparatus.

Date	Time	Temp. (C)	Salinity (ppt)
5/23	1000	17.0	5.1*
5/23	1440	18.0	4.5*
5/23	1530	18.0	4.2
5/24	0300	17.0	-
5/24	0735	17.0	-
5/24	1325	17.0	-
5/25	1225	17.0	4.2
5/27	<1005	17.0	-
5/27	1005	17.0	4.2
5/28	<0935	17.0	-
5/29	0820	17.0	-
5/29	1130	17.0	4.5
5/30	0810	17.0	4.1
5/30	1335	19.0	4.7*
5/30	<1425	19.0	4.1
5/30	1515	19.0**	-
5/31	1010	17.0	-
5/31	1120	17.0	4.3
5/31	1225	17.0	3.5
6/01	1030	17.0	3.5
6/01	1125	17.5	3.9
6/02	0815	16.2	-
6/02	1140	17.0	4.2
6/02	1610	17.0	-
6/03	0830	16.4	-
6/04	0820	16.8	-
6/05	0815	17.0	-
6/05	1405	17.5	4.5
6/06	0830	17.0	-

* Adjusted to correct towards 4 ppt
** Adjusted room temperature

Table 2. Summary of Cumulative Mortality (number of dead fish removed) at various times during the test.

Jar No.	Nominal Conc(mg/L)	24hr	48hr	72hr	96hr
9	Control	0	1	2	4
10	Control	2	4	6	11
6	0.12	0	1	1	2
8	0.12	1	1	2	3
5	0.21	1	1	6	9
11	0.21	2	2	3	8
2	0.38	0	2	5	8
4	0.38	1	3	3	7
1	0.67	1	4	4	12
12	0.67	0	0	5	17
3	1.20	3	15	19	22
7	1.20	3	10	23	26

Table 3. Summary of condition of fish at the end of the 96-hr test. All jars were presumed to have received 25 fish at the start of the test (except No. 10 which received 26 fish.)

Jar No.	Nominal Conc(mg/L)	Dead	Immobile	Weak	OK	Lost	Total "Dead"
9	Control	4	0	1	17	3	7
10	Control	11	0	1	12	1	12
6	0.12	2	0	3	14	6	8
8	0.12	3	1	5	11	5	9
5	0.21	9	0	9	5	2	11
11	0.21	8	0	5	10	2	10
2	0.38	8	2	10	2	3	13
4	0.38	7	1	14	3	0	8
1	0.67	12	9	2	0	2	23
12	0.67	17	4	1	0	3	24
3	1.20	22	2	0	0	1	25
7	1.20	26	0	0	0	0	26

Table 4. Summary of chemistry and conditions in test jars at the termination of the 96-hr test.

Jar No.	Nominal Conc(mg/L)	pH	D.O. (mg/L)	Temperature (C)
9	Control	7.63	8.76	18.2*
10	Control	7.87	9.04	18.2*
6	0.12	7.18	9.48	17.3
8	0.12	7.55	9.00	17.3
5	0.21	7.22	9.36	17.3
11	0.21	7.70	9.00	18.2*
2	0.38	7.04	9.04	17.2
4	0.38	7.31	8.94	17.2
1	0.67	-	9.20	17.2
12	0.67	7.75	9.02	18.2*
3	1.20	7.22	9.08	17.2
7	1.20	7.20	8.80	17.3

*Result of sitting at room temperature following removal from the water bath (see text).

Table 5. Standard lenghts of striped bass larvae at the termination of the 96-hr toxicity test.

	Nominal Thiobencarb Conc. (mg/L) and Jar No.											
Length*	Control		0.12		0.21		0.38		0.67		1.20	
	9	10	6	8	5	11	2	4	1	12	3	7
5.7	1				1				1			
5.8	4		2	3	1	1	3	2			1	
5.9	5		5	7	4	4	5	2	1	1		
6.0	2	2	7	6	2	5	2	5	7	2		
6.1	1	4		2	3		1	2	1			
6.2		1	1		1	3		1	1	1		
6.3							3	4				
6.4		1				1						
6.5	1											
6.6		1										
6.7						1						
Avg.	5.93		5.94		6.00		5.99		5.99		5.90	
Avg.	6.22		5.94		6.07		6.06		6.03		---	

*arbitrary scale units, 1 unit = 0.83 mm
Average lengths converted to millimeters are:

Avg.	4.94	4.95	5.00	4.99	4.99	4.93
Avg.	5.18	4.95	5.06	5.05	5.02	---

Table 6. Results of CF&G thiobencarb analysis of samples from the EPA toxicity test.

	Concentration (mg/L)		
		Measured	Measured
Jar No.	Nominal	Initial	Final
1	0.67	0.80	0.87
2	0.38	0.45	0.44
3	1.20	1.50	1.40
4	0.38	0.45	0.45
5	0.21	0.30	0.31
6	0.12	0.15	0.15
7	1.20	1.40	1.50
8	0.12	0.17	0.17
9	Control	<0.01	0.002
10	Control	<0.01	0.001
11	0.21	0.32	0.33
12	0.67	0.78	0.74

Appendix C. Daily mean quality of water for the static acute toxicity test.

Date	pH	Salinity (o/oo)	D.O. (mg/L)	Temperature (°C)
6-13-86	7.8	2.5	9.6±0.1 [a/] (9.5-9.6)	17.5
6-14-86	--	2.5	9.6±0.0 (9.5-9.6)	17.5±0.0 (17.5-18.0)
6-15-86	--	2.5	9.0±0.1 (8.9-9.2)	18.5±0.0 (18.0-18.5)
6-16-86	--	2.5	9.1±0.1 (9.0-9.3)	18.5
6-17-86	8.0	2.5	9.2±0.1 (9.1-9.3)	17.5

[a/] Mean ± S.E. with range in parentheses.