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TOXICITY OF WEST POINT EFFLUENT TO MARINE INDICATOR ORGANISMS

by

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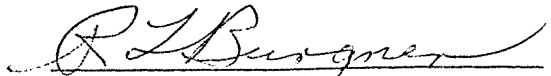

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TABLE OF CONTENTS

		Page
LIST OF TABLES		v
LIST OF FIGURES		vii
1.0	SUMMARY	1
2.0	ACKNOWLEDGEMENTS	5
3.0	INTRODUCTION	7
4.0	MATERIALS AND METHODS	9
4.1	Marine Bioassay Laboratory and Facilities	9
4.1.1	Laboratory	9
4.1.2	Seawater Supply System	9
4.1.3	Seawater Heating System	11
4.1.4	Temperature Control System	13
4.1.5	Additional Systems	15
4.1.6	Laboratory Equipment	15
4.1.7	Sewage Effluent Treatment System	18
4.2	Test Animal Collection, Holding and Acclimation	22
4.3	Bioassay Procedures	23
4.3.1	Proportional Diluter System	23
4.3.2	Acute Bioassays	25
4.3.3	Data Analysis	27
4.3.4	Chronic Bioassays	29
4.4	Water Quality Monitoring Procedures	30
4.4.1	Acute and Chronic Bioassays	30
4.4.2	METRO Effluent Analysis	32
4.5	Biological Sample Collection and Preparation	32
4.5.1	Histology	32
4.5.2	PCB Analysis	34
4.5.3	Trace Metal Analysis	35
5.0	RESULTS	38
5.1	Acute Bioassays	38
5.1.1	LC50 Determinations for Five Resident Species	38
5.1.2	Seasonal Changes in Effluent and Receiving Water Quality	39

Page

5.1.3 Temperature Effects 45
5.1.4 Life Stage Mortality Differences 49
5.1.5 Filtered Effluent 51
5.1.6 Dechlorination 55
5.1.7 Ammonia Removal 58
5.1.8 Excessive Fish Mortality 64
5.1.9 Gill Histopathology 67

5.2 Eight-Week Chronic Bioassays 72

5.2.1 Mortality 72
5.2.2 Trace Metal Bioaccumulation 74
5.2.3 PCB Bioaccumulation 79
5.2.4 Chronic Histopathology 85

6.0 DISCUSSION AND CONCLUSIONS 89

6.1 Acute Bioassays 89

6.1.1 Acute Toxicity of West Point Sewage Effluent . . 89
6.1.2 Acute Toxicity of Treated West Point Effluent . . 91

6.1.2.1 Chlorine Toxicity 91
6.1.2.2 Chlorine Concentrations at METRO outfall 93
6.1.2.3 Dechlorination 94
6.1.2.4 Ammonia Toxicity 96

6.2 Trace Metal Toxicity and Bioaccumulation 98

6.2.1 Requirements of Trace Metals in Aquatic Organisms 98

6.2.1.1 Chemistry of Trace Metals Related to Bioaccumulation and Toxicity 99
6.2.1.2 Mode of Entry of Trace Metals in Some Aquatic Organisms 102

6.2.2 Trace Metal Toxicity of West Point Effluent . . . 103

6.2.2.1 Cadmium 104
6.2.2.2 Chromium 105
6.2.2.3 Copper 106
6.2.2.4 Mercury 107
6.2.2.5 Nickel 109
6.2.2.6 Lead 110
6.2.2.7 Zinc 111

6.2.3 Bioaccumulation Studies Related to Sewage Outfalls 112
6.2.4 Trace Metal Tissue Concentrations from Chronic Bioassays 114

Page

6.3 Histopathology 115

6.3.1 Acute Histopathology 115
6.3.2 Chronic Histopathology 116

7.0 REFERENCES 118

8.0 APPENDIX 124

LIST OF TABLES

Table		Page
1	Equipment and methodology of water chemistry analysis for West Point sewage effluent (WPE) acute and chronic bioassays	31
2	Summary of methodology used by METRO laboratories for chemical analysis of West Point sewage effluent	33
3	Comparison of dissolved oxygen levels in aerated and unaerated dilutions of West Point effluent (WPE) in Bioassays 7 and 8	40
4	Average test tank temperature, total residual chlorine, and dissolved oxygen values for temperature Bioassays 9, 10 and 11, using shiner perch	48
5	Comparison of turbidity and total residual chlorine values in filtered and unfiltered WPE as measured in head tanks and test tanks of Bioassay 13 (shiner perch)	53
6	Average total residual chlorine, sulfur dioxide, pH, and dissolved oxygen values recorded from head tanks and test tanks of dechlorination Bioassays 16 (English sole) and 21 (shiner perch)	57
7	Average ammonia, total residual chlorine, and turbidity in head tanks and test tanks of ammonia removal Bioassays 18 (English sole) and 20 (shiner perch)	62
8	Pattern of unusual fish mortality at 40 hours in Bioassay 17 (English sole)	66
9	Pattern of unusual fish behavior and mortality from 30 to 36 hours in Bioassay 21 (shiner perch)	68
10	Trace metal values in METRO hourly raw sewage and effluent samples collected from 1500 to 2200 hours on July 22, 1976. Unusual fish behavior and mortality was observed in Bioassay 21 from approximately 1900 to 2100 hours	69
11	Results of histopathological examination of gill tissue from juvenile English sole exposed to chlorinated West Point effluent in Bioassay 14	70
12	Results of histopathological examination of gill tissue from shiner perch exposed to chlorinated West Point effluent in Bioassay 19	71

Table		Page
13	Average whole body total copper and zinc in shiner perch, *Cymatogaster aggregata*, and English sole, *Parophrys vetulus* for Bioassay 12. Fish were analyzed individually	75
14	Mean whole body total copper and zinc in shelled, common littleneck clams, *Protothaca staminea*, from Bioassay 15. Five organisms from each dilution were analyzed individually	78
15	Whole body total copper and zinc in juvenile English sole, *Parophrys vetulus*, from Bioassay 15. One composite sample of 5 fish was analyzed from each WPE dilution	80
16	Determination of diluter accuracy during a chronic bioassay. Values are in percent WPE (v/v) and are based on 53 daily measurements of diluter flow rates	83
17	Polychlorinated biphenyl (PCB) and total chlorinated hydrocarbon levels in English sole after 8-week exposure to dilutions of WPE in chronic Bioassay 12	84
18	Results of histopathologic examination of gill tissue from juvenile English sole exposed to sub-acute concentrations of chlorinated WPE for 8 weeks	86
19	Results of histopathologic examination of gill tissue from shiner perch exposed to sub-acute concentrations of chlorinated WPE for 4 weeks and 8 weeks	87
20	Concentration factors of copper and zinc in marine organisms. Adapted from Waldichuck (1974)	100

LIST OF FIGURES

Figure		Page
1	Diagram of marine bioassay laboratory at West Point	10
2	Mechanical design of seawater supply and heating system	12
3	Diagram of the pneumatically-operated seawater temperature control system	14
4	Diagrammatic view of laboratory interior and test facilities	16
5	End and side views of adjustable trough and shelf support system in wet laboratory	17
6	Flow diagram of seawater and sewage effluent filter columns and effluent ammonia removal (by ion exchange) and dechlorination (by sulfur dioxide) columns	20
7	Longitudinal section of an individual filter or treatment column	21
8	Diagram of sewage effluent diluter system. Percentage values are percent effluent in seawater v/v. Rubber drain hoses from overflow drains to floor drains are not shown	24
9	Determination of shiner perch and English sole LC50 in dilutions of unaerated and aerated West Point effluent (WPE) and dilutions of freshwater	41
10	Mean ambient seawater temperature, salinity, and dissolved oxygen as measured in the seawater head tank of the proportional diluter	43
11	Mean West Point effluent temperature and dissolved oxygen as measured in the effluent head tank of the proportional diluter	44
12	Mean and standard deviation salinity values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays. The line is eye-fitted	46
13	Mean and standard deviation dissolved oxygen values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays. The lines are eye-fitted	46

Figure		Page
14	Determination of shiner perch LC50 in dilutions of West Point effluent with seawater temperatures of 8.5, 13.5, and 18.5 C	47
15	Determination of differences in toxicity of West Point effluent to different age classes of English sole and shiner perch	50
16	Determination of shiner perch LC50 in filtered and unfiltered West Point effluent(WPE)	52
17	Mean and standard deviation turbidity values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays	54
18	Determination of English sole and shiner perch LC50 in dilutions of West Point effluent (WPE) and WPE dechlorinated with sulfur dioxide	56
19	Mean and standard deviation total residual chlorine values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays	59
20	Mean and standard deviation pH values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays. The expected line is an estimate based on simple dilution and assumes no buffering capacity for seawater or effluent. Both lines are eye-fitted	59
21	Determination of English sole and shiner perch LC50 in dilutions of West Point effluent (WPE) and WPE after ammonia removal with ion-exchange resin	61
22	Mean and standard deviation ammonia (N) values for dilutions of West Point effluent in head tanks and test tanks for all acute bioassays. The lines are eye-fitted	63
23	Average rainfall, effluent flow, and effluent ammonia nitrogen per month from October 1975 to September 1976 as measured by METRO Water Quality Staff at West Point	65
24	English sole gill tissue after 4 days in seawater control tank in Bioassay 14. Tissue is essentially normal except for *Trichodina sp.* between lamellae. X 250	73
25	English sole gill tissue after 4 days' exposure to 10% effluent in Bioassay 14. Arrows show areas affected by an inflammatory infiltrate. X 250	73

Figure		Page
26	English sole gill tissue from moribund fish exposed to 50% effluent for 6 hours in Bioassay 14. Arrows locate areas of epithelial hyperplasia. X 250	73
27	English sole gill tissue from moribund fish exposed to 70% effluent for 3 hours in Bioassay 14. Arrows show edema and separation of epithelium from vascular tissue (A) and congestion (B). X 250	73
28	Average whole body total copper content in shiner perch following 8-week exposure to West Point effluent in Bioassay 12. Wet ash analysis	76
29	Average whole body total copper content in shiner perch and English sole following 8-week exposure to West Point effluent in Bioassay 12. Dry ash analysis	76
30	Average whole body total zinc content in shiner perch following 8-week exposure to West Point effluent in Bioassay 12. Wet ash analysis	77
31	Average whole body total zinc content in shiner perch and English sole following 8-week exposure to West Point effluent in Bioassay 12. Dry ash analysis	77
32	Average whole body total copper content in shelled, common littleneck clams following 8-week exposure to West Point effluent in Bioassay 15. "Unexposed" data were not used in calculation of the regression line. The slope (b) is not statistically different from zero at the 95% confidence level	81
33	Average whole body total zinc content in shelled, common littleneck clams following 8-week exposure to West Point effluent in Bioassay 15. "Unexposed" data were not used in calculation of the regression line. The slope (b) is not statistically different from zero at the 95% confidence level	82
34	English sole gill tissue after exposure to 1% effluent for 8 weeks in Bioassay 15. Tissue is essentially normal, except for several *Oodinium sp.* embedded at base of filaments (arrows). X 250	88
35	Shiner perch gill tissue after exposure to 2.5% effluent for 4 weeks in Bioassay 22. Arrow indicates extralamellar exudate. X 250	88

Figure | Page

36 Shiner perch gill tissue after exposure to 1% effluent for 4 weeks in Bioassay 22. Arrows point out edema and separation of epithelium from underlying vascular tissue. X 250 88

37 Shiner perch gill tissue after exposure to 1% effluent for 8 weeks in Bioassay 22. Arrows show edema and separation of epithelium from vascular tissue. X 250 88

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1.0 SUMMARY

Toxicity of West Point effluent was determined by utilizing continuous-flow acute and chronic bioassays with 0.5 to 60% volume/volume effluent dilutions in ambient seawater. An on-site laboratory was constructed incorporating proportional diluters; seawater filtration and temperature control; and effluent filtration, dechlorination and ammonia removal systems. Prior to full operation in September 1975, the laboratory was equipped with water quality monitoring instrumentation for temperature, dissolved oxygen, salinity, pH, chlorine, ammonia, turbidity, and sulfur dioxide.

From October 1975 to September 1976, bioassays were conducted with primary chlorinated West Point effluent (WPE) using 5 species of marine organisms indigenous to Puget Sound receiving waters. Toxicity of WPE was not only variable among species tested, but also among days of tests. Juvenile English sole and shiner perch had average 96-hour LC50 values of 16.1 and 15.4% v/v WPE, respectively. These species were most sensitive to WPE and were selected for further tests due to ease of availability. The LC50 for coonstripe shrimp ranged from 15 to 20% v/v WPE and was approximately 30% v/v WPE for Pacific staghorn sculpin. Shore crabs were the most tolerant species tested, with a 120-hour LC50 value of approximately 50% v/v WPE.

Effects of reduced salinity (up to 60% freshwater/40% seawater) and dissolved oxygen in acute bioassays were tested and found to be minimal.

An increase in the temperature of the seawater from 8.5 to 18.5 C (Δt = 10 C) increased toxicity of WPE resulting in an LC50 of 11% v/v for shiner perch.

Filtration of WPE through a graded pea gravel filter resulted in reduction of the toxicity from an LC50 of 15% to 18% v/v WPE for shiner perch. Dechlorination of WPE with sulfur dioxide gas reduced the toxicity of WPE resulting in LC50 values of 32 and 28% v/v dechlorinated WPE for English sole and shiner perch, respectively. Treatment of WPE with the ion-exchange resin clinoptilolite resulted in greater than 95% removal of ammonia and chlorine. This treatment decreased toxicity of WPE resulting in LC50 values of 45 and 26% v/v WPE for English sole and shiner perch, respectively.

The toxicity of WPE was greater to age zero English sole (LC50 = 8% v/v) than to age 1+ (LC50 = 16% v/v). However, toxicity to age zero shiner perch (LC50 = 14% v/v) was essentially the same as for age 1+ individuals (LC50 = 15% v/v), further indicating the utility of shiner perch of all ages for nearshore marine toxicology studies.

The calculated safety factors (concentration of WPE at the outfall after initial dilution (0.7%) ÷ LC50 concentration of WPE) for WPE based on these results are: 0.044 for chlorinated WPE; 0.024 for dechlorinated WPE; and 0.020 for WPE treated for ammonia and chlorine removal with clinoptilolite. These values fall between 0.1 and 0.01, and are the application factors by which the LC50 for a toxicant is generally multiplied to yield an estimated "safe" level of discharge.

Abnormally high mortality was observed in two 96-hour bioassays, resulting in LC50 values of less than 10% v/v WPE. The exact toxic

component(s) remain unknown; however, the mortalities do correlate with a peak in mercury on one occasion and a peak in chromium/copper on another occasion.

Chronic bioassays conducted for 8 weeks with WPE concentrations from 0.5 to 10% did not indicate a significant whole body bioaccumulation of lead or zinc in English sole, shiner perch, or littleneck clams. Whole body analysis of a small sample of English sole from a chronic bioassay indicated depuration of chlorinated hydrocarbons and polychlorinated biphenyls (PCB's) when compared to a background specimen not exposed to the test system.

Histopathological examination of English sole and shiner perch gill tissues revealed extensive damage to the cellular integrity in the form of edema (excess intracellular fluids), epithelial hyperplasia (increase in number of cells), goblet cell hypertrophy (increase in size), inflammation, congestion, and hemorrhaging in acute concentrations of chlorinated WPE. Chronic exposures (8 weeks) to WPE as low as 0.5% resulted in gill tissue edema, inflammation, hemorrhaging, congestion and separation of the epithelial cells from the underlying vascular tissue in shiner perch and English sole. Adverse hematological effects in coho salmon held in 1.1% v/v WPE were reported by Buckley, *et al.* (1976), but no effect was found in 0.3%. We conclude that 0.3% chlorinated WPE is an approximate upper limit for a maximum acceptable concentration (MAC) which should be discharged to Puget Sound, if concentrated short-term discharges of certain trace metals can be controlled (*i.e.*, mercury, chromium, copper).

These tests indicate that toxicity of WPE can be reduced by control of slug discharges of trace metals and other industrially related toxic

constituents, and reduction or elimination of residual chlorine. Only a small reduction in acute toxicity of WPE would be expected by removal of ammonia nitrogen; however, ammonia combines with chlorine to form relatively persistent chloramines which may contribute to chronic toxicity.

Considering the difficulties encountered in analyzing the effects of a complex waste such as sewage, further testing and monitoring will be needed in order to insure the best possible management of domestic and industrial wastes in Puget Sound.

2.0 ACKNOWLEDGEMENTS

This report presents the results of studies conducted by the Fisheries Research Institute, University of Washington, for the Municipality of Metropolitan Seattle (METRO). The Institute personnel responsible for the studies reported herein are as follows:

Dr. Q. J. Stober, Principal Investigator

Dr. R. E. Nakatani, Co-Principal Investigator

Mr. P. A. Dinnel, Project Leader

Mr. M. A. Wert, Research Assistant, Chronic Bioassays

Dr. Marsha Landolt conducted the histopathological analysis of organisms exposed to various concentrations of effluent. Mr. Sam Felton assisted in water quality and tissue analysis for trace metals. Messrs. Ted Isom and Bill Nelson, Marine Engineer and Machinery Master Mechanic, respectively, assisted in construction of the laboratory. Student assistants who aided in various stages of the study were Craig Nightingale, Debbie Ilman, Robert Atterberry, Mark Meyers, Jeff McKay, and Steve Joner.

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A portion of the funding for bioassay laboratory equipment and personnel was supplied by the Nuclear Regulatory Commission (NRC) for work related to the effects of seawater chlorination on marine organisms.

3.0 INTRODUCTION

The Puget Sound Interim Studies Program sponsored by METRO included the bioassay of West Point sewage effluent using marine organisms indigenous to the outfall area. A majority of the Interim Studies was conducted in the marine environment near West Point and in Central Puget Sound to develop an ecological baseline needed for an environmental impact assessment. In order to aid interpretation of the field data, a bioassay program was initiated to investigate the real or potential effects of the effluent under semi-controlled environmental conditions. Bioassays were designed to determine the effects of some of the physical and chemical components of the effluent on selected marine organisms. The environmental interactions of components of a complex waste like sewage were reviewed.

The general objectives of the present study included:

1. Construction of a portable bioassay laboratory at the West Point Sewage Treatment Plant which utilized flowing ambient seawater and sewage effluent with water quality control and effluent treatment capabilities;
2. Lethal and sublethal bioassays of the West Point effluent and specific toxicants which are chemically identified components in the effluent; and
3. Analysis of toxic components in the sewage effluent, verification of concentrations of toxicants tested, and determination of tissue accumulations of trace metals in chronically exposed organisms.

Construction of the laboratory extended from March 1, 1975, through September 1975 when the facility became operational. Acute and chronic toxicity tests were conducted from October 1975 through September 1976. Data analysis, interpretation, and report preparation were conducted during the remaining period.

4.0 MATERIALS AND METHODS

4.1 Marine Bioassay Laboratory and Facilities

A mobile marine laboratory was designed and constructed in 1975 to bioassay sewage effluent from the Municipality of Metropolitan Seattle's (METRO) West Point Sewage Treatment Plant. The seawater distribution system, heating and temperature control systems, and monitoring equipment are similar to those described by Stober (1972). The majority of the work presently involves testing West Point sewage effluent (WPE); however, the laboratory has proven valuable in lending assistance to several other marine research projects.

4.1.1. Laboratory

The laboratory is a 10 x 55-ft mobile home converted into a 10 x 40-ft wet laboratory and a 10 x 15-ft dry laboratory and office (Fig. 1). Adjacent facilities include an 8 x 24-ft cargo container housing water treatment equipment and a small shop, a prefabricated metal shed housing a behavior lab, and several fish holding tanks.

4.1.2 Seawater Supply System

The seawater supply system consists of two 7.5-hp cast iron pumps (ITT Marlow Model # 34EL-15D), with stainless steel impellers which supply approximately 200 gpm of seawater per pump. The pumps are located on a 200-ft dock. The screened intake ports are approximately 20 to 25 ft deep at lower low water and 30 to 35 ft deep at higher high water.

Except for a short section of flexible rubber hose adjacent to the intake ports, the entire seawater supply line and laboratory distribution

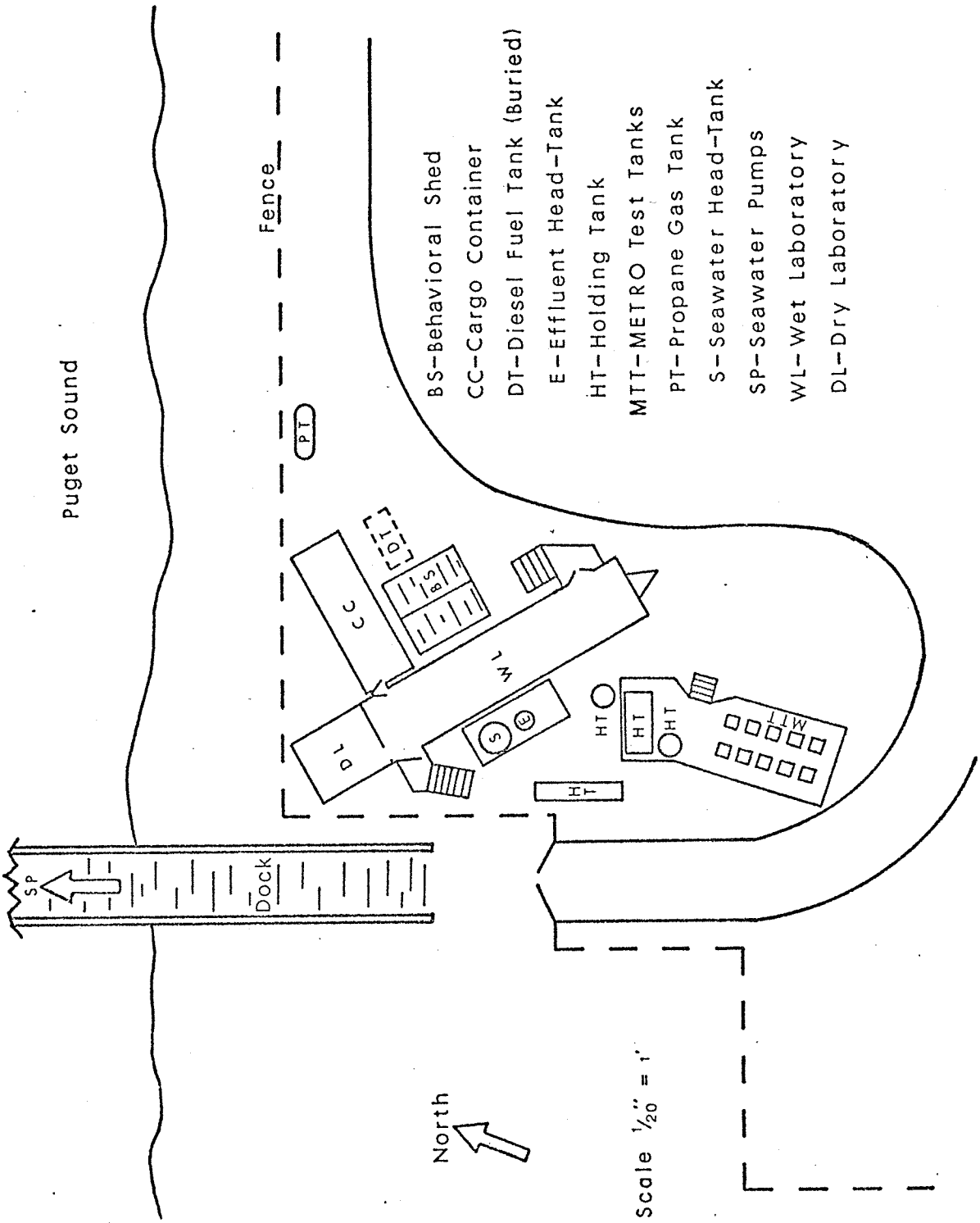


Figure 1. Diagram of marine bioassay laboratory at West Point.

system is constructed of polyvinyl chloride (PVC) pipe, valves and fittings. Seawater is pumped to a 500-gal fiberglass constant-head tank mounted on a platform at laboratory roof level. The head tank is provided with a 4-inch drain, a 6-inch elevated downspout for laboratory distribution, and a 6-inch overflow drain for excess seawater. A 6-inch header line fitted with 10 1.5-inch ball valves distributes ambient seawater to exterior holding tanks, test tanks in the wet laboratory, seawater filters in the cargo container, and the behavior shed (Fig. 2).

The dual pump/intake system allows for continued seawater delivery in case of the failure of a pump or clogging of an intake line. One of the two pumps normally is running with the other pump on "standby". Failure of the primary pump is signalled electrically by closure of a mercury float switch in the constant-head tank. Activation of the float switch automatically sounds an alarm in the wet lab and at METRO main control (manned 24 hours daily) and simultaneously switches on the standby pump.

4.1.3 Seawater Heating System

An automatic, oil-fired boiler (Aldrich Model WHO-47-ID) heats seawater at the rate of 20 gpm with a maximum temperature increase of 20 C. The heating system includes a 380-gal freshwater storage tank, 3/4-hp freshwater circulating pump (ITT Bell & Gossett, 1725 rpm), and a 60-ft^2 shell and tube heat exchanger (Corning Model 600GRB). Freshwater is heated in a closed recirculating system (Fig. 2). The boiler has a captive internal heat exchanger that contains freshwater and requires a 30-gal tank for expansion. Freshwater is continuously circulated through the boiler, storage tank, and outer shell of the

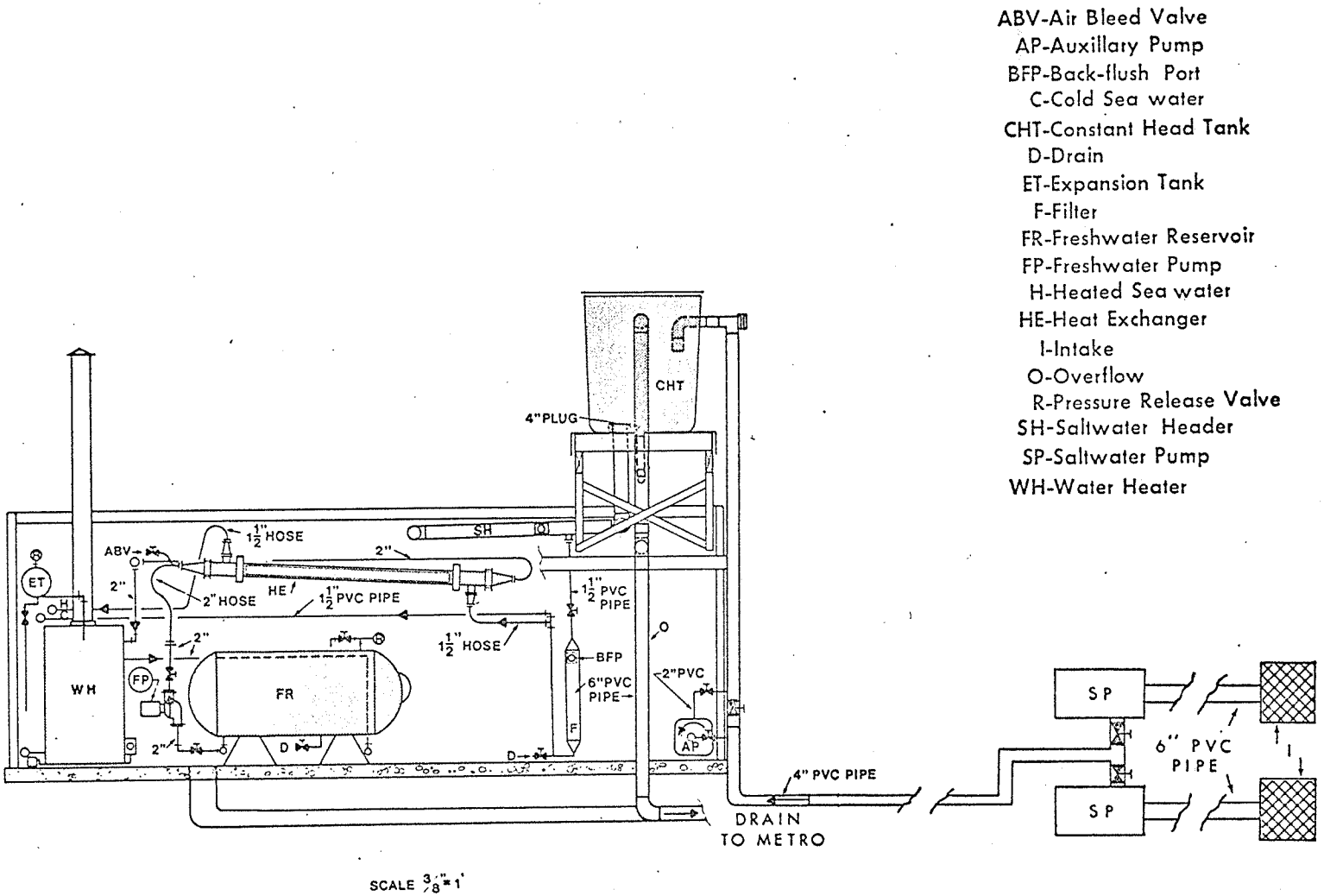
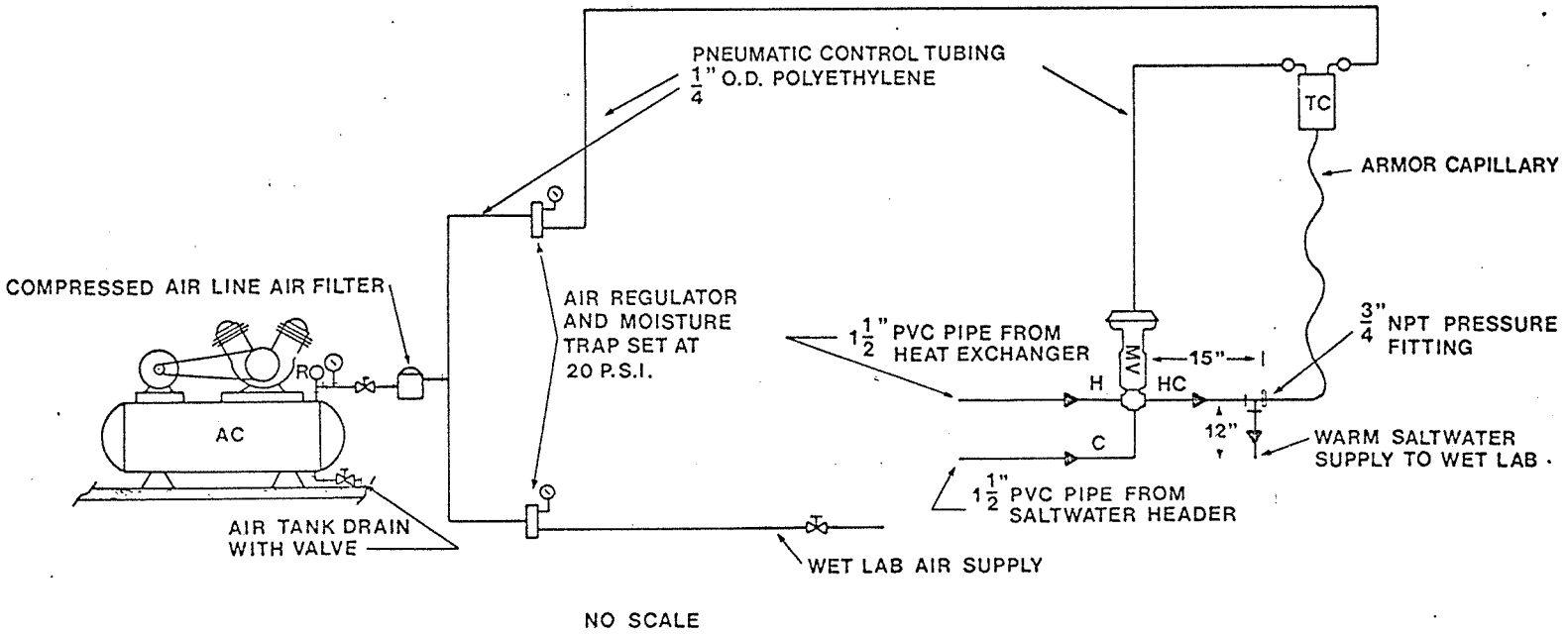


Figure 2. Mechanical design of seawater supply and heating systems.

glass heat exchanger. A 1/4-inch bleeder valve in the upper end of the glass heat exchanger removes air from the recirculating freshwater system when filling. During operation, the boiler thermostat automatically maintains the desired set temperature, which ranges from 49 to 93 C, depending on the quantity and temperature of heated seawater desired. Ambient-temperature seawater flows from the common header pipe through a 1.5-inch PVC line. This water is passed through a filter column (12-inch diameter PVC pipe, 5 ft long) filled with pea gravel for removal of detritus, invertebrates, and small fish. The filtered seawater then flows through the inner tubes of the heat exchanger counter to the flow of heated freshwater. The heated seawater leaving the heat exchanger flows to the mixing valve, where seawater of the desired temperature is selected for experimental use.

4.1.4 Temperature-Control System

A 1-1/4-inch, pneumatically operated, temperature-regulating, cast iron mixing valve (Honeywell Model # 1601) is used to provide thermal control of heated seawater (Fig. 3). This device operates on 20 psi air pressure and consists of a three-way, pneumatic-diaphragm mixing valve and a controller (Honeywell Model TP954A-1293) with a gas-filled, stainless steel sensing bulb. Ambient-temperature seawater enters one inlet port while heated seawater enters the other. The valve ports automatically open or close, mixing heated and ambient-temperature seawater. The sensing bulb is inserted in the outlet port of the mixing valve. The desired temperature is set on the controller and the mixed, heated seawater is regulated at the set point by thermal feedback from the sensing bulb. Thermally regulated seawater flows through a 1.5-inch



AC-Air Compressor
C-Cold Sea water
H-Heated Sea water
HC-Mixed Sea water

MV-Mixing Valve
R-Pressure Release Valve
TC-Temperature Controller

Figure 3. Diagram of the pneumatically-operated seawater temperature control system.

PVC distribution header with five 1/2-inch plastic ball valves, which deliver heated seawater to the desired tanks in the wet laboratory. Temperature is controlled to within ± 0.1 C.

4.1.5 Additional Systems

The compressed-air system (Fig. 3) consists of a 2-hp electric air compressor (Speedaire Model 7Z476). The compressor runs automatically and maintains an air supply of 125 psi in the storage tank. Air is supplied to the pneumatic mixing valve from one pressure regulator at 20 psi while the other regulator supplies any desired pressure for general laboratory use.

Electric power, 110 and 220 v single phase, is supplied by METRO through a 200-amp panel to the laboratory. In case of interruption of this service, emergency power is supplied by an auxilliary generator maintained by METRO.

Ambient air temperature is controlled by a free-standing, forced-air propane furnace located at one end of the wet lab.

4.1.6 Laboratory Equipment

The wet laboratory is equipped with fiberglass-lined plywood troughs and plastic holding trays (Fig. 4). All troughs and trays have removable PVC standpipes for water level control. Troughs, trays, and waterproof plywood shelf units are supported by a system of 2 x 4-inch wooden support units with 1.5-inch dowels which are adjustable to fit a variety of shelf and tank configurations (Fig. 5).

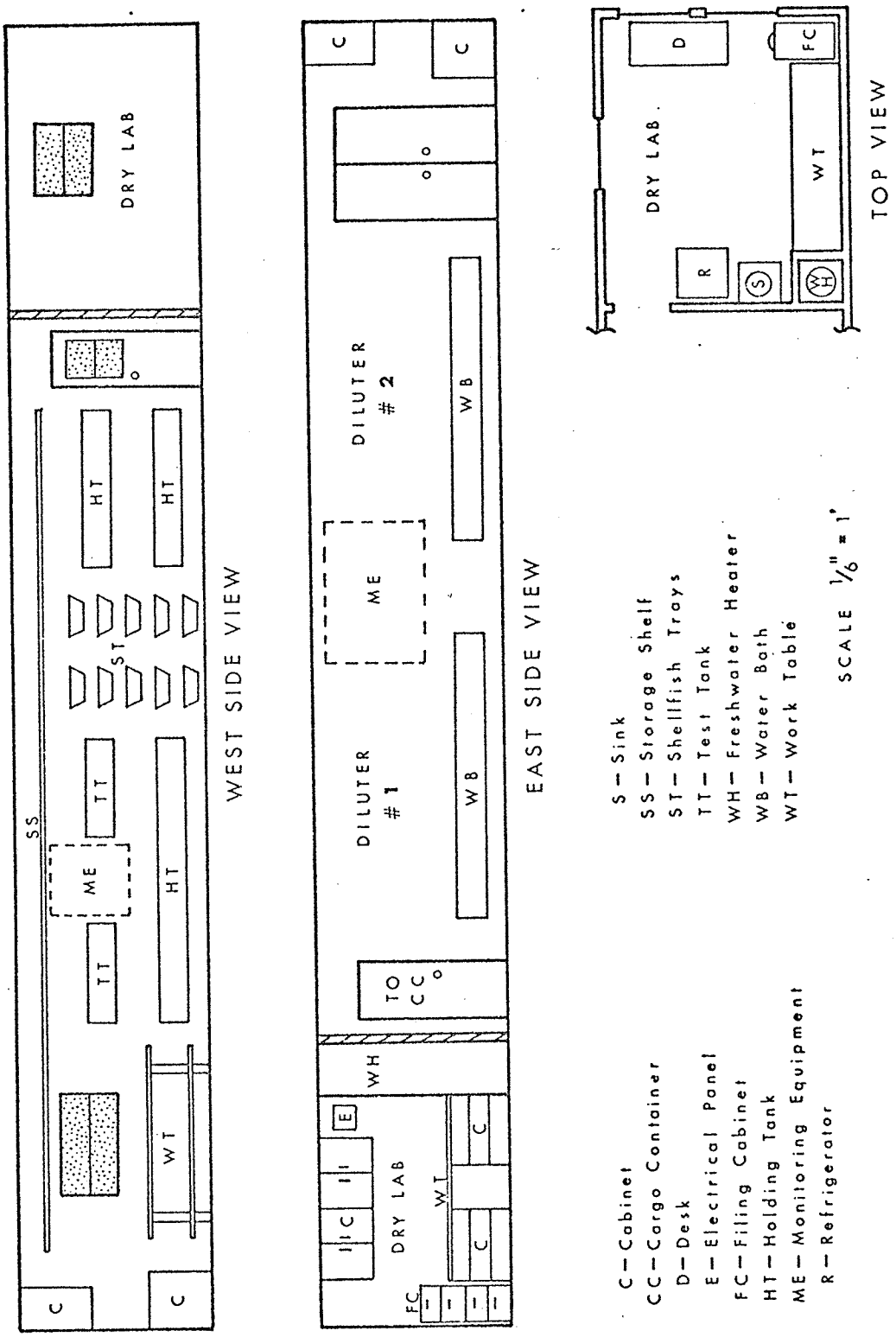


Figure 4. Diagrammatic view of laboratory interior and test facilities.

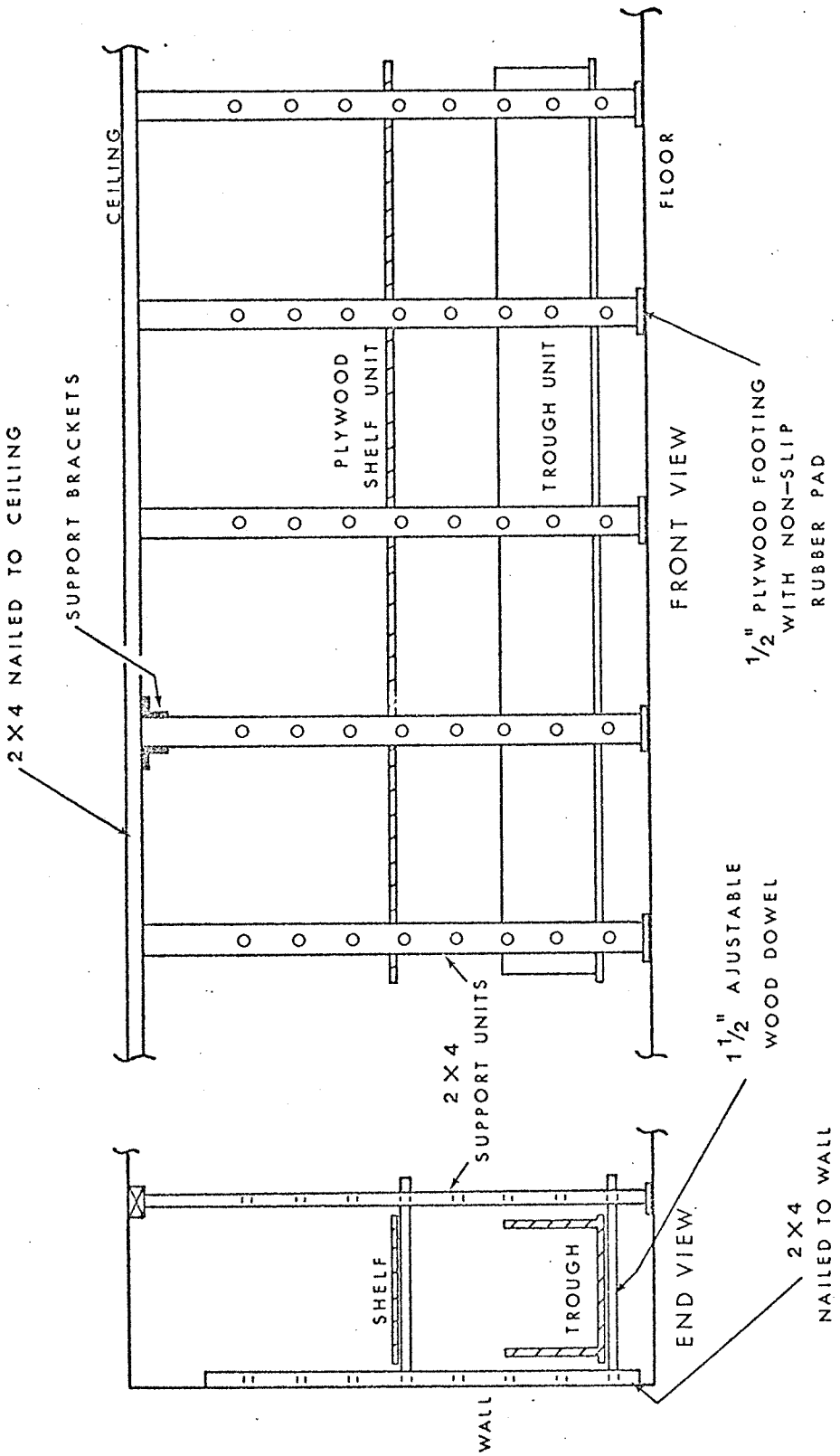


Figure 5. End and side views of adjustable trough and shelf support system in wet laboratory.

A double row of 4-ft fluorescent light fixtures connected to an automatic time switch provides controlled lighting. A workbench is installed near the front of the laboratory for additional work space. Two 280-gal circular tanks (Heath Model 169), one 718-gal fiberglass-lined plywood-reinforced tank, and one 12 x 2 x 1-ft fiberglass-lined plywood trough provide fish and invertebrate holding facilities outside the wet laboratory (Fig. 1).

Monitoring equipment in the laboratory includes an electronic thermometer (ARA-ET100A), a 20-input programmable scanner (ARA-SD20), and two strip chart recorders (ARA-400). Temperatures are detected in the test tanks with hydrographic probes (Model L5U-SF). Salinity is monitored with a portable salinometer (Beckman Model RS5-3). Dissolved oxygen is measured with a YSI model #54 RC-W/4 dissolved oxygen meter and probe. Turbidity is checked with a Hach kit. Chlorine is monitored with a Wallace & Tiernan amperometric titrator and by a rotating platinum electrode amperometric titrator utilizing a milliammeter and strip chart recorder for end point detection. Ammonia, sulfur dioxide, and pH are measured on an Orion Specific Ion Meter (Model 407A) in conjunction with the proper electrodes and standards.

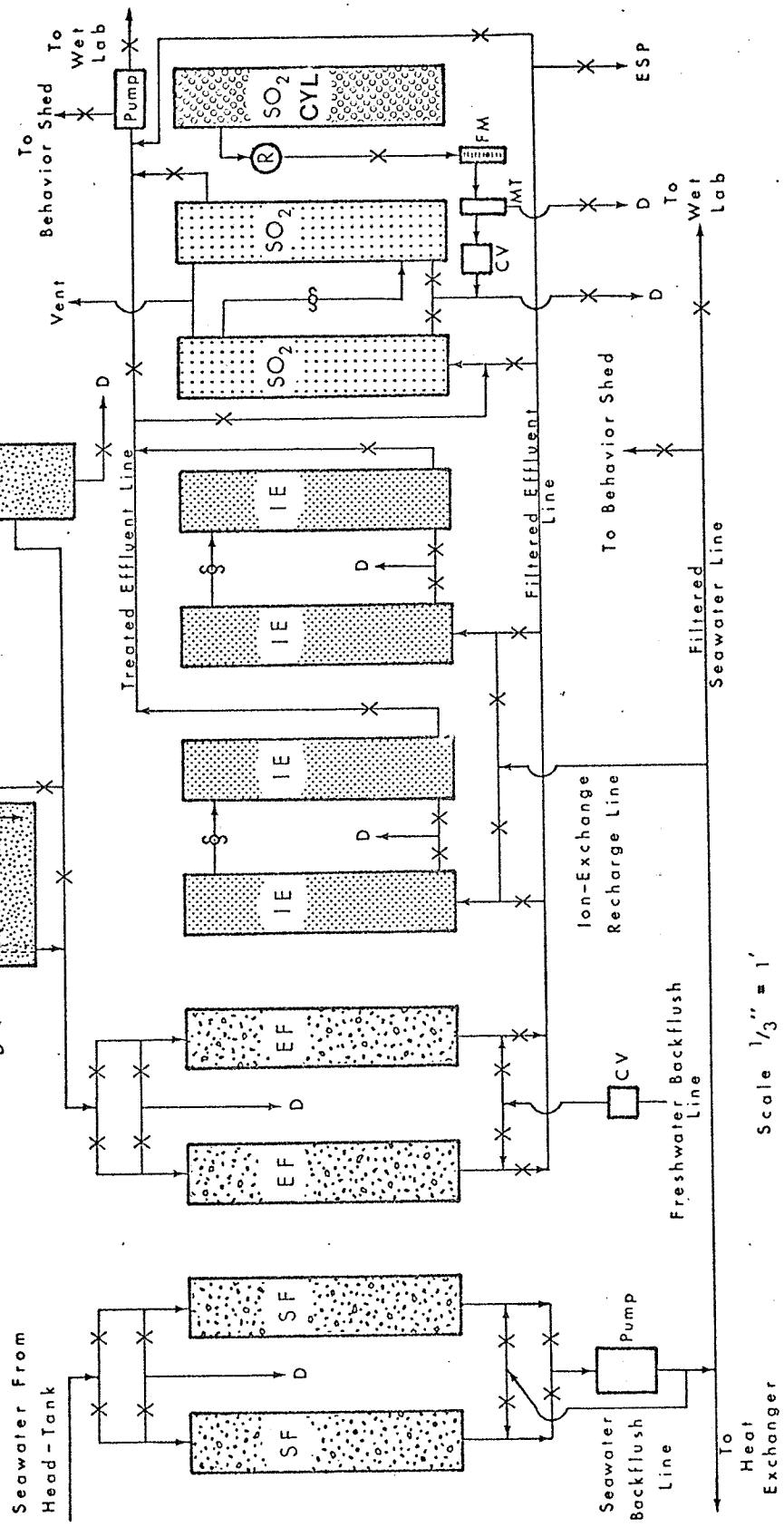
4.1.7 Sewage Effluent Treatment System

Primary treated, chlorinated sewage effluent is pumped to a constant-head tank (30-gal heavy duty plastic trash can) located on the outside platform at laboratory roof level. The effluent head tank has a 1.5-inch intake line, a 2-inch bottom drain, and a 3-inch overflow drain for excess effluent. A 1.5-inch distribution header with four 1-inch distribution

lines supplies effluent to the wet lab, behavior shed, and treatment columns in the cargo container. All pipes and fittings are PVC.

A set of treatment columns was constructed to remove ammonia and/or chlorine, two of the principal toxicants in the WPE (Fig. 6). These columns were constructed of 5-ft lengths of 12-inch diameter, schedule 80 PVC pipe with 3/4-inch plexiglass flanges bolted on each end (Fig. 7). Two columns contain pea gravel to filter seawater and two columns contain pea gravel to filter the effluent. Four columns (2 each, connected in series) contain ion-exchange resin (minus-4 mesh clinoptilolite) for ammonia removal. Two columns were injected with sulfur dioxide (SO_2) gas through a Fischer & Porter gas flow metering valve to dechlorinate the effluent. The SO_2 columns are filled with 1-inch porcelain saddles to insure adequate mixing and contact time with the SO_2. The treated effluent is pumped to the laboratory and behavior shed for distribution to the test tanks.

Each set of filter or treatment columns (except SO_2) is a dual system such that one side is available to supply filtered (or treated) effluent to the lab while the other side is being backflushed or recharged. Seawater is used to backflush the seawater filters and recharge the ion-exchange resin. The effluent filters are backflushed with freshwater. During periods of increased solid content in the WPE (usually summer) a settling tank was added in line between the effluent head tank and the gravel filters. This reduced rapid clogging of the filter bed.



CV — Check Valve
D — Drain
EF — Effluent Filter Column
EHT — Effluent Head-Tank
ESP — Effluent Sampling Point
FM — Gas Flow Meter
IE — Ion-Exchange Column
MT — Moisture Trap
R — Gas Pressure Regulator
SF — Seawater Filter Column
SO_2 — SO_2 Dechlorination Column
SO_2 CYL — SO_2 Gas Supply Cylinder
ST — Effluent Settling Tank
X — PVC Single-Union Valve
§ — PVC Union

Figure 6. Flow diagram of seawater and sewage effluent filter columns and effluent ammonia removal (by ion-exchange) and dechlorination (by sulfur dioxide) columns.

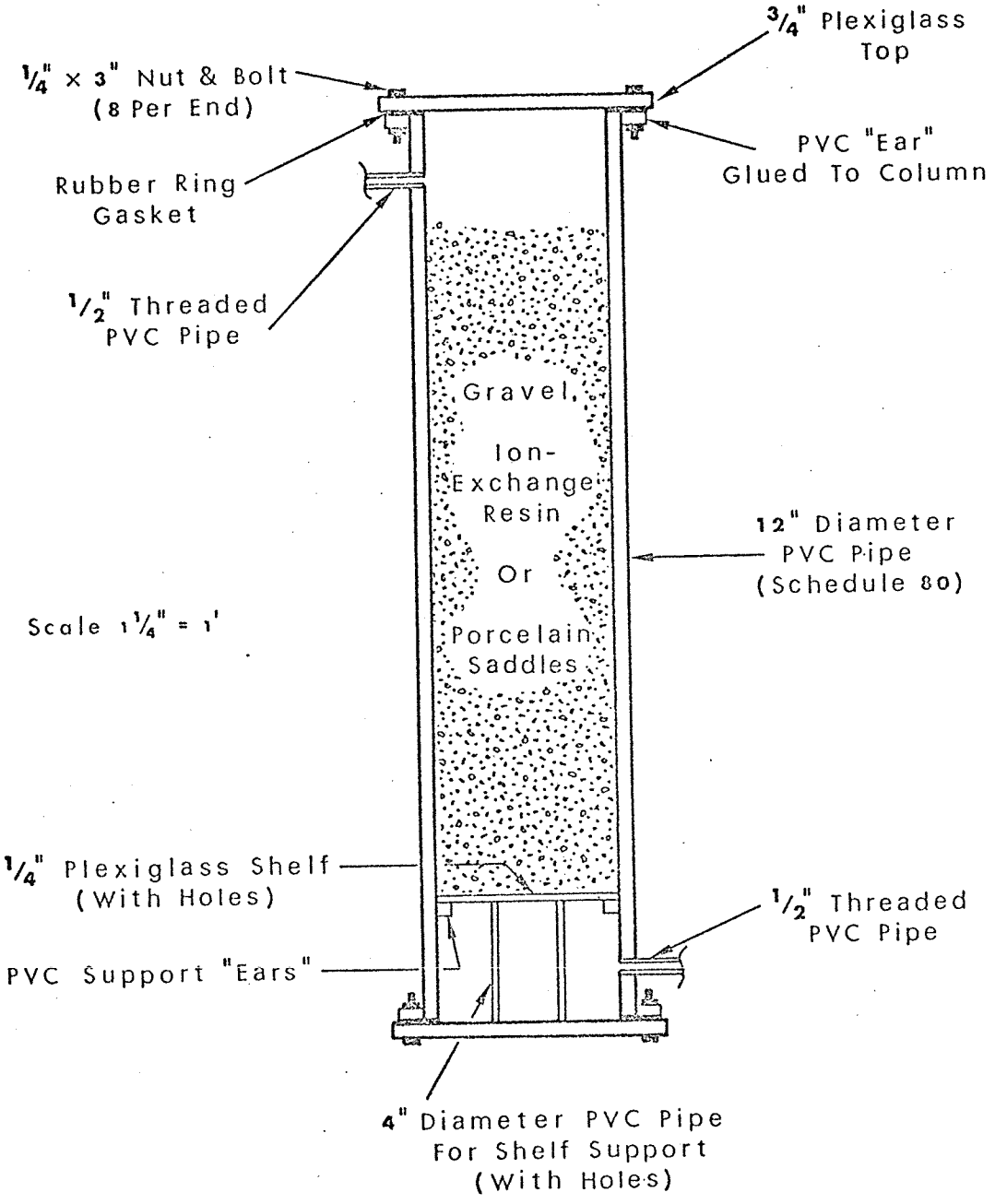


Figure 7. Longitudinal section of an individual filter or treatment column.

4.2 Test Animal Collection, Holding and Acclimation

English sole (*Parophrys vetulus*), Pacific staghorn sculpin (*Leptocottus armatus*), and shiner perch (*Cymatogaster aggregata*) (except Bioassays 19-22) were collected from the north beach at West Point using a 30 and/or 120-ft beach seine. Shiner perch for Bioassays 19-22 were collected by 120-ft beach seine from Kopachuck State Park, located in South Puget Sound approximately ten miles west of Tacoma. Coonstripe shrimp (*Pandalus danae*) for Bioassay 5 were captured by otter trawl towed by the R/V Malka off the north beach at West Point. Shore crabs (*Hemigrapsus nudus*) for Bioassay 6 were collected by hand from a rocky intertidal beach in Shilshole Bay. Common littleneck clams (*Protothaca staminea*) for Bioassay 15 were obtained from commercial stocks at Discovery Bay, located on the northeast side of the Olympic Peninsula.

All test animals were transferred from the capture site to holding tanks in seawater-filled plastic trash cans. Oxygen was added from O_2 cylinders when transport time exceeded 30 minutes. At the laboratory, test animals were held and acclimated in large circular or rectangular fiberglass tanks supplied with a constant flow of ambient seawater. The test animals (except clams) were fed canned clams or shrimp, or University of Washington Moist Pellet daily. Plankton in the ambient seawater served as a food source for the clams. All test animals were acclimated to West Point ambient seawater for at least one week before each test. Test animals were fasted three days prior to use in acute bioassays; but fish used in chronic bioassays were fed throughout acclimation and the 8-week test.

4.3 Bioassay Procedures

4.3.1 Proportional Diluter System

Acute (96-hr) and chronic (8-week) bioassays were conducted with WPE using two calibrated siphon tube, continuous-flow diluter systems (Fig. 8). Seawater and WPE were piped to the diluter constant-head tanks (20-gal glass aquariums) from head-tanks or respective filter/temperature control/treatment systems. Glass siphon tubes delivered the desired proportions of WPE and seawater from the diluter constant-head tanks to 10-gal mixing tanks via funnels and plexiglass tubes. Flow rates were controlled by the diameter of the glass siphon tube and fine-tuned by vertical movement of the siphon until the flow rate was $\pm$ 1% of that desired. Another glass siphon tube delivered the proper dilution of mixed WPE/seawater to the test tanks (14-gal aquariums) located in a water bath (12 x 2 x 1-ft fiberglass-lined trough) for temperature control. Seawater only was provided to the control mixing tank. Head tanks, mixing tanks, and test tanks were provided with overflow drains to maintain constant fluid levels. The siphon tubes maintained proper flow rates within $\pm$ 2% during the 4-day tests, however, during infrequent periods of heavy solids concentration, physical clogging did occur.

The test tanks were 14-gal (53 l) glass aquariums measuring approximately 10 x 20 x 15 inches. The flow-through volume of the test tanks was limited to approximately 40 l, due to the overflow drain. Flow rates through the test tanks ranged between bioassays from 1.0 to 1.4 l/min.

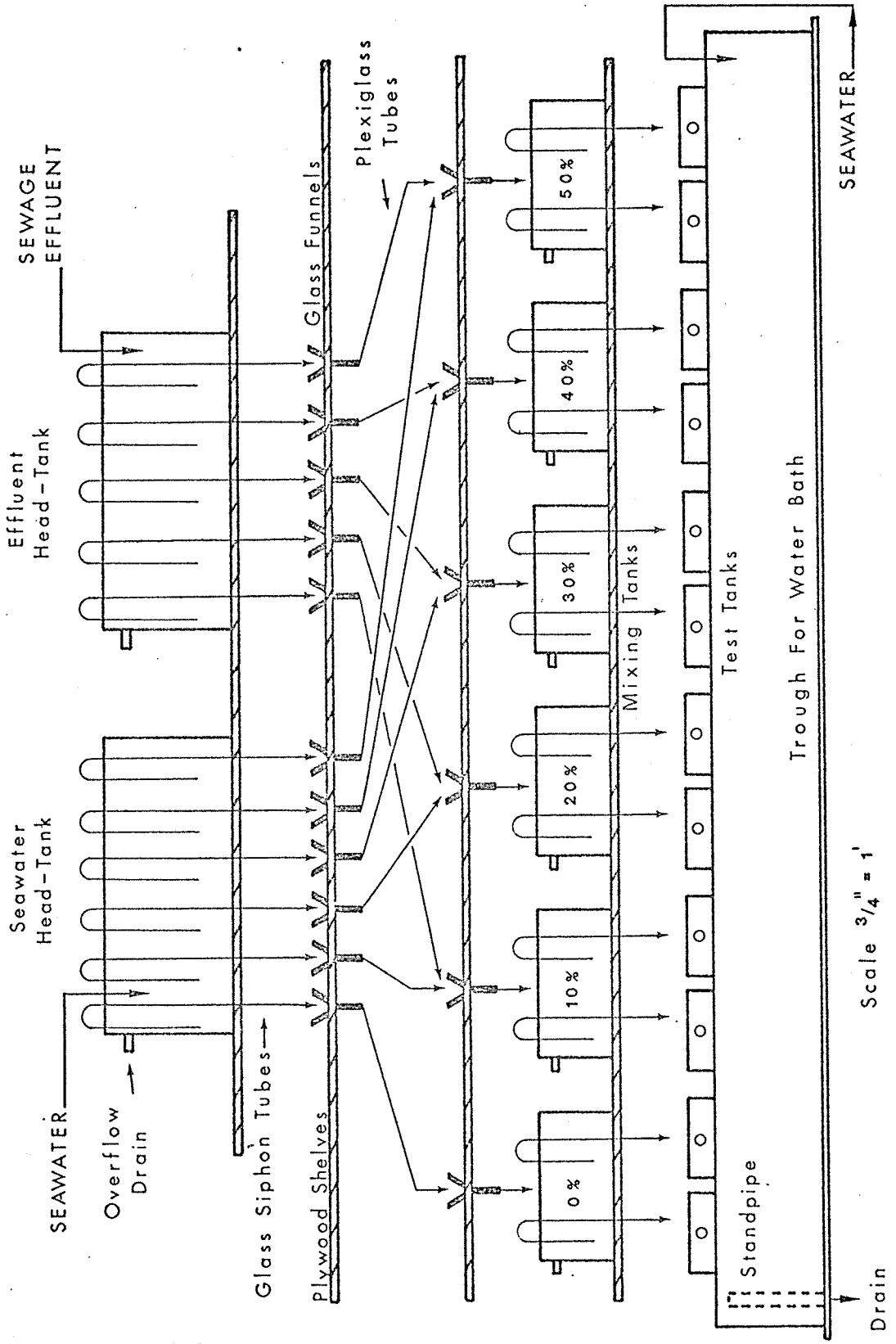


Figure 8. Diagram of sewage effluent diluter system. Percentage values are percent effluent in seawater V/V. Rubber drain hoses from overflow drains to floor drains are not shown.

A 1.0 1/min flow rate provided 36 tank volumes/day, which easily exceeded the recommended flow rate of 10 tank volumes/day for effluent flow-through tests (Stephan, 1975). Fish loading rates in the test tanks varied with species, but never exceeded 0.15 g/1 of the test solution passing through the tank in 24 hours, nor exceeded 5.6 g/1 of the test tank volume. Stephan (1975) allowed up to 2 g/1 and 20 g/1, respectively. The properly calibrated diluter system was allowed to run for several hours prior to the random assignment of test animals to the test tanks (10/tank). Lighting in the laboratory was maintained on for 16 hours and off for 8 hours during testing.

All tanks, funnels, and plexiglass distribution tubes were scrubbed with detergent and fresh water and rinsed with concentrated hydrochloric acid between bioassays. The glass siphon tubes were replaced for each bioassay.

4.3.2 Acute Bioassays

Bioassay 1 was aborted at 48 hours as the flow rates were found insufficient to prevent clogging of the calibrated siphon tubes. The flow system was redesigned to minimize future clogging by increasing all siphon tube flow rates to a minimum of 0.5 1/min. Additionally, fine-mesh, plastic window screen bags were tied on the effluent and seawater diluter head tank supply lines to capture large detritus.

Dilutions of primary chlorinated WPE (0, 1, 5, 10, 25, and 50% v/v) were used in Bioassays 2 through 6 to determine the approximate LC50 for five test animals which commonly occur in the West Point receiving waters: juvenile English sole, Pacific staghorn sculpin, shiner perch, coonstripe shrimp, and shore crab.

Bioassays 7 and 8 tested the effects of reduced dissolved oxygen and salinity in the bioassay test tanks. To assess the effect of dissolved oxygen, one series of 0, 20, 40, and 60% v/v WPE/seawater test dilutions was aerated. Additionally, one series of tanks contained 0, 20, 40 and 60% (v/v) freshwater/seawater dilutions to test the effect of reduced salinity on the test animals.

Bioassays 9, 10 and 11 were conducted to assess the effects of seawater temperature on WPE toxicity. Bioassay 9 was conducted at ambient seawater temperature (8.5 C) while Bioassays 10 and 11 were conducted at Δt = 5 C (12.5 C) and Δt = 10 C (18.5 C), by utilizing the seawater heat exchange/ mixing valve temperature control system. Dilutions of WPE/seawater tested were 0, 20, 40 and 60% v/v.

Life stage mortality differences were investigated in Bioassays 14 and 19. Bioassay 14 exposed age zero (1976) and age 1+ (1975) English sole to identical concentrations of WPE (0, 20, 40 and 50% v/v). Bioassay 19 repeated the same test with age zero (1976) and age 1+ (1975) shiner perch using 0, 5, 10, 20, 40 and 60% v/v dilutions of WPE/seawater.

Bioassay 13 used shiner perch to test the effects of filtering WPE through a 5-ft column of pea gravel (Figs. 6 and 7). Identical concentrations of filtered and unfiltered WPE (0, 10, 20, 40 and 60% v/v) were run side by side. Filtering of the WPE was necessary before it could be run through the ammonia removal or dechlorination columns in future bioassays. This test was designed to assess whether reduction of toxicity was caused by filtration so that tests requiring filtration prior to dechlorination and ammonia removal could be adjusted for any toxicity removed by the filtering process.

Bioassays 16 (English sole) and 21 (shiner perch) tested the differences in mortality between chlorinated WPE and WPE dechlorinated with gaseous sulfur dioxide, which was continuously metered into a porcelain saddle-filled PVC treatment column (Figs. 6 and 7) to produce a sulfur dioxide residual of 1 to 4 ppm. Equivalent dilutions of chlorinated and dechlorinated WPE (0, 10, 20, 40 and 60% v/v) were run side by side in the diluter system.

Bioassays 17 and 18 (English sole) and 20 (shiner perch) were conducted with identical concentrations (0, 10, 20, 40 and 60% v/v) of chlorinated WPE and WPE with ammonia removed by passage through a natural zeolite ion-exchange resin (clinoptilolite) in a pair of treatment columns (Figs. 6 and 7).

Test animal mortality in acute Bioassays 1 through 6 was monitored at 24, 72, and 96 hours, and dead fish were removed and measured at these times. To further elucidate the shape of mortality curves, mortality in acute Bioassays 7 through 21 was monitored at 1- to 2-hour intervals for the first 6 hours, 2-hour intervals for the next 6 hours, 4-hour intervals up to 48 hours, and 24-hour intervals for the following 48 hours. This schedule allowed for definition of mortality curves in the higher dilutions of effluent where the test animals were usually killed in the 1- to 24-hour time span.

4.3.3. Data Analysis

Four-day continuous-flow bioassays were conducted to determine geometric mean survival times (GMST) and approximate 96-hr median lethal concentrations (96-hr LC50) of WPE for various test animals under a

variety of conditions. An LC50 value is the statistically derived best estimate of the concentration of toxicant in dilution water that is lethal to 50% of the test organisms during continuous exposure for a specified period of time, based on data from one experiment (Stephan, 1975). The calculation of the GMST provides a rapid technique for accurate estimation of a median response (survival) of regular log-normal data (Brown, *et al.*, 1967; Sprague, 1969). It is calculated by:

$$GMST = (T_1 \times T_2 \ldots \times T_n)^{\frac{1}{n}}$$

where T_1 = time to first death in each test tank and T_n = time to second to last death. The times to zero and 100% mortality do not enter into the calculation, in order to keep the distribution uniform (Davis and Mason, 1973). For ease of calculation, the following alternative formula may be used (Freund and Williams, 1966):

$$\text{Ln GMST} = \frac{\Sigma \text{ Ln } T_1 + T_2 \ldots T_n}{n}$$

The next step in the analysis is to plot the GMST for each test on log-log or semi-log graph paper to determine a lethal threshold concentration. An approximate lethal threshold concentration may be estimated by visual inspection of the resulting toxicity curves. Straight or curved lines are fitted to the points by eye. Curved lines are made asymptotic to the time axis either gradually or abruptly depending on the data. The asymptote marks the approximate value of the incipient LC50 (Sprague, 1969). The intersection of a straight line plot with the 96-hr abscissa also marks the approximate LC50.

4.3.4 Chronic Bioassays

Three 8-week continuous-flow bioassays were conducted from February to November 1976. Organisms tested were shiner perch, English sole, and common littleneck clam. These organisms are all endemic to Central Puget Sound, and represent three different life styles and feeding habits. Shiner perch are pelagic fish usually associated with pier pilings and eelgrass beds. English sole are demersal bottom feeders. Littleneck clams are benthic filter feeders.

Primary treated WPE flowed from the constant head tank at laboratory roof level to a diluter head tank in the wet lab. Continuous-flow WPE concentrations (0, 0.5, 1, 2.5, 5 and 10% v/v) were achieved with the proportional diluter system and delivered to the 40-l test aquariums contained in a constant-temperature water trough (Fig. 8). Daily checks of the diluter flow rates were taken during one of the bioassays to determine the accuracy of the diluter system over time.

All organisms used in chronic bioassays were acclimated for at least 2 weeks in ambient West Point seawater. A sample of acclimated organisms was frozen prior to each bioassay for later analysis. These organisms served as controls which had been unexposed to the diluter system. To begin each test the groups of organisms were carefully removed from the holding tanks and randomly placed in the various 40-l test aquariums. Flow rates to the test aquariums were 1.5 l/min, sufficient for a 95% replacement in approximately 1 hour (Sprague, 1969). The daily flow into the test aquariums was at least 15 l/g of organism/day. This was in excess of the 2 to 3 l/g of fish/day recommended by Sprague (1969). The shiner perch and English sole were kept in the test aquariums without

substrate. The littleneck clams were kept in glass marble-filled, 1/4-inch mesh vexar baskets suspended midway in the test tanks. The glass marbles provided a chemically inert substrate for the clams. Because of the 8-week duration of the chronic bioassays, it was necessary to feed the fish. Commercial frozen paneid shrimp (from Alaska) and moist food pellets prepared at the University of Washington fish hatchery were used as fish food. Plankton in the ambient dilution seawater served as a food source for the littleneck clams. Fish were starved for 4 days prior to sampling in order to eliminate any residual food in the gut.

Occasional mortality occurred in the 8-week chronic bioassays. These dead test animals were noted and removed from the test tanks each day.

4.4 Water Quality Monitoring Procedures

4.4.1 Acute and Chronic Bioassays

The methodology and equipment used for monitoring the physical-chemical parameters of the bioassays are summarized in Table 1. Temperature, salinity and dissolved oxygen (DO) were measured directly in each tank with as little disturbance to the test animals as possible. Appropriate size samples for turbidity, pH, total residual chlorine, ammonia, and sulfur dioxide were collected from the calibrated siphon tubes for head tank analysis and from 1/4-inch plastic tygon tubing extending into the middle of each test tank.

Acute bioassays generally were sampled and analyzed at 0, 24, 48, 72, and 96 hours. However, bioassays which included ammonia removal (Bioassays 17, 18, 20) or dechlorination (Bioassays 16, 21) were sampled

Table 1 . Equipment and methodology of water chemistry analysis for West Point Sewage Effluent (WPE) acute and chronic bioassays.

Test		Equipment	Type Measurement	Standard	Detection† Limits	Units
Temperature	1.	C^{o} Thermometer	Direct		S = 0.1 R = 0-100	Degrees Centigrade (^{o}C)
	*2.	ARA electronic thermometer with scanner and recorder	Direct	C^{o} thermometer	S = 0.25 R = 0-55	
Salinity	1.	Hydrometer	Conversion of reading by tables		S = .01	Parts per Thousand ($^{o}/oo$)
	*2.	Portable Beckman salinometer and probe	Direct	Hydrometer	S = .01 R = 0-99	
Dissolved Oxygen (DO)	1.	DO bottle and titration equipment	Winkler Titration	0.025ON PAO	S = .01	Parts per Million (ppm)
	*2.	YSI DO meter and probe	Direct, after calibration in sat. air	Winkler Titration	R = 0-20 S = 0.05	
Turbidity		Hach kit	Direct, based on light transmission	Distilled water blank	R = 0-500 S = 1	Jackson Turbidity Units (JTU)
pH		Orion specific ion meter with pH and reference probes	Direct, after calibration with pH standards	Orion pH standard solutions	R = 0-14 S = 0.05	Standard pH Units
Total Residual Chlorine (TRC)		Wallace and Tiernan amperometric titrator	Back titration	Distilled water blank	R = 0.05-10 S = 0.05	Parts per Million (ppm)
Ammonia (NH_3-N)		Orion specific ion meter with gas sensing electrode	Direct, after calibration with 2 standards	Orion ammonium chloride standard solution	R = 0.017-17,000 S = variable	Parts per Million (ppm)
Sulfur Dioxide (SO_2)		Orion specific ion meter with gas sensing electrode	Direct, after calibration with 2 standards	Orion sulfur dioxide standard solution	R = 0.1-1000 S = variable	Parts per Million (ppm)

† R = Range; S = Sensitivity

*Normal monitoring method

for chlorine and ammonia (or chlorine and sulfur dioxide) on a schedule identical to the mortality monitoring as described above.

4.4.2 METRO Effluent Analysis

Additional physical-chemical sampling data for the WPE from 24-hr composite samples was supplied by the METRO water quality laboratories. METRO sampling and analytical methodology are summarized in Table 2 and include daily information on rainfall, sewage flow rate, temperature, pH, DO, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids, volatile suspended solids, and settleable solids. Weekly information was obtained on ammonia as nitrogen (NH_3-N), total nitrogen, phosphate as phosphorus (PO_4-P), and grease. TRC was monitored continuously. Additionally, the trace metals cadmium, chromium, copper, mercury, nickle, lead, zinc, and hexavalent chromium were measured on a daily basis from 24-hour composite samples.

4.5 Biological Sample Collection and Preparation

4.5.1 Histology

Samples of gill tissue were collected from shiner perch and English sole exposed to lethal and sublethal concentrations of WPE in seawater and to 100% seawater (control). Collections of gill tissue from fish in lethal concentrations of WPE were made from moribund fish. Fish in sublethal concentrations of WPE and control fish were sacrificed for gill tissue at the conclusion of 96-hr acute bioassays. Additionally, gill tissue from shiner perch and English sole and gill and body tissue of littleneck clams were collected from several chronic bioassays after

Table 2. Summary of methodology used by METRO laboratories for chemical analysis of West Point Sewage Effluent.

Test	Method	Units
Rainfall	Rain gauge at West Point Treatment Plant	inches
Average Flow Rate	Flowmeter	million gallons/day (MGPD)
Temperature	Thermometer	^{o}C
Dissolved Oxygen (DO)	DO meter and probe	ppm
pH	Continuous monitor pH meter and probe	pH units
Biological Oxygen Demand (BOD)	Standard Methods, p. 474*	mg/l
Chemical Oxygen Demand (COD)	Dichromate reflux method, Standard Methods, p. 495*	mg/l
Total Residual Chlorine (TRC)	Continuous monitor amperometric titrator	ppm
Ammonia (NH_3 as N)	Phenolhypochlorite method	ppm
Total Nitrogen (N)	Kjeldahl Digestion	ppm
Phosphate (PO_4 as P)	Vanadomolybdophosphoric acid colorimetric method	ppm
Grease	Freon 113 extractable	ppm
Suspended Solids	Standard Methods, p. 537*	mg/l
Settleable Solids	Standard Methods, p. 539*	mg/l
Volatile Suspended Solids	Standard Methods, p. 538*	mg/l
Hexavalent Chromium	1-5 diphenolhydrocarbohydrazide direct wet test method	mg/l
Other Trace Metals	Atomic absorbtion spectrophotometry	mg/l

* Standard Methods for the Examination of Water and Wastewater. 13th ed., American Public Health Association, New York, NY. (1971).

4 or 8-week exposures to 0.5 to 10% WPE in seawater and control tanks. In all cases, the gills and body tissues of the clams were quickly excised from the animal and placed in Bouins fixative for 24 hours. Samples were subsequently dehydrated in 30 and 50% ethyl alcohol for 4-hour intervals and then stored in 70% alcohol. Later laboratory preparation of the tissues included dehydration, clearing and embedding in paraffin. The tissues were cut at approximately 5 microns, stained in hematoxylin and eosin, and permanently mounted on glass slides for examination with a light microscope.

4.5.2 Polychlorinated Biphenyl (PCB) Analysis

Several English sole from Bioassay 12 were analyzed for PCB bioaccumulation after an 8-week exposure to a range from 0.5 to 10% WPE. At the end of the chronic exposure period, the sole were starved for 4 days and then carefully removed, rinsed with ambient seawater, placed in zip-lock polyethylene bags and frozen. A subsample of the fish food (frozen shrimp) was also frozen for analysis. Additionally, a pre-test batch of fish was frozen. This sample of fish was randomly drawn from the holding tank prior to the start of Bioassay 12 and provided an approximation of background levels of PCB commonly found in Puget Sound populations. Fish from the bioassay seawater control tanks served to monitor any PCB uptake from the ambient seawater.

The English sole were analyzed for PCB bioaccumulation by the METRO Water Quality Laboratory. The fish tissues were prepared for gas chromatographic analysis by initial extraction with petroleum ether in beakers followed by secondary extraction with 6 and 15% ethylether in petroleum ether (Ray Dalseg, personal communication).

4.5.3 Trace Metal Analysis

English sole, shiner perch, and littleneck clams from Bioassays 12, 15 and 22 were analyzed for bioaccumulation of copper and zinc after 8-week exposures to a range from 0.5 to 10% WPE. At the conclusion of the test period, the fish were starved for 4 days (clams continued to feed), rinsed in seawater and frozen along with a food sample for later analysis. A pre-test sample of fish or clams was also frozen.

Trace metal analysis consisted of drying each organism to a constant weight, dry ashing in a muffle furnace at 550 C, or chemically oxidizing (wet ashing) the tissue in nitric and perchloric acids, and measuring the resulting metal concentration on a Perkin-Elmer Model 303 atomic absorption spectrophotometer (AAS) equipped with a digital readout.

In order to insure accuracy in the final results, special handling procedures were utilized to minimize the risk of possible metal contamination during the analysis procedures. All glassware and equipment which came in contact with the specimens during analysis was washed with detergent, rinsed in tap water, and distilled water, soaked at least 16 hours in 1:1 nitric acid, rinsed in distilled de-ionized water, and air dried. All nitric acid was reagent grade which was re-distilled prior to use. The dry ashing technique consisted of homogenizing individual fish in ethyl alcohol in a food blender; drying the mixture to a constant weight; ashing a weighed sample in a porcelain crucible at 550 C in a muffle furnace; adding 1 N HCl to put trace metals in solution; diluting the solution to 50 ml with distilled de-ionized water in a volumetric flask, and aspirating the solution into the AAS.

The wet ash technique consisted of a number of steps. Individual organisms were partially thawed and weighed by difference in tared 250-ml glass beakers. Beakers containing the specimens were then covered with watch glasses and placed in an oven at 95 C for 16 hours or until a constant weight was attained. Beakers were then removed to a dessicator until cool and then re-weighed to determine the dry weight of the specimen. Chemical oxidation of the specimens was conducted in a perchloric acid hood by a modification of the method used by Smith (1953). Covered beakers containing the dried specimens as well as a reagent blank, a spiked blank, a biological standard (National Bureau of Standards powdered bovine liver), and spiked liver standard each received equal volumes of concentrated 16 M nitric acid (approximately 10 ml/g dry weight). After the initial foaming subsided, the beakers with watch glasses were placed on a hot plate, boiled to a low volume, and then removed from the hot plate. Into each beaker was then added (in order) 5 ml of 16 M nitric acid and 15 ml of 70% perchloric acid. Beakers were then returned to the hot plate to exotherm until dense white vapors and a clear solution were formed. At this time, the inner walls of the beakers were rinsed with distilled de-ionized water and allowed to reheat until the reformation of dense white vapors and a clear solution. Finally the solutions were transferred to 50 ml volumetric flasks and diluted to volume with 0.1 M nitric acid (pH 1.5). Solutions were transferred to 2-oz polypropylene bottles with tight-fitting lids for storage until analyzed by AAS.

Analysis of the diluted sample was obtained by aspirating it directly into the acetylene flame of the AAS and reading the absorbence on the digital readout. A standard curve, using known concentrations of the

metals which bracketed the concentrations of the diluted samples, was used to convert absorbence to mg/l (ppm) of the metal. The metal solutions used to establish the standard curve and spiked reagent blanks were cross checked with EPA trace metal standard solutions.

Reagent blanks, spiked reagent blanks, a biological standard (NBS powdered bovine liver) and spiked bovine liver standards were used to determine the accuracy of the trace metal measurements. The reagent blanks served to determine the presence or absence of trace metal contamination during analysis. The spiked reagent blank was used to determine the percent recovery of known quantities of trace metals from the digestion chemicals. Biological standards were digested to determine the accuracy and recovery ability of the wet ashing technique for biological materials. When biological materials were chemically digested, various body salts became unavoidably present in the final solution. These salts may have affected the matrix of the solution which was analyzed by AAS. By comparing spiked reagent blanks to biological standards, both of known trace metal concentration, one could determine the changes which resulted from the biological-salt-induced matrix effects. Metal recovery was at least 90% in all wet ash digestions.

Detection limits of the AAS were 0.05 ppm for copper and zinc. Trace metal analyses were determined to be accurate when there was no significant contamination as determined by reagent blanks and when the analyzed concentration of the NBS bovine liver standard fell within the NBS 95% confidence limits for the particular metal. All wet ashed data reported are based on accurate determinations as defined above.

5.0 RESULTS

5.1 Acute Bioassays

5.1.1 LC50 Determinations for Five Resident Species

Five preliminary 96-hour bioassays were conducted to assess the response of five resident species to WPE dilutions ranging from 5 to 60% v/v with ambient seawater. Juvenile English sole, shiner perch and coonstripe shrimp were approximately equal in sensitivity to chlorinated WPE with estimated LC50 values between 15 and 20% v/v. Staghorn sculpin were more tolerant with an LC50 approximating 30% v/v. Shore crabs proved the most tolerant, with a 120-hour LC50 of approximately 50% v/v. The biological, physical and chemical sampling data for each of these tests are summarized in Appendix Tables 2 through 6.

Shiner perch and English sole were selected as primary species for further bioassay tests, based on sensitivity to WPE and availability. Shiner perch are a common inshore inhabitant of the Pacific Coast from Southern California to Southern Alaska (Hart, 1973). These schooling fish spend their entire life history in shallow coastal and estuarine waters where they may be affected by various types of waste discharge. Thatcher, *et al.* (1976) and Nakatani, *et al.* (1977) have found shiner perch to be very sensitive to chlorine. This species has also been recommended by Stephan (1975) for use as a marine bioassay test animal. English sole is a commercially important flatfish found in nearshore waters from Southern California to the Gulf of Alaska (Hart, 1973). Juvenile English sole spend their first year of life in or near the intertidal zone feeding on various benthic invertebrates. Shiner perch and juvenile English sole are common inhabitants of Puget Sound and are relatively abundant in the vicinity of the West Point sewage outfall.

5.1.2 Seasonal Changes in the Effluent and Receiving Water Quality

The determination of the toxicity of sewage effluent using conventional fish bioassays is complicated due to the ever-changing concentrations of both known and unknown toxicants. Primary sewage effluent is typically low in dissolved oxygen. When conducting bioassays with marine species in dilutions of sewage effluent, the depression of salinity in the test tanks might confound the results, especially at high concentrations of effluent or when testing stenohaline marine species. The potential for confounding of the experimental design by reduced DO and salinity was specifically tested.

A series of 96-hour bioassays was conducted with WPE and aerated WPE in order to determine if differences in toxicity would occur. Results of 96-hour tests presented in Figure 9 indicate the LC50 values for English sole were 15% v/v for WPE and 16% v/v for aerated WPE. LC50 values for shiner perch were 18% v/v for WPE and 19% for aerated WPE. The aerated WPE had an average increase in dissolved oxygen of approximately 1 ppm in the lower concentrations (20%) of WPE and up to 2 ppm in higher concentrations (60%) of WPE, when compared to unaerated WPE (Table 3). The test animals generally survived longer in the aerated WPE but the final LC50 values were essentially equal. It was apparent that conducting tests in unaerated effluent up to 60% v/v dilution would not yield results confounded by dissolved oxygen stress.

The potential effects of reduced salinity on marine test organisms was determined by making a series of dilutions ranging up to 60% v/v freshwater. No mortality was observed with English sole or shiner perch in 96-hour bioassays. The behavior of the test fish did not indicate any signs of obvious stress.

Table 3. Comparison of dissolved oxygen levels in aerated and unaerated dilutions of West Point Effluent (WPE) in Bioassays 7 and 8.

Bioassay	Treatment	% WPE	Average Dissolved Oxygen (ppm)
7 (English sole)	Control	0	8.4
	Unaerated	60	5.5
	Unaerated	40	6.6
	Unaerated	20	7.6
	Aerated	60	7.2
	Aerated	40	7.8
	Aerated	20	8.5
8 (Shiner perch)	Control	0	8.2
	Unaerated	60	4.8
	Unaerated	40	5.9
	Unaerated	20	7.3
	Aerated	60	6.8
	Aerated	40	7.8
	Aerated	20	8.2

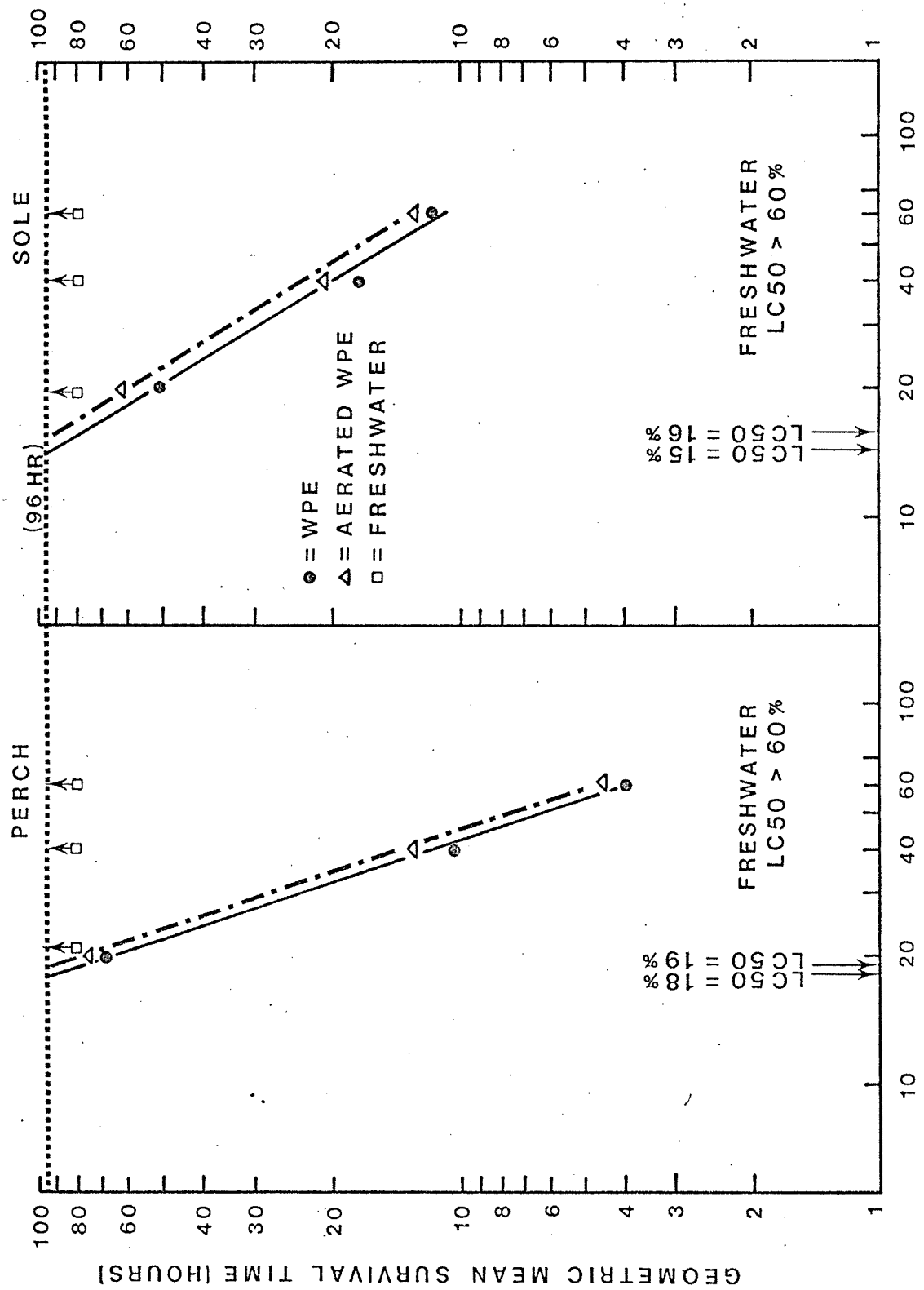


Figure 9. Determination of shiner perch and English sole LC50 in dilutions of unaerated and aerated West Point Effluent (WPE) and dilutions of freshwater.

Biological, physical, and chemical sampling data for Bioassays 7 and 8 are summarized in Appendix Tables 7 and 8.

Seasonal change in the ambient temperature, salinity, and dissolved oxygen of the marine receiving waters was apparent during the annual period over which tests were conducted. The average monthly temperature, salinity, and dissolved oxygen in the ambient seawater utilized in the bioassays are presented in Figure 10 for the period October 1975 through September 1976.

Minimum average ambient temperature (7.7 C) occurred in early March while maximum ambient temperature (12.6 C) occurred in August. Mean salinity was generally lowest (26.9%) during the winter when maximum runoff of freshwater occurred. Maximum salinity was observed in September 1976 at 31.2% . Average dissolved oxygen was lowest during summer (6.5 ppm) and fall (7.2 ppm) when higher temperatures tended to depress the oxygen-holding capacity of seawater.

Similar fluctuations of dissolved oxygen and temperature occurred in the WPE (Fig. 11). Average dissolved oxygen remained at levels less than 1 ppm during the summer but fluctuated widely during winter when storm runoff and low temperatures resulted in an average maximum of 3.2 ppm. The temperature of the effluent ranged from a mean minimum of 11.8 C in early April to a maximum of 19.5 C in August. The temperature of the WPE averaged 4.5 C above the winter minimum ambient and 7.1 C above the summer maximum ambient temperature. Mean daily effluent monitoring data for numerous additional parameters monitored are presented in Appendix Table 1. By conducting repetitive bioassays throughout an annual period the changes in the quality of the WPE and the receiving waters were integrated into the determinations of toxic and/or chronic effects of the effluent.

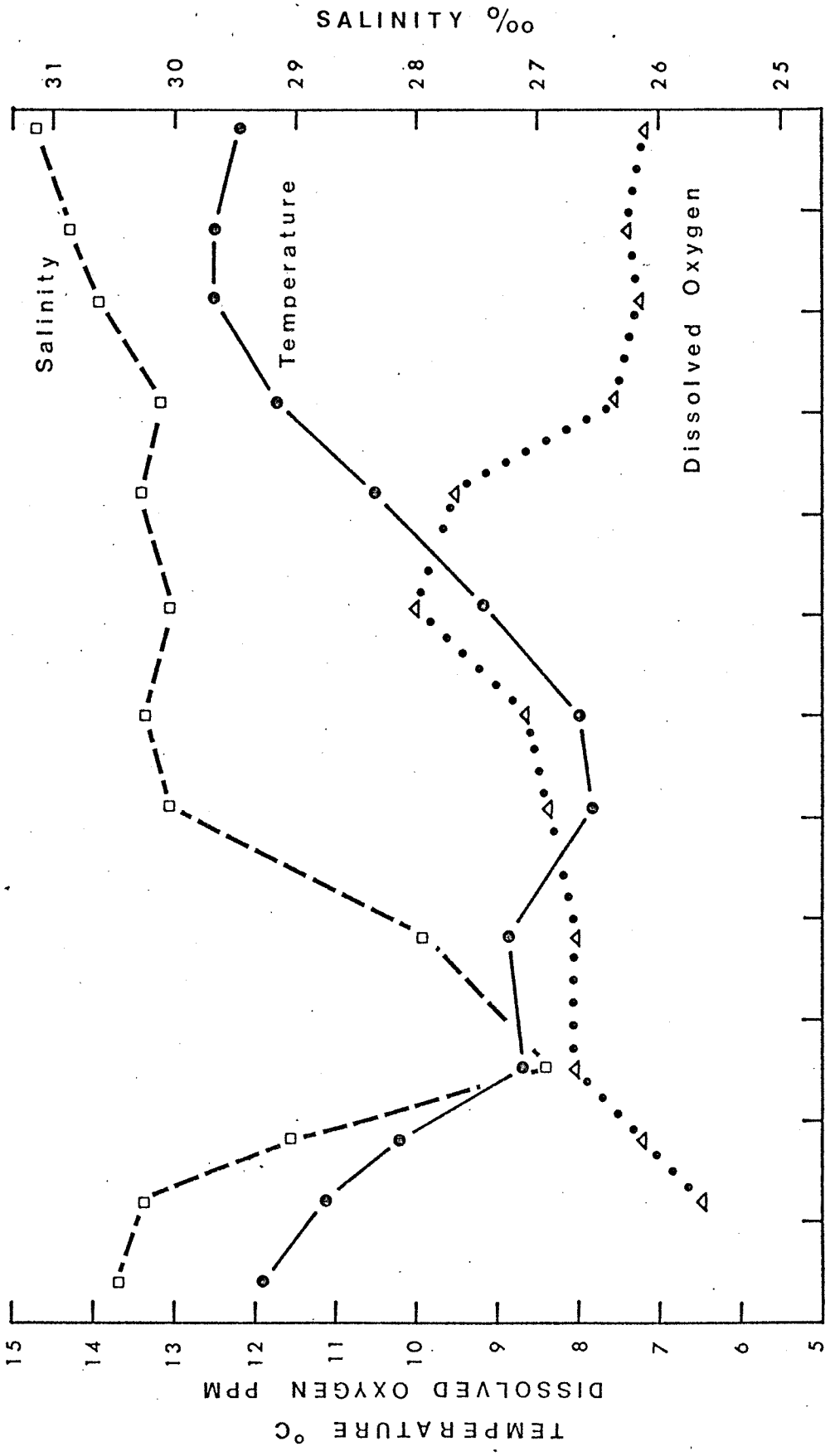


Figure 10. Mean ambient seawater temperature, salinity, and dissolved oxygen as measured in the seawater head-tank of the proportional diluter.

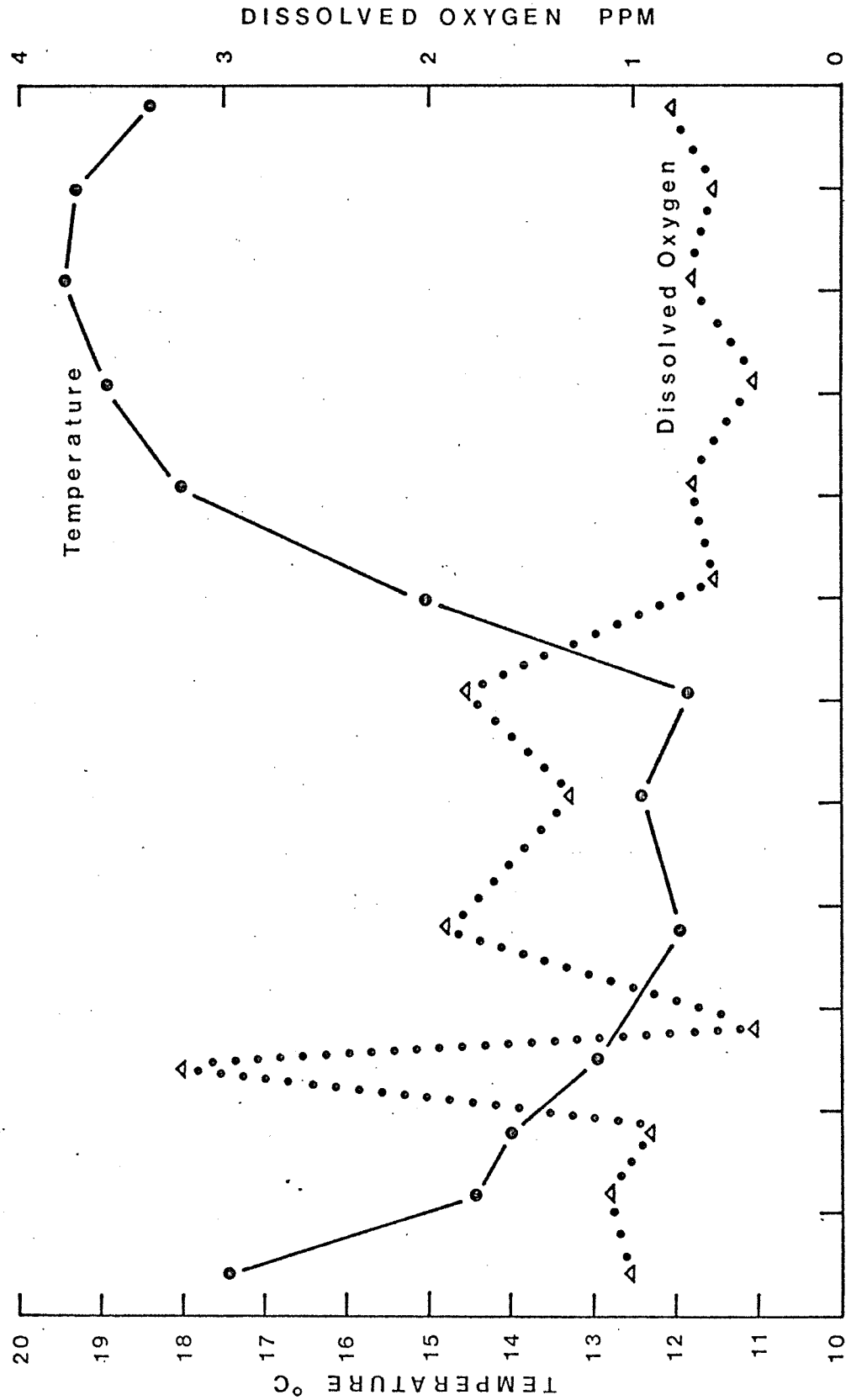


Figure 11. Mean West Point effluent temperature and dissolved oxygen as measured in the effluent head-tank of the proportional diluter.

A graphic illustration of the effectiveness of the diluter system to deliver and maintain a predetermined volume/volume dilution of WPE and seawater in the combined tests is shown in Figure 12. Mean values fall almost exactly on the expected curve. The relatively small variance indicated by the standard deviations was probably due to a combination of diluter error and changes in the salinity of the ambient seawater.

The observed concentrations of dissolved oxygen in the tanks following dilution of WPE in all acute tests combined indicated the mean was consistently above 4.6 ppm at 60% v/v WPE (Fig. 13). Observed values were greater than the expected at dilutions ranging from 10 to 60% effluent. The increase in the dissolved oxygen was probably due to the flow of seawater and sewage through the diluter system, which aerated the mixture prior to introduction into the test tanks.

5.1.3 Temperature Effects

The effect of seawater temperature on the toxicity of the WPE was tested in three 96-hour bioassays. Shiner perch were tested at 8.5 C (ambient temperature), 13.5 C (Δt = 5 C), and 18.5 C (Δt = 10 C). The LC50 values were 18, 18, and 11% WPE (v/v), respectively (Fig. 14). Average test tank temperatures for each test are presented in Table 4, along with TRC and DO measurements. There was no difference in mortality rates between the two lower temperatures. However, the highest temperature (18.5 C) produced a substantially lower LC50 value. The average chlorine level increased in the WPE head tanks with increasing test temperatures; however, the samples from the test tank dilutions showed a decrease in total residual chlorine with increasing test temperatures (Table 4). The

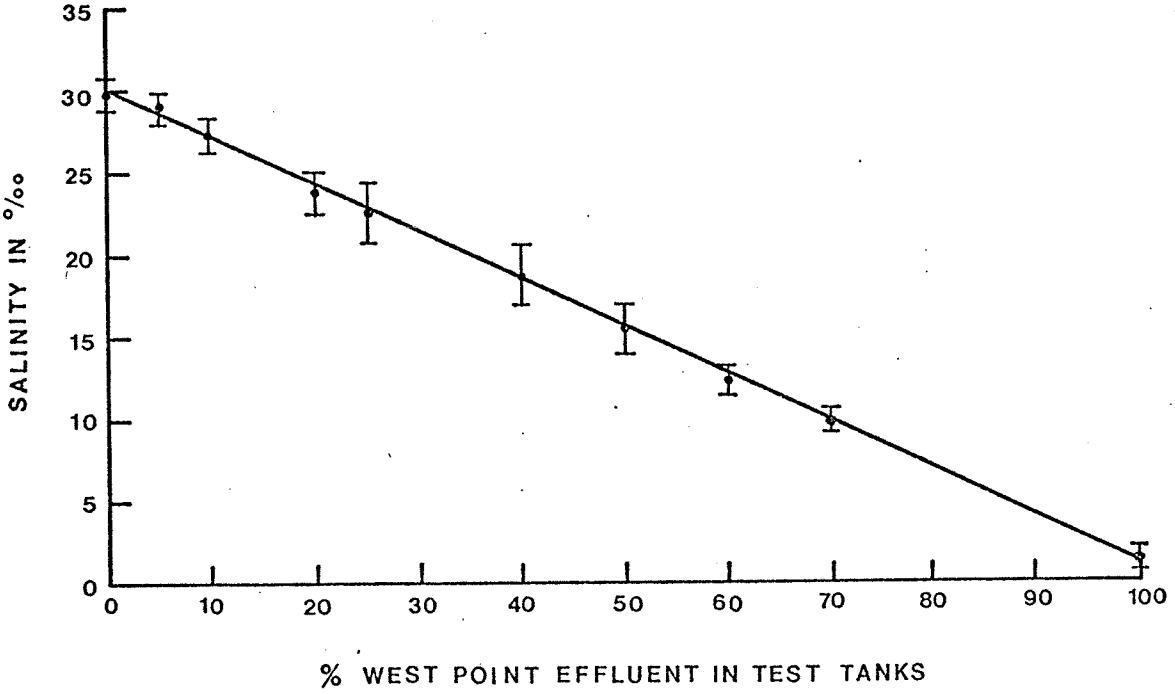


Figure 12. Mean and standard deviation salinity values for dilutions of West Point Effluent in head-tanks and test tanks for all acute bioassays. The line is eye-fitted.

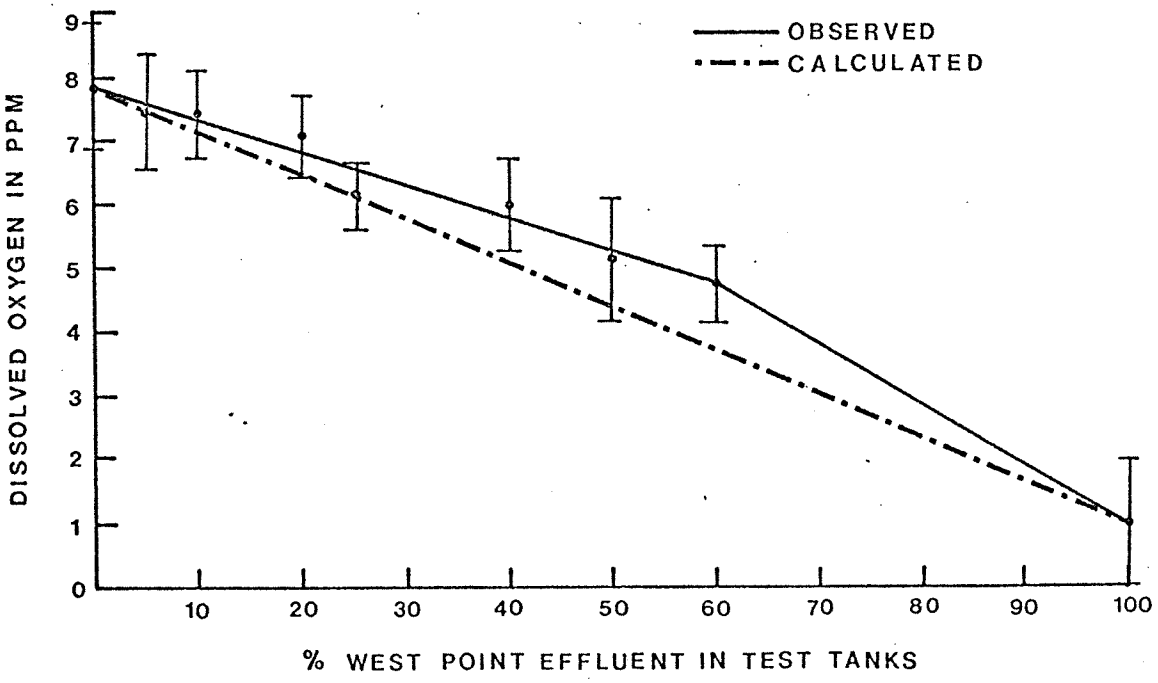


Figure 13. Mean and standard deviation dissolved oxygen values for dilutions of West Point Effluent in head-tanks and test tanks for all acute bioassays. The line is eye-fitted.

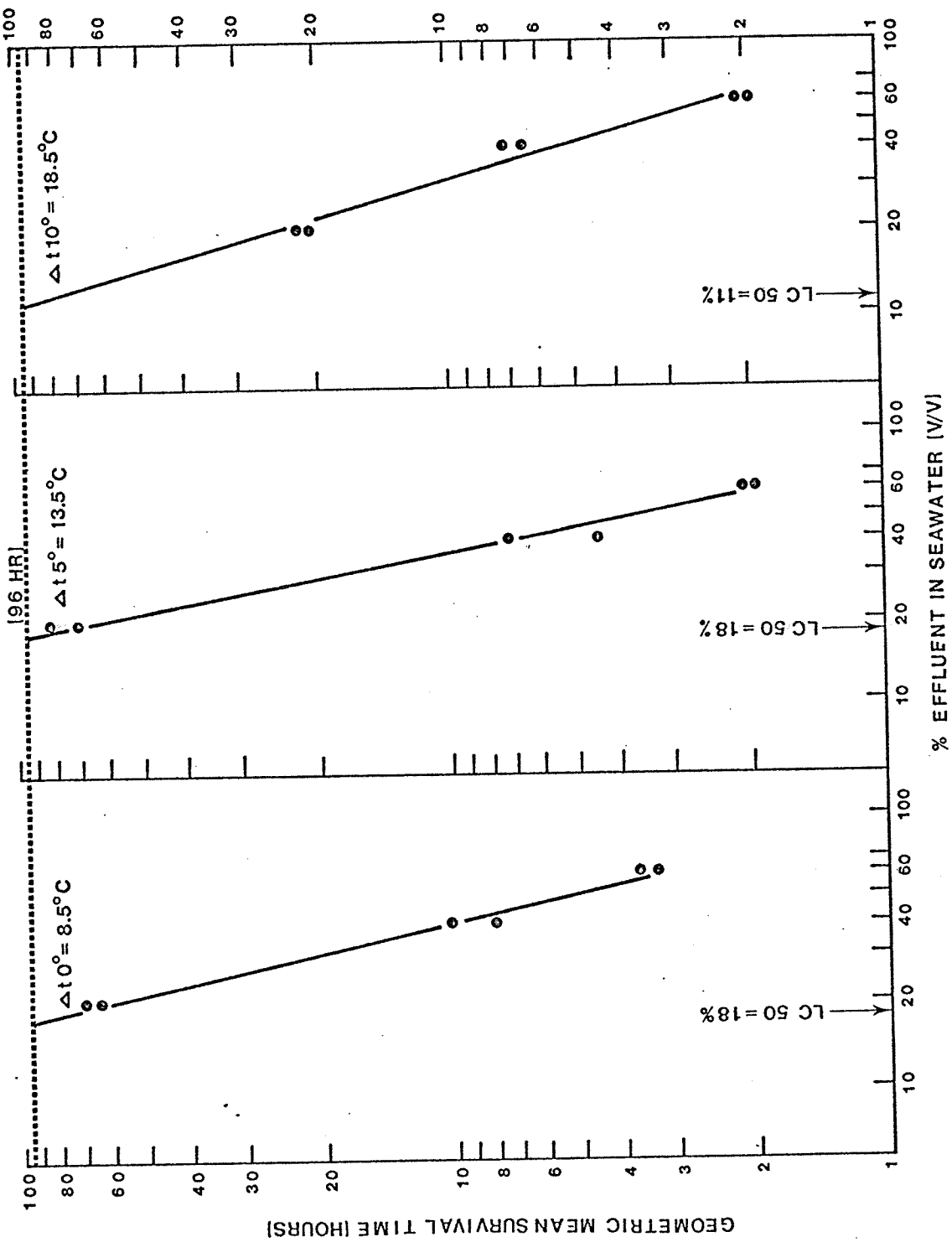


Figure 14. Determination of shiner perch LC50 in dilutions of West Point Effluent with seawater temperatures of 8.5, 13.5, and 18.5 C.

Table 4. Average test tank temperature, total residual chlorine, and dissolved oxygen values for temperature Bioassays 9, 10 and 11, using shiner perch.

Δt	Bioassay #	% WPE	Temperature	Total Residual Chlorine (ppm)	Dissolved Oxygen (ppm)
0 C	9	100 (Head Tank)	11.9	0.79	1.9
		0 (Control)	8.8	0	8.4
		60	10.0	0.64	5.4
		40	9.7	0.56	6.6
		20	9.1	0.45	7.1
5 C	10	100 (Head Tank)	13.2	0.95	1.1
		0 (Control)	13.6	0	7.5
		60	13.3	0.58	4.7
		40	13.4	0.32	5.8
		20	13.6	0.12	7.1
10 C	11	100 (Head Tank)	13.2	1.35	0.6
		0 (Control)	17.5	0	7.2
		60	16.2	0.45	4.4
		40	16.7	0.35	5.4
		20	17.2	0.10	6.2

reasons for this seemingly inconsistent observation of chlorine concentrations in the test tanks with increasing temperature may be partly due to increased volitization or chemical reaction of chlorine. Dissolved oxygen also decreased with an increase in temperature (Table 4) and may be partly responsible for increased mortality in the Δt = 10 C bioassay. The effects of increased summer temperature in the receiving water could be expected to increase the toxicity of the effluent; however, due to an array of toxic components in the effluent, synergistic effects are suggested. Specific effects of temperature alone would require considerable additional testing.

Biological, physical, and cnemical data for Bioassays 9, 10 and 11 are summarized in Appendix Tables 9, 10 and 11.

5.1.4 Life Stage Mortality Differences

Age zero and 1+ English sole and shiner perch were exposed side by side to equal dilutions of WPE to assess life stage differences in their sensitivity to WPE (Fig. 15). The age zero (1976) English sole (average length = 28.6 $\pm$ 6.6 mm) were tested against the age 1+ (1975) English sole (average length = 109.8 $\pm$ 16.6 mm). The age zero group proved more sensitive than the age 1+ age group, with LC50 values of 8 and 16% v/v WPE, respectively.

A group of age zero (1976) shiner perch (average length = 49.4 $\pm$ 3.4 mm) was tested against age 1+ shiner perch (average length = 124.4 $\pm$ 1.5 mm). The age zero group of shiner perch was generally as tolerant of WPE as the older age group with LC50 values of 14 and 15% v/v WPE, respectively. This further indicates the value of the shiner perch as a test animal for Puget Sound waters, since it appears to remain at the same sensitivity throughout its life cycle.

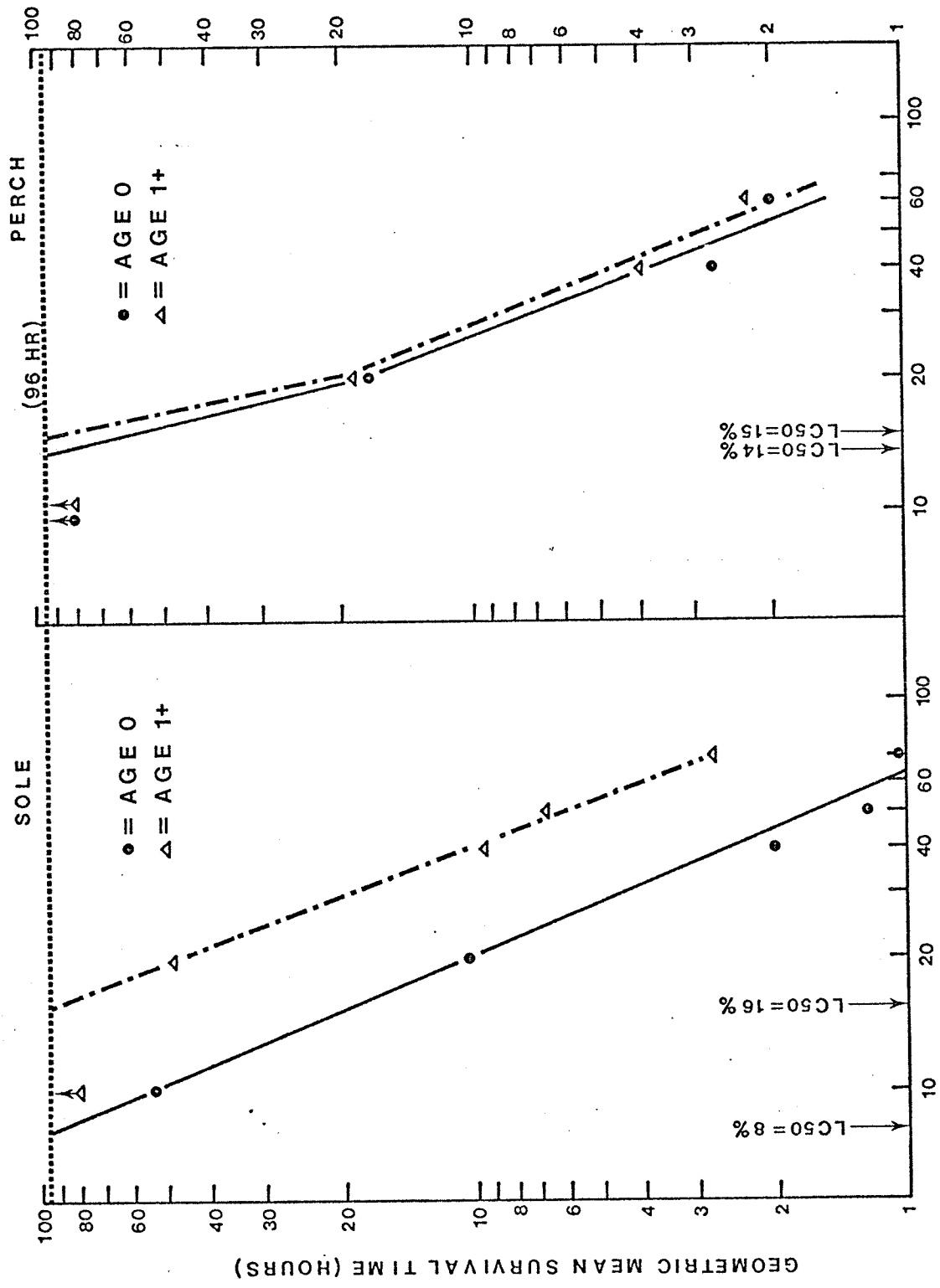


Figure 15. Determination of differences in toxicity of West Point Effluent to different age classes of English sole and shiner perch.

Biological, physical and chemical sampling parameters are summarized in Appendix Tables 14 and 19.

5.1.5 Filtered Effluent

One bioassay was conducted with shiner perch using side-by-side dilutions of WPE and WPE filtered through a column of graded pea gravel. Filtration of the effluent was necessary for other tests to avoid clogging the dechlorination and ammonia removal treatment columns. This test was designed to determine if toxicity would be reduced due to filtration so that subsequent testing in the dechlorination and ammonia removal bioassays could be adjusted for the effect due to filtration. LC50 values for shiner perch in filtered and unfiltered WPE were 21 and 19% v/v, respectively (Fig. 16). The small reduction in mortality in filtered WPE correlated closely with reductions in turbidity and chlorine (Table 5). It is hypothesized that a small amount of chlorine and other toxicants (such as trace metals) may have been physically or chemically complexed with the detrital or suspended solids and removed by the gravel filter, resulting in a reduction in mortality in the filtered effluent.

The mean values for turbidity in each test tank concentration for all acute bioassays show close agreement with the expected values at dilutions of WPE up to 50% v/v. A slight reduction from the expected values occurred at high dilutions (Fig. 17). This slight reduction may have been due to settling out of some of the settleable solids in concentrations from 60 to 100% v/v WPE. Variation in each dilution is partly due to diluter error and partly due to WPE changes in turbidity throughout the year.

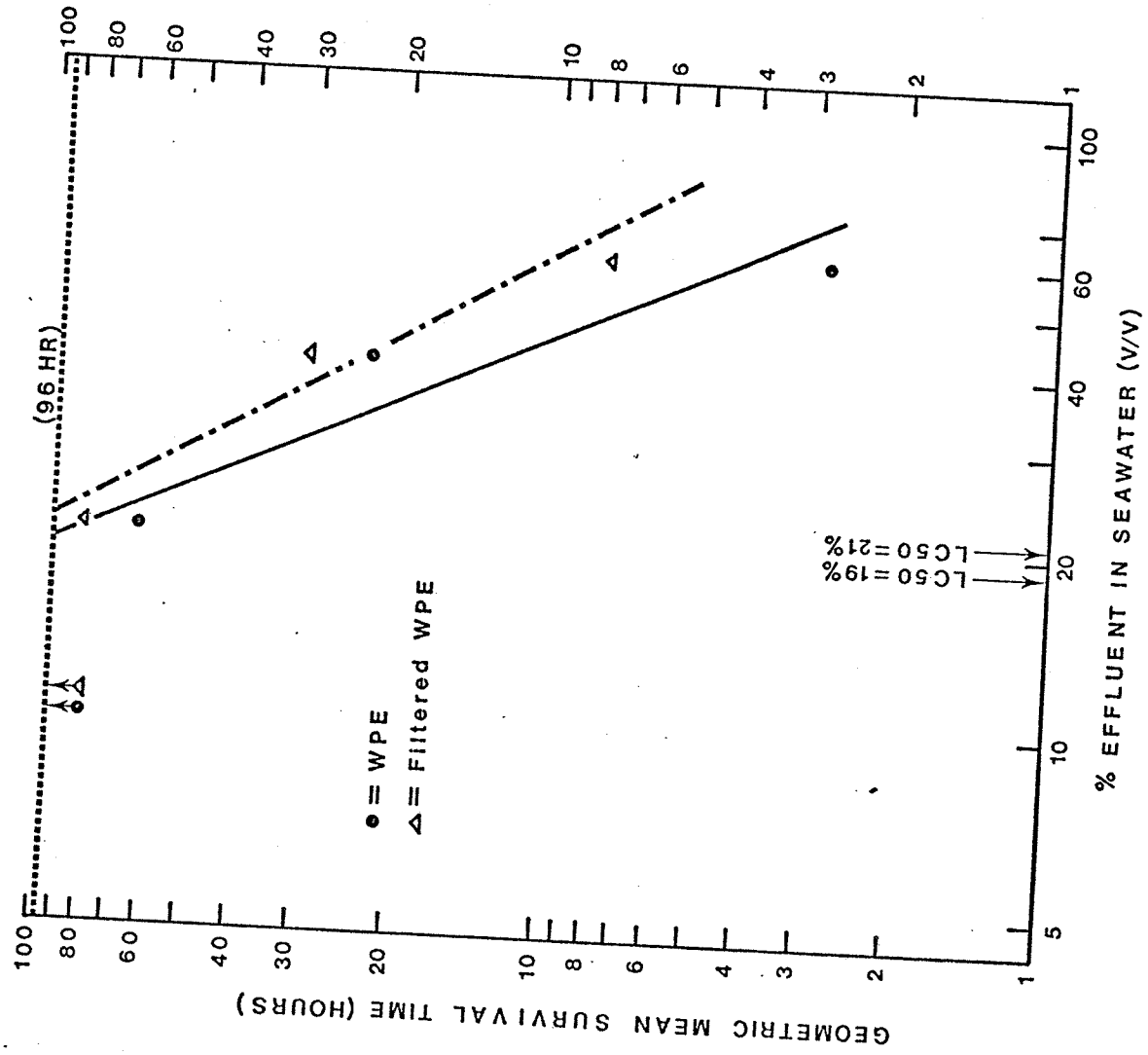


Figure 16. Determination of shiner perch LC50 in filtered and unfiltered West Point Effluent (WPE).

Table 5. Comparison of turbidity and total residual chlorine values in filtered and unfiltered WPE as measured in head tanks and test tanks of Bioassay 13 (shiner perch).

Treatment	% WPE	Turbidity (JTU)	Total Residual Chlorine (ppm)
Unfiltered WPE	100 (Head Tank)	82.8	0.98
	10	12.3	0.02
	20	19.7	0.11
	40	34.2	0.24
	60	45.7	0.45
Filtered WPE	100 (Head Tank)	55.2	0.93
	10	12.2	0.01
	20	16.7	0.08
	40	30.2	0.25
	60	38.8	0.39

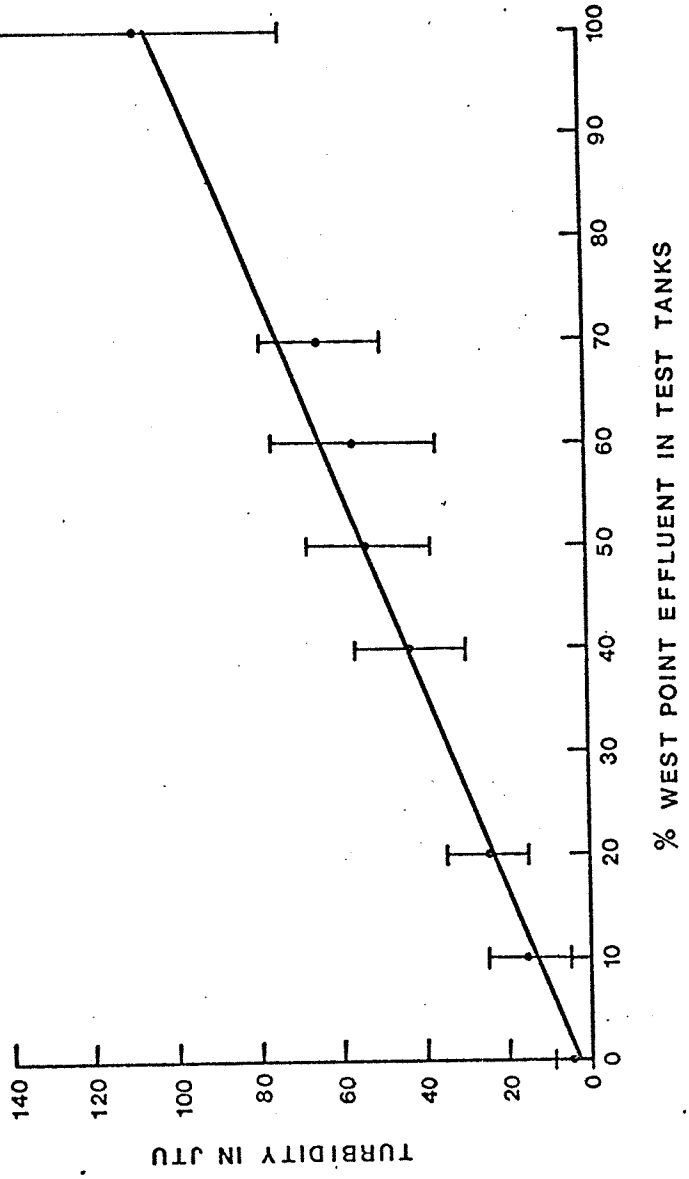


Figure 17. Mean and standard deviation turbidity values for dilutions of West Point Effluent in head-tanks and test tanks for all acute bioassays. The line is eye-fitted.

Biological, physical, and chemical sampling data are summarized in Appendix Table 13.

5.1.6 Dechlorination

Bioassays were conducted with English sole and shiner perch to determine the toxicity of WPE dechlorinated with gaseous sulfur dioxide. Side-by-side dilutions of chlorinated WPE and filtered WPE dechlorinated with SO_2 yielded LC50 values of 14 and 32% v/v, respectively for English sole and 15 and 28% v/v, respectively, for shiner perch (Fig. 18). In each case, the concentration of dechlorinated WPE which could be tolerated by 50% of the fest fish was approximately double the amount of chlorinated WPE.

The average total residual chlorine, sulfur dioxide, pH and dissolved oxygen values for each dilution of WPE are summarized in Table 6. Additional biological, physical, and chemical sampling data are summarized in Appendix Tables 16 and 21.

The mean TRC concentrations in the test tanks for all acute bioassays were generally less than the calculated amount, based on simple dilution (Fig. 19). Seawater typically exhibits a demand for chlorine as does sewage. The chlorine demand for seawater was much less than the chlorine demand for sewage which ranges typically from 8 to 15 ppm for primary sedimentation effluent (White, 1970). Seawater chlorine demand can vary with pH, ammonium ion, temperature, and concentration of various reducing agents and organic substances. The lower than calculated values for chlorine in the test tanks was possibly the result of the chlorine demand of the dilutent seawater in the test tanks. Volatization of chlorine in the diluter system may also have been responsible for some of the chlorine loss.

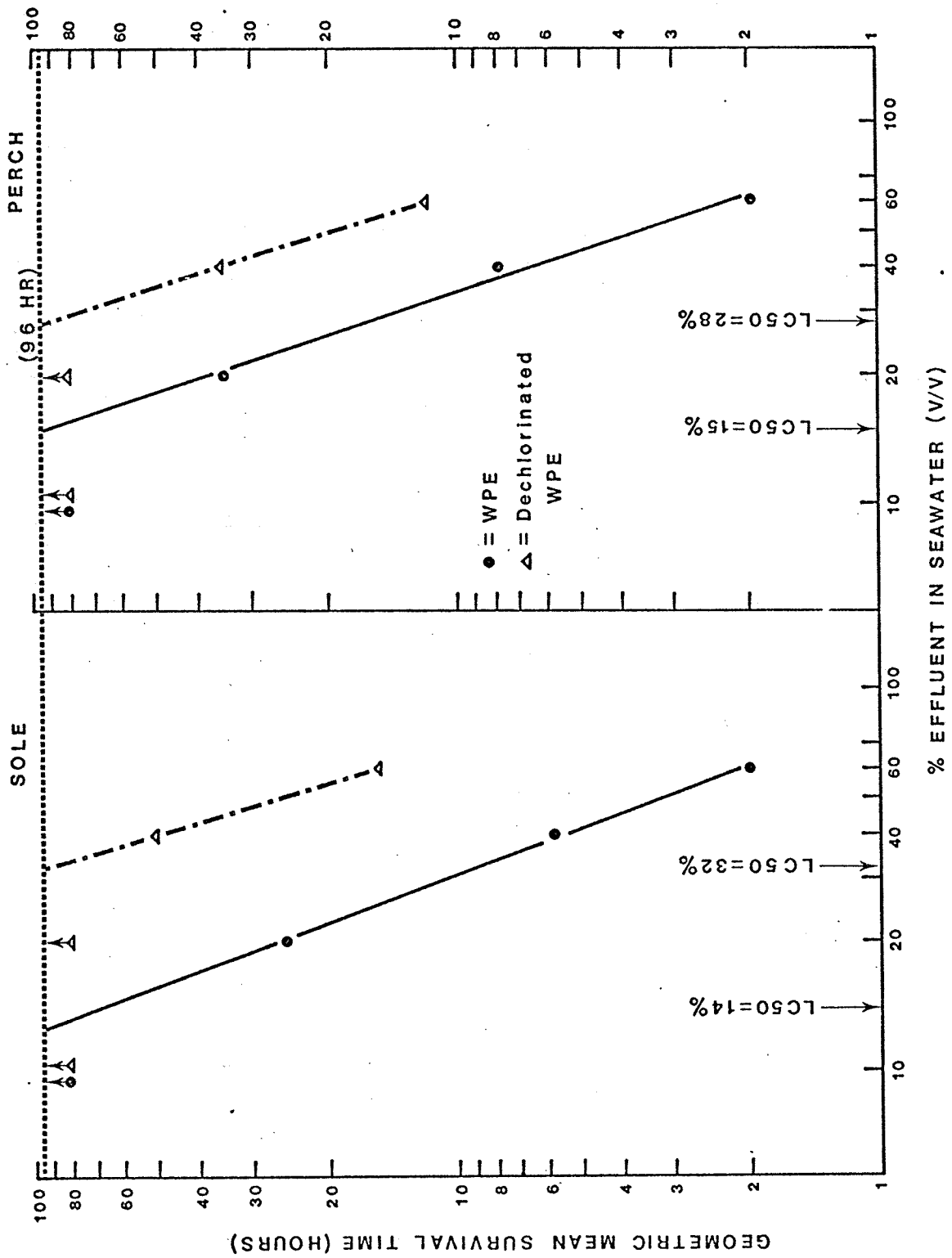


Figure 18. Determination of English sole and shiner perch LC50 in dilutions of West Point Effluent (WPE) and WPE dechlorinated with sulfur dioxide.

Table 6. Average total residual chlorine, sulfur dioxide, pH and dissolved oxygen values recorded from head tanks and test tanks of dechlorination Bioassays 16 (English sole) and 21 (shiner perch).

Bioassay #	Treatment	% WPE	Total Residual Chlorine (ppm)	Sulfur Dioxide (ppm)	pH	Dissolved Oxygen (ppm)
16	WPE	100 (Head Tank)	1.15	0		
	Dechl. WPE	"	<0.05	1.35		
21	WPE	100 (Head Tank)	1.31	0	6.7	0.8
		10	0.01	0	7.7	7.3
		20	0.05	0	7.5	6.9
		40	0.11	0	7.2	6.2
		60	0.30	0	7.0	4.9
	Dechl. WPE	100 (Head Tank)	<0.05	2.57	6.6	0.6
		10	<0.05	0.52	7.6	7.2
		20	<0.05	0.82	7.4	6.5
		40	<0.05	1.22	7.1	5.6
		60	<0.05	1.72	6.9	4.9

Figure 19 shows the wide and variable standard deviations around the means which resulted from the large fluctuations in the automatic chlorination of WPE.

The mean values for pH in each test tank concentration for all acute bioassays were well below the values that would have been expected from a simple dilution of two non-buffered solutions (Fig. 20). The buffering action of a solution is dependent on the amount of weak acid and its salts or weak base and its salts in that solution. Seawater contains carbonic and boric acids and their salts and is, therefore, a buffer solution (Sverdrup, *et al.*, 1942). However, sewage effluents are generally composed of a complex mixture of many ingredients including many acids and bases and their salts, and can also be expected to act as a buffering solution. The deviation of observed pH below that expected (Fig. 20) with increasing percentage of WPE illustrates that the buffering capacity of WPE may be greater than the buffering capacity of seawater. The wide standard deviations around the means suggest the buffering capacity of WPE was subject to wide fluctuations, depending on the constituents present in the effluent at any one time.

5.1.7 Ammonia Removal

Three acute bioassays were conducted with side-by-side dilutions of WPE and WPE treated to remove the ammonia by passage through a column of clinoptilolite ion-exchange resin. This natural zeolite resin has been shown by several investigators to be effective at removing in excess of 95% of the ammonia in freshwater fish systems (Williams, no date) and sewage effluents (Mercer, *et al.*, 1970; Esvelt, *et al.*, 1973). Clinoptilolite is highly selective for ammonia and can be recharged repeatedly with a NaCl-lime slurry or with seawater.

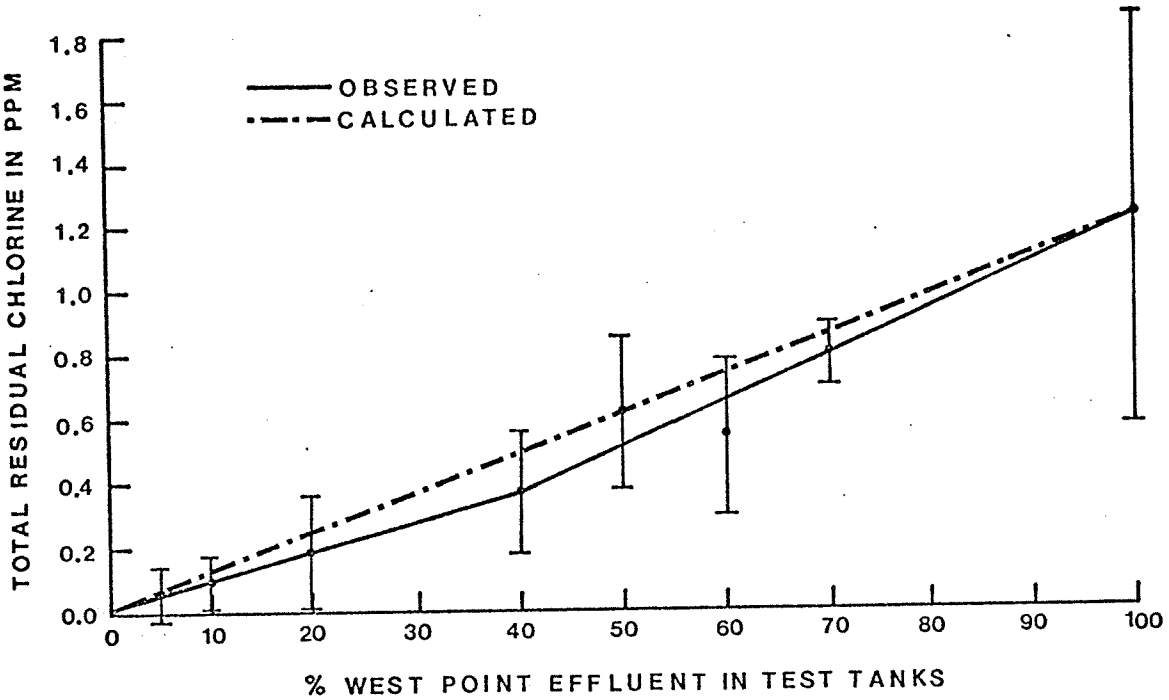


Figure 19. Mean and standard deviation total residual chlorine values for dilutions of West Point Effluent in head-tanks and test tanks for all acute bioassays.

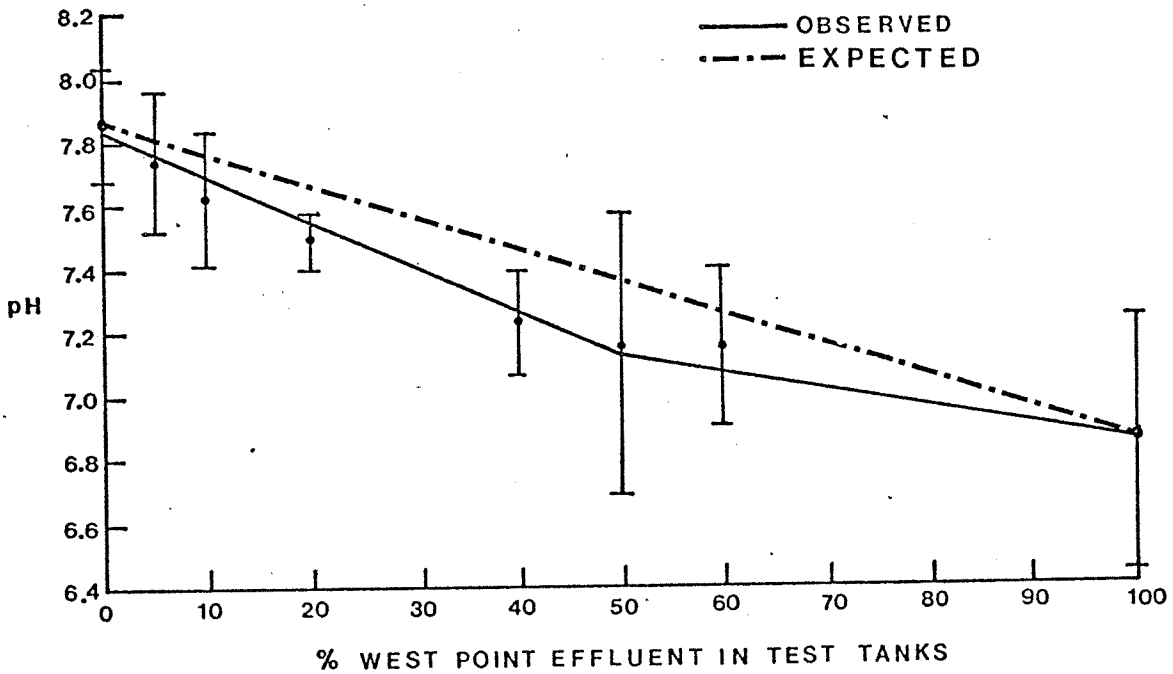


Figure 20. Mean and standard deviation pH values for dilutions of West Point Effluent in head-tanks and test tanks for all acute bioassays. The expected line is an estimate based on simple dilution and assuming no buffering capacity for seawater or effluent. Both lines are eye-fitted.

Bioassays 17 and 18 were conducted with English sole. Bioassay 17 was aborted at 40 hours due to total sudden mortality in all test tanks (except controls). This occurrence is discussed in Section 5.1.8. Bioassay 18 yielded LC50 values of 14% v/v WPE and 45% v/v WPE with ammonia removed. Bioassay 20, conducted with shiner perch yielded LC50 values of 12% v/v WPE and 26% v/v WPE with ammonia removed (Fig. 21).

Clinoptilolite was effective in reducing the WPE ammonia levels by more than 95% (Table 7). Coincidental to ammonia removal, the clinoptilolite resin also reduced the turbidity in the treated WPE head tank by 48% versus the 33% reduction achieved when only using the graded gravel filter. The clinoptilolite proved to be an effective treatment to dechlorinate the WPE with chlorine removal efficiency greater than 95% (Table 7). The chlorine apparently remained in a combined form with the ammonia (as chloramine) even though the ammonia was selectively removed by the clinoptilolite.

Biological, physical, and chemical sampling data are summarized in Appendix Tables 18 and 20.

The mean ammonia values measured in the test tanks for all acute bioassays were very close to the values predicted for simple dilution (Fig. 22). Evidently, the ammonia in the WPE was relatively stable so that significant losses did not occur in the diluter system due to volatization or chemical reactions with the dilutent seawater. The wide standard deviations around the means are indicative of the fluctuating ammonia concentrations in WPE throughout the year.

The ammonia nitrogen concentration in WPE was inversely correlated with the average effluent flow (Pearson Correlation Coefficient = -0.84),

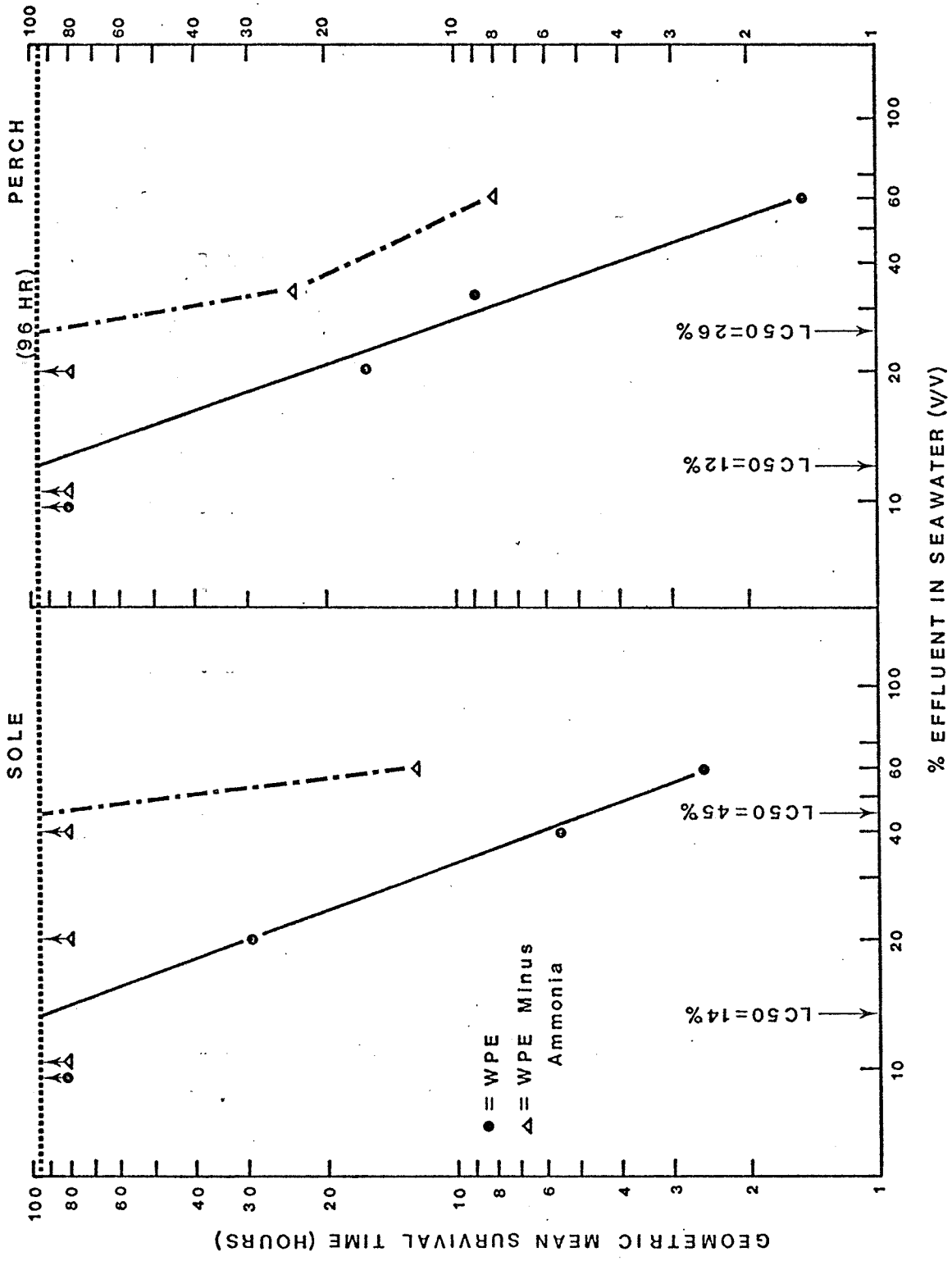


Figure 21. Determination of English sole and shiner perch LC50 in dilutions of West Point Effluent (WPE) and WPE after ammonia removal with ion-exchange resin.

Table 7. Average ammonia, total residual chlorine, and turbidity in head tanks and test tanks of ammonia removal Bioassays 18 (English sole) and 20 (shiner perch).

Bioassay #	Treatment	%WPE	Ammonia (ppm)	Total Residual Chlorine (ppm)	Turbidity (JTU)
18	WPE	100 (Head Tank)	18.0	1.56	147.0
		10	1.8	0.08	15.5
		20	4.0	0.20	31.2
		40	7.6	0.42	53.2
		60	11.2	0.68	92.2
18	WPE minus ammonia	100 (Head Tank)	0.2	< 0.05	77.2
		10	< 0.05	< 0.05	13.5
		20	0.02	< 0.05	19.5
		40	0.05	< 0.05	35.2
		60	0.05	< 0.05	50.5
20	WPE	100 (Head Tank)	15.9	1.25	120.4
		10	1.9	0.02	11.4
		20	4.2	0.09	29.4
		40	5.5	0.20	42.8
		60	9.5	0.39	74.8
20	WPE minus ammonia	100 (Head Tank)	0.1	< 0.05	62.8
		10	< 0.05	< 0.05	8.4
		20	< 0.05	< 0.05	13.2
		40	< 0.05	< 0.05	28.8
		60	< 0.05	< 0.05	40.0

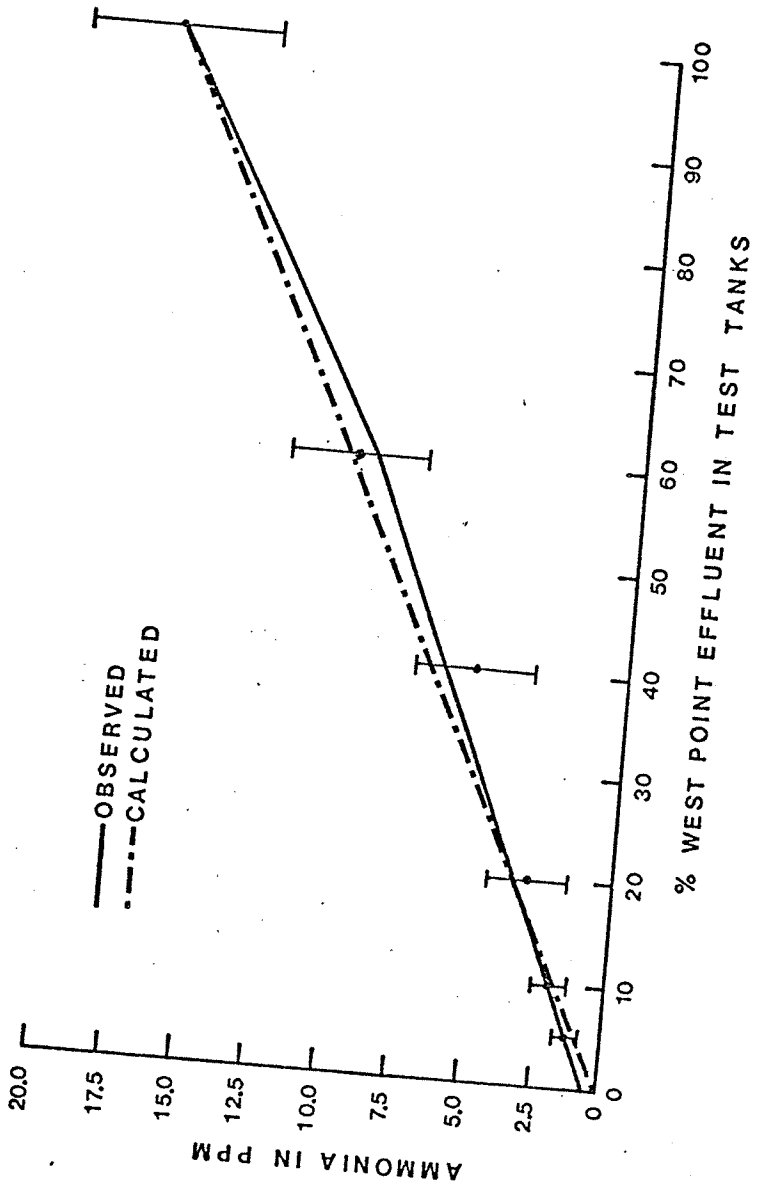


Figure 22. Mean and standard deviation ammonia (N) values for dilutions of West Point Effluent in head tanks and test tanks for all acute bioassays. The lines are eye-fitted.

which in turn was directly related to rainfall and storm water runoff (Pearson Correlation Coefficient = 0.75). Figure 23 shows that average monthly rainfall was closely related to the monthly average effluent flow, while ammonia increased during the summer months due to a reduction in rainfall and flow. Rainfall acts as a dilutent, thereby decreasing the concentration of toxicants such as ammonia in WPE. Ammonia nitrogen was also directly correlated with the effluent BOD and COD (Pearson Correlation Coefficient = 0.79 and 0.73, respectively). Thus, high summer ammonia concentrations probably accounted for a large proportion of the dissolved oxygen depletion in WPE during periods of low surface runoff.

5.1.8 Excessive Fish Mortality

Two bioassays produced unusual fish mortality and/or behavior. Bioassay 17 (ammonia removal with English sole) was abruptly terminated at 40 hours when all but three fish were suddenly killed in 10% v/v WPE (Table 8). While cause of mortality must remain somewhat speculative, the METRO 24-hour composite sample for mercury and the peak concentration for TRC showed unusually high values of 0.0037 ppm (July 2, 1976) and 2.65 ppm, respectively. This mercury value was substantially higher than the effluent monthly average (for July 1976) of 0.0006 ± 0.0007 ppm. It appears that a slug of mercury was received at the West Point treatment plant prior to the observed fish mortality. The increase in TRC may have resulted due to interference with the continuous chlorine monitor. However, chlorine was probably not the primary toxicant since fish in the WPE with ammonia and chlorine removed suffered mortality similar to those in untreated WPE. Clinoptilolite resin was found to effectively remove more than 95% TRC along with the ammonia in previous tests.

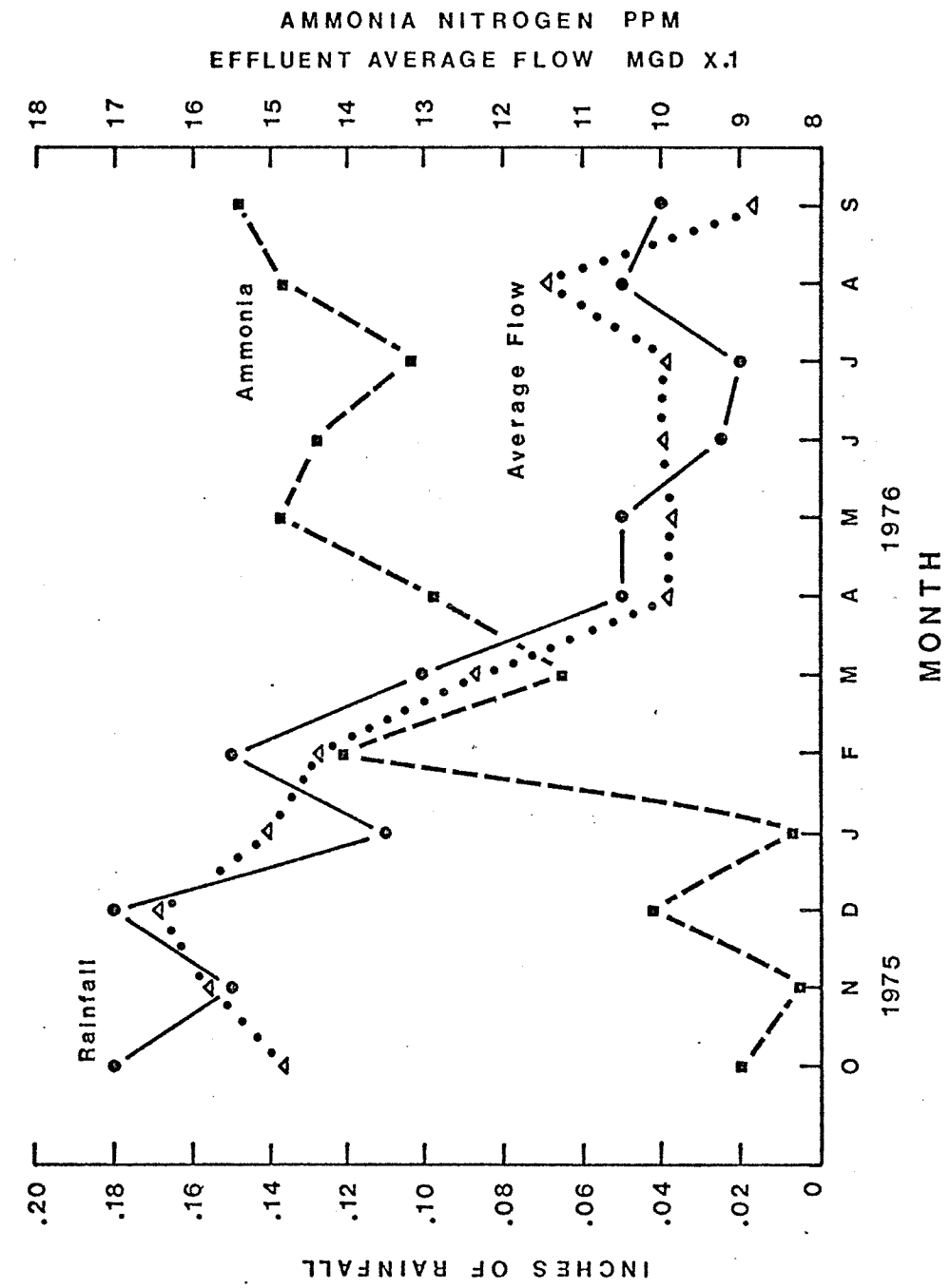


Figure 23. Average rainfall, effluent flow, and effluent ammonia nitrogen per month from October 1975 to September 1976, as measured by METRO Water Quality Staff at West Point.

Table 8. Pattern of unusual fish mortality at 40 hours in Bioassay 17 (English sole).

Treatment	% WPE	Status at 40 hours
WPE	60	Died earlier in test
	40	Died earlier in test
	20	Killed at 40 hours
	10	Killed at 40 hours
WPE minus ammonia	60	Died earlier in test
	40	Killed at 40 hours
	20	Killed at 40 hours
	10	7 of 10 killed at 40 hours (3 lived to 96 hours)
Control	0	No mortality in 96 hours
Control	0	No mortality in 96 hours

Shiner perch in Bioassay 21 (dechlorination) likewise suffered a similar fate (Table 9). Unusual behavior was observed in 10% WPE and 20% dechlorinated WPE dilutions, while early mortality occurred in 20% WPE and 40% dechlorinated WPE dilutions. During this same time span, METRO personnel were collecting hourly grab samples for trace metal analysis in raw sewage and primary effluent. These hourly samples bracketing the time of the observed fish morbidity are summarized in Table 10. It was apparent that one or two slug discharges containing elevated levels of chromium and copper were received at West Point between 1700 and 1900 hours on July 22, 1976. These slugs coincided closely with the peculiar fish behavior and subsequent mortality. In conjunction with this time period there occurred another peak of total residual chlorine of between 2.0 and 3.4 ppm lasting approximately three hours. The primary toxicant was probably not chlorine since fish mortality or abnormal behavior occurred in both chlorinated and dechlorinated WPE.

5.1.9 Gill Histopathology

Histological examination of gill tissues from English sole and shiner perch in various concentrations of chlorinated WPE indicates a progression of pathological symptoms (Tables 11 and 12). The control fish in 100% seawater were essentially normal except for some minor focal congestion and hemorrhaging in the gill tissue of shiner perch. With increasing WPE concentrations of 10, 50, and 70%, some or all of the following tissue responses were noted: edema (excess intracellular fluid), epithelial hyperplasia (increase in number of cells), goblet cell hypertrophy (increase in size), epithelial desquamation (sloughing off of cells),

Table 9. Pattern of unusual fish behavior and mortality from 30 to 36 hours in Bioassay 21 (shiner perch).

Treatment	% WPE	Fish Reaction
WPE	60	Died earlier in test
WPE	40	Died earlier in test
WPE	20	All dead at 34 hours
WPE	10	All appeared dead at 30 hours, but recovered fully by 32 hours
Dechlorinated WPE	60	Died earlier in test
	40	All dead at 36 hours
	20	All showed loss of equilibrium; recovered fully by 32 hours
	10	All showed loss of equilibrium; 2 dead, 8 recovered by 32 hours
Control	0	All survive to 96 hours
Control	0	All survive to 96 hours

Table 10. Trace metal values in METRO hourly raw sewage and effluent samples collected from 1500 to 2200 hours on July 22, 1976. Unusual fish behavior and mortality was observed in Bioassay 21 from approximately 1900 to 2100 hours.

Sample Hour	Trace Metal (ppm)					
	Cd	Cr	Cu	Ni	Pb	Zn
			Raw Sewage			
1500	0.020	0.10	0.23	0.10	0.25	0.46
1600	0.016	0.28*	0.37*	0.10	0.20	0.45
1700	0.020	0.21*	0.38*	0.07	0.23	0.46
1800	0.018	0.22*	0.39*	0.08	0.20	0.47
1900 (fish	0.022	0.11	0.76*	0.07	0.13	0.55
2000 morbidity)	0.022	0.09	0.20	0.08	0.16	0.38
2100 "	0.022	0.09	0.19	0.06	0.14	0.38
2200	0.018	0.09	0.17	0.10	0.14	0.37
2-day average	0.015	0.16	0.21	0.08	0.18	0.48
			Effluent			
1500	0.016	0.07	0.17	0.06	0.19	0.36
1600	0.020	0.08	0.18	0.09	0.19	0.36
1700	0.025	0.31*	0.22	0.10	0.18	0.46
1800	0.026	0.32*	0.22	0.10	0.18	0.41
1900 (fish	0.020	0.07	0.32*	0.08	0.18	0.38
2000 morbidity)	0.023	0.13	0.20	0.09	0.18	0.34
2100 "	0.018	0.06	0.20	0.07	0.17	0.37
2200	0.018	0.05	0.14	0.08	0.15	0.27
2-day average	0.013	0.09	0.13	0.05	0.11	0.29

* Peaks possibly due to one or more trace metal-containing slugs discharged to the West Point treatment plant.

Table 11 Results of histopathological examination of gill tissue from juvenile English sole exposed to chlorinated West Point Effluent in Bioassay 14.

WPE Concentration	Length of Exposure (hrs)	Fish Length (mm)	Histologic Finding
0% (Control)	96	130	normal
10%	96	150	minimal epithelial hyperplasia with some edema and sub-epithelial inflammatory infiltrate primarily in the mid- and basal sections of the lamellae.
20%	48	125	extreme epithelial desquamation with hyperplasia at the apical tips of the lamellae and some lamellar fusion. Sub-epidermal mononuclear infiltrate generally present. Pronounced exudate exterior to the gill tissue containing bacterial colonies and fungal hyphae. Hyperplasia of goblet cells.
40%	8	120	desquamation of epithelial cells in basal segments of the gill lamellae with distal fusion and epithelial hyperplasia. Sub-epidermal mononuclear infiltrate with minimal congestion. Few goblet cells.
50%	6	120	pronounced epithelial hyperplasia in gill lamellae with sub-epithelial mononuclear infiltrate. Focal epithelial sloughing in some proximal ends of lamellae and focal hemorrhaging in some areas. Few goblet cells.
70%	3	135	marked congestion of branchial vessels. Large number of sub-epidermal infiltrating mononuclear cells. Marked diffuse desquamation of epithelial cells in proximal ends of the lamellae coupled with some distal end fusion. Diffuse hypertrophy of goblet cells. External exudate of mononuclear cells, red blood cells, and desquamated epithelial cells.

Table 12. Results of histopathological examination of gill tissue from shiner perch exposed to chlorinated West Point Effluent in Bioassay 19.

WPE Concentration	Length of Exposure (hrs)	Fish Length (mm)	Histological Finding
0% (Control)	96	120	some focal congestion and hemorrhage.
5%	96	125	minimal congestion and hemorrhage.
10%	96	130	congestion, hemorrhage, focal edema, and mucous cell hypertrophy generally throughout tissue.
20%	16	125	general presence of congestion and inflammatory infiltrate. Epithelial hyperplasia and focal clubbing of lamellae. Epithelial desquamation and focal edema. Mucous gland hypertrophy.
40%	4	120	extreme basal edema of lamellae coupled with diffuse loss of epithelium. Multiple exposed capillaries with focal hemorrhaging and some vascular congestion. Minimal inflammatory infiltrate present with extra lamellar exudate composed of white blood cells, red blood cells, and epithelial debris.
60%	2	150	extreme basal edema of the lamellae resulting in distortion of the filaments. Focal loss of lamellae epithelium with focal inflammatory infiltrate at base of lamellae. Complete exposure of some capillaries due to extreme edema with some focal epithelial hypertrophy.

sub-epidermal mononuclear cell infiltration, congestion, inflammation, and hemorrhaging. Some of these responses are illustrated in a selected series of Figures, 24 through 27. Apparently incidental protozoan parasites and parasitic granulomas were also noted in many of the gill tissue samples.

5.2 Eight-Week Chronic Bioassays

5.2.1 Mortality

Shiner perch mortalities in chronic Bioassay 12 were 40, 80 and 90% in 1, 5 and 10% WPE, respectively. No English sole mortality occurred. Mortality of littleneck clams in Bioassay 15 approximated 20% in each test concentration (0.5 to 10% WPE) while all English sole survived. No mortality of shiner perch occurred in Bioassay 22 in WPE concentrations of 0.5 to 5%.

Some of the shiner perch mortalities associated with Bioassay 12 probably occurred due to insufficient preliminary data indicating the maximum sublethal concentration. Later studies have shown the maximum sublethal concentration to be somewhat less than 10% for shiner perch. Some mortality in Bioassay 12 was probably associated with WPE toxicity; however, it was believed that some mortalities may have been induced by handling and a somewhat irregular feeding schedule. No mortalities occurred in Bioassay 22 where the fish were fed on a daily basis throughout the experiment. Clam mortalities which occurred in Bioassay 15 may have been due, in part, to the stress resulting from a lack of adequate burrowing material. It was thought that the inert glass marbles, which were used as a

Figure 24. English sole gill tissue after 4 days in seawater control tank in Bioassay 14. Tissue is essentially normal except for *Trichodina sp.* between lamellae. X 250.

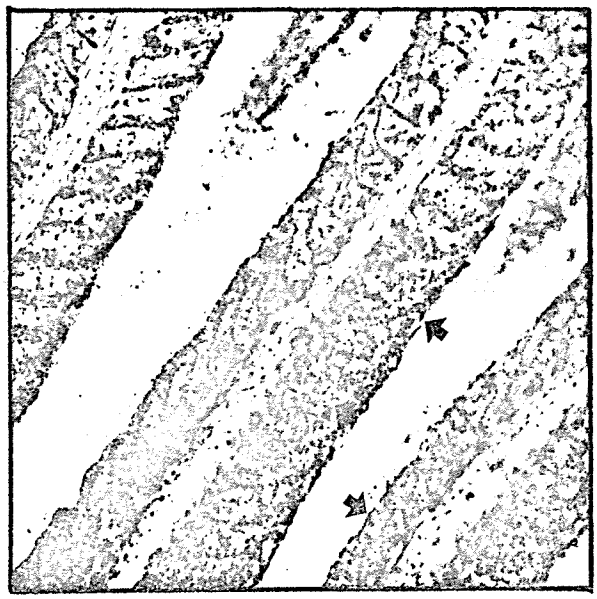


Figure 25. English sole gill tissue after 4 days' exposure to 10% effluent in Bioassay 14. Arrows show areas affected by an inflammatory infiltrate. X250.

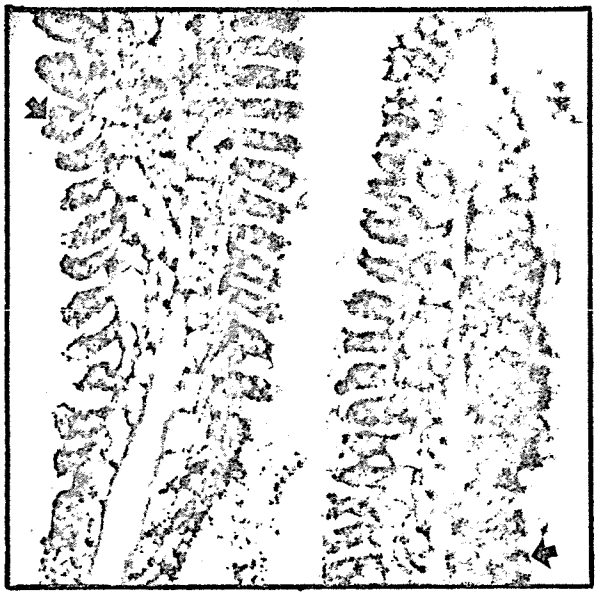


Figure 26. English sole gill tissue from moribund fish exposed to 50% effluent for 6 hours in Bioassay 14. Arrows locate areas of epithelial hyperplasia. X250.

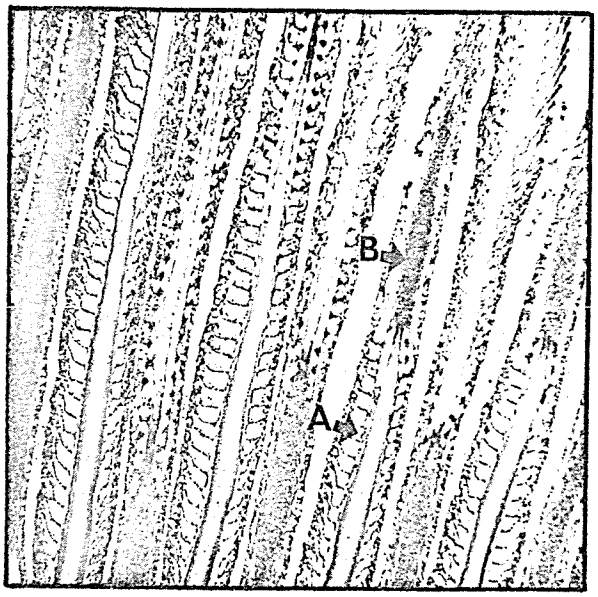


Figure 27. English sole gill tissue from moribund fish exposed to 70% effluent for 3 hours in Bioassay 14. Arrows show edema and separation of epithelium from vascular tissue (A) and congestion (B). X250.

burrowing substrate, would allow the clams to function effectively. It is possible that the marbles did not completely satisfy this need.

5.2.2 Trace Metal Bioaccumulation

Thirty-eight fish from Bioassay 12 were analyzed (singly and some as composites) using two ashing methods. The dry ashing method yielded results which indicated in most cases higher whole body concentrations of zinc and copper than did the wet ashing method (Table 13). The results based on dry ashing also showed observable differences in the whole body concentrations of the metals in fish exposed to different WPE dilutions. In contrast, the wet ash method yielded lower results which did not appear to vary among fish from different effluent dilutions as shown by comparing Figure 28 to Figure 29, and Figure 30 to Figure 31. No consistent change in whole body total copper and zinc concentrations was found with exposure to increasing concentrations of WPE during 8-week chronic bioassays.

The food (shrimp) used during Bioassay 12 was analyzed by the dry ash method and found to contain a whole body concentration of copper in the mid-range of that found for the shiner perch and English sole. The whole body concentration of zinc for the shrimp was less than one-half of the lowest whole body concentration of zinc found in any of the fish analyzed in this bioassay. Only in one case (copper in shiner perch from dry ash analysis) did the unexposed fish (direct from Puget Sound) contain a metal at a concentration which appeared appreciably different from that obtained for the seawater controls.

Thirty clams from Bioassay 15 were analyzed individually by the wet ash method (Table 14), and twenty-five juvenile English sole were analyzed

Table 13. Average whole body total copper and zinc in shiner perch, *Cymatogaster aggregata*, and English sole, *Parophrys vetulus*, for Bioassay 12. Fish were analyzed individually.

% WPE Dilutions	DRY ASHING			WET ASHING				
	No. Fish	mg Cu/kg Dry Wt.	mg Zn/kg Dry Wt.	No. Fish	mg Cu/kg Dry Wt.	mg Cu/kg Wet Wt.	mg Zn/kg Dry Wt.	mg Zn/kg Wet Wt.
Shiner Perch								
Unexposed	2	30.2	138	5	5.0	1.2	118	29
Seawater Control	2	20.6	135	4	4.4	1.0	94	22
0.5	2	22.3	120	5	4.4	1.0	106	24
1.0	2	13.9	126	-	-	-	-	-
5.0	2	17.2	148	2	5.7	1.2	120	24
English Sole								
Unexposed	2	5.3	88.5					
Seawater Control	2	9.2	104.5					
0.5	2	8.4	105.0					
1.0	2	14.6	139.5					
5.0	2	6.3	95.5					
10.0	2	15.2	132.5					

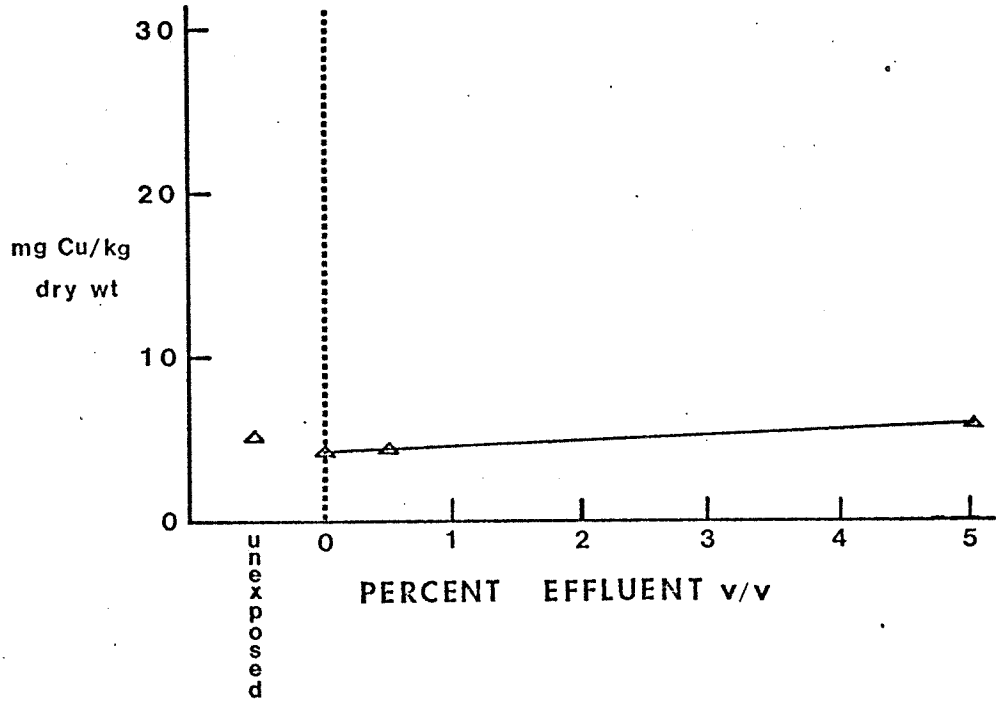


Figure 28. Average whole body total copper content in shiner perch following 8-week exposure to West Point effluent in Bioassay 12. Wet ash analysis.

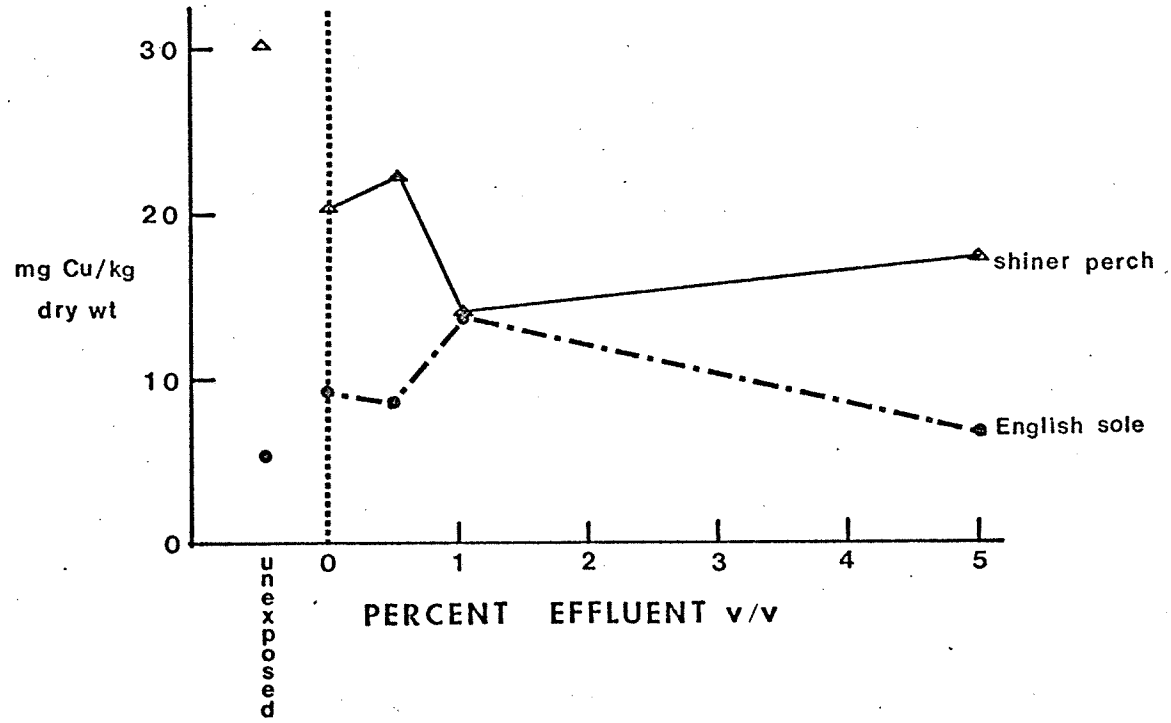


Figure 29. Average whole body total copper content in shiner perch and English sole following 8-week exposure to West Point effluent in Bioassay 12. Dry ash analysis.

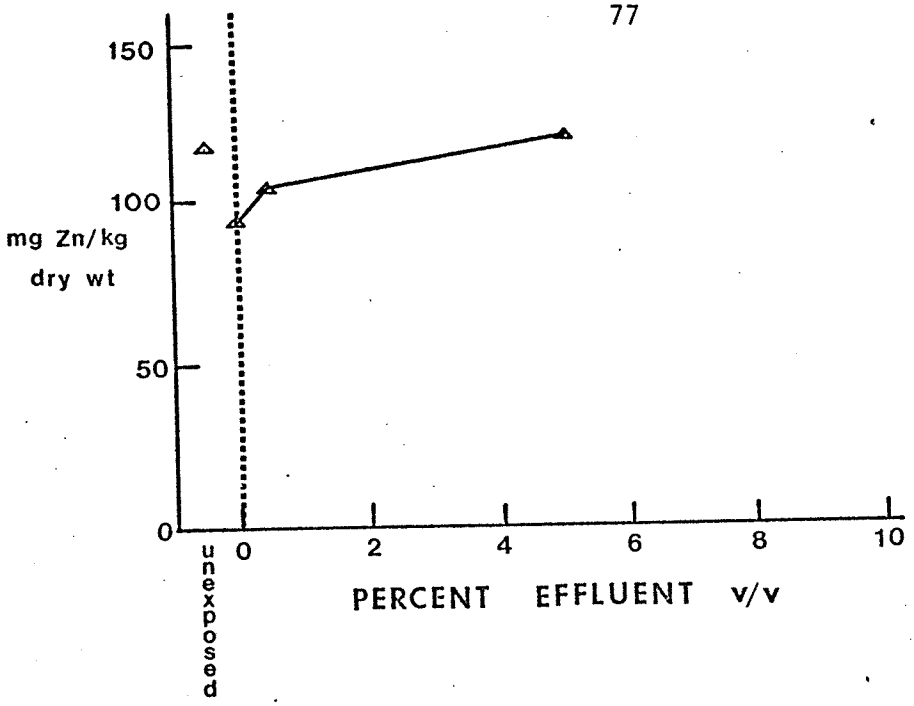


Figure 30. Average whole body total zinc content in shiner perch following 8-week exposure to West Point effluent in Bioassay 12. Wet ash analysis.

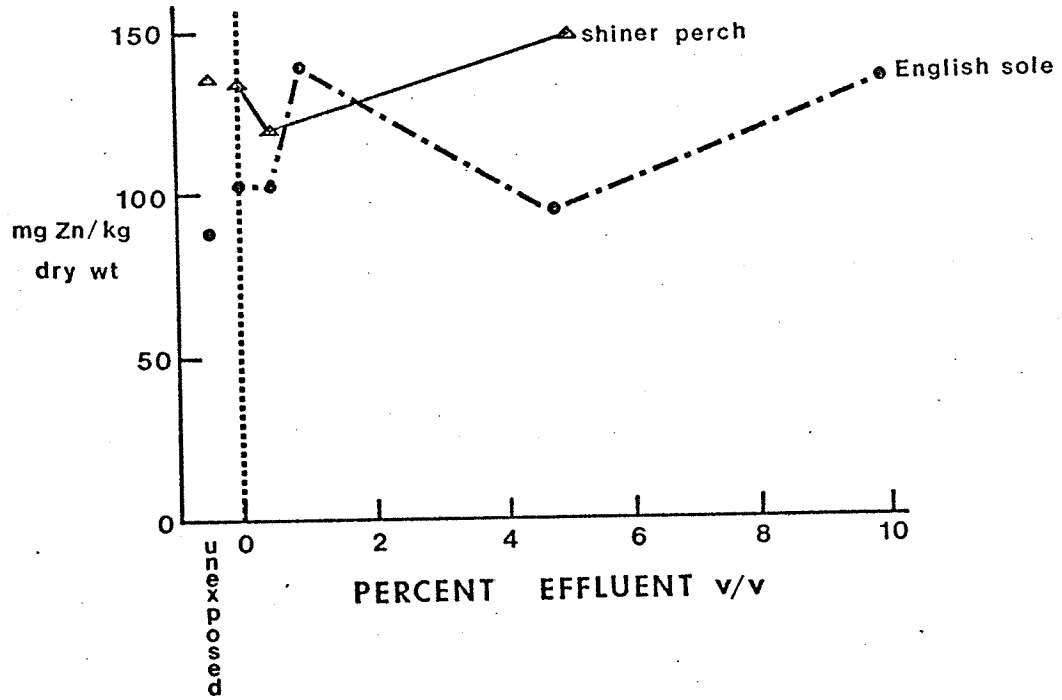


Figure 31. Average whole body total zinc content in shiner perch and English sole following 8-week exposure to West Point effluent in Bioassay 12. Dry ash analysis.

Table 14. Mean whole body total copper and zinc in shelled, common littleneck clams, *Protothaca staminea*, from Bioassay 15. Five organisms from each dilution were analyzed individually.

% WPE Dilution	mgCu/kg Dry Weight		mgCu/kg Wet Weight	
	Mean	95% Confidence Limit	Mean	95% Confidence Limit
Unexposed	6.1	3.95 - 8.24	1.00	0.72 - 1.28
Seawater Control	8.8	6.63 - 10.92	1.28	1.00 - 1.56
0.5	11.8	9.51 - 13.90	1.46	1.18 - 1.74
1.0	11.8	9.69 - 13.98	1.52	1.24 - 1.80
5.0	12.3	10.19 - 14.48	1.56	1.28 - 1.84
10.0	10.3	8.15 - 12.44	1.28	1.00 - 1.56
	mgZn/kg Dry Weight		mgZn/kg Wet Weight	
Unexposed	102.4	66.0 - 138.7	17.0	12.7 - 21.2
Seawater Control	111.2	74.8 - 147.5	16.2	11.9 - 20.4
0.5	156.6	120.2 - 175.5	19.4	15.1 - 23.6
1.0	139.2	102.8 - 175.5	17.6	13.3 - 21.8
5.0	161.6	125.5 - 197.9	20.6	16.3 - 24.8
10.0	129.2	92.8 - 165.5	15.0	10.7 - 19.2

in composites of 5 fish each (Table 15). Two-way analysis of variance showed no statistically significant change in whole body total copper and zinc content among clams at the different effluent dilutions including seawater controls. This was also true for the juvenile English sole. Linear regression analysis of the data for the clams yielded a slope which was statistically not different from zero for both copper and zinc (Figs. 32 and 33). The concentrations of zinc in the unexposed clams were not statistically different (at the 5% level) from that of the seawater controls. The concentration of copper in the unexposed clams was significantly lower than seawater controls. Shiner perch tissues from Bioassay 22 are still in the process of analysis. Results of this analysis will be available at a later date.

Summaries of the results of bi-weekly physical and chemical measurements of the WPE and seawater dilutions during each bioassay can be found in Appendix Tables 12, 15 and 22. Daily checks of the diluter system flow rates have been summarized in Table 16 to show the accuracy of the diluter.

5.2.3 PCB Bioaccumulation

A small group of juvenile English sole from chronic Bioassay 12 (8-week) was sent to METRO's Water Quality Laboratory for whole body analysis for polychlorinated biphenyls (PCB). This group consisted of one fish each from 10, 5, 1, and .5% v/v WPE dilutions, and one fish from the seawater control tank. Additionally, one control fish was sacrificed from the acclimation tank just prior to the start of Bioassay 12. The PCB and total chlorinated hydrocarbon content of each fish are presented in Table 17. Although the sample size was small, the results suggest that

Table 15. Whole body total copper and zinc in juvenile English sole, *Parophrys vetulus*, from Bioassay 15. One composite sample of 5 fish was analyzed from each WPE dilution.

% WPE Dilutions	mgCu/kg Dry Wt.	mgCu/kg Wet Wt.	mgZn/kg Dry Wt.	mgZn/kg Wet Wt.
Seawater Control	5.8	0.8	139	20
0.5	4.7	0.7	134	21
1.0	4.2	0.7	133	21
5.0	5.0	0.8	129	20
10.0	7.3	1.1	139	21

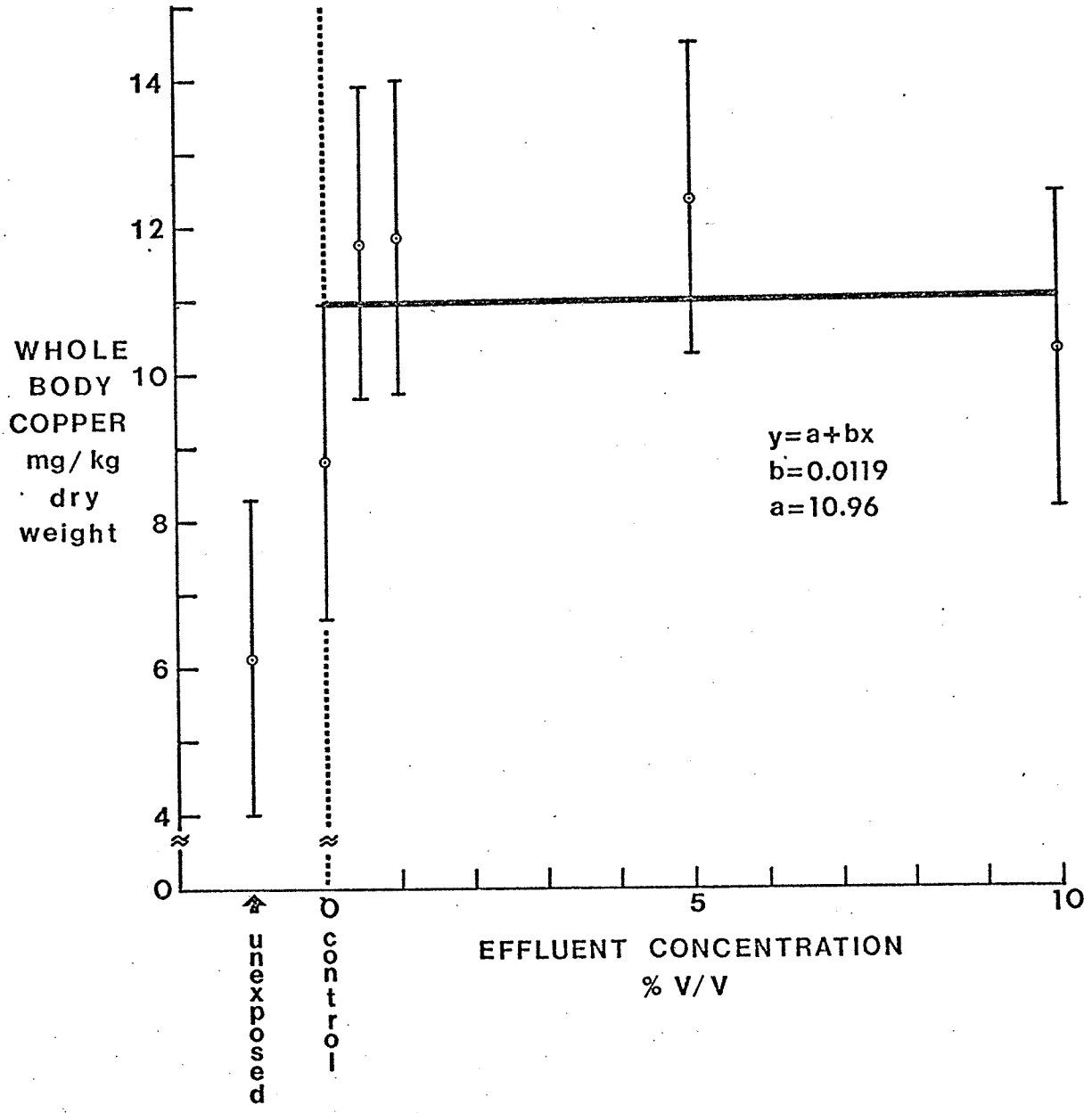


Figure 32. Average whole body total copper content in shelled, common littleneck clams following 8-week exposure to West Point effluent in Bioassay 15. "Unexposed" data were not used in calculation of the regression line. The slope (b) is not statistically different from zero at the 95% confidence level.

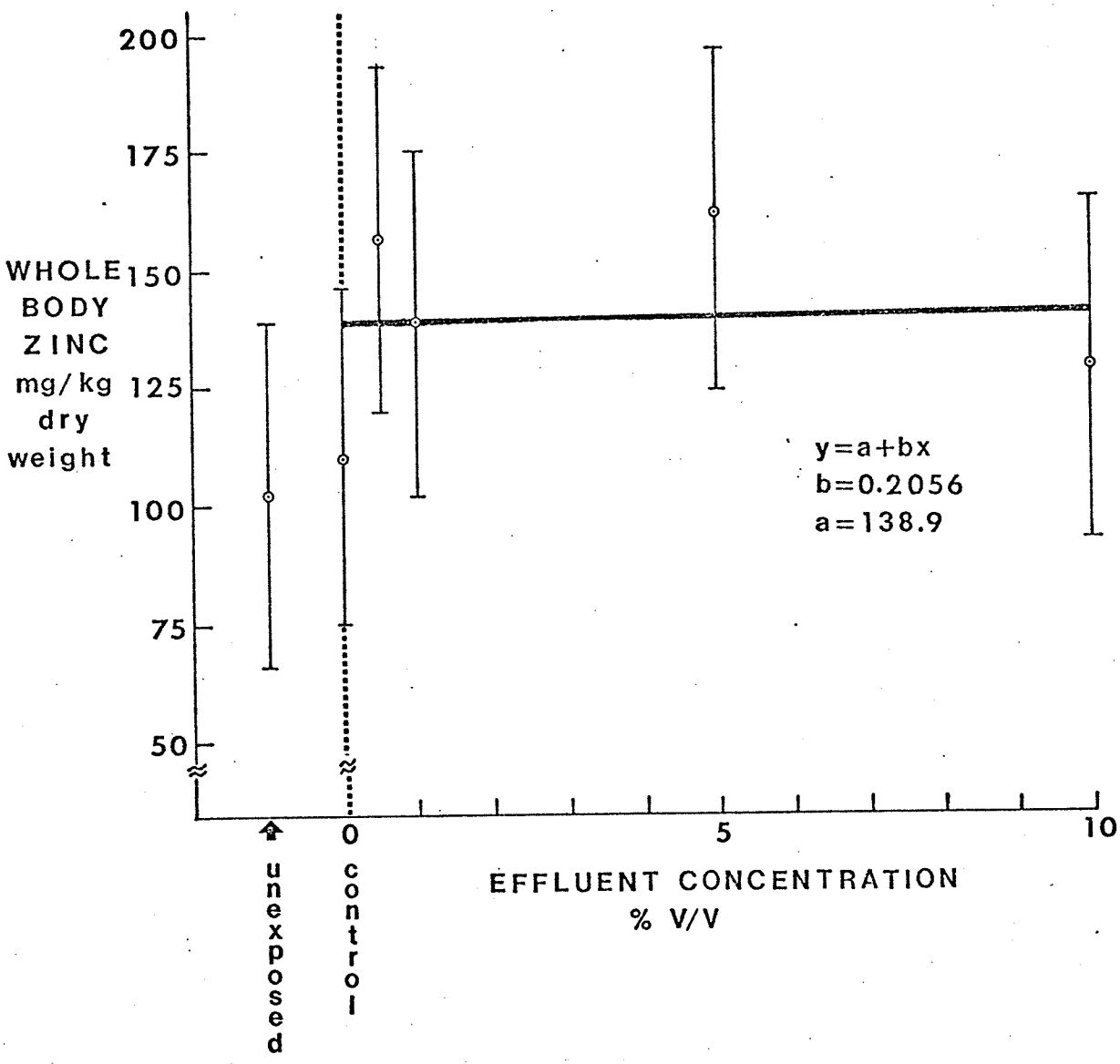


Figure 33. Average whole body total zinc content in shelled, common littleneck clams following 8-week exposure to West Point effluent in Bioassay 15. "Unexposed" data were not used in calculation of the regression line. The slope (b) is not statistically different from zero at the 95% confidence level.

Table 16. Determination of diluter accuracy during a chronic bioassay. Values are in percent WPE (v/v) and are based on 53 daily measurements of diluter flow rates.

Theoretical Concentration of WPE	Mean	95 % Confidence Interval
0.5	.50	0.48 - 0.51
1.0	1.00	.99 - 1.01
2.5	2.40	2.33 - 2.48
5.0	4.87	4.74 - 5.00

Table 17. Polychlorinated biphenyl (PCB) and total chlorinated hydrocarbon levels in English sole after 8-week exposure to dilutions of WPE in chronic Bioassay 12.

Treatment	% WPE	PCB (1260) (µg/kg)	Total Chlorinated Hydrocarbons (µg/kg)
Background	0	82.0	6.24
Control	0	28.8	2.57
WPE	0.5	46.0	3.05
WPE	1.0	20.0	1.60
WPE	5.0	55.0	2.98
WPE	10.0	0.0	0.11
Fish Food (shrimp)	-	5.2	0.50

the fish subjected to the 8-week exposure in the diluter system lost PCB's and chlorinated hydrocarbons. Also of interest is the fact that the PCB in the fish tissue was the 1260 form while none of the 1254 form was found. The primary form in the West Point effluent was 1254 (Ray Dalseg, personal communication). It appeared that the test fish not only lost background levels of PCB and chlorinated hydrocarbons, but did not bioaccumulate these compounds from dilutions of the WPE. It may be possible that the PCB and chlorinated hydrocarbons in the WPE are in a chemical state which is not readily assimilated by the fish. Bioaccumulation of these products might only be possible by assimilation from the natural food chain after these products are converted to biologically active forms by lower trophic levels (possibly bacteria in the sediment). Should this be true, the observed depuration of PCB and chlorinated hydrocarbons in the test fish may be explained due to removal from a contaminated natural food source during the 8-week bioassay. These fish received only shrimp meat which was found to be relatively low in PCB and chlorinated hydrocarbons. Further investigations are required to confirm these results and the tentative conclusions indicated.

5.2.4 Chronic Histopathology

Histological examination of gill tissues from English sole and shiner perch from 8-week exposures to low concentrations of WPE revealed sub-acute pathological changes which ranged from congestion and inflammation to edema, hyperplasia and hypertrophy (Tables 18 and 19). Selected photo micrographs of these pathological conditions are illustrated in Figures 34 through 37. Histologic examination of gill and gut tissues of common littleneck clams exposed to 0.5 to 10% WPE for 8 weeks did not show noticeable pathologic symptoms.

Table 18. Results of histopathologic examination of gill tissue from juvenile English sole exposed to sub-acute concentrations of chlorinated WPE for 8 weeks.

WPE Concentration	Histologic Finding
0% Control	normal.
0.5%	extreme congestion and focal hemorrhaging throughout tissue; goblet cell hypertrophy and some epithelial compression.
1.0%	minimal vascular congestion and focal epithelial hyperplasia.
5.0%	focal inflammation between some gill lamellae.
10.0%	focal proliferation of some basal epithelial cells.

Table 19. Results of histopathologic examination of gill tissue from shiner perch exposed to sub-acute concentrations of chlorinated WPE for 4 weeks and 8 weeks.

WPE Concentration	Histologic Finding
Exposure Time - 4 Weeks	
0% Control	normal.
0.5%	focal edema and separation of epithelium from underlying vascular tissue in mid- and basal sections of gill filaments.
1.0%	same as 0.5%.
2.5%	same as 0.5%. Additionally, intralamellar exudate consisting of large eosinophilic macrophages, red blood cells, fibrin, and desquamated epithelial cells present.
5.0%	same as 0.5%. Additionally, focal proliferation of basal cells and focal intralamellar exudate consisting of red blood cells, desquamated epithelial cells, and macrophages.
Exposure Time - 8 Weeks	
0% Control	normal.
0.5%	focal edema and separation of epithelial cells from underlying vascular tissue in mid- and basal sections of the gill filaments.
1.0%	same as 0.5%.
2.5%	same as 0.5%.
5.0%	same as 0.5%.

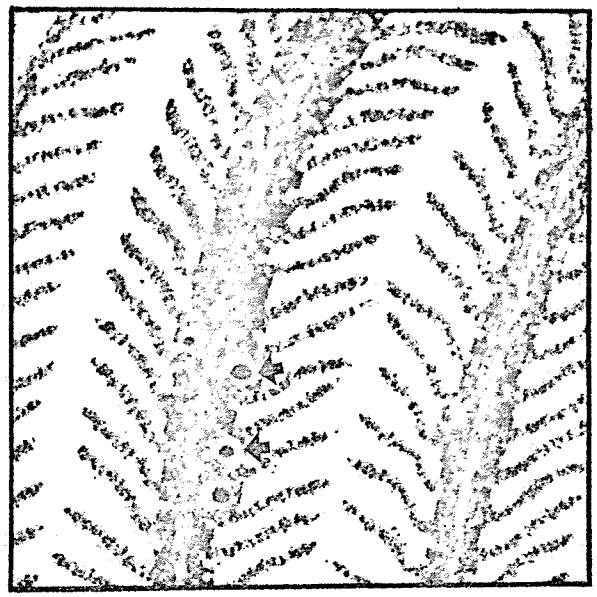


Figure 34. English sole gill tissue after exposure to 1% effluent for 8 weeks in Bioassay 15. Tissue is essentially normal, except for several *Oodinium sp.* embedded at base of filaments (arrows). X250.

Figure 35. Shiner perch gill tissue after exposure to 2.5% effluent for 4 weeks in Bioassay 22. Arrow indicates extralamellar exudate. X250.

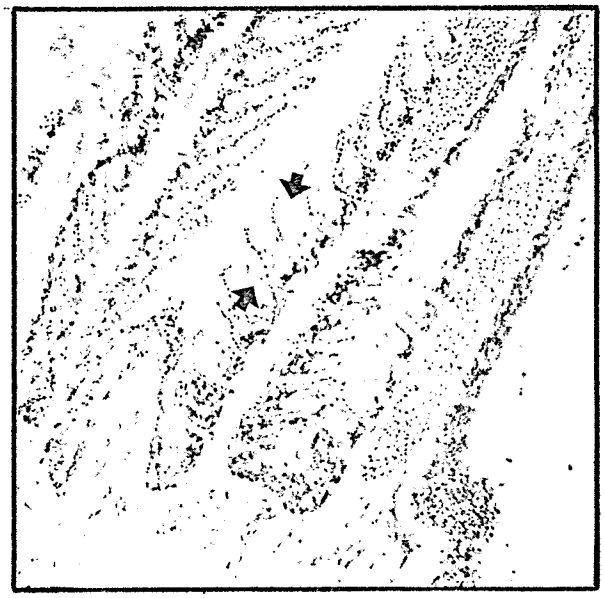


Figure 36. Shiner perch gill tissue after exposure to 1% effluent for 4 weeks in Bioassay 22. Arrows point out edema and separation of epithelium from underlying vascular tissue. X250.

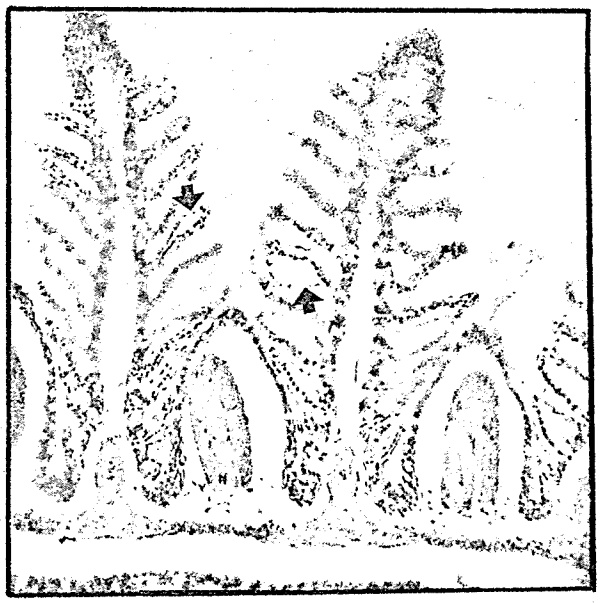


Figure 37. Shiner perch gill tissue after exposure to 1% effluent for 8 weeks in Bioassay 22. Arrows show edema and separation of epithelium from vascular tissue. X250.

6.0 DISCUSSION AND CONCLUSIONS

6.1 Acute Bioassays

6.1.1 Acute Toxicity of West Point Sewage Effluent

Acute toxicity tests of primary chlorinated West Point effluent showed that of five marine species tested, shiner perch and English sole were the most sensitive. Coonstripe shrimp were equally as sensitive, but difficult to obtain easily and inexpensively. Pacific staghorn sculpin and shore crab were relatively insensitive when exposed to West Point effluent during initial testing.

The mean 96-hour LC50 values for shiner perch (7 tests) and English sole (5 tests) in chlorinated WPE were 16.1 and 15.4% v/v WPE, respectively. Based on these average LC50's, a "safety factor" of 0.044 was calculated for the West Point effluent, using the following formula:

$$\text{Safety Factor} = \frac{\text{Concentration of WPE at outfall after initial dilution}}{\text{LC50 concentration of WPE}}$$

with an initial dilution of 140:1 (Bendiner and Ewart, 1976). Generally, acceptable "safety factors" (also called application or dilution factors) for discharge of a toxic effluent range from 0.1 to 0.01. The calculated safety factor falls approximately in the middle of this acceptable range based on average toxicity to shiner perch and juvenile English sole. However, slug discharges of such things as trace metals may drastically alter the toxicity of WPE, thereby decreasing the margin of safety. This will be discussed in a later section.

The toxicity of sewage effluent can be highly variable depending on sewage source, type of treatment, quality of the receiving water, amount

of dilution at the point of discharge, and degree of chlorination for disinfection. Martens and Servizi (1976) report LC50's for fingerling sockeye salmon (*Oncorhynchus nerka*) between 40 and 45%, 25 and 40%, and 17 and 25% v/v for primary effluent from three treatment plants in the Vancouver, British Columbia area. Esvelt, *et al.* (1973) found toxicity to golden shiners (*Notemigonous chrysoleucas*) to average 45% for primary sewage from four treatment plants in the San Francisco area. Gill and Toor (1975) working in India found municipal sewage effluent toxic to *Puntius ticto* at 20.8% in 48-hour bioassays. A 10% dilution of primary sewage from Pacific Grove, California, was a weak inhibitor of sea urchin (*Strongylocentrotus purpuratus*) egg fertilization, while chlorinated sewage was much more detrimental to fertilization success, significantly reducing fertilization at 0.5% chlorinated sewage (Muchmore and Epel, 1973).

Adverse effects of WPE due to reduced salinity and dissolved oxygen in the test tanks were minimal. Shiner perch and English sole suffered no apparent ill effects in 60% freshwater/40% seawater after 96 hours. Increasing dissolved oxygen in test tanks by aeration appeared to increase survival time slightly; however, mortality at 96 hours was essentially the same in aerated and unaerated dilutions of WPE.

Toxicity of WPE was similar at ambient seawater temperature (8.5 C) and at Δt = 5 C(13.5 C). However, toxicity increased at Δt = 10 C (18.5 C). Temperature and solubility of oxygen are inversely related. Temperature is also a critical factor affecting respiration. Respiratory and metabolic rates of aquatic animals increase with temperature, creating a higher oxygen demand in the tissues (Spotte, 1970). Thus, reduced

solubility of dissolved oxygen coupled with a higher test animal oxygen demand at increased temperature probably accounts for a major portion of the increased toxicity of WPE in the Δt = 10 C bioassay. Additionally, it is possible that the constituents of WPE may be more toxic at higher temperatures due to increased physical or chemical reactivity. Stober and Hanson (1974) found that temperature has a considerable influence on the reaction rate of chlorine in seawater while Emerson, *et al.* (1975) have shown that the concentration of un-ionized ammonia (the form toxic to fish) increases with both pH and temperature.

The effect of increased temperature of WPE on organisms in the receiving waters is probably minimal. The maximum ambient seawater temperature recorded between October 1975 and September 1976 was 13.0 C. Based on a WPE dilution at the outfall of 140:1 (Bendiner and Ewart, 1976) the maximum resulting receiving water temperature would be about 13.05 C (also based on the 7 C Δt of the effluent as noted in Section 5.1.3). The LC50 for shiner perch in WPE/seawater heated to 13.5 C was essentially the same as in 8.5 C.

6.1.2 Acute Toxicity of Treated West Point Effluent

6.1.2.1 Chlorine Toxicity. Chlorination of the West Point effluent is controlled by a continuous monitoring amperometric titrator with feedback system for continuous control of upstream chlorine addition to a pre-set total chlorine residual level of 1.0 - 1.5 ppm. The amount of chlorine required to produce a particular residual level varies with the chlorine demand of the wastewater. This demand primarily varies with the concentration of ammonia nitrogen and organic nitrogen in the wastewater. Addition of normal levels of chlorine to wastewater generally produces

monochloramine almost instantaneously by the following reaction (White, 1970):

$$NH_3 + HOCl \rightarrow NH_2Cl + H_2O$$

Subsequent additions of chlorine can produce dichloramine and nitrogen trichloride. Although little data exists, it is known that chlorinated hydrocarbons may also be formed in wastewaters. Some of these compounds are known carcinogens (EPA, 1976).

Ample data exist to show that chlorine is toxic to aquatic life in concentrations at the ppb level. Based on a review of the toxic effects of chlorine by many investigators, Brungs (1973) has recommended a criterion of 0.01 mg/l for warm water fish and 0.002 total residual chlorine for cold water fish in freshwater. Similarly, an EPA task force report (EPA, 1976) has concluded that free residual chlorine in excess of 0.01 mg/l can be hazardous to marine life. Little information is available on the toxicity of combined chlorine in seawater. However, Muchmore and Epel (1973) have determined that chlorinated sewage had a significant detrimental effect on sea urchin fertilization at 0.05 ppm total residual chlorine for a 5-minute exposure time. Buckley and Matsuda (1972) and Buckley (1974) have determined chlorinated WPE 96-hour LC50 values for coho salmon (*Oncorhynchus kisutch*) in static and continuous flow tests to be 0.1 and 0.07 mg/l total residual chlorine, respectively.

Toxicity of residual chlorine to fish appears to be related to chloramine formation at the gills. Katz (1975) found that chlorine can instantaneously combine with ammonia at the gills, which then disrupts the process of ionic and osmotic regulation. Formation of chloramine interferes with the normal ammonia (NH_3)$\leftrightarrow$ammonium ion (NH_4^+) equilibrium

by enhancing the formation of the more toxic NH_3. Additionally, it is postulated that chloramine is more easily diffuseable through the gill membrane than the relatively less toxic ammonium ion (see next section for a discussion of ammonia toxicity). Katz also found that the presence of sodium ions in the ambient water decreased the toxic effect of chlorine. Evidently, chloramine can disrupt the normal active transport of sodium ions across the outer membrane. Larger concentrations of sodium ions in the ambient water (as in seawater) help overcome the sodium efflux to restore the active transport system.

6.1.2.2 Chlorine Concentrations at METRO Outfall. The average total residual chlorine found in WPE between October 1975 and November 1976 was 1.10 ± 0.34 ppm. However, peaks of chlorine to 2 or 3 ppm were not uncommon while peaks in excess of 5 ppm occurred occasionally. Based on a dilution factor of 140:1 at the diffuser, the calculated average chlorine residual at the outfall would be 0.008 ppm with peaks occasionally in excess of 0.04 ppm. These figures might be slightly lower due to the small but variable seawater demand for chlorine. However, until further data are collected on seawater demand for combined chlorine, an estimation of this factor cannot be made.

Based on simple dilution of the chlorine in the West Point effluent, we conclude that chlorine probably is not acutely toxic to marine life in Puget Sound. However, chlorine concentrations may occasionally approach or exceed 0.05 ppm, which is the level reported by Muchmore and Epel (1973) to significantly reduce fertilization success of sea urchin eggs after a 5-minute exposure to chlorinated primary sewage effluent. Additionally, Buckley, *et al.*, (1976) report that 40% mortality occurred in

3.6% chlorinated WPE when total residual chlorine reached a peak value of 0.094 mg/l in a chronic bioassay. Further study of the chronic effects of combined chlorine in seawater is needed to refine the estimation of toxicity of chlorinated WPE to organisms in Puget Sound.

6.1.2.3 Dechlorination. Dechlorination of municipal wastewater is currently being practiced by several sewage treatment plants which are unable to meet chlorine discharge standards due to excess chlorine residuals and/or minimal dilution at the outfall. Dechlorination can be accomplished with activated carbon or by the addition of sodium bisulfite or sulfur dioxide. Sulfur dioxide is the best direct reacting chemical agent available for wide-scale use in dechlorinating wastewater.

The reaction of sulfur dioxide with chlorine has been well documented (White, 1972; Martens and Servizi, 1975; EPA, 1976). Sulfur dioxide combines with water to form a weak sulfurous acid solution which then combines with both free and combined chlorine almost instantaneously. The stoichiometric relationship of sulfur dioxide to chlorine is 0.9 mg/l SO_2 to dechlorinate 1.0 mg/l chlorine. This reaction converts chlorine to the chloride ion by the following reactions (White, 1972):

Free Chlorine (HOCl)

$$SO_2 + H_2O \rightarrow H_2SO_3 + HOCl \rightarrow H_2SO_4 + HCl$$

Chloramine

$$NH_2Cl + H_2SO_3 + H_2O \rightarrow NH_4HSO_4 + HCl$$

Sulfur dioxide also reacts with dissolved oxygen in the following manner (Martens and Servizi, 1975):

$$2SO_2 + 2H_2 + O_2 \rightarrow 2H_2SO_4$$

which may act to deplete dissolved oxygen content and lower pH if excessive amounts of SO_2 are used.

Minimal information exists on the toxicity of sulfur dioxide. McKee and Wolf (1963) report trout mortality at 5 mg/1 SO_2, and Martens and Servizi (1975) found that excessive sulfonation of sewage effluent to a level of 2.20 mg/1 SO_2 had no toxic effect on salmon in 96-hour bioassays. They also reported no noticeable effect on pH or dissolved oxygen caused by dechlorination with sulfur dioxide. However, our chemical sampling data show a slight reduction of pH and dissolved oxygen for the bioassay in which this data was collected (Table 4). The sulfur dioxide level during this test was 2.57 ppm, approximately 2 ppm higher than the residual level of 0.5 ppm recommended by White (1972). It is unlikely that a significant depression of dissolved oxygen or pH will occur at the recommended SO_2 residual level.

Dechlorination of WPE substantially reduced the toxicity of WPE. Shiner perch and English sole were able to survive in approximately twice the concentration of WPE dechlorinated with SO_2, with LC50 values of 32 and 28% v/v, respectively. Thus, based on an average LC50 value of 30%, the "safety factor" for WPE discharged at the outfall would increase from 0.044 to 0.024 if dechlorinated with SO_2. Several investigators have recorded results similar to ours when dechlorinating wastewater. Esvelt, *et al.* (1973) concluded that dechlorination (with sodium bisulfite) consistently removed all chlorine induced toxicity. Furthermore, the toxicities of primary and chemical precipitation effluents were consistently less after than before chlorination-dechlorination. Martens and Servizi (1975) similarly concluded that "chlorine induced toxicity was removed by application of sulfur dioxide. Furthermore, dechlorinated primary

sewage was less toxic than primary chlorinated sewage, suggesting degradation of some toxic constituents by the chlorination-dechlorination process." Tests with unchlorinated primary WPE were not conducted at West Point, thus we do not have a measure of toxicity of primary versus chlorinated-dechlorinated effluent.

One other factor which should be considered with chlorinated wastewater is the formation of toxic chlorinated organics. Jolley (1975) found evidence that approximately 1% of the chlorine applied during treatment of municipal sewage occurs as stable chlorine-containing organic constituents. Gehrs, *et al.* (1974) have shown that concentrations of 5-chlorouracil and 4-chlororesorcinol as low as 0.001 mg/l significantly decreased hatchability of carp (*Cyprinus carpio*) eggs. Our study did not address this problem, thus we have no measure of the contribution of toxicity due to chlorinated organics in the WPE.

6.1.2.4 Ammonia Toxicity. The toxicity of ammonia to aquatic life has been documented by various investigators. Acute toxicity in 96-hour bioassays (for un-ionized ammonia) has ranged from 0.29 mg/l for perch (*Perca*)(Ball, 1967) to 2.8 mg/l for striped bass (*Morone saxatalis*) (Hazel, *et al.*, 1971), while Burrows (1964) found progressive gill hyperplasia in fingerling chinook salmon during a 6-week exposure to 0.006 mg/l.

Ammonia toxicity is primarily dependent on chemical state. Ammonium hydroxide readily dissociates into ammonium and hydroxyl ions as follows (NAS, 1972):

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

This equilibrium is dependent primarily on pH, temperature, and to a small extent, the hardness or salinity of the water (Emerson, *et al.*,1975). Un-ionized ammonia has been shown to be the toxic component of ammonia solutions, as ionized ammonia (NH_4^+) apparently is unable to pass tissue barriers and thus enter an aquatic animal from the external medium (Milne, *et al.*,1958). Concentrations of un-ionized ammonia increase with increasing pH and temperature and decreasing salinity or hardness of the water. Wuhrman (1952) and Downing and Merkens (1955) have demonstrated an increase in NH_3 toxicity with decreasing dissolved oxygen.

The use of ion-exchange resins for ammonia removal was recently evaluated by Williams (no date). He was primarily concerned with extending the efficiency of ammonia removal beyond the capabilities of bacterial nitrification in closed-system fish rearing facilities. He found that the natural zeolite resin, clinoptilolite, was 97 to 99% effective in removing ammonia. Clinoptilolite has also been used by Mercer, *et al.* (1970) for 99%+ removal of ammonia from secondary effluent and by Esvelt, *et al.* (1973) to reduce toxicity of primary effluent.

Removal of ammonia from WPE was responsible for substantially decreasing the toxicity of WPE to shiner perch and English sole (LC50 values in WPE minus ammonia = 26 and 45% v/v, respectively). Based on an average LC50 value of 36% for these two species, the calculated "safety factor" of discharged WPE without ammonia is 0.020, which is a slightly greater margin of safety than dechlorinated effluent at 0.024. Clinoptilolite-treated effluent is also effectively dechlorinated as well as deammoniated. Thus, the reduction of toxicity is due both to chlorine and ammonia removal.

Daily composite samples of the effluent taken by METRO showed an average value of 12.1 mg/l total ammonia (as nitrogen) from September 1975 to October 1976. However, daily grab samples of effluent from the proportional diluter head tank commonly yielded values closer to 20 mg/l ammonia. These figures are not inconsistent since the grab samples were usually collected during mid-day when ammonia levels were elevated due to increased domestic discharges. Based on a total ammonia value of 20 mg/l and a pH of 8.1 and temperature of 11 C (as monitored by Environmental Quality Analysts, Inc., 1974) around the outfall on August 27 and 28, 1974, the calculated un-ionized ammonia concentration (after Emerson, *et al.*, 1975) at the West Point outfall would approximate 0.004 mg/l. This is a reasonable level only if the effluent were free of chlorine, otherwise much of the ammonia would be combined with the chlorine and thus would not be available as NH_3 but as chloramine. No acute mortality would be expected to occur at the outfall; however, a concentration of 0.004 mg/l approaches the level of un-ionized ammonia reported by Burrows (1964) to cause gill hyperplasia in fingerling chinook salmon during a 6-week exposure period.

6.2 Trace Metal Toxicity and Bioaccumulation

6.2.1 Requirements of Trace Metals in Aquatic Organisms

Trace metals are essential to all forms of life. They are vital in the formation of metallo-enzymes, such as cytochrome oxidase, and as metal-ion-activated enzymes (Davies, 1972). Mollusks require copper for the formation of the respiratory protein hemocyanin. Trace metals have a

natural ability to accumulate in organisms due in part to enzymatic requirements. Increasing proportions in organisms have been shown to reflect the relative abundance of trace metals in the environment (Waldichuck, 1974; Eisler, *et al.*, 1972). While required for the normal functioning of an organism, excessive quantities can cause deleterious effects or even death (Bowen, 1966). Numerous studies have shown that macroinvertebrates as well as fish and other aquatic life are capable of concentrating trace metals into their tissues from the seawater environment (Seymour and Lewis, 1964; Eisler, *et al.*, 1972; Sherwood and Wright, 1976; Oshida, 1976; Young and Jan, 1976). The extent to which such concentrations occur is expressed as a concentration factor. This factor, the ratio of the wet weight concentration of a trace element in an organism to that in ambient water, is usually based on radionuclide studies using labeled isotopes of trace elements. Table 20 has been adapted from Waldichuck (1974) and lists the concentration factors of copper and zinc for various types of marine organisms.

6.2.1.1 Chemistry of Trace Metals Related to Bioaccumulation and Toxicity. The chemical state or species of trace metals is an important factor affecting the biological availability for bioaccumulation. According to Chapman (1973), the physical and chemical factors in the aquatic environment such as pH, temperature, dissolved oxygen, turbidity, carbon dioxide, magnesium salts and phosphates, influence the toxicity of trace metals to fish by influencing the rate of accumulation or uptake of the trace metal. This is done by either changing the biological availability of the metal, its chemical state, or by altering the rate

Table 20. Concentration factors of copper and zinc in marine organisms. Adapted from Waldichuck (1974).

Metal	Natural Concentration in Seawater (ppb)	Bioaccumulation Concentration Factor			
		Plankton		Macro-	
		Phyto-	Zoo-	inverts.	Fish
Copper	2	38	437	24,000 - 35,000	50 - 250
Zinc	2	113	1800	172,000 - 290,000	1600 - 2100

and volume of ventilation of the fish. There presently exists a minimal literature describing which chemical state(s) of individual trace metals are toxic and/or biologically available for accumulation in various aquatic organisms at various stages of development. The literature which is available suggests that while soluble forms of trace metals appear to be the primary toxic chemical state, suspended forms also indicate toxic qualities. Pagenkopf (1974) indicates that $Copper^{2+}$ and CuOH are toxic chemical species of this trace metal. His information is based on detailed equilibrium calculations as well as information derived from reports of bioassays from the literature. Suspended zinc (as hydroxide) as well as dissolved zinc have been reported as toxic (Lloyd, 1960; Mount, 1966). Mount has theorized that zinc hydroxide could be converted to dissolved zinc at the surface of the gills where CO_2 excretion lowers the pH. Sprague (1964 a, b), has shown suspended zinc to be non-toxic.

Trace metals exhibit the ability to complex with both natural and artificial agents in the aquatic environment. Schmidt and Wildung (1975) in a review of copper in seawater and marine biota, state that organic ligands strongly influence the chemistry of this metal in seawater. Furthermore, they state that organic complexing agents may lower the effective concentration of Cu^{2+} below a required nutrient level or potentially toxic concentration. Natural complexing agents such as organic ligands (Barber, 1973) and humic substances (Prakash, *et al.*, 1973) have been observed to reduce trace metal toxicity in marine phytoplankton. Pagenkopf, *et al.*, (1974) state that "copper is highly complexed by carbonate and hydroxide ions in natural waters and this complexation determines the concentration of copper species in solution." According to

Schmidt and Wildung (1975) while carbohydrates, peptides, amino acids, lipids and humic substances compose the majority of potential organic complexing agents in seawater, analysis of Cu speciation in model and natural systems indicate that the bulk of added Cu^{2+} is present as amino acid and polypeptide complexes. Furthermore, they state that Cu, as well as other trace metals, has been found to concentrate in natural seawater at the water surface in a thin microlayer containing enriched concentrations of surface-active organic substances such as organic acids and proteinaceous material. Sewage effluent contains many of the previously mentioned complexing agents. Organic ligands and amino acids from sewage plant effluent have been shown to reduce the toxicity of copper by forming copper-organic complexes which do not contribute to lethal toxicity (Bender, *et al.*, 1970; United Kingdom Ministry of Technology, 1969; Lewis, *et al.*, 1972). Artificial complexing agents also exist which render certain trace metals less toxic. Nitrilotriacetic acid (NTA) has been shown to reduce or eliminate zinc toxicity in fish (Sprague, 1968).

6.2.1.2 Mode of Entry of Trace Metals in Some Aquatic Organisms. In studying the effects of trace metals or any toxic substance, it is necessary to be aware of the mode of entry of the toxicant into the organism. Pentreath (1973 a, b) has shown, using ^{65}Zn and other labelled isotopes, that in the accumulation in the mussel, *Mytilus edulis*, and the plaice, *Pleuronectes platessa*, the transfer of metal radionuclides occurs mostly through the food and only to a minor extent from the water. Marine fish consume a considerable amount of water (as compared to freshwater species) for osmoregulation which may act as a source to some extent for

trace metal bioaccumulation. Exposure to the large surface area of gill lamellae in aquatic organisms may allow an exchange (uptake) of free ionic forms of trace metals from the environment to occur. Eisler, *et al.* (1972) has shown increases in tissue content of Cd in the marine mummichog *Fundulus heteroclitus*, over that of controls, after 3 weeks' exposure to 10 ppb Cd^{2+} when both experimental and control groups were fed the same diet. This seems to indicate that uptake took place via the water and not through the food. For this same study, Eisler observed an 82% increase in Cd content in gill tissues of the subadult lobster, *Homarus americanus*, over that of controls. Schmidt and Wildung (1975) state that "the high uptake of copper in the gills of oysters can be related to the binding of the metal by ligands in the mucous sheets." Chapman (1973) mentions that trace metal accumulation differences among fish species may result from different uptake rates through the gills or different rates of trace metal excretion. In general it appears that the mode of entry of trace metals in aquatic organisms is dependent upon the particular metal and organism in consideration.

6.2.2 Trace Metal Toxicity of West Point Effluent

Information on the toxicity of trace metals in sewage is virtually non-existent. Lawrence and McCarty (1965) mention that "heavy metals" have long been known to cause retardation or complete cessation of the anaerobic process of biological sewage treatment. Esvelt, *et al.* (1973) speculated that as much as 33% of the toxicity observed in San Francisco primary sewage was due to heavy metals or reduced substances.

Bioassays of the West Point effluent did not include direct analysis of metal toxicity. Toxicity of the metals in WPE can only be analyzed by relating WPE concentrations and fluctuations of the various metals (as total metal) to toxicity work done by other investigators, and by relating expected concentrations of metals at the outfall to previously determined acceptable levels for discharge. Mortalities in two bioassays were found to correlate with observed peaks of trace metals in the WPE.

6.2.2.1 Cadmium. Cadmium is an extremely dangerous cumulative poison. In animals there is an insidious, progressive, chronic poisoning because there is almost no excretion of the metal (NAS, 1972). Cadmium has been found toxic in 1 to 6 days to rainbow trout (*Salmo gairdneri*) at 10 µg/1 (Ball, 1967), while Biesinger and Christensen (1971) found *Daphnia magna* reproduction was reduced in 3-week exposures to 0.5 µg/1. NAS (1972) criteria recommends that habitats should be safe for crustaceans or eggs and larvae of salmon if levels of cadmium do not exceed 3 µg/1 in hard water at any time or place. The EPA discharge limits for cadmium in West Point effluent were (through October 1976) 10 µg/1 average (monthly average) and 20 µg/1 maximum (for daily 24-hour composite samples).

The average cadmium concentration in WPE from September 1975 to October 1976 was 6 µg/1, which would be diluted at the outfall to approximately .04 µg/1. The average cadmium in WPE is safely below recommended levels and the concentration reported to affect *Daphnia magna*. Daily cadmium levels exceeded the EPA discharge limit once during this time with a value of 24 µg/1, which would be diluted to approximately 0.17 µg/1 at the outfall. This level still does not exceed NAS criteria for

receiving waters of 3 µg/1. Cadmium in WPE is probably causing no acute adverse effect on Puget Sound biota.

6.2.2 Chromium. Toxicity tests with chromium have produced wide-ranging LC50 values of 17 to 118 mg/1 (hexavalent chromium) for 4 species of fish (Pickering and Henderson, 1966) and 50 µg/1 (hexavalent chromium) for *Daphnia* (Biesinger and Christensen, 1971). Hervey (1949) found decreased growth of diatoms at 32 µg/1 chromium. NAS (1972) criteria recommend that "mixed aquatic populations should be protected where the concentration of total chromium in water does not exceed 50 µg/1 at any time or place." EPA standards for West Point effluent dictate 10 µg/1 average and 200 µg/1 maximum chromium and 5 µg/1 average and 50 µg/1 maximum for hexavalent chromium.

West Point effluent contains an average of 50 µg/1 chromium and < 5 µg/1 hexavalent chromium as measured by METRO from September 1975 to October 1976. The projected outfall concentrations after dilution would approximate 0.36 µg/1 chromium and < 0.05 µg/1 hexavalent chromium. These values are below those recommended by NAS for receiving waters. But, the average chromium discharge is 5 times higher than the average allowable limit set by EPA for the West Point effluent. However, the new EPA standards for WPE dictate an average allowable limit for chromium of 70 µg/1, somewhat higher than the average WPE value.

Peak values of chromium exceeded EPA standards once from September 1975 to October 1976 with a value of 220 µg/1. Additionally, fish in Bioassay 21 were adversely affected in 20% WPE (death) and 10% WPE (loss of equilibrium). These adverse effects are correlated with a 2-hour peak in chromium of

320 µg/l and a 1-hour peak in copper of 320 µg/l. It is impossible to say whether or not this chromium-copper slug was responsible for the observed fish reactions or if some other unknown component of the same discharge affected the fish. It is of interest to note that high peaks of metals occurring over a short period of time are effectively smoothed out by reporting concentrations of metals in 24-hour composite samples. A 320-µg/l slug of chromium of 2-hour duration only raised the average chromium value for the 24-hour composite sample from 50 µg/l to 80 µg/l. Based on this relationship of grab sample to composite sample ratio, it is plausible to conclude that a peak of chromium as high as 1760 µg/l (based on a 2-hour slug duration) could have occurred on August 26, 1976, when METRO's 24-hour composite yielded a value of 220 µg/l. This hypothetical slug is 5.5 times higher than the slug which was correlated with the observed mortality/equilibrium loss. However, correlations do not prove relationships. They do suggest, however, that metal toxicity in a complex effluent such as sewage needs further investigation. Synergistic effects of metals with other sewage constituents is an important area for further investigation.

6.2.2.3 Copper. Copper has been shown to be toxic to American lobsters (*Homarus americanus*) at 56 µg/l (McLeese, 1974) and sea urchin (*Hemicentrotus*) embryos at 32 µg/l (Okubo and Okubo, 1962), while Atlantic salmon (*salmo salar*) have demonstrated avoidance to copper concentrations of 4 µg/l in the laboratory (Sprague, 1964). NAS (1972) criteria recommend applying an application factor of 0.1 to each determined LC50 for each receiving water. No absolute criterion is set because the available toxic form of

copper (Cu^{+2}) (Pagenkopf, *et al.*, 1974) can fluctuate greatly in receiving waters depending on pH, hardness, and alkalinity.

Average copper in WPE (September 1975 to October 1976) was 159 µg/l producing an approximate outfall concentration of 1.1 µg/l. EPA standards for WPE dictate that the effluent not exceed a monthly average of 100 µg/l nor a daily maximum of 200 µg/l. Copper concentrations in WPE exceeded this monthly standard all 12 months, while the daily maximum was exceeded approximately 90 times. New EPA standards now call for monthly and daily levels no greater than 250 µg/l and 400 µg/l, respectively. By these new standards, copper only exceeded the monthly limits once and the daily standards once. It does not appear that copper in WPE is a cause for concern. The highest daily value (460 µg/l) was safely below reported toxic levels (after dilution at the outfall) for lobster and sea urchin embryos. However, copper is implicated with mortality and loss of equilibrium in 20 and 10% WPE in conjunction with chromium as explained above. More information is needed on toxicity and synergistic effects of copper in a complex effluent.

6.2.2.4 Mercury. The public concern for metals in the environment was intensified with the occurrence of "Minamata disease" in Japan. Since its first occurrence in 1953, 168 cases resulting in 52 deaths due to mercury poisoning (from eating fish and shellfish) were recorded from the Minamata and Niigata areas of Japan (Takuchi, 1970).

Mercury in the environment exists in both inorganic and organic forms. Organic methylmercury is the most toxic form and undergoes the greatest biological magnification. However, inorganic and organic forms

will be discussed here as total mercury since microbes are capable of synthesizing methylmercury from mercury ions (Jensen and Jernelov, 1969; Wood, *et al.*, 1969), and the chemical form of methylmercury administered to fish makes little difference in its toxic effect (Miettinen, *et al.*, 1970).

NAS (1972) reports that recent experiments at the National Water Quality Laboratory have shown that 0.2 µg/l methlymercury killed fathead minnows (*Pimphales promelas*) in 6 to 8 weeks and 0.1 µg/l decreased both photosynthesis and growth in several species of marine and freshwater phytoplankton (Harriss, *et al.*, 1970). NAS (1972) criteria recommendations for mercury are: "(1) the concentration of total mercury should not exceed a total body burden of 0.5 µg/l wet weight in any aquatic organism; (2) the total mercury concentrations in unfiltered water should not exceed 0.2 µg/l at any time or place; and (3) the average total mercury concentration in unfiltered water does not exceed 0.05 µg/l." The EPA standards for WPE until October 1976 were 5 µg/l monthly average and 10 µg/l daily maximum, which would be approximately 0.04 µg/l and 0.1 µg/l at the outfall after dilution. Concentrations of mercury recorded by METRO in WPE exceeded the monthly standards once and the daily maximum 6 times from September 1975 to October 1976. However, the new EPA standards for WPE (after October 1976) have been significantly lowered to 0.7 µg/l monthly and 3.3 µg/l daily maximums. Based on these figures, the WPE for this same time period would have exceeded the legal maximums 5 times for monthly average and 12 times for the daily maximum levels.

Unusual mortality of juvenile English sole in Bioassay 17 was correlated with a peak of mercury in METRO's daily composite sample of 3.8 µg/l mercury. Fish mortality in the test tanks was swift and almost complete in

the lowest dilutions of 10% WPE. Only 3 fish survived in 10% WPE which had been filtered and treated for removal of ammonia and chlorine with clinoptilolite. Results of this bioassay indicate that the safety factor for WPE discharge had approached or fallen below the generally acceptable 0.1 level. The observed increase in total residual chlorine during the period of rapid fish mortality could have been triggered by the chlorine demand of the mercury. Mercury in a concentrated short-term discharge may combine with chlorine to produce water soluble mercuric chloride ($HgCl_2$). This compound is highly poisonous and is used as an effective germicide in dilute solutions of about 1% (King and Caldwell, 1954). The occurrence of fish mortality after removal of TRC and ammonia suggested this compound may have been the lethal agent which prematurely ended Bioassay 17, since it would have passed through the clinoptilolite resin unaltered.

Coupled with these observations is the fact that recorded daily mercury levels in METRO 24-hour composite samples exceeded 3.8 µg/l 9 times from September 1975 to October 1976 with one sample as much as 10 times higher (37.3 µg/l) than the level which was correlated with the observed fish kill. Based on a hypothetical slug discharge time of 2 hours' duration, the actual peak concentration of mercury in WPE may have been as much as 400 µg/l. This information suggests that mortality of marine organisms may have occurred in the vicinity of the West Point outfall during periods of excessive mercury discharge.

6.2.2.5 Nickel. As a pure metal, nickel is not a pollutant because it is not affected by, or soluble in, water. Many nickel salts, however, are highly soluble in water and are used in metal plating. Nickel salts may be discharged to surface or ground waters (McKee and Wolf, 1963).

Nickel salts are reported toxic as low as 700 µg/1 to *Daphnia magna* (as nickel chloride) in 64 hours (Anderson, 1948) and the lethal concentration limit of nickel nitrate for sticklebacks is 800 µg/1 (Murdock, 1953). NAS (1972) criteria recommend a maximum level of nickel be determined for each receiving water based on an application factor of 0.02 times the LC50 value of the most sensitive species. EPA standards for WPE prior to October 1976 were 50 and 100 µg/1 for monthly average and daily maximum values, respectively. Subsequent to October 1976 the new EPA standards are 60 and 65 µg/1 for monthly average and daily maximum levels, respectively. The yearly average (84 µg/1) and 11 of 12 monthly average values of nickel in WPE exceed both the old and new EPA standards prescribed for WPE (September 1975 to October 1976). Daily maximum values exceeded the pre-October 1976 standard 89 times and the post-October 1976 standard 9 times. However, even the highest recorded daily value of 310 µg/1 should not cause an acute problem at the outfall, especially after a 140-fold dilution, as the acutely toxic levels for *Daphnia* and sticklebacks are 700 to 800 µg/1 nickel.

6.2.2.6 Lead. Lead can occur in natural waters as elemental lead and in wastewaters as various lead salts. Lead toxicity varies greatly with changes in water hardness. Pickering and Henderson (1966) have reported 96-hour LC50's for lead of 4 to 5 µg/1 for soft water and 442 µg/1 for hard water using brook trout (*Salvelinus fontinalis*). However, Biesinger and Christensen (1971) have recorded that reproduction of *Daphnia magna* was affected at 30 µg/1.

NAS (1972) criteria for a safe level of lead in receiving waters is 30 µg/1 maximum at any time or place. EPA standards for WPE have been 100

and 200 µg/l for monthly average and daily maximum, respectively. The post-October 1976 standards are similar at 90 and 250 µg/l. Lead concentrations in WPE never exceeded these standards from September 1975 to October 1976. The yearly average for WPE was 66 µg/l, which would be diluted to approximately 0.47 µg/l at the outfall. This amount was much less than would be expected to cause either acute or chronic effects.

6.2.2.7 Zinc. Naturally occurring zinc and industrially related zinc salts are relatively non-toxic to human beings. However, zinc can be toxic to aquatic life at levels similar to those presented for some other metals. The LC50 for fathead minnow was 0.87 mg/l and 33 mg/l for soft and hard water, respectively (Pickering and Henderson, 1966), while Brungs (1969) found fathead minnow reproduction reduced 83% in a chronic test at 0.18 mg/l zinc.

NAS (1972) criteria for total zinc is 0.005 times the LC50 value for the most sensitive species in each receiving water. EPA standards for WPE have been (pre-October 1976) 300 and 500 µg/l for monthly and daily samples, respectively. The post-October 1976 standards are 550 and 780 µg/l, respectively. Zinc concentrations in WPE exceeded the old monthly standard 5 times from September 1975 to October 1976, but did not exceed the new standard once. Likewise, daily samples exceeded the old standard 12 times and the new standard 5 times with a maximum daily value of 1,140 µg/l. However, even this high peak for zinc was diluted at the outfall to approximately 8.14 µg/l, which is about 20 times less than the level reported to affect fathead minnow reproduction. Thus, zinc concentrations normally found in WPE probably do not have an adverse effect on Puget Sound biota.

6.2.3 Bioaccumulation Studies Related to Sewage Outfalls

Recently, two comprehensive studies concerning the environmental assessment of the effects of municipal wastewater outfalls have been conducted near two major cities of the West Coast. The effects of trace metal emissions from these outfalls on the marine environment have been included as a portion of both reports. The Southern California Coastal Water Research Project (SCCWRP, 1976) studied the coastal area surrounding several major outfalls near Los Angeles, California. The other study is part of the METRO Interim Studies by Olsen (1976). Following is a summary of the trace metal bioaccumulation studies contained within these reports.

Young and Jan (1976) found that purplehinged rock scallops (*Hinites multirugosus*) living inshore from one of the major sewage diffusers accumulated concentrations of all (7) trace metals measured in excess of those for controls in at least one of the tissues measured. The tissues measured were digestive gland, gonad, and adductor muscle. While most increases were minor, chromium levels in all three tissues of specimens collected near the outfall averaged 7 times greater than those of controls (significant at the 0.05% level). Cadmium levels in gonads and digestive gland in outfall specimens were lower than controls. This depression, the report states, may be due to increased concentrations of DDT residues in the area.

Sherwood and Wright (1976) discussed the uptake and effects of chromium in the speckled sanddab (*Citharichthys stigmaeus*). The conclusions based on their findings are that (1) dissolved hexavalent chromium at concentrations as low as 16 ppb was biologically available for accumulation in this organism while the trivalent hydroxide precipitate was not; (2) accumulation was proportional to the exposure concentration; (3) the levels of dissolved hexavalent chromium that

affect feeding behavior, limit growth, disrupt tissue structure, or cause mortality, were substantially higher than those likely to be encountered in the ocean.

Oshida (1976) conducted a study involving the effects of Cr^{6+} and Cr^{3+} on polychaete reproduction. The results have shown no negative effects on reproduction at 10 ppb Cr^{6+} or at greater concentrations of Cr^{3+}. While no effects on reproduction were reported, tissue accumulation was measured and determined to be proportional to the chromium concentrations in the toxicant solutions. Each successive generation of polychaetes contained a higher tissue concentration of chromium than the previous generation. A study conducted by McDermott, *et al.* (1976) for SCCWRP involved the determination of metal contamination in Dover sole (*Microstomus pacificus*) living in proximity to a major submarine sewage effluent diffuser in Southern California. The conclusions of this study are that outfall-resident Dover sole showed no overall pattern of tissue uptake for silver, cadmium, chromium, copper, nickel, lead and zinc, when compared with control specimens. Statistically significant increases in liver and gonad chromium levels and flesh silver levels were reported. Statistically significant depressions were reported for liver cadmium and gonad silver concentrations.

In the METRO study, Olsen (1976) reported that plankton were found to contain significantly higher concentrations of Cu and Zn in the vicinity of METRO sewage outfalls as compared to those collected from control areas. Organs of English sole from METRO outfall areas contained possible higher concentrations of several of the trace metals measured than did those from control locations. Dover sole contained 6.5 and 3 times the concentration of Pb in liver and muscle, respectively, in outfall area specimens as compared to controls. Several of the mollusc species analyzed also appeared to contain elevated levels of a number of trace metals when comparing METRO outfall areas to control areas.

6.2.4 Trace Metal Tissue Concentrations from Chronic Bioassays.

The dry ash analysis of organisms for Bioassay 12 was conducted by a support team on a one-time basis. Because of the small numbers representing each data point, it is difficult to make statistically based conclusions for this bioassay. It is not certain at this time how the two different ashing techniques yielded such obviously different results, as no quality control information is available for the dry ashing analysis.

In Bioassay 15, the lack of change in whole body content of copper or zinc in the common littleneck clams and juvenile English sole indicate that these metals were not biologically available directly from the sewage effluent during the time period that the experiment was conducted. The most reasonable explanation for this is that the metals contained within the effluent had been transformed into a complexed state. While this may seem to be obvious, the complicated dynamic nature of sewage effluent makes such a prediction speculative.

Detailed chemical analyses of the trace metal chemical states of the effluent are required to confidently understand the complexities of the problem. Such analyses would be required for the entire duration of the experiment and may only apply to the time period from which they were made. While results of Bioassay 15 indicate that no trace metal bioaccumulation directly from WPE occurred, it should be recalled that there are two major mechanisms responsible for trace metal uptake: (1) directly from the water, and (2) from the food chain, Trace metals accumulate at different rates in different organisms. The plankton which the clams filter from the seawater may not have contained sufficiently large enough concentrations of trace metals to induce elevated levels in the clams.

Sediment-feeding organisms such as polychaetes may increase their trace metal body burden in a bioassay such as was conducted in this study. Predation by successive trophic levels can allow trace metals which are biologically unavailable directly from the water to become available. This may explain why the organisms analyzed in the vicinity of sewage outfalls in the SCCWRP study and by Olsen (1976) showed increases (some statistically significant) over the levels for the same species from uncontaminated control locations. It is not certain at this time what the ecological significance is of such low levels of bioaccumulation. Further studies in controlled laboratory situations as well as in the field with more marine species and different trace metals may allow for a better understanding of the toxicological significance of trace metal bioaccumulation.

6.3 Histopathology

6.3.1 Acute Histopathology

Chlorine has been shown to be primarily responsible for acute mortality of fishes in chlorinated WPE. The physiological mode of toxicity of chlorine is not fully understood. However, several authors report that chlorine is associated with gill tissue hyperplasia and cell necrosis (Servizi and Martens, 1974), and that 0.1 ppm causes sloughing off of the gill epithelium (Penzes, 1971). Hughes and Morgan (1973) suggest that damages to the gill epithelium by toxicants may affect gas exchanges, extra-renal excretion, or ion-exchange functions. Forbes (1971) and Katz and Cohen (1975) have shown that oxygen uptake by fish gills is not impaired by lethal

concentrations of chlorine. Katz (1975) subsequently demonstrated that chlorine exerts a marked effect on fish gills by disrupting the process of ionic and osmotic regulation.

Histological examination of gill tissues from moribund English sole and shiner perch from acutely toxic concentrations of chlorinated WPE showed extensive damage to the integrity of the gill tissue. Edema, hemorrhaging and epithelial hypertrophy suggest osmotic distress. Goblet cell hypertrophy, inflammation and the presence of abnormal extralamellar exudates further suggest that chlorine (or combined chlorine) acts as a cellular irritant. Ionic transport and respiration certainly must be compromised in the presence of such extensive gill damage.

6.3.2 Chronic Histopathology

"Safety" or "application" factors for dilutions of a toxicant are generally estimated by multiplying an LC50 value by a more-or-less arbitrary value, usually in the range of 0.1 to 0.01. This is done to provide a margin of "safety" when sub-lethal effects are unknown. However, when sub-lethal effects have been quantified the "safety factor" is often replaced by a "maximum acceptable concentration" (MAC) which approximates the highest concentration of a toxicant which does not produce any long-term sub-lethal effects.

An analysis of gill tissue histology of English sole and shiner perch exposed for 8 weeks to chlorinated WPE suggests that the MAC is less than 0.5% WPE. Congestion, hemorrhaging, edema, and separation of epithelium from underlying vascular tissue were found in gill tissues of fish exposed to concentrations of WPE as low as 0.5%. These observations are in close

agreement with Buckley, *et al.* (1976) who found that the blood chemistry and blood cell morphology of yearling coho salmon were adversely affected in concentrations of chlorinated WPE of 1.1 and 3.6%. The salmon were not affected in 0.3% WPE. Buckley, *et al.* thus concluded that the MAC for chlorinated WPE was between 0.3 and 1.1%. Based on gill pathology, we conclude that 0.3% chlorinated WPE approximates an upper limit for a maximum acceptable concentration for discharge to Puget Sound receiving waters, if concentrated short-term discharges of certain trace metals (*i.e.*, mercury, chromium, copper) can be controlled.

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8.0 APPENDIX

Summary of Units for Constituent Values Reported in Appendix Tables 1-22.

Constituent	Unit
Rain	Inches
Average Flow	MGD
Temperature	C^o
Dissolved Oxygen (DO)	ppm
pH	pH units
Biological Oxygen Demand (BOD)	mg/l
Chemical Oxygen Demand (COD)	mg/l
Chlorine	ppm
Ammonia	ppm
Total Nitrogen	ppm
Phosphate	ppm
Grease	ppm
Suspended Solids	mg/l
Settleable Solids	mg/l
Volatile Solids	mg/l
Cadmium (Cd)	mg/l
Chromium (Cr)	mg/l
Copper (Cu)	mg/l
Mercury (Hg)	mg/l
Nickel (Ni)	mg/l
Lead (Pb)	mg/l
Zinc (Zn)	mg/l
Hexavalent Chromium	mg/l
Turbidity	JTU
Sulfur Dioxide (SO_2)	ppm

For further explanation of these units, see Text Tables 1 and 2.

The number of test animals per tank for all acute bioassays was 10. The number of test animals per tank for chronic bioassays (12, 15 and 22) was variable.

Appendix

Table 1. Average daily values for West Point Sewage Effluent constituents compiled by month and year for METRO's daily composite samples from 1 October 1975 to 30 September 1976.*

Month	Rain	Avg. Flow	Temp	DO	pH	BOD	COD	Total Residual Chlorine	Ammonia (NH_3-N)	Total Nitrogen	Phosphate (PO_4-P)	Grease
1975												
October	0.19	148.5	16.9	2.2	7.2	74.6	189.5	0.98	9.0	19.0	3.2	19.8
November	0.14	157.5	14.0	2.4	7.1	53.6	167.3	1.20	8.2	17.2	4.4	24.0
December	0.18	163.5	12.6	2.7	7.0	59.4	153.5	1.17	10.1	20.4	4.0	16.2
1976												
January	0.11	150.2	12.0	2.4	7.1	62.6	164.6	1.03	8.3	17.3	3.8	17.4
February	0.15	142.7	11.8	2.7	7.2	71.2	172.8	0.85	14.1	21.0	4.1	16.8
March	0.10	123.3	11.9	3.6	7.2	71.9	182.2	1.07	11.1	19.3	3.8	19.2
April	0.05	98.7	13.5	2.4	7.2	81.2	183.5	1.17	12.8	22.2	5.0	20.2
May	0.05	98.1	16.0	1.7	7.4	104.0	212.0	1.27	14.8	23.6	6.0	20.2
June	0.03	99.6	17.3	1.8	7.3	113.8	244.6	1.14	14.4	22.4	6.8	25.4
July	0.02	98.9	19.1	2.3	6.7	109.8	221.6	1.19	13.2	21.7	6.2	25.4
August	0.05	113.5	19.2	2.6	7.1	101.0	232.8	1.11	14.8	24.4	6.3	23.8
September	0.02	87.3	18.9	1.9	7.1	106.6	255.3	0.97	15.4	24.4	6.8	38.0
Yearly Average	0.09	123.6	15.3	2.4	7.1	83.8	197.8	1.10	12.1	21.0	4.9	21.6

*Units are as recorded in Table 2.

Appendix
Table 1, (Continued.)

Month	Susp. Solids	Set. Solids	Vol. Solids	Chromium	Hexavalent Chromium	Cadmium	Copper	Mercury	Nickle	Lead	Zinc
1975											
October	70.8	0.24	50	0.047	0.006	0.006	0.141	0.0005	0.068	0.083	0.332
November	56.1	0.20	35	0.044	0.008	0.006	0.142	0.0011	0.063	0.070	0.248
December	58.6	0.20	37	0.061	<0.005	0.005	0.137	0.0006	0.047	0.073	0.250
1976											
January	53.8	0.15	35	0.055	<0.005	0.005	0.128	0.0005	0.072	0.046	0.254
February	64.3	0.24	38	0.039	<0.005	0.007	0.134	0.0011	0.084	0.080	0.294
March	54.3	0.12	35	0.042	<0.005	0.004	0.141	0.0009	0.081	0.055	0.444
April	51.5	0.10	33	0.040	<0.005	0.005	0.145	0.0010	0.099	0.063	0.265
May	65.3	0.10	50	0.055	<0.005	0.005	0.143	0.0012	0.125	0.050	0.304
June	90.6	0.33	64	0.049	<0.005	0.006	0.170	0.0054	0.119	0.076	0.354
July	92.0	0.30	65	0.050	<0.005	0.006	0.189	0.0007	0.124	0.084	0.311
August	82.6	0.27	60	0.086	<0.005	0.007	0.209	0.004	0.066	0.069	0.287
September	97.1	0.54	75	0.043	<0.005	0.007	0.231	0.0005	0.062	0.049	0.291
Yearly Average	69.7	0.23	44	0.050	<0.005	0.006	0.159	0.0012	0.084	0.066	0.303

Appendix

Table 2. Biological, physical, and chemical data for Bioassay #2.

Determination of English sole LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	.5	0		
WPE	.5	0		
WPE	1.0	0		
WPE	1.0	0		
WPE	10.0	0		
WPE	10.0	0		
WPE	25.0	90	48.0	48
WPE	25.0	100	50.2	48 - 72
WPE	50.0	100	38.1	24 - 48
WPE	50.0	100	24.0	24

Estimated LC50 = 15 - 20% WPE

Average Fish Length = 91.9 $\pm$ 17.4 mm

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	17.4	11.9	Turb	89.0	11.0
Sal	0.4	30.4	Chlorine		
DO	1.0	8.9	Ammonia		
pH			SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.17	Total N		Cd	0.005
Avg. Flow	127.2	Phosphate		Cr	0.036
Temp	17.6	Grease		Cu	0.132
DO	1.5	Sus. Solids	68.8	Hg	0.0004
pH	7.3	% Reduction	66.0	Ni	0.058
BOD	85.6	Set. Solids	0.14	Pb	0.054
COD	205.8	% Reduction	98.0	Zn	0.350
Chlorine	0.84	Vol. Solids	41.6	Hex Cr	<0.005
Ammonia		% Reduction	66.4		

Appendix
Table 3. Biological, physical, and chemical data for Bioassay # 3

Determination of staghorn sculpin LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	10		96
Control	0	0		
WPE	1.0	0		
WPE	1.0	0		
WPE	5.0	0		
WPE	5.0	0		
WPE	10.0	0		
WPE	10.0	0		
WPE	25.0	20		48
WPE	25.0	30		48 - 96
WPE	50.0	100	30.24	24 - 48
WPE	50.0	100	38.10	24 - 48

Estimated LC50 = 30% WPE

Average Fish Length = 128.0 $\pm$ 15.9 mm; Average Fish Weight = 23.1 $\pm$ 9.3 g

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	15.8	11.6	Turb	84.8	3.8
Sal	0.4	30.6	Chlorine		
DO	0.7	7.6	Ammonia		
pH			SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.21	Total N	14.2	Cd	0.005
Avg. Flow	146.9	Phosphate	3.1	Cr	0.052
Temp	16.4	Grease	15.8	Cu	0.152
DO	2.6	Sus. Solids	58.0	Hg	0.0008
pH	7.3	% Reduction	65.6	Ni	0.092
BOD	61.4	Set. Solids	0.14	Pb	0.066
COD	181.6	% Reduction	95.6	Zn	0.318
Chlorine	0.87	Vol. Solids	42.4	Hex Cr	0.011
Ammonia	5.6	% Reduction	65.0		

Appendix
Table 4. Biological, physical, and chemical data for Bioassay #4.

Determination of shiner perch LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	1.0	0		
WPE	1.0	0		
WPE	5.0	0		
WPE	5.0	0		
WPE	10.0	0		
WPE	10.0	0		
WPE	25.0	100	48.0	48
WPE	25.0	100	48.0	48 - 72
WPE	50.0	100	24.0	24
WPE	50.0	100	24.0	24

Estimated LC50 = 15 - 20% WPE

Average Fish Length = 82.4 $\pm$ 4.0 mm; Average Fish Weight = 6.8 $\pm$ 1.0 g

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	14.4	11.1	Turb	95.2	5.5
Sal	0.4	30.2	Chlorine		
DO	1.1	6.4	Ammonia		
pH			SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.32	Total N	16.2	Cd	0.008
Avg. Flow	177.0	Phosphate	4.6	Cr	0.044
Temp	15.0	Grease	23.8	Cu	0.166
DO	2.5	Sus. Solids	64.4	Hg	0.0009
pH	7.0	% Reduction	64.8	Ni	0.100
BOD	53.6	Set. Solids	0.34	Pb	0.104
COD	196.4	% Reduction	92.2	Zn	0.294
Chlorine	1.26	Vol. Solids	40.4	Hex Cr	<0.005
Ammonia	7.1	% Reduction	66.0		

Appendix

Table 5. Biological, physical, and chemical data for Bioassay # 5.

Determination of coonstripe shrimp LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	1.0	10		96
WPE	1.0	0		
WPE	5.0	0		
WPE	5.0	0		
WPE	10.0	10		96
WPE	10.0	10		96
WPE	25.0	100	24.0	24
WPE	25.0	100	24.0	24 - 72
WPE	50.0	100	24.0	24
WPE	50.0	100	24.0	24

Estimated LC50 = 15-20% WPE

Average Fish Length = (none)

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	15.5	10.8	Turb	93.6	6.8
Sal	0.3	29.9	Chlorine		
DO	1.1	7.0	Ammonia		
pH			SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.02	Total N	19.1	Cd	0.009
Avg. Flow	132.0	Phosphate	5.1	Cr	0.032
Temp	15.3	Grease	25.4	Cu	0.142
DO	2.1	Sus. Solids	60.0	Hg	0.0003
pH	6.9	% Reduction	68.8	Ni	0.010
BOD	57.8	Set. Solids	0.34	Pb	0.080
COD	198.6	% Reduction	94.4	Zn	0.254
Chlorine	1.24	Vol. Solids	42.4	Hex Cr	0.010
Ammonia	9.9	% Reduction	69.0		

Appendix

Table 6. Biological, physical, and chemical data for Bioassay # 6.

Determination of shore crab LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality * (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	1.0	0		
WPE	1.0	0		
WPE	5.0	0		
WPE	5.0	0		
WPE	10.0	0		
WPE	10.0	0		
WPE	25.0	0		
WPE	25.0	0		
WPE	50.0	50		96 - 120
WPE	50.0	60		96 - 120

Estimated LC50 = 50% WPE * * = 120 hours

Average Carapace Width = 19.6 $\pm$ 4.1 mm; Average Crab Weight = 5.0 $\pm$ 3.0 g

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	14.0	10.2	Turb	94.3	3.7
Sal	0.4	29.0	Chlorine		
DO	0.9	7.2	Ammonia		
pH	7.1	7.6	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.12	Total N	19.0	Cd	0.004
Avg. Flow	146.5	Phosphate	4.6	Cr	0.065
Temp	14.2	Grease	26.4	Cu	0.133
DO	1.8	Sus. Solids	56.0	Hg	0.0002
pH	7.2	% Reduction	69.5	Ni	0.063
BOD	62.5	Set. Solids	0.12	Pb	0.057
COD	154.5	% Reduction	98.0	Zn	0.237
Chlorine	1.10	Vol. Solids	35.7	Hex Cr	0.010
Ammonia	9.8	% Reduction	72.8		

Appendix

Table 7. Biological, physical, and chemical data for Bioassay #7.

Determination of English sole LC50 in West Point Effluent (WPE) with adjusted dissolved oxygen and salinity, and freshwater.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
FW	60	0		
FW	40	0		
FW	20	0		
WPE	60	100	12.0	12
WPE	40	100	16.8	16 - 20
WPE	20	100	51.5	36 - 72
Aerated WPE	60	100	13.2	12 - 16
Aerated WPE	40	100	21.2	20 - 24
Aerated WPE	20	100	62.1	36 - 96

Estimated LC50 = > 60% FW; 15% WPE; 16% aerated WPE.

Average Fish Length = 125.1 ± 21.9 mm; Average Fish Weight = 17.9 ± 10.0 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Freshwater	Seawater		WPE	Freshwater	Seawater
Temp	12.9	17.3	9.4	Turb	64.7	0.7	4.3
Sal	0.4	0.0	27.7	Chlorine			
DO	3.2	8.0	8.6	Ammonia			
pH	7.1	6.3	7.7	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.09	Total N	18.6	Cd	0.005
Avg. Flow	151.0	Phosphate	3.7	Cr	0.080
Temp	12.9	Grease	17.6	Cu	0.140
DO	2.5	Sus. Solids	49.6	Hg	0.0010
pH	7.2	% Reduction	64.6	Ni	0.028
BOD	52.2	Set. Solids	0.10	Pb	0.064
COD	162.8	% Reduction	97.8	Zn	0.226
Chlorine	1.28	Vol. Solids	37.6	Hex Cr	<0.005
Ammonia	7.8	% Reduction	63.6		

Appendix

Table 8. Biological, physical, and chemical data for Bioassay # 8.

Determination of shiner perch LC50 in West Point Effluent (WPE) with adjusted dissolved oxygen and salinity, and freshwater.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
FW	60	0		
FW	40	0		
FW	20	0		
WPE	60	100	4.0	4
WPE	40	100	10.5	8 - 12
WPE	20	100	67.8	20 - 96
Aerated WPE	60	100	4.7	4 - 8
Aerated WPE	40	100	13.7	8 - 16
Aerated WPE	20	100	75.8	48 - 96

Estimated LC50 = > 60% FW; 18% WPE; 19% aerated WPE.

Average Fish Length = 84.7 ± 6.7 mm; Average Fish Weight = 7.7 ± 2.1 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Freshwater	Seawater		WPE	Freshwater	Seawater
Temp	12.9	15.9	8.7	Turb	101.7	2.0	1.0
Sal	0.4	0.0	26.9	Chlorine			
DO	0.6	9.0	8.0	Ammonia	14.4	<0.05	<0.05
pH	7.1	6.5	7.8	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.01	Total N	18.2	Cd	0.006
Avg. Flow	125.8	Phosphate	4.3	Cr	0.054
Temp	12.5	Grease	10.5	Cu	0.150
DO	1.3	Sus. Solids	50.8	Hg	0.0004
pH	7.0	% Reduction	71.6	Ni	0.032
BOD	70.6	Set. Solids	0.10	Pb	0.078
COD	162.4	% Reduction	96.8	Zn	0.232
Chlorine	1.08	Vol. Solids	37.2	Hex Cr	< 0.005
Ammonia	9.0	% Reduction	73.0		

Appendix
Table 9. Biological, physical, and chemical data for Bioassay # 9.

Determination of shiner perch LC50 in West Point Effluent (WPE) at ambient temperature (8.5 C).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE - 8.5 C	60	100	2.7	2 - 4
WPE - 8.5 C	60	100	2.3	2 - 4
WPE - 8.5 C	40	100	11.1	8 - 16
WPE - 8.5 C	40	100	8.2	4 - 16
WPE - 8.5 C	20	100	65.9	40 - 96
WPE - 8.5 C	20	100	70.1	44 - 96

Estimated LC50 = 18% WPE - 8.5 C

Average Fish Length = 86.9 $\pm$ 7.5 mm; Average Fish Weight = 7.6 $\pm$ 2.2 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	11.9	8.8	Turb	75.8	6.0
Sal	0.4	27.9	Chlorine	0.79	< 0.05
DO	1.9	8.0	Ammonia	17.4	< 0.05
pH	7.0	7.8	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.07	Total N	16.0	Cd	0.004
Avg. Flow	136.0	Phosphate	3.6	Cr	0.062
Temp	11.8	Grease	18.0	Cu	0.122
DO	2.4	Sus. Solids	57.6	Hg	0.0007
pH	6.8	% Reduction	65.2	Ni	0.076
BOD	75.6	Set. Solids	0.20	Pb	0.044
COD	165.2	% Reduction	96.6	Zn	0.264
Chlorine	0.61	Vol. Solids	39.2	Hex Cr	< 0.005
Ammonia	7.4	% Reduction	62.0		

Appendix

Table 10. Biological, physical, and chemical data for Bioassay # 10.

Determination of shiner perch LC50 in West Point Effluent (WPE) at Δt = 5 C (13.5 C).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE - 13.5 C	60	100	2.0	2
WPE - 13.5 C	60	100	2.0	2
WPE - 13.5 C	40	100	7.4	4 - 8
WPE - 13.5 C	40	100	4.6	2 - 8
WPE - 13.5 C	20	100	84.0	84 - 96
WPE - 13.5 C	20	100	70.8	44 - 96

Estimated LC50 = 18% WPE - 13.5 C

Average Fish Length = 85.1 $\pm$ 5.3 mm; Average Fish Weight = 7.1 $\pm$ 1.2 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	13.2	13.8	Turb	85.5	4.2
Sal	0.6	28.3	Chlorine	0.95	< 0.05
DO	1.1	7.5	Ammonia	14.5	< 0.05
pH	7.3	7.5	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.00	Total N		Cd	0.005
Avg. Flow	122.0	Phosphate		Cr	0.042
Temp	12.6	Grease		Cu	0.127
DO	1.6	Sus. Solids	55.0	Hg	0.0004
pH	7.0	% Reduction	71.0	Ni	0.080
BOD	73.2	Set. Solids	0.10	Pb	0.038
COD	183.5	% Reduction	98.8	Zn	0.242
Chlorine	0.88	Vol. Solids	37.2	Hex Cr	< 0.005
Ammonia		% Reduction	74.0		

Appendix

Table 11. Biological, physical, and chemical data for Bioassay # 11.

Determination of shiner perch LC50 in West Point Effluent (WPE) at Δt = 10 C (18.5 C).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE - 18.5 C	60	100	2.0	2
WPE - 18.5 C	60	100	2.0	2 - 4
WPE - 18.5 C	40	100	7.2	4 - 8
WPE - 18.5 C	40	100	6.4	4 - 8
WPE - 18.5 C	20	100	22.5	16 - 34
WPE - 18.5 C	20	100	21.6	16 - 24

Estimated LC50 = 11% WPE - 18.5 C.

Average Fish Length = 85.6 $\pm$ 5.7 mm; Average Fish Weight = 6.9 $\pm$ 1.4 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	13.2	18.1	Turb	105.0	6.5
Sal	0.4	28.0	Chlorine	1.35	< 0.05
DO	0.6	7.6	Ammonia	13.3	< 0.05
pH	7.0	7.6	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.19	Total N	28.0	Cd	0.010
Avg. Flow	139.5	Phosphate	5.8	Cr	0.057
Temp	12.8	Grease		Cu	0.133
DO	1.9	Sus. Solids	68.7	Hg	0.0037
pH	7.3	% Reduction	63.7	Ni	0.083
BOD	31.7	Set. Solids	0.20	Pb	0.123
COD	204.0	% Reduction	97.0	Zn	0.337
Chlorine	1.11	Vol. Solids	45.3	Hex Cr	< 0.005
Ammonia	17.6	% Reduction	66.3		

Appendix

Table 12. Biological, physical, and chemical data for Bioassay #12.

Sixty-day subacute bioassay for trace metal and PCB bioaccumulation analysis, using English sole and shiner perch.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (days)
Control - Perch	0	20		11 - 19
Control - Sole	0	0		
WPE - Perch	.5	0		
WPE - Sole	.5	0		
WPE - Perch	1.0	40		32 - 46
WPE - Sole	1.0	0		
WPE - Perch	5.0	80		29 - 43
WPE - Sole	5.0	0		
WPE - Perch	10.0	90		10 - 19
WPE - Sole	10.0	0		

Estimated LC50 = None

Average Fish Length = Shiner Perch: 80.6 $\pm$ 7.9 mm; Avg. Weight: (Shiner Perch) 7.4 $\pm$ 2.3 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	12.2	8.0	Turb	87.7	3.0
Sal	0.5	29.6	Chlorine	1.19	< 0.05
DO	1.6	8.5	Ammonia	13.1	< 0.05
pH	7.1	7.8	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.13	Total N	19.8	Cd	0.006
Avg. Flow	132.8	Phosphate	3.8	Cr	0.039
Temp	11.8	Grease	18.4	Cu	0.138
DO	3.2	Sus. Solids	60.6	Hg	0.0012
pH	7.2	% Reduction	65.3	Ni	0.083
BOD	70.8	Set. Solids	0.19	Pb	0.069
COD	173.5	% Reduction	97.1	Zn	0.388
Chlorine	0.94	Vol. Solids	36.4	Hex Cr	< 0.005
Ammonia	12.2	% Reduction	67.6		

Appendix

Table 13. Biological, physical, and chemical data for Bioassay #13.

Determination of shiner perch LC50 in filtered and unfiltered West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
WPE	10	0		
WPE	20	100	62.2	44 - 96
WPE	40	100	23.1	16 - 28
WPE	60	100	2.8	2 - 4
Filtered WPE	10	10		96
Filtered WPE	20	90	81.8	72 - 96
Filtered WPE	40	100	30.0	24 - 36
Filtered WPE	60	100	7.8	5 - 16

Estimated LC50 = 19% WPE; 21% Filtered WPE

Average Fish Length = 83.9 $\pm$ 6.3 mm; Average Fish Weight = 7.0 $\pm$ 1.9 g.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Filtered WPE	Seawater		WPE	Filtered WPE	Seawater
Temp	12.4	12.4	7.8	Turb	82.8	55.2	3.4
Sal	0.8	0.8	30.0	Chlorine	0.98	0.93	< 0.05
DO	1.3	1.6	8.3	Ammonia	14.4	15.6	< 0.05
pH	7.3	7.2	7.8	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.02	Total N	20.1	Cd	0.004
Avg. Flow	125.8	Phosphate	4.5	Cr	0.042
Temp	11.7	Grease	15.4	Cu	0.144
DO	3.3	Sus. Solids	50.8	Hg	0.0042
pH	7.2	% Reduction	71.6	Ni	0.080
BOD	73.2	Set. Solids	0.10	Pb	0.048
COD	187.4	% Reduction	98.8	Zn	0.318
Chlorine	1.26	Vol. Solids	35.6	Hex Cr	< 0.005
Ammonia	11.6	% Reduction	73.8		

Appendix

Table 14. Biological, physical, and chemical data for Bioassay #14.

Determination of 1975 and 1976 year class English sole LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control - 1975	0	0		
WPE - 1975	10	10		96
WPE - 1975	20	100	48.0	48 - 72
WPE - 1975	40	100	9.7	8 - 16
WPE - 1975	50	100	6.8	6 - 8
WPE - 1975	70	100	2.9	2 - 4
Control - 1976	0	0		
WPE - 1976	10	100	55.0	48 - 72
WPE - 1976	20	100	10.4	6 - 16
WPE - 1976	40	100	2.0	2 - 3
WPE - 1976	50	100	1.2	1 - 2
WPE - 1976	70	100	1.0	1

Estimated LC50 = 16% WPE - 1975; 8% WPE - 1976.

Average Fish Length = 1975: 109.8 $\pm$ 16.6 mm; 1976: 28.6 $\pm$ 6.6 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	11.8	8.0	Turb	87.2	0.8
Sal	0.6	30.2	Chlorine	1.74	< 0.05
DO	1.8	8.6	Ammonia	9.8	< 0.05
pH	6.9	7.8	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.14	Total N		Cd	0.005
Avg. Flow	132.4	Phosphate		Cr	0.060
Temp	11.7	Grease		Cu	0.130
DO	3.7	Sus. Solids	40.0	Hg	0.0006
pH	7.4	% Reduction	77.7	Ni	0.067
BOD	66.2	Set. Solids	0.10	Pb	0.077
COD	165.5	% Reduction	98.0	Zn	0.257
Chlorine	1.56	Vol. Solids	30.5	Hex Cr	< 0.005
Ammonia		% Reduction	77.7		

Appendix

Table 15. Biological, physical, and chemical data for Bioassay #15.

Sixty-day subacute bioassay for trace metal bioaccumulation analysis of English sole and littleneck clams.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (days)
Control - Clam	0	13		60
Control - Sole	0	0		
WPE - Clam	.5	40		32 - 52
WPE - Sole	.5	0		
WPE - Clam	1.0	27		18 - 32
WPE - Sole	1.0	0		
WPE - Clam	5.0	7		12
WPE - Sole	5.0	0		
WPE - Clam	10.0	7		39
WPE - Sole	10.0	0		

Estimated LC50 = None.
Average Weight: Sole = 1.2 $\pm$ 2.2 g; Clam = (without shell) 10.5 $\pm$ 2.7 g.
Average Sole Length = 51.6 $\pm$ 7.0 mm; Clam Shell Width = 47.2 $\pm$ 2.3 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	15.8	9.8	Turb	104.4	7.1
Sal	0.6	30.2	Chlorine	1.76	< 0.05
DO	0.6	9.4	Ammonia	17.1	< 0.05
pH	7.0	8.0	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.04	Total N	23.6	Cd	0.005
Avg. Flow	97.0	Phosphate	6.1	Cr	0.047
Temp	16.1	Grease	21.1	Cu	0.152
DO	1.8	Sus. Solids	71.2	Hg	0.0025
pH	7.3	% Reduction	67.3	Ni	0.123
BOD	104.3	Set. Solids	0.16	Pb	0.054
COD	220.3	% Reduction	98.4	Zn	0.301
Chlorine	1.16	Vol. Solids	51.0	Hex Cr	< 0.005
Ammonia	14.6	% Reduction	69.6		

Appendix

Table 16. Biological, physical, and chemical data for Bioassay # 16.

Determination of English sole LC50 in chlorinated and dechlorinated West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	10	0		
WPE	20	90	25.2	30 - 46
WPE	40	100	5.8	4 - 8
WPE	60	100	2.0	2
Dechlorinated WPE	10	0		
Dechlorinated WPE	20	0		
Dechlorinated WPE	40	100	51.6	38 - 96
Dechlorinated WPE	60	100	15.2	14 - 22

Estimated LC50 = 14% WPE; 32% Dechlorinated WPE

Average Fish Length = 47.2 ± 7.1 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Dechlorinated WPE	Seawater		WPE	Dechlorinated WPE	Seawater
Temp				Turb			
Sal				Chlorine	1.15	< 0.05	< 0.05
DO				Ammonia			
pH				SO_2	< 0.05	1.35	< 0.05

Average Values for METRO Composite Effluent Samples

Rainfall	0.12	Total N		Cd	0.006
Avg. Flow	110.0	Phosphate		Cr	0.076
Temp	16.9	Grease		Cu	0.168
DO	1.5	Sus. Solids	99.0	Hg	0.0005
pH	7.2	% Reduction	54.2	Ni	0.120
BOD	127.7	Set. Solids	0.12	Pb	0.112
COD	232.8	% Reduction	98.5	Zn	0.406
Chlorine	1.07	Vol. Solids	72.5	Hex Cr	< 0.005
Ammonia		% Reduction	56.0		

Appendix

Table 17. Biological, physical, and chemical data for Bioassay #17.

Determination of English sole LC50 in West Point Effluent (WPE) and West Point Effluent with ammonia removed.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	10	100	39.5	36 - 40
WPE	20	100	40.0	40
WPE	40	100	8.8	6 - 10
WPE	60	100	2.3	2 - 4
WPE minus ammonia	10	70		40
WPE minus ammonia	20	100	40.0	40
WPE minus ammonia	40	100	40.0	40
WPE minus ammonia	60	100	17.2	16 - 20

Estimated LC50 = < 10% WPE; 7% WPE minus ammonia.

Average Fish Length = 74.6 + _ 11.4 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	WPE - Ammonia	Seawater		WPE	WPE - Ammonia	Seawater
Temp	18.7	18.9	11.7	Turb	159.8	85.5	3.5
Sal	0.5	0.5	30.1	Chlorine	1.38	0.10	< 0.05
DO	0.4	0.7	7.5	Ammonia	17.8	0.1	< 0.05
pH	6.5	6.6	8.0	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.01	Total N		Cd	0.007
Avg. Flow	96.6	Phosphate		Cr	0.050
Temp	18.2	Grease		Cu	0.168
DO	1.4	Sus. Solids	106.0	Hg	0.0016
pH	7.1	% Reduction	50.0	Ni	0.126
BOD	108.8	Set. Solids	0.44	Pb	0.056
COD	203.4	% Reduction	95.0	Zn	0.320
Chlorine	1.20	Vol. Solids	74.4	Hex Cr	< 0.005
Ammonia		% Reduction	53.8		

Appendix

Table 18. Biological, physical, and chemical data for Bioassay #18.

Determination of English sole LC50 in West Point Effluent (WPE) and West Point Effluent with ammonia removed.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	10	0		
WPE	20	90	28.7	19 - 72
WPE	40	100	5.6	5 - 6
WPE	60	100	2.6	2 - 3
WPE minus ammonia	10	0		
WPE minus ammonia	20	0		
WPE minus ammonia	40	20		72 - 96
WPE minus ammonia	60	100	12.1	9 - 19

Estimated LC50 = 14% WPE; 45% WPE minus ammonia.

Average Fish Length = 91.6 $\pm$ 14.1 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	WPE - ammonia	Seawater		WPE	WPE - ammonia	Seawater
Temp	19.7	19.8	12.7	Turb	147.0	77.2	3.5
Sal	0.5	0.7	29.9	Chlorine	1.56	< 0.05	< 0.05
DO	0.5	0.5	7.9	Ammonia	18.0	0.2	< 0.05
pH	6.6	6.6	8.0	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.00	Total N		Cd	0.006
Avg. Flow	96.8	Phosphate		Cr	0.052
Temp	19.3	Grease		Cu	0.186
DO	1.8	Sus. Solids	92.0	Hg	0.0005
pH	6.5	% Reduction	61.0	Ni	0.138
BOD	115.2	Set. Solids	0.36	Pb	0.120
COD	231.5	% Reduction	96.2	Zn	0.318
Chlorine	1.22	Vol. Solids	66.4	Hex Cr	< 0.005
Ammonia		% Reduction	64.0		

Appendix

Table 19. Biological, physical, and chemical data for Bioassay # 19.

Determination of 1975 and 1976 year class shiner perch LC50 in West Point Effluent (WPE).

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control - 1975	0	0		
WPE - 1975	5.0	0		
WPE - 1975	10.0	10		28
WPE - 1975	20.0	100	18.4	16 - 28
WPE - 1975	40.0	100	3.9	2 - 8
WPE - 1975	60.0	100	2.3	2 - 3
Control - 1976	0	10		28
WPE - 1976	5.0	0		
WPE - 1976	10.0	20		24 - 96
WPE - 1976	20.0	100	17.1	10 - 24
WPE - 1976	40.0	100	2.7	2 - 4
WPE - 1976	60.0	100	2.0	2

Estimated LC50 = 15% WPE - 1975; 14% WPE - 1976.

Average Fish Length = 1975: 124.4 ± 11.5 mm
1976: 44.4 ± 3.4 mm

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	19.4	12.5	Turb	117.0	2.8
Sal	0.5	30.6	Chlorine	1.48	< 0.05
DO	0.7	7.2	Ammonia	19.6	< 0.05
pH	6.8	7.9	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.02	Total N	27.4	Cd	0.007
Avg. Flow	106.4	Phosphate	7.2	Cr	0.083
Temp	19.8	Grease	25.6	Cu	0.185
DO	3.0	Sus. Solids	97.0	Hg	0.0006
pH	6.8	% Reduction	56.5	Ni	0.070
BOD	118.2	Set. Solids	0.25	Pb	0.090
COD	264.2	% Reduction	96.8	Zn	0.320
Chlorine	1.12	Vol. Solids	75.5	Hex Cr	< 0.005
Ammonia	16.3	% Reduction	56.0		

Appendix

Table 20. Biological, physical, and chemical data for Bioassay # 20.

Determination of shiner perch LC50 in West Point Effluent and West Point Efflue with ammonia removed.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	10.0	0		
WPE	20.0	100	15.6	14 - 22
WPE	33.0	100	8.8	8 - 10
WPE	60.0	100	1.5	1 - 2
WPE minus ammonia	10.0	0		
WPE minus ammonia	20.0	0		
WPE minus ammonia	40.0	100	23.6	14 - 34
WPE minus ammonia	60.0	100	8.0	5 - 10

Estimated LC50 = 12% WPE; 26% WPE minus ammonia.

Average Fish Length = 53.9 $\pm$ 4.0 mm.

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	WPE - Ammonia	Seawater		WPE	WPE - Ammonia	Seawater
Temp	19.3	19.2	12.5	Turb	120.4	62.8	2.6
Sal	0.5	0.5	30.8	Chlorine	1.25	< 0.05	< 0.05
DO	0.6	0.9	7.3	Ammonia	15.9	0.1	< 0.05
pH	6.7	6.8	7.9	SO_2			

Average Values for METRO Composite Effluent Samples

Rainfall	0.07	Total N	23.5	Cd	0.008
Avg. Flow	118.0	Phosphate	5.7	Cr	0.074
Temp	18.6	Grease	23.5	Cu	0.207
DO	2.9	Sus. Solids	76.6	Hg	0.0004
pH	7.0	% Reduction	60.0	Ni	0.069
BOD	97.4	Set. Solids	0.26	Pb	0.067
COD	194.7	% Reduction	96.7	Zn	0.240
Chlorine	1.11	Vol. Solids	56.5	Hex Cr	< 0.005
Ammonia	14.6	% Reduction	65.0		

Appendix

Table 21. Biological, physical, and chemical data for Bioassay # 21.

Determination of shiner perch LC50 in chlorinated and dechlorinated West Point Effluent (WPE)

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (hours)
Control	0	0		
Control	0	0		
WPE	10.0	0		
WPE	20.0	100	34.2	32 - 36
WPE	40.0	100	7.8	6 - 8
WPE	60.0	100	1.9	1 - 3
Dechlorinated WPE	10.0	20		36
Dechlorinated WPE	20.0	0		
Dechlorinated WPE	40.0	100	36.0	36
Dechlorinated WPE	60.0	100	12.0	12

Estimated LC50 = 15% WPE; 28% Dechlorinated WPE

Average Fish Length = 63.0 $\pm$ 4.1 mm

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Dechlorinated WPE	Seawater		WPE	Dechlorinated WPE	Seawater
Temp	18.4	17.6	12.2	Turb	138.5	70.8	4.8
Sal	1.0	0.8	31.1	Chlorine	1.31	< 0.05	< 0.05
DO	0.8	0.6	7.1	Ammonia	20.6	19.6	< 0.05
pH	6.7	6.6	7.9	SO_2	< 0.05	2.57	< 0.05

Average Values for METRO Composite Effluent Samples

Rainfall	0.08	Total N	22.7	Cd	0.007
Avg. Flow	111.9	Phosphate	5.6	Cr	0.076
Temp	18.9	Grease	18.5	Cu	0.198
DO	2.7	Sus. Solids	68.4	Hg	0.0004
pH	7.2	% Reduction	67.4	Ni	0.056
BOD	97.2	Set. Solids	0.10	Pb	0.048
COD	221.2	% Reduction	98.4	Zn	0.242
Chlorine	1.13	Vol. Solids	50.0	Hex Cr	< 0.005
Ammonia	13.9	% Reduction	70.0		

Appendix

Table 22. Biological, physical, and chemical data for Bioassay # 22.

Eight-week subacute bioassay for trace metal bioaccumulation analysis of shiner perch.

Treatment	Tank Concentration	Mortality (%)	GMST (hours)	Range in Mortality (days)
Control	0	0		
Control	0	0		
WPE	.5	0		
WPE	.5	0		
WPE	1.0	0		
WPE	1.0	0		
WPE	2.5	0		
WPE	2.5	0		
WPE	5.0	0		
WPE	5.0	0		

Estimated LC50 = None

Average Fish Length = None

Physical - Chemical Data

Average Values for Bioassay Head Tank Grab Samples

	WPE	Seawater		WPE	Seawater
Temp	18.9	11.7	Turb	153.2	1.9
Sal	0.6	31.4	Chlorine	0.70	< 0.05
DO	0.4	7.0	Ammonia	22.0	< 0.05
pH	6.5	8.0	SO_2		

Average Values for METRO Composite Effluent Samples

Rainfall	0.04	Total N	27.7	Cd	0.008
Avg. Flow	94.6	Phosphate	7.1	Cr	0.061
Temp	18.4	Grease	35.6	Cu	0.276
DO	2.2	Sus. Solids	118.5	Hg	0.0004
pH	7.0	% Reduction	53.1	Ni	0.077
BOD	126.8	Set. Solids	0.74	Pb	0.095
COD	294.4	% Reduction	97.1	Zn	0.360
Chlorine	1.08	Vol. Solids	90.7	Hex Cr	< 0.005
Ammonia	16.7	% Reduction	54.6		