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TOXICITY OF WEST POINT EFFLUENT TO MARINE INDICATOR ORGANISMS

PART II

by

Q. J. Stober, P. A. Dinnel, M. A. Wert,
D. H. DiJulio, and R. E. Nakatani

FINAL REPORT

March 1, 1977 to September 30, 1977
for
Municipality of Metropolitan Seattle (METRO)
Seattle, Washington

Approved

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Director

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1.0 SUMMARY

Eight-week continuous flow, chronic bioassays with shiner perch in dilutions (0, 0.5, 1, 2.5, and 5% volume/volume in seawater) of West Point sewage effluent were conducted to assess the potential for bioaccumulation of copper and zinc from the effluent. Generally, the whole-body burden of copper did not change significantly throughout the 8-week test period. A significant change in the whole-body burden of zinc was determined. In general, as time of exposure increased, there was a reduction in whole-body zinc which was inversely related to the per cent effluent concentration to which the fish were exposed. The general trend through time was an increase in tissue zinc concentration at 2 weeks with an ensuing reduction at 4, 6, and 8 weeks exposure.

Behavior tests were conducted to determine avoidance/preference responses of shiner perch to dilutions of West Point effluent in seawater (0, 1, 5, 10, 15, and 20% v/v). Shiner perch exhibited a significant preference for 1, 5, and 10% effluent and a significant avoidance of 15 and 20% effluent.

Echinoderm fertilization bioassays were conducted by exposing sand dollar sperm to seawater dilutions of 0.5 to 25% chlorinated or composite (slightly prechlorinated) West Point effluent for 15 min. The sperm were

subsequently added to sand dollar eggs and the per cent successful fertilization tabulated as the biological response. Twenty-one tests with chlorinated West Point effluent yielded a 50% decrease of fertilization in an average effluent concentration of 2.1 $\pm$ 1.5% with a range of 0.1 - 5.1%. Composite effluent was approximately one-half as toxic with a 50% decrease of fertilization in an average effluent concentration of 4.4 $\pm$ 3.7% with a range of 0.1 - 14.3%. Based on these tests, echinoderm sperm exposed to West Point effluent for 15 minutes were approximately 7-8 times more sensitive than shiner perch or juvenile English sole in 96-h acute bioassays.

In conclusion, the mean 96-h LC50 values for shiner perch and English sole in chlorinated effluent were 16.1 and 15.4% v/v, respectively. Dechlorination and ammonia removal decreased toxicity by about two. Shiner perch were attracted to effluent concentrations from 1 to 10% but avoided 15 and 20%. Histopathologic effects were observed when fish were chronically exposed to 0.5% chlorinated effluent. Whole-body trace metal analysis showed no bioaccumulation of Cu but body burden of Zn increased. Zn may have been accumulated largely from the food. However, as time of exposure to the effluent increased, whole-body burden of Zn decreased. The maximum acceptable concentration of chlorinated effluent based on histopathologic effects on chronically exposed shiner perch was 0.5%. These data do not adequately account for the potential effects of slug discharges of trace metals or other industrial toxicants nor have the potential effects of chronic exposure to persistent toxicants been addressed. Further testing and monitoring with more refined techniques will be required.

2.0 ACKNOWLEDGEMENTS

This report presents the results of the second phase of the West Point toxicity 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. D. H. DiJulio, Research Technician

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

Mr. Sam Felton assisted in water quality and tissue analysis for trace metals. Student assistants who aided in various stages of the study were Robert Atterberry, Jeff McKay, Steve Joner, Joseph Johovich, and Matt Oshiro. Keith Pierson aided in data analysis.

Project coordination received from Messrs. Ralph Domenoske and Rich Tomlinson of METRO is appreciated. The patience and assistance of Mr. Tom Rice, West Point Plant Superintendent, and his staff is also greatly appreciated. Mr. Dennis Brown and the West Point maintenance crews provided valuable assistance with the seawater supply system. Messrs. Carl Hunter and Mike Mar provided occasional support needed in water quality analysis.

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 PREFACE

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 first phase of the West Point toxicity studies was conducted between March 1, 1975, through February 29, 1977. The general objectives of the first phase 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. Acute and chronic toxicity tests were conducted from October, 1975, through September, 1976. The results of these tests are reported in Stober, et al. (1977a). Generally, the results yielded the following 96-h LC50 values (the concentration which kills 50% of the test organisms in 96 hours) for 5 marine species in flow-through tests of chlorinated West Point effluent (in seawater): shiner perch *(Cymatogaster aggregata)* = 15.4%; juvenile English sole *(Parophyrs vetulus)* = 16.1%; Pacific staghorn sculpin, *(Leptocottus armatus)* = 30%; coonstripe shrimp *(Pandalus danae)* = 15-20%; and shore crabs *(Hemigrapsus nudus)* = 50%. Dechlorination of the effluent with sulfur dioxide or removal of ammonia and chlorine with the ion-exchange resin clinoptilolite decreased the toxicity of West Point effluent by a factor of approximately 2. Abnormally high mortalities were observed in two 96-h bioassays (LC50 <10%). The exact toxic components were unknown; however, the mortalities were correlated with peaks of mercury or copper/chromium.

Chronic bioassays conducted for 8 weeks with West Point effluent concentrations of 0.5 to 10% did not indicate a significant whole body accumulation of copper or zinc in English sole, shiner perch, or littleneck clams, *(Protothaca staminea)*. Whole body analysis of a small sample of English sole from an 8-week test suggested possible depuration of chlorinated hydrocarbons and polychlorinated biphenyls (PCB's).

Histopathological examination of English sole and shiner perch gill tissues from chronic bioassays revealed damage to the cellular integrity in concentrations of chlorinated effluent as low as 0.5%.

The second phase of study was conducted from March 1, 1977, through September 30, 1977, on an extension of the original contract at no additional cost to METRO. The objectives of the second phase included:

1) Supplemental analysis of whole-body bioaccumulation patterns of copper and zinc by shiner perch exposed to chronic levels of West Point effluent for 60 days,
2) Bioassays of echinoderm gametes using "success of fertilization" as the biological response, and
3) Behavioral tests with shiner perch to determine avoidance/preference responses in the presence of various concentrations of chlorinated West Point effluent.

The results of the second phase are the subject of this report.

4.0 BIOACCUMULATION OF COPPER AND ZINC IN SHINER PERCH *(Cymatogaster aggregata)* FROM CHRONIC EXPOSURE TO SUBLETHAL DILUTIONS OF SEWAGE EFFLUENT

4.1 Introduction

The purpose of this section of study was to conduct a sublethal bioassay which would indicate whether or not exposure to dilutions of primary treated sewage plant effluent was capable of altering the whole-body concentrations of copper and zinc in a marine teleost.

Trace metals are emitted into marine waters from numerous industrial and domestic sources. The uptake of trace metals into marine organisms from elevated environmental levels has been documented in field surveys (Bryan and Hummerstone, 1971; Wright, 1976; Greig, et al., 1976; Ikuta, 1967 and 1968; McDermott, et al., 1976; Frazier, 1976; and Young and Jan, 1976) and laboratory investigations (Eisler, et al., 1972; Sherwood and Wright, 1976; Oshida, 1976; Pentreath, 1972, 1973*a*, *b*, *c*; Milanovich, et al., 1976; Bryan, 1964). Prior to their discharge into the marine environment, some trace metals may pass through municipal sewage treatment plants. Plants equipped with adequate advanced primary, secondary, or tertiary treatment can effectively reduce or eliminate trace metals from their effluent. The decision as to whether or not a plant requires advanced treatment or an appropriate modification should be based, at least in part, on a reasonable analysis of the capacity of the receiving water to tolerate continued emissions of trace metals. Such an analysis, a part of which is the subject of this study, requires a thorough examination and understanding of the numerous interactions of the wastewater constituents, including trace metals, with the ecology of the receiving waters. Information from baseline studies concerning the trace metal content of biota and seawater near the sewage outfall as well as the determination of the acute toxicity and sublethal effects from exposure to the sewage effluent should be of significance in the evaluation and prediction of the effects of trace metals and other sewage effluent components on marine life at a particular sewage outfall site.

An evaluation of the ecological effects of trace metals discharged into Puget Sound from the Municipality of Metropolitan Seattle (METRO) West Point sewage treatment plant at Seattle, Washington, was initiated as an interdisciplinary study in 1974. Baseline studies of trace metals in the water and biota near several sewage outfalls on central Puget Sound were conducted by Huntamer (1976) and Olsen (1976). Studies concerning the acute toxicity and sublethal effects of sewage effluent were conducted by Stober, et al. (1977*a*) on site at the METRO West Point treatment plant.

The study reported here is a continuation of the investigations conducted by Stober, et al. (1977*a*). It is concerned with sublethal changes in the whole-body copper and zinc content of shiner perch *(Cymatogaster aggregata)* resulting from exposure to volume/volume dilutions of primary treated sewage effluent in seawater in a continuous flow bioassay. Due to the known fluctuating concentrations of copper and zinc in the West Point effluent (WPE) no attempt was made to monitor the trace metal concentrations or to relate their concentration to the accumulation patterns displayed by the fish. METRO data is presented, however, and indicates the change in the copper and zinc concentrations in the undiluted effluent for the duration of the study.

The term "bioaccumulation" as it appears in this study refers to the uptake of trace metals, whether at normal or abnormal levels, into an organism either directly from the water or from the ingestion of food.

The objective of this section of the study was to measure the temporal change of the whole-body copper and zinc concentrations in shiner perch *(Cymatogaster aggregata)* held at four sublethal dilutions of primary treated sewage effluent (and seawater control) for 8 weeks in a continuous flow bio-assay.

4.2 Literature Review

4.2.1 Historical

Historically, many of the studies of trace metal pollution in the aquatic environment have been conducted following the loss of aquatic and sometimes human life. Prior to the twentieth century, such pollution was generally associated with the extraction of metal ores from the earth. In England in the nineteenth century lead, silver, and zinc mining operations discharged toxic metal salts into adjacent rivers as the metal ores were crushed and washed. Such contamination resulted in the loss of large numbers of fish and wildlife as well as domestic animals (Jones, 1964; Carpenter, 1926). The use of trace metals has become widespread among the modern industrialized nations of the world within the past three or four decades. Such use has resulted in many more recent contaminations and losses of aquatic life. One of the most noted recent mishaps occurred in Japan. Mercury from a plastics manufacturing plant contaminated Minamata Bay from 1950-1960. Fish and shellfish accumulated the pollutant in high levels. Persons eating the contaminated sea life died or suffered serious neurological damage (Irukayama, 1967).

Most of the studies presented in the reviews and annotated bibliographies of trace metal pollution in the aquatic environment (Doudoroff and Katz, 1961;

NAS, 1972; Saha, 1972; Eisler, 1973) were conducted since 1950. This is due in part to the recent development of analytical procedures required for accurate measurement of trace metals at low levels. A greater number of the studies deal with the freshwater environment as compared to the marine environment. This is due in part to the complex nature of seawater which makes trace metal measurements relatively more difficult. Comparison of data among studies is oftentimes not possible due to variations in experimental techniques, variations among the test species used, variations among the life stages of the test species and lack of information defining which chemical state(s) of the trace metal were measured.

4.2.2 Recent Field Studies of Trace Metal Bioaccumulation in the Marine Environment

Field studies present an assessment of trace metal uptake in aquatic life under "real world" conditions. Because of the understanding that trace metal bioaccumulation is dependent on numerous environmental factors, recent studies, having available more accurate equipment and techniques, have learned to combine the laboratory approach with field conditions (Frazier, 1976). Control areas for such field studies are usually carefully selected so that they can be realistically compared with polluted experimental test sites. Duplications of many of the environmental conditions measured in the field are sometimes conducted in laboratory experimentation of the dynamics of trace metals. Other field studies of trace metal bioaccumulation are conducted for the sole purpose of indicating environmental hot spots resulting from industrial or domestic contamination.

The literature contains studies on various organisms subject to trace metal contamination in different locations around the world. Ikuta (1967, 1968) reported increased levels of copper and zinc in oysters in contaminated waters of Japan. Bryan and Hummerstone (1971) have reported that the survival of the polychaete *(Nereis diversicolor)* in copper-rich sediments in England (as high as 900 μg/g) is partially based on the regulatory mechanism(s) which the worm has developed allowing it to tolerate elevated levels of at least one trace metal. Wright (1976) reported high levels of trace metals, especially zinc, in various coastal and marine fish near England. Greig, et al. (1976) reported that deep-water marine finfish contained lower levels of trace metals in their musculature than did the species along the continental shelf. American oysters *(Crassostrea virginica)* from a trace metal contaminated region of Chesapeake Bay have been reported to contain 2.4 and 7.5 times as much zinc and copper, respectively, than controls (Frazier, 1976). In this same study, uptake was found to be seasonally dependent with contaminated oysters having shells 16% thinner than controls. Chum salmon fry *(Oncorhynchus keta)* exposed to *in situ* seawater concentrations of 2.5 to 5.0 μg/ℓ Cu for 42 days have been reported to have accumulated no significant levels of copper in the muscle tissue (Thompson and Paton, 1976).

4.2.3 Trace Metal 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 (Olsen, 1976) is part of the METRO Interim Studies conducted by the University of Washington for the Municipality of Metropolitan Seattle. 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 polychaeta 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 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 *(Parophrys vetulus)* from METRO outfall areas contained 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 mollusk species analyzed also appeared to contain elevated levels of a number of trace metals when comparing METRO outfall areas to control areas. Working in conjunction with Olsen, Huntamer (1976), surveyed the trace metal concentrations of the water column at the METRO sewage outfalls and found no significant concentration differences as compared to control waters of Puget Sound.

4.2.4 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; Ikuta, 1967 and 1968; Frazier, 1976; Bryan, 1971). While required for normal functioning of an organism, expressive quantities can cause deleterious effects or even death.

4.2.5 Chemistry of Trace Metals as Related to Bioaccumulation and Toxicity

The chemical state or species of trace metals is an important factor affecting the biological availability for bioaccumulation (Frazier, 1976). According to Chapman (1973), physical-chemical factors in the aquatic environment such as pH, temperature, dissolved oxygen, turbidity, carbon dioxide, magnesium salts and phosphates may influence the toxicity of trace metals to aquatic life by altering their chemical state, making them more or less available for accumulation.

There presently exists minimal literature describing which chemical state(s) of individual trace metals in water are toxic and/or biologically available for accumulation in the various stages of development of aquatic organisms. 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, et al. (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. Windom and Smith (1972) indicate that under normal seawater conditions, copper and zinc occur in solution as dissociated ions or labile complexes in the +2 valence state.

4.2.6 Complexation of Trace Metals in the Aquatic Environment

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. The importance of organics in controlling the biological availability of copper in the marine environment has also been demonstrated by Lewis (1976) and Williams, et al. (1976). In the latter study, although elevated levels of cupric ion (Cu^{2+}) were measured in the waters of San Diego Harbor, no increased levels were found in the phytoplankton. The authors indicated that the complexation of copper with organic matter was the major mechanism preventing high cupric ion activity which would otherwise have reached toxic proportions. 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, amino acids, humic substances and suspended organics typical of sewage plant effluent have been shown to reduce the toxicity of copper by forming copper-organic complexes which do not contribute to lethal toxicity (United Kingdom Ministry of Technology, 1969; Lewis, et al., 1972; Brown, et al., 1974; Zitko, et al., 1973).

Complexing agents have been shown to deter sublethal effects of trace metals in the aquatic environment. Humic acid has been found to decrease or eliminate avoidance reactions of Atlantic salmon *(Salmo salar)* to various copper concentrations (Carson and Carson, 1973). In addition to natural complexing agents, artificial agents such as nitrolotriacetic acid (NTA) have been shown to reduce or eliminate zinc toxicity in fish (Sprague, 1968).

4.2.7. Mode of Entry of Trace Metals in Some Marine Biota

In studying the effects of trace metals (or any toxic substance) on aquatic life, it is necessary to be aware of the mode of entry of the toxicant into the organism. The accumulation, retention, distribution and depuration of various trace metals in aquatic life have been studied using both labeled isotopes and stable elements. The accumulation of trace metal radionuclides including ^{65}Zn have been shown to occur chiefly through food intake and to only a minor extent directly from water in the mussel *(Mytilus edulis)* (Pentreath, 1973*a*), in the elasmobranch thornback ray *(Raja clavata)* (Pentreath, 1973*b*), and in the marine teleost plaice *(Pleuronectes platessa)* (Pentreath, 1973*c*). Pentreath (1972) states that the sites of entry of the trace metals which are accumulated directly from the water in marine teleosts include the gills, the tissues of the opercular cavity and the gut. Bryan and Hummerstone (1971) report that, while it is uncertain as to the extent that copper is absorbed from food in the marine polychaete *(Nereis divesicolor)* copper is absorbed directly from solution presumably across the body surface.

Trace metals have been demonstrated to accumulate in aquatic life exposed to elevated seawater concentrations of metals while trace metal food levels were held constant. Eisler, et al. (1972) has shown increases in tissue cadmium in the marine teleost *(Fundulus heteroclitus)*, the scallop *(Aquipecten irradians)*, the oyster *(Crassostrea virginica)*, and subadult lobsters *(Homarus americanus)* after 3 weeks' exposure to 10 mg/ℓ Cd^{2+}. All experimental and control groups were fed the same diet. It was concluded from this study that trace metal accumulation can occur via water.

In general it appears that the mode of entry and ultimate bioaccumulation of trace metals in aquatic life is dependent upon the particular metal, its chemical state, and the organism in consideration. The anatomy of one organism may allow that organism to be more adept at accumulating trace metals than another organism. 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 into the organism or different rates of trace metal excretion. Bryan and Hummerstone (1971) have demonstrated that some marine organisms may posses regulatory mechanisms which can allow such organisms to accumulate and withstand levels of trace metals much greater than those which can be accumulated or tolerated by other organisms.

4.2.8 Global Sources and Modes of Transport of Trace Metals into the Marine Environment

Naturally occurring trace metals become available to the marine environment from the biogeochemical breakdown of igneous, metamorphic and sedimentary rock. The trace metals are transported to the oceans mainly by rivers, the atmosphere, and glaciers. According to Garrels and Mackenzie (1971), 90% of the trace metals added to the oceans are derived from rivers.

Various anthropogenic sources resulting from man's impact on nature have in the past few decades contributed significant amounts of trace metals to the oceans. A study by French (1976) suggests that trace metal pollutants which enter the oceans directly or are wind or river transported are transferred readily from the waters to the sediments. Anthropogenic trace metal sources include manufacture and use of industrial products, domestic wastes, agriculture, forest and urban runoff, ocean dumping and vessel wastes (Zafiropoulos, 1976). Manufacture and use of industrial products and domestic wastes are the two most important anthropogenic sources of trace metals entering the oceans (GESAMP, 1974).

Specific examples of anthropogenic copper sources which may enter the oceans include its use in algicides, bacteriocides, marine antifouling paints, the formation of currency, electrical high-tension wires, food processing utensils and containers and various plating operations. Zinc sources include its use as pigment in paints (ZnO), electrodeposition in galvanizing operations, electrical cells, formation of brass, die castings, pulp mill operations (ZnO), fertilizer and fruit tree fungicide ($ZnSO_4$).

4.2.9 Sources of Copper and Zinc Entering Puget Sound

A survey of the concentrations of trace metals in waters and effluents entering Puget Sound, based on values derived from the literature and environmental agencies was conducted by Zafiropoulos (1976). Total amounts of copper and zinc entering Puget Sound from various sources were calculated (Table 1). The study concluded that rivers seemed to contribute the greatest amount of copper and zinc to Puget Sound. Atmospheric input was found to be the second greatest contributor of copper and zinc. METRO's West Point sewage treatment plant combined with all other municipal sewage treatment plants were determined to contribute approximately 2% each of the total copper and zinc entering Puget Sound. The total mass emission of copper and zinc from the West Point outfall for 1976 was 28 and 46 metric tons, respectively (Table 2).

4.3 Materials and Methods

4.3.1 The Bioassay Laboratory

The mobile bioassay laboratory used for this study was constructed for toxicity tests involving the determination of the acute and chronic effects of primary treated sewage effluent on marine organisms. Stober, et al. (1977*a*) describe the details of the laboratory facility.

Table 1. Total copper and zinc inputs to Puget Sound (metric tons/yr). From Zafiropoulos, 1976

Source	Copper	Zinc	Estimated standard deviation
Rivers (Lake Washington ship Canal included)	787	1624	50%
METRO's West Point Plant	29	56	10%
Other municipalities in the Puget Sound area	22	26	50%
Atmospheric input	450	880	50%
Vessels protective measures and fuel consumption	360–590	140–240	–
Urban runoff (Seattle)	15	50	–
Advective transport	306	874	50%

Table 2. Total mass and mean daily concentration of copper and zinc discharged into Puget Sound from West Point sewage effluent for 1976*

	Cu	Zn
Total mass of element discharged (kg/yr)	28,170	45,919
Effluent concentration (μg/l)	206	324
Concentration at diffuser (μg/l) (100:1 dilution)	2.06	3.24

*Based on values obtained from METRO's NPDES discharge permit performance records for the period January 1, 1976 to December 31, 1976.

4.3.2 Seawater and Sewage Effluent Intake Systems

Ambient seawater was pumped by two 200-gpm stainless steel impeller pumps which were located at the end of a pier projecting approximately 60 m offshore. This location was on the opposite side of the point of land from which the sewage effluent was discharged; a shoreline distance of approximately 1.6 km. The screened seawater intake pipe was located 7.6 m deep at lower low tide and 10.7 m deep at higher high tide. The seawater was pumped to a 1892-l fiber glass constant-head-tank located on a platform above and adjacent to the laboratory (Fig. 1).

Primary treated, chlorinated West Point effluent (WPE) was tapped off of the main discharge line and pumped to a constant-head-tank located above and adjacent to the laboratory. All plumbing delivering the seawater and WPE to the wet lab was constructed of polyvinyl chloride (PVC) pipe.

4.3.3 Test Fish: Shiner Perch

4.3.3.1 General Description. Shiner perch belong to the family *Embiotocidae*, the surfperches. According to Hart (1973), these fish are distributed from northern Baja California to southern Alaska. They can be found generally in shallow waters during the summer, moving to deeper water in winter. Adults may attain lengths to about 15 cm, with females usually larger than males. The breeding season in Puget Sound waters appears to occur from April to July. Approximately 1 year following breeding, the ovoviviparous fish give birth to 5 to 17 young per female. At birth, the young range from 5.6 to 7.8 cm, with males being sexually mature. Food consists of copepods for the young, while later stages and adults feed on mussels, algae, and barnacle appendages.

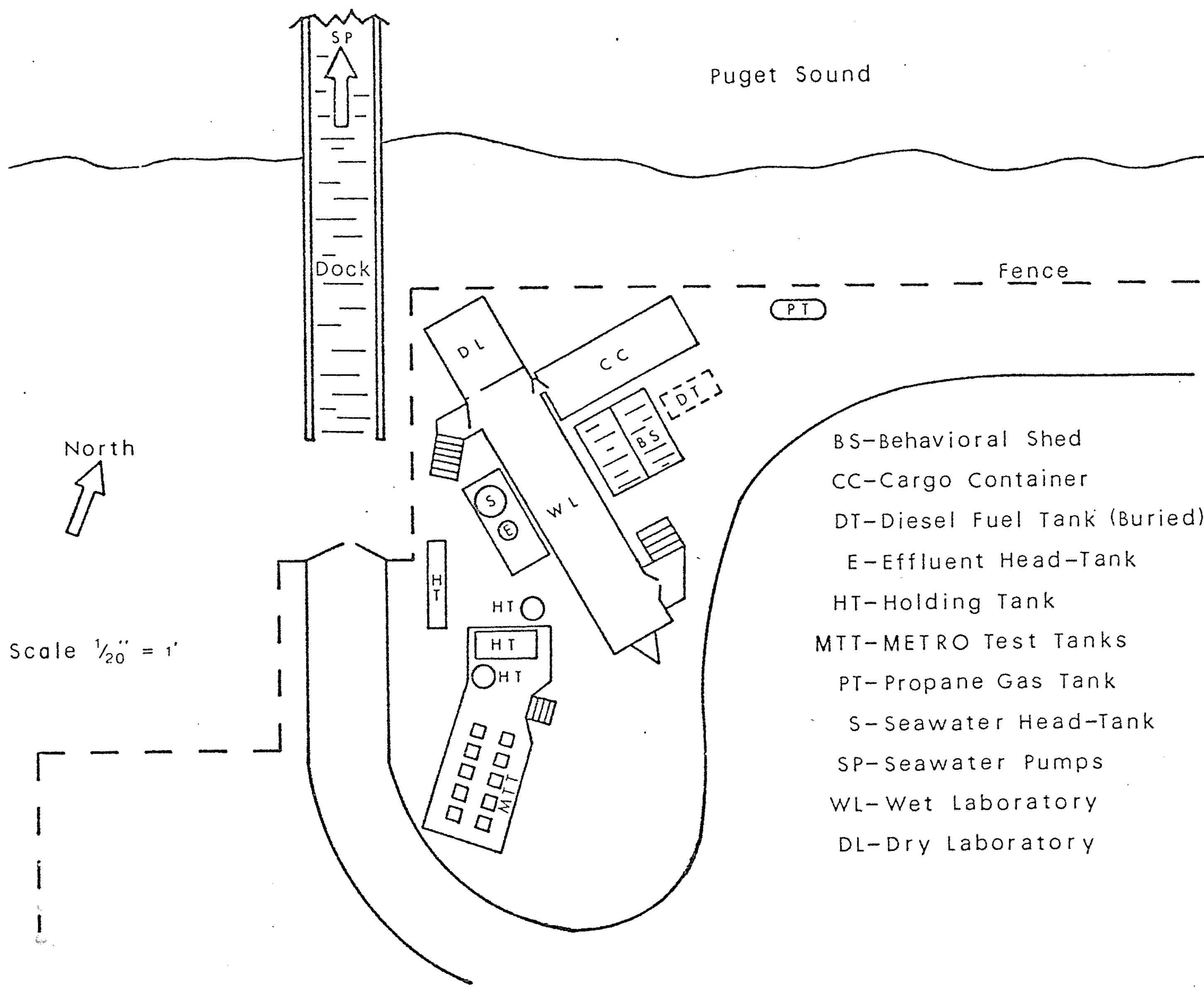

Figure 1. Diagram of marine bioassay laboratory at West Point.

A description of the methods of collection of shiner perch can be found in Shaw, *et al*. (1974).

4.3.3.2 Selection for Experimental Use. Shiner perch were selected for use in this study due to 1) their importance as a forage species for common food fish (possible food web magnification); 2) their ability to adapt to the laboratory environment; 3) their seasonal presence in the West Point fish assemblage (Miller, *et al*., 1977), and 4) their availability for collection.

4.3.3.3 Collection. Specimens were collected in South Puget Sound at Kopachuck State Park, approximately 16 km west of Tacoma (Fig. 2), in August, 1976. This site was selected due to its relative remoteness from industrial and domestic pollution. It was theorized that fish from such a location would have experienced relatively less pollution-related environmental stress and have a lower body burden of trace metals than those fish in the vicinity of the West Point outfall. A 37-m sinking beach seine set parallel to shore from the stern of a 3.7-m rowboat was used for collection. Most of the shiner perch captured were young-of-the-year, approximately 3- to 4-months old. After capture, the fish were carefully transferred to 114-liter plastic garbage cans containing seawater and transported to the University of Washington's Marine Bioassay Lab at West Point. During transport, the seawater was oxygenated from a compressed oxygen cylinder.

4.3.4 Bioassay Laboratory Procedures

4.3.4.1 Acclimation and Feeding of Fish. Collected fish were held in round fiber glass tanks (diameter 1.5 m by height 1 m) supplied with constant flowing ambient West Point seawater. On the day of their capture 16 fish were rinsed

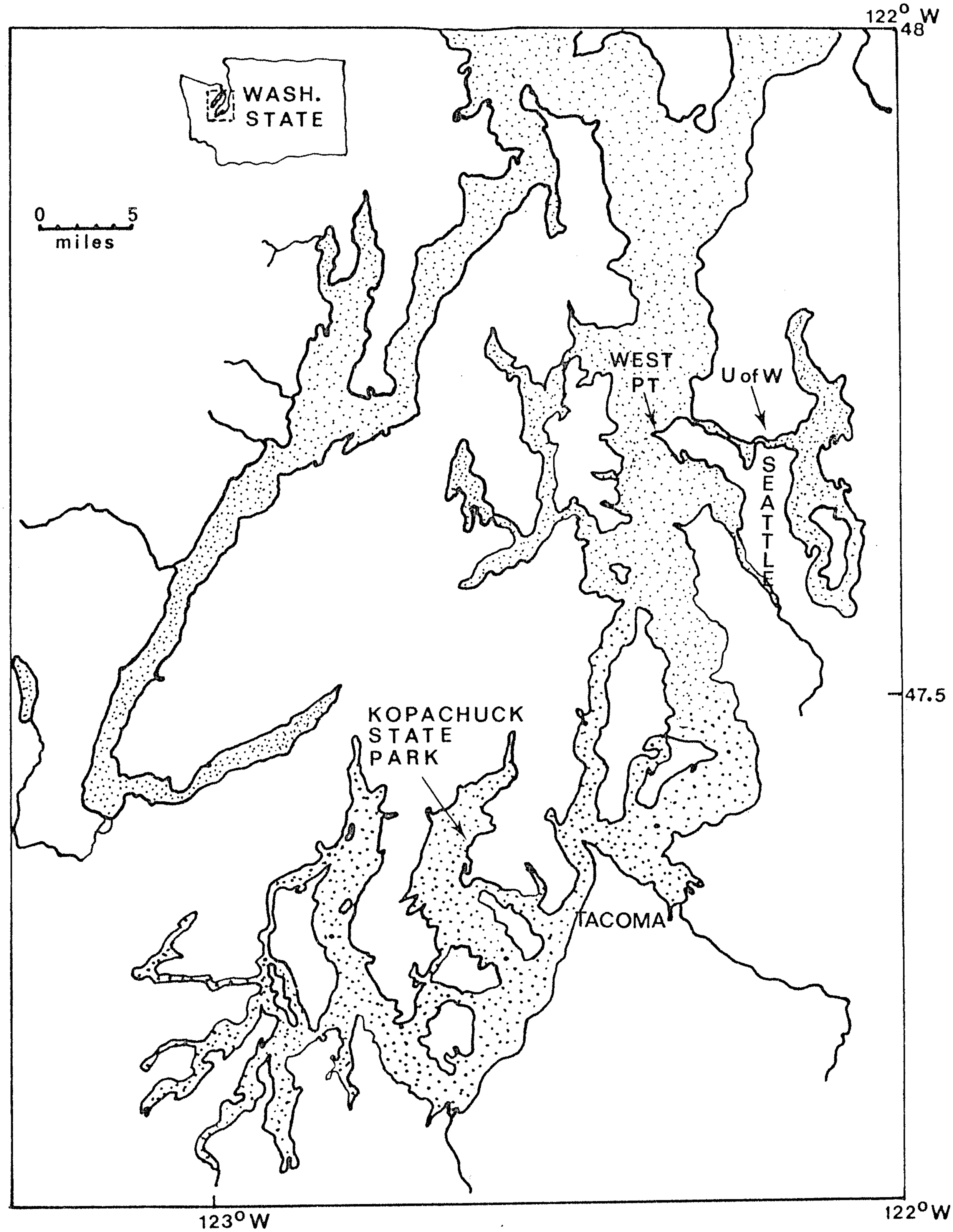

Fig. 2. Map of central and southern Puget Sound.

in seawater, placed in polyethylene bags and frozen for later analysis to determine the total copper and zinc body burden of the fish direct from the collection site.

Because some of the metals measured from such an analysis may include unassimilated amounts which may pass through the gut, the fish were starved for 3 days. After 3 days a random sample of 10 fish were dissected and were observed to contain no food in their digestive system. A sample of 16 starved fish was frozen to determine whether a 3-day starvation significantly reduced the whole-body total copper and zinc content to reflect only assimilated copper and zinc.

Fish to be used in the bioassay were acclimated in the holding tanks for 3 weeks prior to initiating the bioassay. Because of the time duration involved (11 weeks including acclimation), it was necessary to feed the fish. The standard hatchery diet from the Fisheries College at the University of Washington was used throughout the bioassay. Samples of food used during the 11-week period were frozen, composited and later analyzed for total copper and zinc content. The amount of food the fish were fed during the bioassay was equivalent to 4.4% of the estimated total weight of fish per tank, and was provided twice daily. This amount is based on an individual weight of 2.8 g and water temperatures of 12-13°C.[1]

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[1]Based on Oregon Pellet Feeding Chart by R. V. Moore Company, Inc., Box M., LaConner, Washington 98257

4.3.4.2 The Diluter System. The test dilutions of primary treated sewage effluent in seawater were obtained through the use of a continuous flow diluter modified from that described by Stober, et al. (1977*a*). Seawater and sewage effluent from the respective head-tanks adjacent to the lab were piped to the diluter head-tanks comprised of 76-ℓ glass aquariums. Dilutions of 10% and 1% sewage effluent in seawater (v/v) were prepared by delivering the appropriate flow rates of sewage effluent and seawater to two additional dilution head tanks (Fig. 3). The flow rates were controlled by 4 non-metallic submersible pumps fitted with plexiglass tubing, with ends containing either PVC valves or calibrated diameter glass tubing. Glass siphon tubes of various diameters delivered the appropriate flow rates from the 4 head-tanks to 5 mixing tanks to yield dilutions of 5.0, 2.5, 1.0. 0.5% (v/v) WPE/seawater and a seawater control. Adjustments to within $\pm$ 1% of the rate of flow from any siphon tube could be made by raising or lowering the tube. Similar adjustments could be made in the head tanks by the PVC valves. The dilutions were transferred from the mixing tanks to the 53-liter test aquariums (two test aquariums per dilution) by another series of glass siphon tubes. The flow rates from these tubes were maintained at 1.5 liters/min, sufficient for a 95% replacement in approximately 1 hour (Sprague, 1973). Based on a volume of 40 liters, this rate provided 54 tank volumes/day. Stephan (1975) recommends a minimum of 10 tank volumes/day for tests using continuous flow sewage effluent. The test aquariums were located in a fiberglas trough (water bath) containing flow-through seawater at ambient Puget Sound temperature. All tanks used in the diluter system had overflow drains in order to maintain constant volumes. Daily monitoring of all flow rates was conducted throughout the bioassay in order to calculate the final

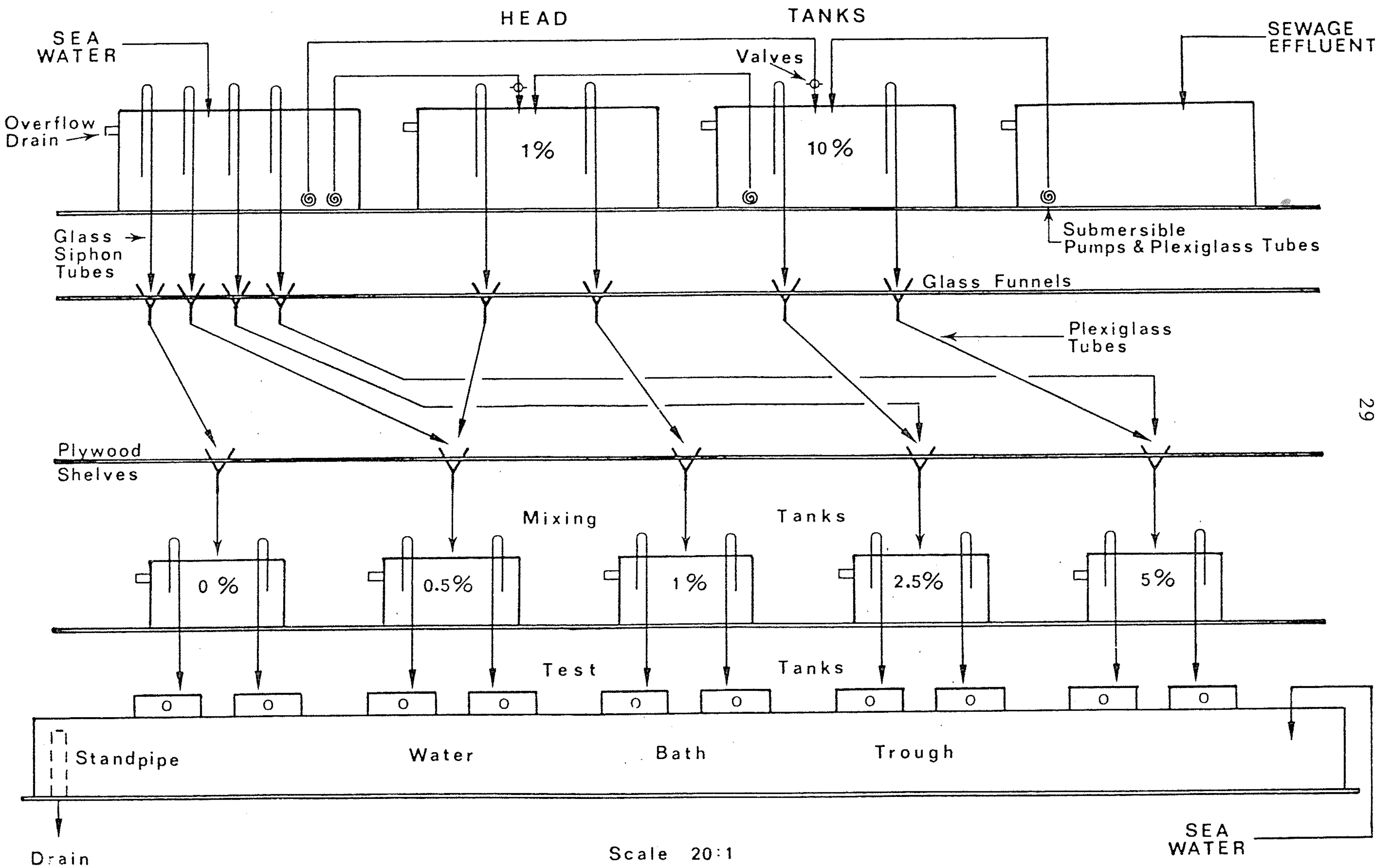

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Fig. 3. Continuous-flow diluter system.

exposure concentrations in the test aquariums. Siphon tubes were adjusted when individual tube flow rates exceeded $\pm$ 1% of their desired value.

4.3.4.3 The Bioassay. All fish used in this study were of the same age class (3-4 months old). To begin the bioassay, the fish, which had been acclimated for 3 weeks in seawater and starved for 3 days, were transferred from the holding tank to a plastic bucket of seawater (5 fish at a time) and placed in one of the 10 aquariums selected at random. This was repeated until all the aquariums contained 25 fish (50 fish per dilution). An additional 16 acclimated and starved fish were rinsed in seawater, placed in a polyethylene bag, and frozen for later analysis. These 3-week acclimated fish were compared to the 3-day acclimated fish to determine whether there had been any change in the whole body content of copper and zinc during the acclimation. These fish, labeled "unexposed group," were also compared to the fish in the diluter seawater control throughout the bioassay to see if the seawater control fish were increasing their metal content from the diluter system. At 2, 4, 6, and 8 weeks of exposure, 10 fish (which had been previously starved for 3 days) were removed from each of the sublethal dilutions and seawater control (5 fish from each aquarium), rinsed in seawater, and placed in polyethylene bags which had been labeled as to the date removed and the concentration of WPE from which they came. A total of 256 fish were analyzed in the experiment.

4.3.4.4 Water Quality Measurements. Water quality measurements were taken biweekly for temperature, salinity, dissolved oxygen, pH, turbidity, total residual chlorine and ammonia in aquariums containing all sewage effluent dilutions, seawater and undiluted sewage effluent. The equipment and methodology of the water quality measurements are summarized in Table 3. Daily

Table 3. 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° Thermometer	Direct		S = 0.1 R = 0-100	Degrees Centigrade (°C)
	*2.	ARA electronic thermometer with scanner and recorder	Direct	C° thermometer	S = 0.25 R = 0-55	
Salinity	1.	Hydrometer	Conversion of reading by tables		S = .01	Parts per thousand (°/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	0.0250N 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 blacnk	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.

physical-chemical measurements of 24-h composite samples of sewage effluent was conducted by METRO water quality laboratory personnel at West Point. The sampling methodology is summarized in Table 4.

4.3.4.5 Dilution of Copper and Zinc in the Diluter System. In order to determine how total copper and zinc contained in the WPE was diluted by the diluter, 5 replicate 1-liter samples of WPE, seawater, and 10% and 1% v/v WPE in seawater were collected in polypropylene bottles with screw caps and acidified with 30 mℓ of 16M redistilled reagent-grade nitric acid. The samples were taken to the Water Quality Lab of the Fisheries Research Institute at the University of Washington, where 700 mℓ of the samples were concentrated to 50 mℓ in an all-glass evaporator (Buchi Rotavapor-R, Model KRvr 65/45). After concentration, the solutions were transferred to 60-mℓ polypropylene bottles and aspirated into a Perkin-Elmer Model 303 flame atomic absorption spectrophotometer (AAS) equipped with digital readout.

4.3.4.6 Special Handling Procedures. In order to minimize the possibility of trace metal contamination in the bioassay system, all aquariums, plexiglass tubing and glassware were washed in detergent, rinsed in tap water, soaked in concentrated hydrochloric acid for at least 24 hours and rinsed in times with tap water. The nets used to capture the fish at the various sampling intervals had vinyl-coated handles.

4.3.5 Laboratory Procedures for Trace Metal Analysis

All trace metal analyses were conducted at the Fisheries College, University of Washington. In order to assure accuracy in the final results and minimize trace metal contamination, special handling procedures were

Table 4. 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	^oC
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).

utilized. Trace metal analysis consisted of drying whole fish to a constant weight, chemically digesting (wet ashing) the fish in nitric and perchloric acids and measuring the resulting concentration of copper and zinc on a Perkin-Elmer Model 303 atomic absorption spectrophotometer (AAS) equipped with a digital readout.

4.3.5.1 Equipment Cleaning. Before use, all glassware, polypropylene bottles and other non-metallic equipment used in the analyses were cleaned thoroughly. The cleaning process involved 1) washing with detergent; 2) several rinses with hot tap water; 3) several rinses with distilled water; 4) a minimum 16-hour soak in an 8M nitric acid bath; 5) several rinses with deionized distilled water; 6) air drying. No metallic equipment came in contact with the fish during sample preparation or analysis.

4.3.5.2 Sample Preparation. To begin the preparation, the frozen fish contained within the polyethylene bags were allowed to partially thaw. The fish were then removed with plastic forceps one at a time, rinsed with copious amounts of deionized distilled water and placed in clean, tared 250-mℓ glass beakers. Two fish were placed in each beaker. The beakers were then reweighed and the fish wet weight calculated by differences. The beakers were covered with watch glasses and placed in an oven at 95°C to attain a constant weight. After approximately 16 hours, the beakers were removed from the oven and allowed to cool in a dessicator. After cooling, the watch glasses were removed and the beakers reweighed to determine the dry weight of the fish. The same procedure was used for preparing the National Bureau of Standards (NBS) powdered bovine liver standard and the University of Washington fish food.

4.3.5.3 Wet Ashing. Wet ashing was conducted in a perchloric acid fume hood using a method adapted from Smith (1953). All nitric acid used in wet ashing was reagent grade which had been redistilled. The perchloric acid was reagent grade, but due to the explosive nature of the concentrated acid, it was not further distilled. Covered beakers containing fish food or dried fish, NBS bovine standard, reagent blank, and spiked reagent blank each received 20 mℓ of 16 M HNO_3. All beakers were then placed on a Thermolyne (Type 2200) hot plate at the highest setting. The watch glasses were positioned to one side on the beakers to allow escape of gases. During the initial foaming, if the foam neared the top of the beaker, the beaker was either removed for a short time period or a few mℓs of dilute (0.1M) HNO_3 was added and the beaker swirled. The solutions in the beakers were boiled down to low volume and then removed from the hot plate. Five mℓs of 16M HNO_3 and 15 mℓ of 70% $HClO_3$ (in that order) were then added to each beaker. The beakers were then returned to the hot plate to exotherm until dense white vapors and a clear solution were present. During this stage, if the reaction began to react greatly, a few mℓs of 16M HNO_3 was added to reduce the reaction. Next, the walls of each beaker were rinsed with deionized distilled water and allowed to reheat until dense white vapors reappeared. The beakers were then removed from the hot plate and the solutions transferred to 50-mℓ volumetric flasks, using small glass funnels, and diluted to volume with 0.1M HNO_3 (approximate pH 1.5). Solutions were then transferred to 2-oz polypropylene bottles with tight fitting screw caps for storage until analysis by AAS.

4.3.5.4 Quality Control. Quality control was required in this study to determine the accuracy of the results as well as the presence of possible trace metal contaminants. For each wet ash analysis (approximately 15 beakers of fish samples), one reagent blank, one spiked reagent blank and three NBS bovine standards were analyzed. The reagent blank was used to detect trace metal contaminants which might be present in the wet ash chemicals. The spiked reagent blank was used to determine the accuracy of the recovery of known quantities of copper and zinc from the wet ash chemicals. The biologically derived salts present in the wet ashed fish solutions compose a solution matrix which is different from that of the spiked blank standards. Certain matrices may affect the accuracy of AAS measurements. For this reason NBS powdered bovine liver standard was analyzed to determine the accuracy of the measurement of known concentrations of copper and zinc from a wet ashed solution having a final matrix similar to that of the fish wet ashed solutions.

4.3.5.5 Atomic Absorption Spectrophotometry (AAS). Analysis of a diluted sample was obtained by aspirating the solution from a polypropylene storage bottle directly into the acetylene flame of the AAS and observing the absorbance in the digital readout. An average of twelve readings was recorded for each sample. The trace metal concentration was calcualted from a standard curve by simple linear regression analysis. The equation for the least squares line was based on the observed absorbance values and the known concentration of copper and zinc in a series of fresh standard solutions. The concentrations of copper and zinc in the standard solutions bracketed the concentration of the trace metals in the diluted fish samples in order to assure accuracy in the measured range. The standard solutions were re-analyzed and a new standard curve established after groups of 10 samples were measured. This procedure was used to determine any drifting of the standard curve through time.

4.3.5.6 Calculations. The determination of the concentration of copper and zinc in the fish was calculated from the copper and zinc concentration of the wet ashed, diluted sample by the equation:

Equation 1 $$C_1 = C_2 \cdot W_2 \cdot K_1 \cdot K_2 \cdot (W_1^{-1})$$

Where

C_1 = the trace metal concentration of the fish (mg Cu or Zn/kg wet or dry weight)

C_2 = the trace metal concentration of the wet ashed and diluted fish sample as calculated from the equation for the least squares line from AAS measurements (mg Cu or Zn/ℓ)

W_2 = the volume to which the wet ashed sample was diluted (mℓ)

K_1 = the equivalency 1/1000 mℓ

K_2 = the equivalency 1000 g/kg

W_1 = the wet or dry weight of the fish prior to wet ashing (g)

For example, a fish composite which had a dry weight equal to 1.10 g and which had been wet ashed and diluted to 50 mℓ was calculated from the equation of the least square line of the standard curve to contain 1.96 mg Zn/ℓ of sample solution from equation 1 was calculated to equal 89/09 mg Zn/kg dry weight of fish.

Example 1

$$C_1 = \frac{1.96 \text{ mg Zn}}{\ell \text{ sol'n}} \times 50 \text{ ml} \times \frac{1\ \ell}{1000 \text{ m}\ell} \times \frac{1000 \text{ g}}{\text{kg}} \times \frac{1}{1.10 \text{ g dry wt.}} = 89.09 \text{ mg Zn/kg dry wt.}$$

Analysis of the data was determined using two SPSS (Statistical Package for the Social Sciences) programs (Nie, et al., 1970). The programs used were "ONEWAY" (a specialized program for one-way ANOVA) and "REGRESSION" (simple linear regression analysis). The "ONEWAY" program was modified to include *a posteriori* contrasts using Tukey's test.

4.4 Results

4.4.1 Water Quality Measurements

Results of the water quality of the diluter head tanks and test aquariums are presented in Table 5. The increase in D O (+ 0.5 mg/ℓ) from the seawater head tank to the seawater control aquariums is theorized to result from the aeration of the seawater as it passes through the diluter. The average concentration of chlorine was below the measurable level (0.05 mg/ℓ) for all test aquariums except those at 5% WPE. Results of the analyses of WPE by METRO laboratory personnel are presented in Table 6. The average concentrations of copper and zinc do not reflect the variation of concentrations which occurred during the bioassay. For this reason, the concentrations (based on daily composite samples) of copper and zinc in WPE for the entire test period were plotted (Fig. 4). Copper ranged from 114 to 480 μg/ℓ. Zinc ranged from 220 to 560 μg/ℓ. The daily composited WPE samples also vary throughout the day. On July 22, 1976, the METRO laboratory made 8 hourly measurements of trace metals in WPE (Table 7). While the daily composited average concentrations of copper and zinc for that day were 180 and 200 μg/ℓ, respectively, copper was measured on an hourly basis as high as 320 μg/ℓ and zinc as high as 460 μg/ℓ.

Table 5. Water quality analysis of head tank and test aquariums. Values are averages of all biweekly measurements from September 15 to November 10, 1976

		Head tanks		% WPE in seawater (v/v) in test aquariums				
	Units	Seawater	WPE	0%	.5%	1.0%	2.5%	5%
Temperature	^{o}C	11.7	18.9	11.7	11.7	11.8	12.0	12.2
Salinity	$^{o}/oo$	31.4	0.6	31.4	31.3	31.3	30.9	30.3
DO	mg/1	7.0	0.4	7.5	7.2	7.3	6.8	6.6
% DO saturation	%	80.9	0.05	86.7	83.2	84.5	79.1	77.2
pH	pH units	8.00	6.50	8.00	7.98	7.95	7.88	7.79
Turbidity	JTU's	1.9	153.2	3	3	3	5	8
Chlorine	mg/1	< 0.05	0.70	< 0.05	< 0.05	< 0.05	< 0.05	.05
Ammonia	mg/1	< 0.05	22.0	< 0.05	.05	.28	.67	1.38

Table 6. METRO analysis of composite sewage effluent samples. Values are average from September 15 to November 10, 1976

Rainfall	0.04	Inches
Average flow	94.6	MGD
Temperature	18.4	^oC
DO	2.2	mg/1
pH	7.0	pH units
BOD	126.8	mg/1
COD	294.4	mg/1
Chlorine	1.08	mg/1
Ammonia	16.7	mg/1
Total N	27.7	mg/1
Phosphate	7.1	mg/1
Grease	35.6	mg/1
Suspended solids	118.5	mg/1
% reduction	53.1	
Set. solids	0.74	mg/1
% reduction	97.1	
Vol. solids	90.7	mg/1
% reduction	54.6	
Cd	0.008	mg/1
Cr	0.061	mg/1
Cu	0.282	mg/1
Hg	0.0004	mg/1
Ni	0.077	mg/1
Pb	0.095	mg/1
Zn	0.351	mg/1
Cr^{6+}	< 0.005	mg/1

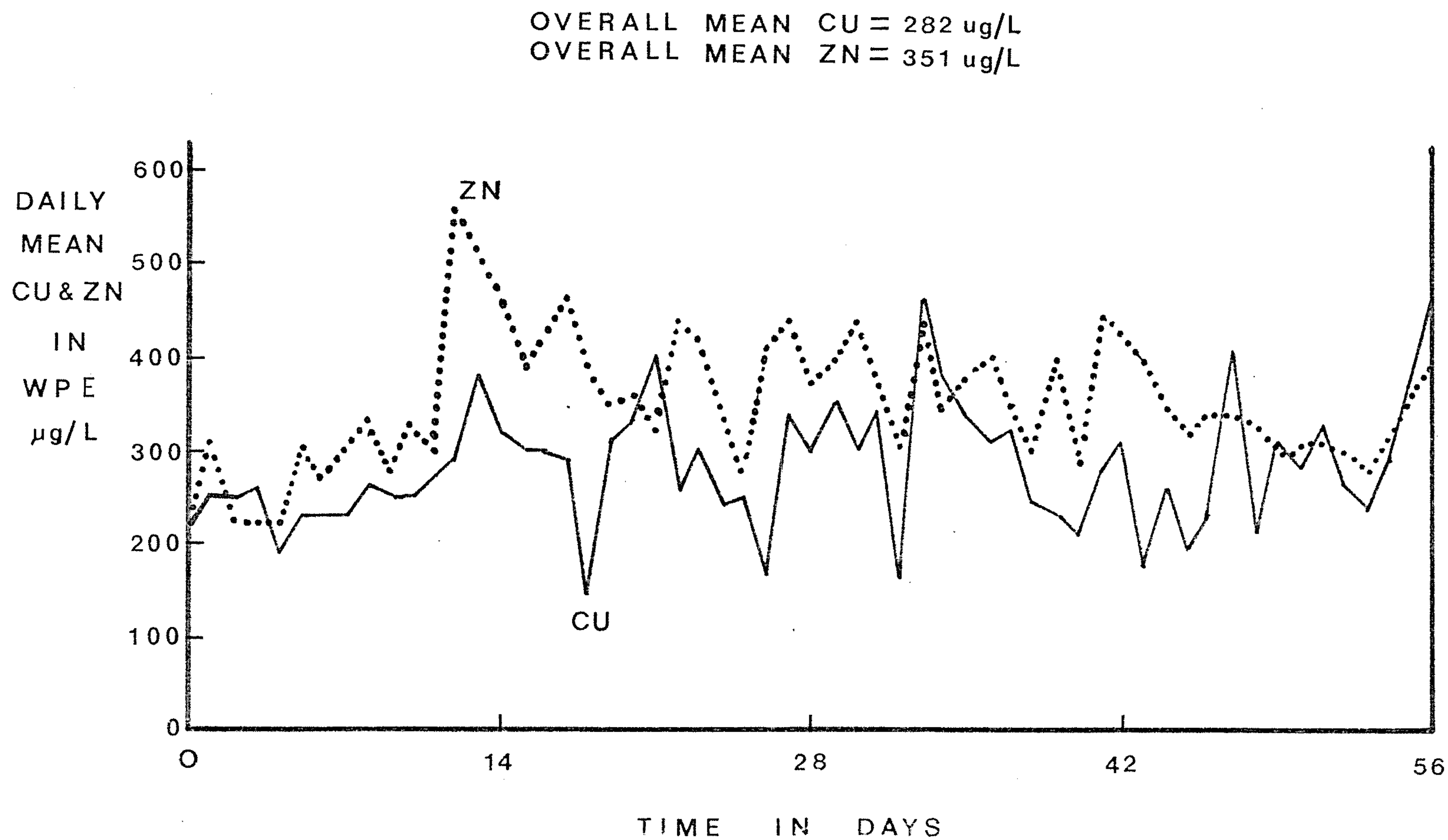

Fig. 4. Daily mean concentrations of total copper and zinc in undiluted West Point sewage effluent (WPE) during the 56-day chronic bioassay (September 15 to November 10, 1976).

Table 7. Copper and zinc concentration in METRO hourly raw sewage and effluent samples from 1500 to 2200 hr on July 22, 1976

	Raw sewage		Effluent	
Sample hour	Cu (μg/l)	Zn (μg/l)	Cu (μg/l)	Zn (μg/l)
1500	230	460	170	360
1600	370	450	180	360
1700	380	460	220	460
1800	390	470	220	410
1900	760	550	320	380
2000	200	380	200	340
2100	190	380	200	370
2200	170	370	140	270
24 hr composite average	N.A.*	N.A.	180	200

*Data not available.

4.4.2 Diluter Accuracy

Calculated concentrations of WPE in the test aquariums (Table 8) are based on daily measurements of flow rates both leaving the head tanks and entering the mixing tanks. While the calculated ranges for 0.5 and 1.0% WPE tightly bracketed the theoretical values, the calculated ranges for 2.5 and 5.0% WPE fell just below the theoretical. The depressed ranges occurred as a result of build-up of scum and algae which were more prevalent in the tubes siphoning these greater two concentrations of WPE.

4.4.3 Copper and Zinc Concentrations in Diluter

Results of the analysis of total copper and zinc content of diluter head tanks (seawater and WPE) and 2 test dilutions indicated that the trace metal concentrations may not have been diluted accurately (Tables 9 and 10). The mean copper and zinc concentrations obtained from the 5 replicate samples of WPE from the diluter head tank were approximately one-third and one-half, respectively, of the concentration of the 24-h composite WPE sample obtained by METRO laboratory personnel. Whether the difference of the means are statistically significant is uncertain as individual replicate values of the composite samples are not available. The 10% dilution of WPE contained 9.4% of the zinc concentration of undiluted WPE. This, however, was based on METRO analysis of 24-h composited WPE and may not be indicative of the actual concentration of the undiluted WPE at the time which the 10% WPE samples were taken.

4.4.4 Quality Control

The standard curves established throughout the period of use of the AAS

Table 8. Determination of diluter accuracy. Values are calculated percent WPE in seawater (v/v) and are based on 53 measurements (1 per day) of diluter flow rates

Intended concentration of WPE	Mean measured concentration of WPE	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 9. Copper content of concentrated and unconcentrated waters from head tanks and two test dilutions. Means are based on five replicates

Sample	Mean copper content of concentrate (mg/l)	S.E.	Calculated mean copper content of un-concentrated sample (mg/l)	S.E.
WPE	107.0	2.1424	.058	.0012
10% WPE	50.0	1.9849	.026	.0011
1% WPE	8.6	.4899	.003	.0004
Seawater	6.2	.3082	.002	< .0001
METRO WPE†			.150	

†24 hr composite sample analyzed by METRO laboratory personnel.

Table 10. Zinc content of concentrated and unconcentrated waters from head tanks and two test dilutions. Means are based on five replicates

Sample	Mean zinc content of concentrate (mg/l)	S.E.	Calculated mean zinc content of unconcentrated sample (mg/l)	S.E.
WPE	2.53	.2672	.181	.0190
10% WPE	.45	.0545	.032	.0038
1% WPE	<.05	–	<.0036	–
Seawater	<.05	–	<.0036	–
METRO WPE †			.340	

†24 hr composite sample analyzed by METRO laboratory personnel.

showed no drifting with time in the range in which samples were measured.

The mean concentration of copper and zinc in the NBS powdered bovine liver standard samples agreed statistically with the values at which the standard was certified.

4.4.5 Copper Content of Shiner Perch

The results of the mean whole-body copper concentrations of shiner perch are presented in Table 11. The mean whole-body copper concentration of shiner perch directly from Kopachuk State Park was significantly higher than that of the shiner perch which had been acclimated for 3 days without food. This may suggest that starvation eliminated a portion of gut contents which might otherwise have been considered part of the assimilated whole-body copper content. Shiner perch which had been acclimated and fed daily for 3 weeks prior to initiation of the experiment were analyzed to determine their background copper levels. These fish were also starved for 3 days prior to sampling. Results showed that these fish contained a significantly higher copper content than the 3-day acclimated group. This may indicate that the background level was elevated from increased copper levels from their diet or from the holding water. The copper concentration of the University of Washington moist food pellets was relatively higher than the concentration measured in most fish samples.

Results of the one-way ANOVA's of whole fish copper content by per cent WPE at 2, 4, 6, and 8 weeks and of whole fish copper content by weeks of exposure in the 4 test dilutions and seawater control are presented in Table 12. In general, the copper body burden did not change throughout the 8-week bioassay. Three of the nine ANOVA's, however, were determined to vary significantly

Table 11. Whole body copper concentration in shiner perch at the capture site, during acclimation and during 8-week bioassay

WPE † Concentration % v/v	Weeks of exposure	Number of 2 fish composites analyzed	Mean whole body Cu (mg/kg dry weight)	S.E.	Mean whole body Cu (mg/kg wet weight)	S.E.
Direct/Kopachuck	0	8	5.420	.676	1.114	.119
3 day acclimation	0	8	4.260	.467	.793	.097
3 week acclimation	0	8	4.693	.325	.896	.062
Seawater control	2	5	4.680	.997	.857	.146
.5	2	5	3.836	1.114	.701	.221
1.0	2	5	4.915	1.130	.880	.161
2.5	2	5	5.376	.882	.953	.109
5.0	2	5	4.737	.261	.924	.023
Seawater control	4	5	4.995	.840	.959	.120
.5	4	5	4.752	.986	.921	.189
1.0	4	5	3.243	.323	.696	.083
2.5	4	5	4.395	.294	.918	.084
5.0	4	5	4.609	.336	.885	.047
Seawater control	6	5	4.918	.986	1.114	.221
.5	6	5	4.787	.827	1.080	.220
1.0	6	5	4.990	.726	.934	.182
2.5	6	5	5.391	.570	1.056	.107
5.0	6	5	5.023	.604	.980	.113
Seawater control	8	5	4.213	.761	.811	.117
.5	8	5	4.415	.284	.876	.049
1.0	8	5	4.463	.842	.913	.126
2.5	8	5	5.027	.517	1.057	.092
5.0	8	5	4.746	.405	1.004	.046
UW Food Pellets		3*	6.618	.210	4.020	.157

† WPE = West Point effluent
*Three, 3-g samples.

Table 12. Results of SPSS (Statistical Package for the Social Sciences) "One-way" ANOVA of whole body copper content in shiner perch. Two asterisks (**) indicate that ANOVA was significant at the .05 level

Weeks of exposure	Percent WPE	One-way ANOVA	Significance of F	Tukey derived *a posteriori* contrasts
2	0–5	Cu by % WPE	.170	
4	0–5	Cu by % WPE	.003**	0, .5, 5% WPE
6	0–5	Cu by % WPE	.777	
8	0–5	Cu by % WPE	.271	
0–8	Seawater	Cu by weeks	.551	
0–8	0.5 % WPE	Cu by weeks	.240	
0–8	1.0 % WPE	Cu by weeks	.004**	6,2,0 weeks
0–8	2.5 % WPE	Cu by weeks	.023**	
0–8	5.0 % WPE	Cu by weeks	.536	

† WPE = West Point effluent.

among their respective groups. The variance appears to be attributable to whole-body copper concentration depressions which occurred at 4 weeks of exposure. Whether this depression was caused as a result of a change in the composition of the WPE prior to the 4-week sampling or whether it reflects individual variability is uncertain at this time. Another possibility is that feeding activity may have been reduced, but this was not observed to have occurred. Linear regression analysis did not yield any lines whose slopes were significant at the 0.05 level.

4.4.6 Zinc Content of Shiner Perch

The results of the mean whole-body zinc concentrations of shiner perch are presented in Table 13. There was no significant difference in whole-body zinc concentration between fish direct from Kopachuk State Park and fish which had been acclimated for 3 days without food at West Point. The gut contents may not have been of long enough duration for gut zinc to have been significantly excreted. There was no significant difference in whole-body zinc content between the 3-day acclimated fish and those fish which had been acclimated and fed for 3 weeks. The lack of change may have resulted from low dietary intakes due to competition in the populated holding tank.

The zinc concentration of the University of Washington food pellets was above the range of the zinc concentration measured for day-0 fish.

Results of the one-way ANOVA's of whole fish zinc content by per cent WPE at 2, 4, 6, and 8 weeks and of zinc content by weeks of exposure in the test dilutions of WPE are presented in Table 14. A significant change in the whole-body zinc content was determined. In general as time of exposure increased, there was a reduction in whole body zinc which was inversely related

Table 13. Whole body zinc concentration in shiner perch direct from capture site, during acclimation and during 8-week bioassay

WPE † concentration % v/v	Weeks of exposure	Number fish composites analyzed	Mean whole body Zn (mg/kg dry weight)	S.E.	Mean whole body Zn (mg/kg wet weight)	S.E.
Direct/Kopachuck	0	8	81.5	6.209	16.84	1.474
3-day acclimation	0	8	77.8	5.754	14.48	1.198
3-week acclimation	0	8	76.6	14.767	14.61	2.699
Seawater control	2	5	92.5	7.815	17.00	.923
.5	2	5	114.8	14.089	20.79	1.294
1.0	2	5	107.4	8.213	19.32	.633
2.5	2	5	103.6	7.639	18.03	2.488
5.0	2	5	109.6	12.470	21.36	1.877
Seawater control	4	5	112.1	8.708	21.66	1.917
.5	4	5	109.9	16.921	21.21	2.423
1.0	4	5	98.9	13.054	21.09	1.911
2.5	4	5	98.5	7.406	20.51	.514
5.0	4	5	103.2	10.674	19.75	1.077
Seawater control	6	5	111.470	9.4203	25.23	1.903
.5	6	5	103.518	16.430	23.31	4.352
1.0	6	5	96.034	8.7854	18.86	1.961
2.5	6	5	90.572	9.5684	17.77	1.880
5.0	6	5	94.220	9.3728	18.38	1.772
Seawater control	8	5	110.9	12.519	21.42	1.770
.5	8	5	103.8	20.753	20.42	2.628
1.0	8	5	105.5	11.932	21.66	1.878
2.5	8	5	81.4	23.676	16.87	3.452
5.0	8	5	83.6	12.630	17.62	2.035
UW food pellets		3*	101.0	6.894	61.33	4.073

† WPE = West Point effluent.
*Three, 3-g samples

Table 14. Results of SPSS (Statistical Package for the Social Sciences) "One-way" ANOVA of whole body zinc content in shiner perch. Two asterisks (**) indicate that the ANOVA was significant at the .05 level

Weeks of exposure	Percent WPE †	One-way ANOVA	Significance of F	Tukey derived *a posteriori* contrasts
2	0–5	Zn by % WPE	.034**	.5% WPE
4	0–5	Zn by % WPE	.270	
6	0–5	Zn by % WPE	.052	
8	0–5	Zn by % WPE	.036**	
0–8	Seawater	Zn by weeks	.001**	4,6,8 weeks
0–8	0.5% WPE	Zn by weeks	.003**	2,4 weeks
0–8	1.0% WPE	Zn by weeks	.001**	2,8,4 weeks
0–8	2.5% WPE	Zn by weeks	.016**	2 weeks
0–8	5.0% WPE	Zn by weeks	.001**	2,4 weeks

† WPE = West Point effluent.

to the per cent WPE concentration to which the fish were exposed. The general trend through time was an increase in the fish zinc concentration at 2 weeks with an ensuing reduction at 4, 6, and 8 weeks exposure. At 1% WPE an exception to this trend was noted where at 8 weeks an increase in zinc reached the 2-week level. Linear regression analysis yielded slopes which showed significant reductions of whole fish zinc content from the test dilutions at 6 and 8 weeks exposure.

4.5 Discussion

4.5.1 Copper Content of Shiner Perch

The data obtained from this study indicated, in general, no significant change in the whole-body copper content of shiner perch exposed to sublethal concentrations of sewage effluent for 8 weeks (Fig. 5). These results support the conclusions of other copper bioaccumulation studies using marine teleost fish. Thompson and Paton (1976) found no appreciable bioaccumulation of copper in muscle tissue of chum salmon fry *(Oncorhynchus keta)* in a controlled marine ecosystem study where nominal copper concentrations were 2.5 to 5.0 μg/ℓ (roughly 10 times greater than ambient levels). Saward, et al. (1975) found no appreciable bioaccumulation of copper in muscle tissue of the marine plaice *(Pleuronectes platessa)* in tank experiments with copper concentrations of 10, 30, and 100 μg/ℓ. Ambient copper levels for Saward's study were 3 to 5 μg/ℓ. McDermott, et al. (1976) analyzed the metal content in Dover sole *(Microstomus pacificus)* collected near a large marine sewage diffuser and also found no overall increase of tissue copper in the outfall-resident fish as compared to controls.

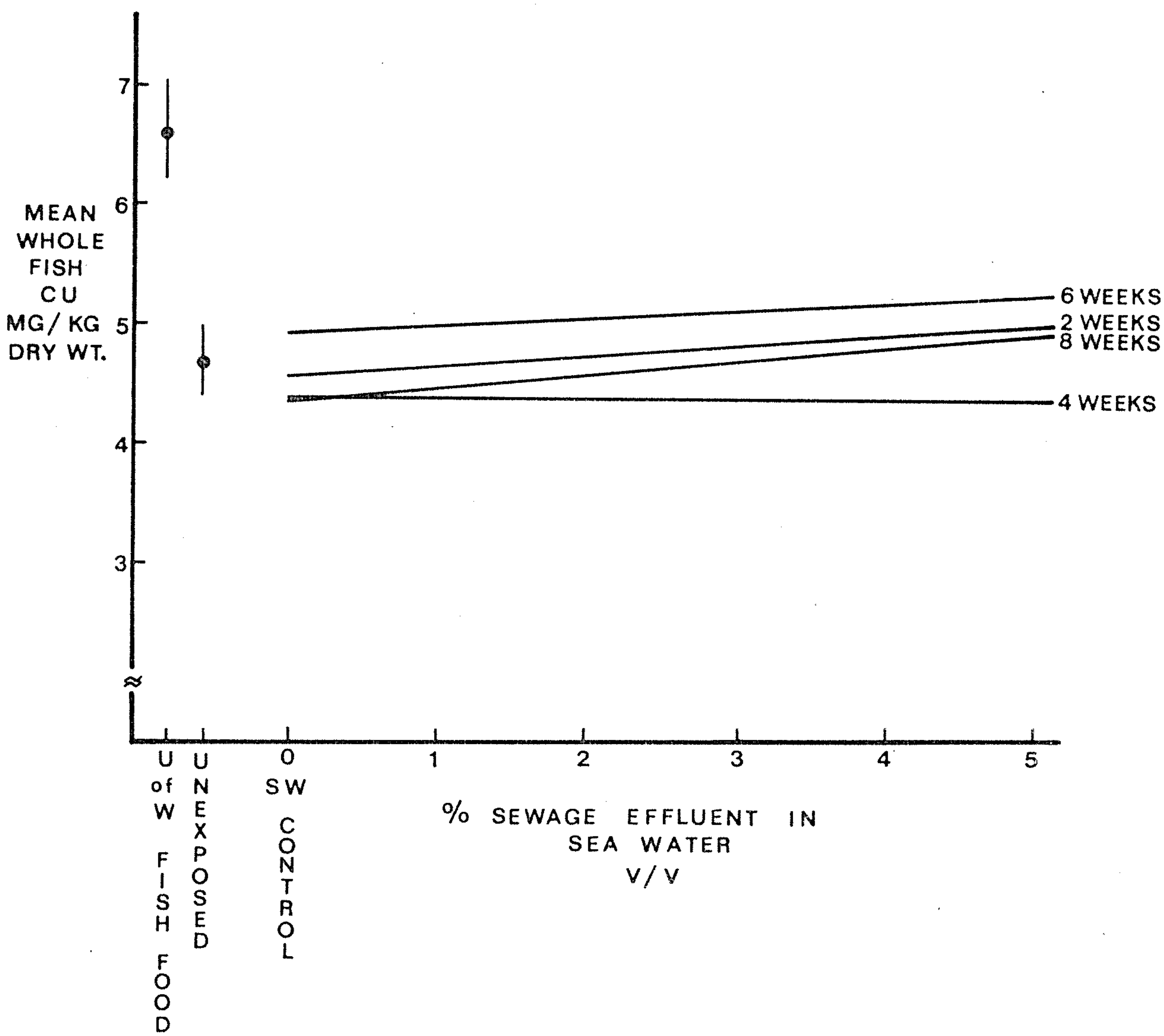

Fig. 5. Summary of least square lines of mean whole fish copper content vs. exposure to dilutions of primary treated sewage effluent for 2, 4, 6, and 8 weeks. Unexposed fish were acclimated and fed for 18 days then starved for three days.

The biological availability of copper from water has been shown to be reduced or eliminated by complexation in the presence of materials common in sewage plant effluent (Section 3.6). Even if complexation had not controlled the amount of seawater derived biologically available copper, then, with respect to at least this source, copper may still have not been accumulated. Preliminary acute toxicity studies of shiner perch in WPE showed that sublethal dilutions should not exceed 5% WPE in seawater. METRO data revealed that while the mean concentration of total copper in 100% WPE during the bioassay was 282 μg/ℓ (564 times the ambient concentration of Puget Sound seawater; 0.5 μg/ℓ [Huntamer, 1976]) the theoretical total copper concentration in the highest WPE dilution (5% WPE) was only 14.1 μg/ℓ. This may not have been great enough to allow excessive uptakes to occur. Thompson and Paton (1976) state that

> " . . . copper and other heavy metals may have threshold limits below which toxic and/or bioaccumulative effects are not produced. At threshold and higher levels, which will vary for a given organism and given set of ecological parameters, impairment or alteration of a detoxifying mechanism may occur. This might be the result of the inability of a system to clear the metal by protein production and subsequent enzymatic inhibition."

The theoretical levels of copper in the WPE dilutions used in this study were most likely well below the 100 μg/ℓ level in which plaice were not found to bioaccumulate copper (Saward, et al., 1975). Metallothionein proteins which are thought to regulate excess quantities of trace metals in fish and other aquatic and land organisms (Olafson and Thompson, 1974; Winge, 1975) may have effectively maintained the normal body levels of copper in the fish at the low levels of copper in the WPE dilutions.

Food has been cited as the major source of trace metal uptake in fish (Pentreath, 1972). Because retention of trace metals from food cannot exceed 100% and because the rate of feeding in fish is less than 10% of the body weight per day, the food (prey) of a predator fish must contain trace metal levels which are of equal magnitude or greater than that of the predator in order to maintain normal trace metal body levels (Pentreath, 1972). As stated earlier, some or all of the copper which is normally available to the shiner perch from seawater may have been complexed and made biologically unavailable in the WPE dilutions. The level of copper in the food pellets, however, which was notably higher than that measured in most test fish, was apparently great enough to offset the reduction of uptake from the water. This level of copper in the food was not apparently too high, however, to cause an unregulated increase in whole-body copper content through time.

4.5.2 Zinc Content of Shiner Perch

The results of this study document the whole body accumulation of zinc in shiner perch. The accumulation appears to be only temporary in experimental fish as elevated levels subsequently recede after a 2-week increase (Fig. 6 and 7). Such a recession may be evidence of a detoxification mechanism attempting to regulate the zinc body burden at a tolerable level. As mentioned earlier, marine teleosts have been reported to accumulate zinc directly from

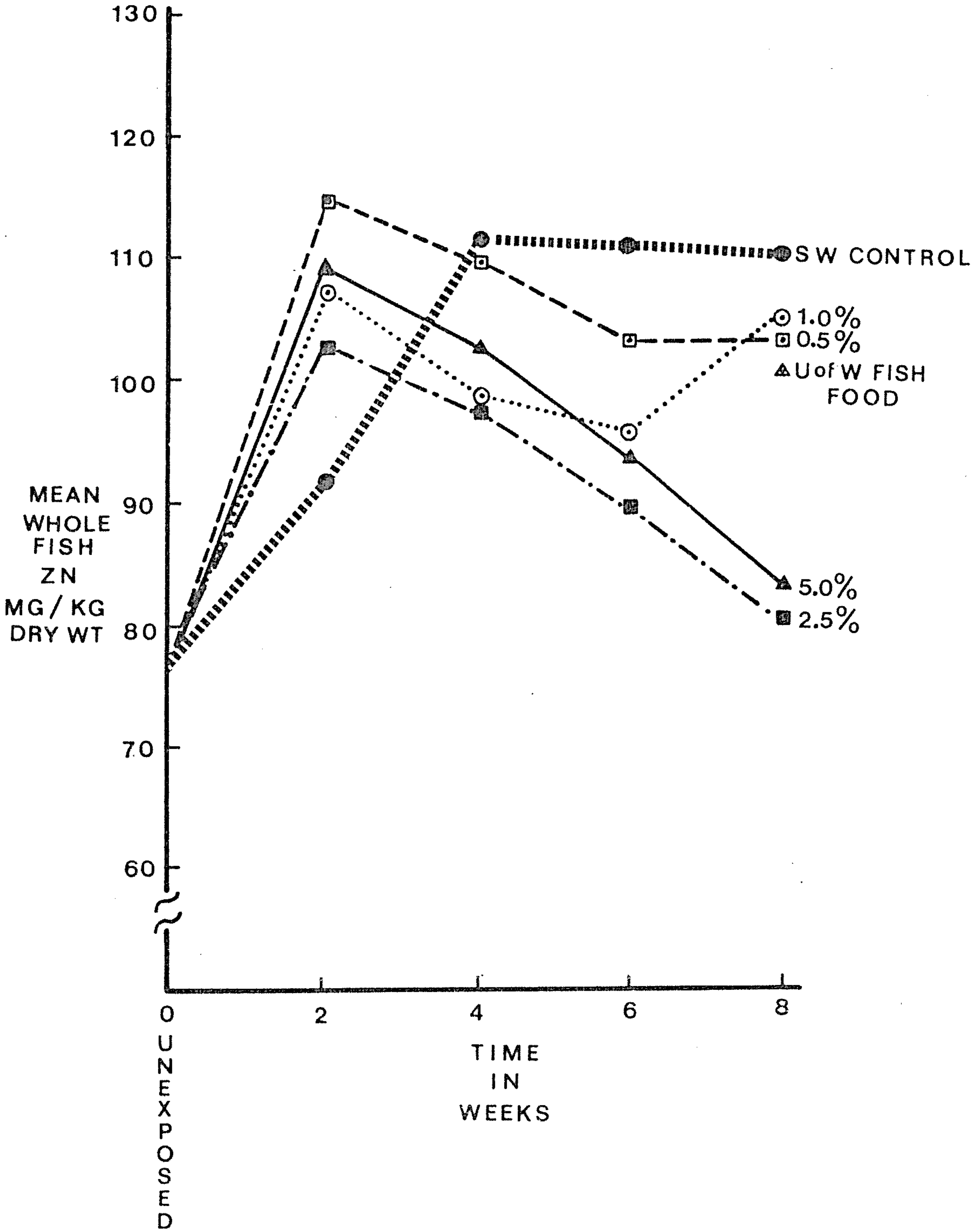

Fig. 6. Summary of mean whole fish zinc content vs. time of exposure to seawater and 0.5, 1.0, 2.5, and 5.0 percent primary treated sewage effluent in seawater (v/v). Unexposed fish were acclimated and fed for 18 days then starved for three days.

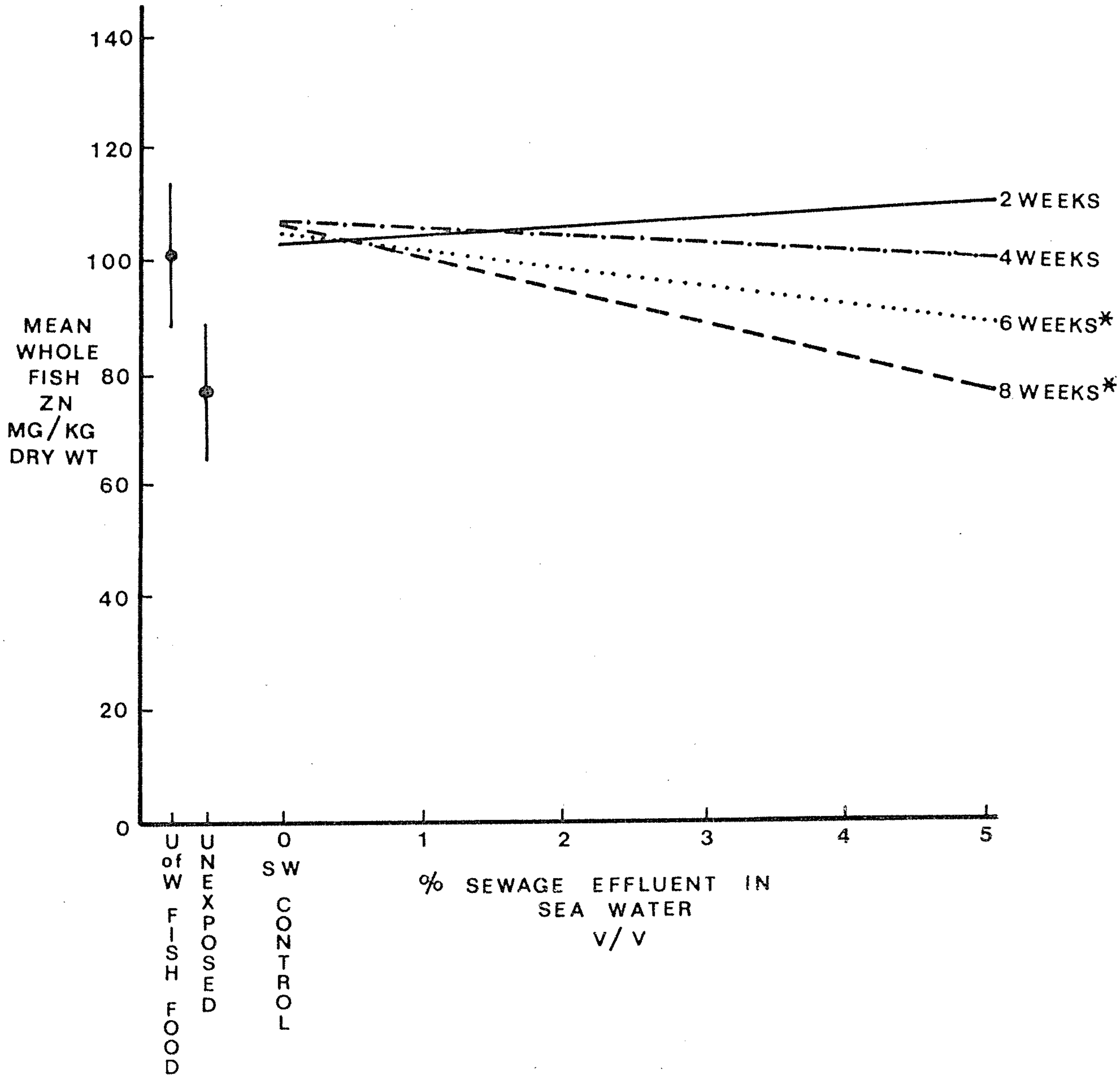

Fig. 7. Summary of least squares lines of mean whole fish zinc content vs. exposure to dilutions of primary treated sewage effluent for 2, 4, 6, and 8 weeks. An asterisk (*) indicates the slope of the line was statistically significant at the .05 level of significance. Unexposed fish were acclimated and fed for 18 days then starved for three days.

seawater but the amount is relatively minor compared to that taken up from the diet (Pentreath, 1973*c*). In this study, experimental as well as control shiner perch were fed a diet which contained levels of zinc greater than the mean zinc body burden of day-0 fish. While both groups accumulated elevated levels of whole-body zinc, at least partially derived from food, the rate of accumulation was greater in the experimental fish exposed to the various effluent dilutions. It is uncertain whether such an increased accumulation rate was the result of the additional uptake of available zinc from the effluent dilutions (which should theoretically have been complexed and unavailable) or resulted from the differences of physical and chemical water quality between the experimental dilutions and the seawater control. The physical and chemical quality of holding water has been reported to affect the rate of trace metal uptake in fish (Chapman, 1973).

The general differences occurred between control and experimental fish after peak levels of whole body zinc were reached. The controls, which reached peak zinc levels at a more gradual rate at 4 weeks, maintained the elevated level for the duration of the study. Perhaps in the absence of stress from the sewage effluent these fish were able to adjust to the elevated zinc body burden. Such a tolerance and maintenance of an elevated trace metal body burden has been reported for polychaetes (Milanovich, et al., 1976). In contrast, the zinc body burden of the experimental fish receded after reaching peak levels at a more rapid rate at 2 weeks. It may have been that such an action was the result of a rate of accumulation which was too rapid and physiologically intolerable.

It appears that an excretory and/or detoxification mechanism(s) were initiated in the experimental fish but not in the control fish. Zinc excretion in marine teleosts has been reported to occur primarily through the kidney and possibly to some extent through the feces (Pentreath, 1973*c*). Reduction of the zinc body burden continued for the duration of the study in the fish exposed to the greater concentrations of sewage effluent (2.5 and 5.0% v/v WPE) and ultimately reached the day-0 level. Reduction of the zinc body burden did not continue for as long in the fish from the 0.5 and 1.0% v/v WPE but maintained at whole-body concentrations between the peak and day-0 levels. Apparently, the detoxification mechanism may have been terminated sooner in the fish from the lower effluent concentrations and allowed the body burden to reach a tolerable yet elevated level.

4.6 Conclusions

Copper concentrations in whole shiner perch exposed to sublethal dilutions of primary treated sewage effluent showed no general change from that exhibited by seawater control fish for the same period of time. Suspected low levels of copper and complexing agents present in the effluent dilutions appear to have been responsible for the lack of accumulation.

The rate of accumulation and retention behavior of whole-body zinc concentrations in shiner perch appears to indicate the action of a detoxification mechanism. The action seems to be triggered by sewage effluent dilutions $\leq$ 5% v/v.

4.7 Recommendations

Several recommendations for further laboratory study are offered in order to more fully understand the effects of trace metal emissions from primary treated sewage effluent on the marine biota of West Point. The recommendations are:

1. Investigate the accumulation of trace metals at the tissue level. It is possible that measurements at the tissue level may indicate significant bioaccumulation where no overall uptake was apparent from previous whole-body analyses. Such an investigation should help to understand the mechanisms controlling the distribution and detoxification of elevated levels of trace metals in aquatic life. Organisms of greater size or of greater number than those used in this study would be required.

2. Investigate trace metal accumulation through the food chain. Food chain magnification may prove to be more responsible for increased levels of trace metals in biota near the outfall than is direct accumulation from the water. An example of such a study might be to feed a species of fish, endemic to the West Point area (such as English sole), a prey species (such as polychaetes) which has a higher trace metal body burden than control prey from an uncontaminated region. This could also be conducted while the predator species is undergoing sublethal exposure to sewage effluent dilutions.

3. Monitor the concentration and chemical states of trace metals in the test dilutions during bioassays using pulse polarography or other appropriate techniques.

4. Investigate the seasonal changes in trace metal uptake from food or seawater/effluent dilutions.

5. Investigate the trace metal uptake in other aquatic species and other stages of development, especially larval stages.

6. Relate laboratory bioaccumulation data to field data, such as that collected by Olsen (1976) for the same species.

5.0 SHINER PERCH BEHAVIORAL RESPONSES IN CHLORINATED WEST POINT SEWAGE EFFLUENT

5.1 Introduction

Our toxicity bioassays of chlorinated West Point sewage effluent have shown that the effluent is acutely toxic to several marine fish species at approximately 15% effluent in seawater and chronically toxic in concentrations of effluent as low as 0.5% (Stober, et al., 1977*a*). However, the actual impact of the sewage effluent in Puget Sound receiving waters is difficult to assess because fish and many invertebrates are capable of movement into or out of a discharge plume. If organisms are attracted to plumes of sewage effluent, the toxic effects in the field could approximate those seen in the laboratory. If, however, organisms avoid sewage plumes, the toxic effects may be minimized, but the plume area must then be considered an uninhabitable area for those organisms avoiding it and the ecological impacts caused by the plume must be assessed in a different manner. Thus, the behavioral responses (avoidance or attraction) of the resident species become important when considering the ecological impact of each sewage discharge.

5.2 Materials and Methods

The behavior responses of shiner perch to dilutions of chlorinated West Point sewage effluent were tested in a behavior trough located in a 10 x 10 ft metal shed. The test trough design was a modification (Meldrim, et al., 1974) of an avoidance trough first used by Shelford and Allee (1913) which employed a counter-flow water pattern. The test and control solutions entered from opposite ends of the tank and flowed toward a central drain (Fig. 8). The test trough had replicate sides which provided for simultaneous duplication of each test with reversal of test and control solutions to minimize any end preference by the fish not related to the test/control solutions. The control and test solutions were prepared by a small diluter system based on adjustable glass siphon tubes. The solutions were actively stirred in the mixing tanks and delivered to the test tank by pumps regulated by flow meters to deliver approximately 2ℓ/min. The water level in the test trough was maintained at a depth which allowed fish to swim freely over the central drain and was regulated by a pump and valve on the drain line. The test trough was shielded on all sides and top by sheets of white plastic and indirectly lighted from above through the plastic blind. Fish movements were remotely monitored by closed circuit television and video tape equipment with time-lapse capabilities (Fig. 9).

Behavior tests were conducted by placing 5 shiner perch in each replicate side and determining the amount of time the perch spent in the test and control solutions after a 15-min acclimation period. Twenty replicated tests (n = 40) were conducted at 0% effluent (control seawater in all 4 quadrants) and 10 replicated tests (n = 20) each in 1, 5, 10, 15, and 20% v/v chlorinated effluent. Physical and chemical sampling for temperature, salinity, pH, ammonia, turbidity, and total residual chlorine was conducted prior to each test run. Fish responses (avoidance or preference) were tested for deviation from randomness by the t-test.

5.3 Results

Shiner perch showed a statistical preference for 1, 5, and 10% chlorinated effluent, avoidance of 15 and 20% effluent, and random movement in the test

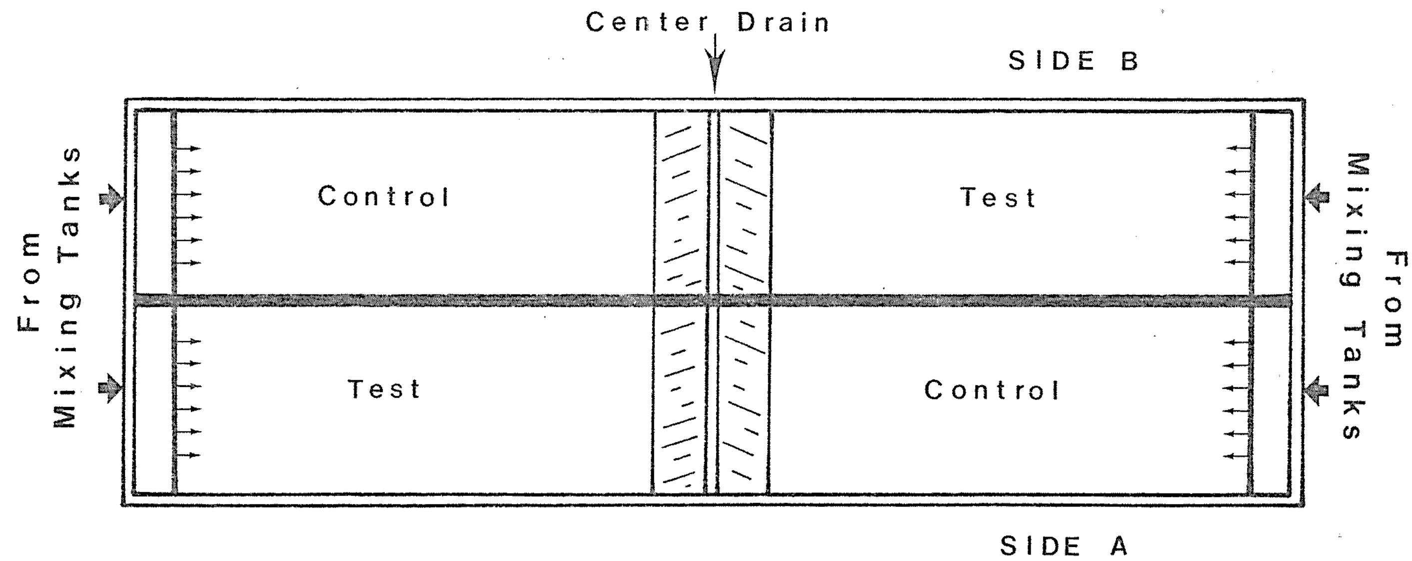

Figure 8. Diagram of the behavior test tank.

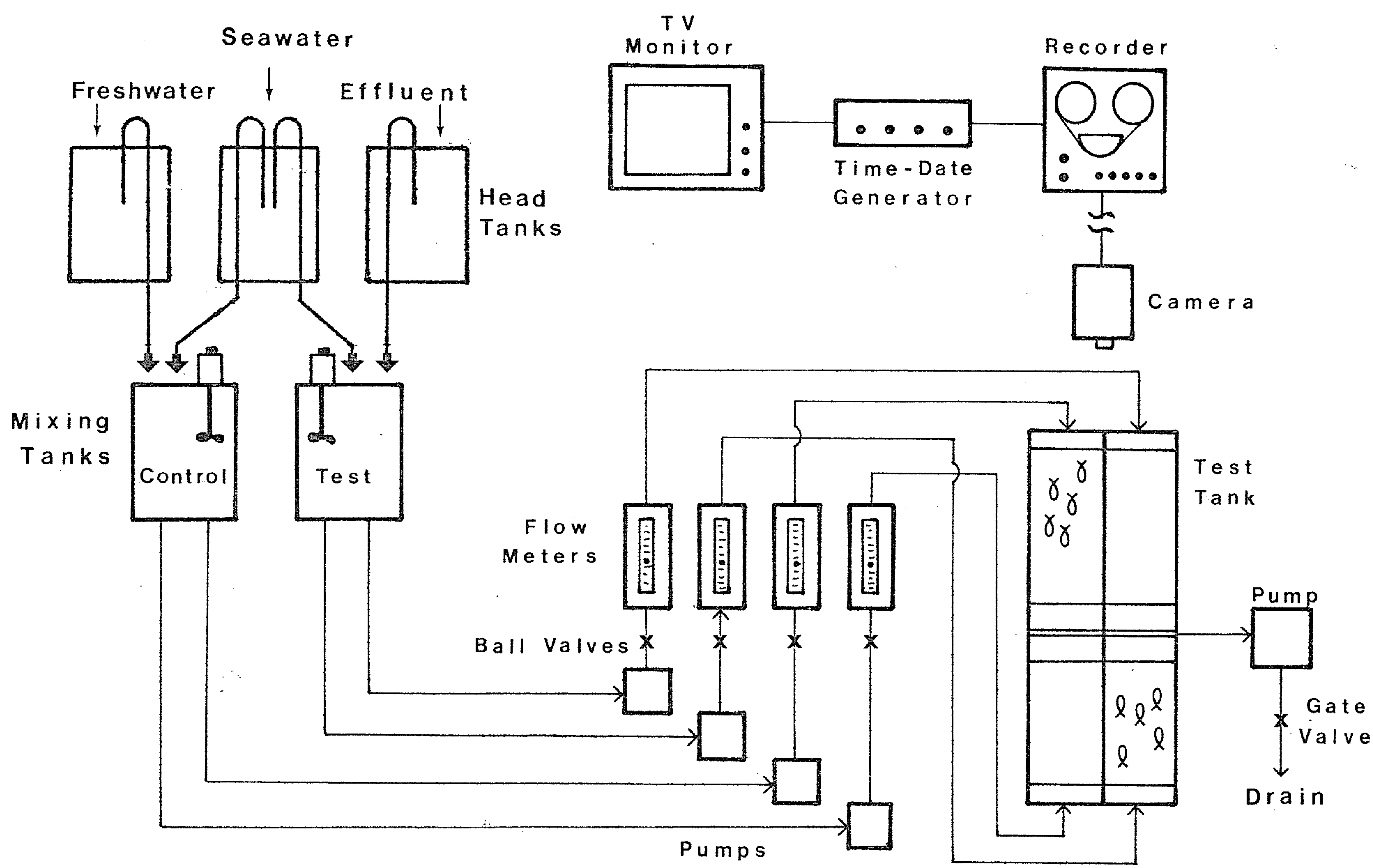

Figure 9. Schematic diagram of the behavior test system located in a 10 x 10-ft metal shed. The TV monitor, time-date generator, and video tape recorder are remotely located in the main laboratory. The white visqueen blinds around the test tank and overhead lights are not shown.

trough when effluent was not present (0%) (Table 15, Fig. 10).

Subjective analyses of shiner perch behavior from video tape recordings yielded similar conclusions. The perch generally moved freely and without hesitation on both ends of the test trough when effluent was not present. Addition of 1, 5, or 10% effluent caused fish to linger on the effluent end longer than normal. Shiner perch obviously did not like 15 or 20% effluent. These concentrations caused the perch to hesitate or turn away from the test/control solution interface at the center drain or caused them to return promptly to the clean seawater if they did venture into the effluent end.

Average temperature, salinity, pH, ammonia, turbidity, and total residual chlorine values are summarized in Table 16. The average total length of all shiner perch used in the behavior tests was 74.6 mm. As noted in Table 16, salinity values on both the control and test ends decreased with an increasing concentration of effluent. The effluent was essentially a freshwater discharge, thus resulting in a reduction of salinity on the test end. The equivalent reduction of salinity on the seawater control end was due to the addition of an equivalent amount of freshwater to maintain an equal density interface at the center drain. Preliminary tests revealed that density differences greater than a few tenths ppt salinity resulted in unacceptable mixing of the test and control solutions with a resulting lack of a distinct interface at the center drain. Additionally, equal salinities on each end were needed to minimize behavior responses due to osmotic stress.

5.4 Discussion

Shiner perch avoided levels of chlorinated sewage which would have been lethal to them in 96 hours. However, they were attracted to effluent concentrations which have been shown to produce sublethal damage to the integrity

Table 15. Average times spent by shiner perch in dilutions of chlorinated West Point effluent and t-test levels of significance of prererence or avoidance responses.

% v/v chlorinated effluent on test side	Number of replicated tests	Avg. time spent in test solution (10-min test)	Level of significance*
0 (control)	20	4.85	
1	10	6.20	PP
5	10	6.18	PP
10	10	5.76	P
15	10	3.00	AA
20	10	2.82	AA

*P = Preference significant at $p \leq 0.05$; PP = $p \leq 0.01$
A = Avoidance significant at $p \leq 0.05$; AA = $p \leq 0.01$

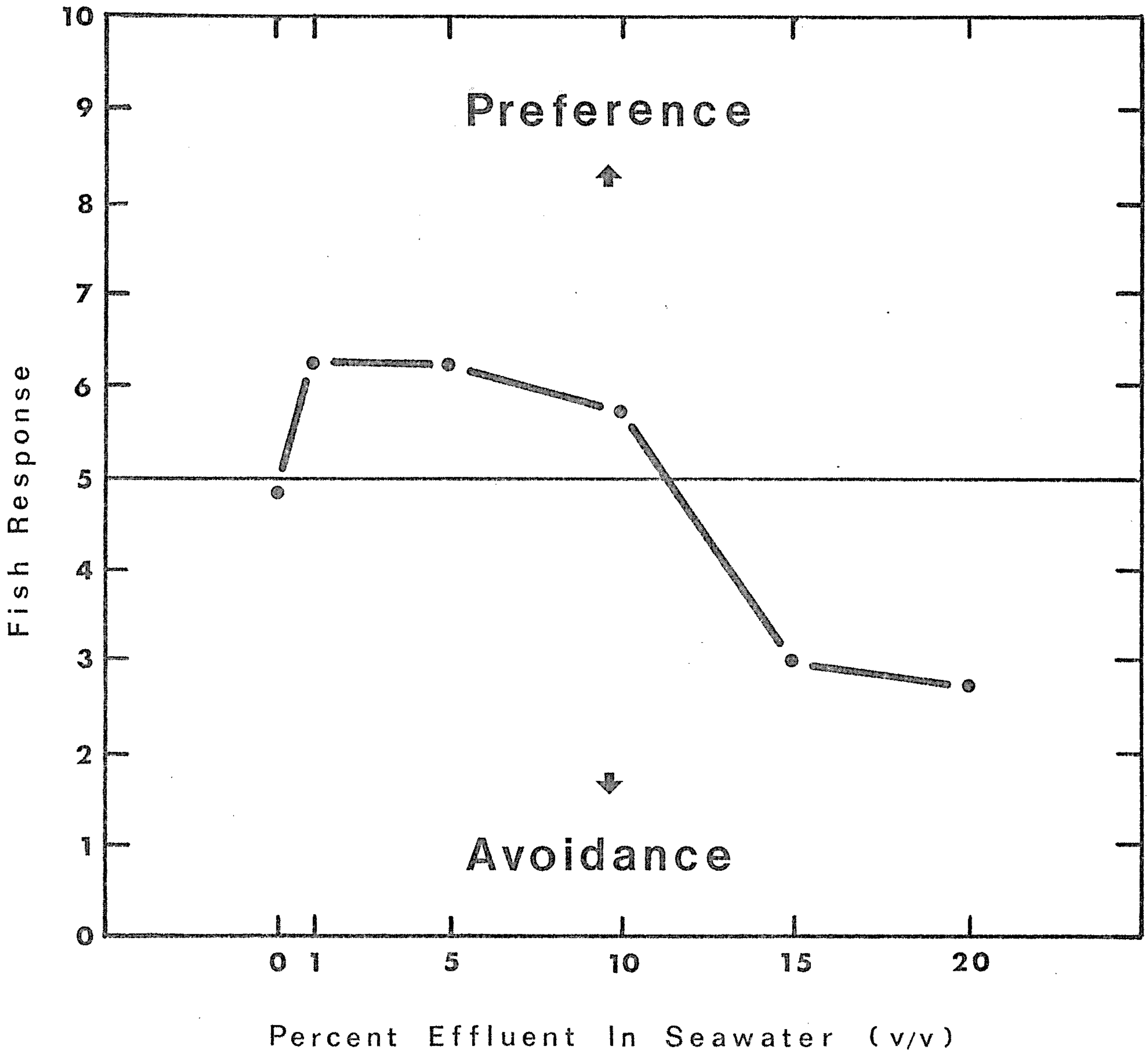

Fig. 10. Pattern of preference or avoidance responses of shiner perch to dilutions of chlorinated West Effluent in seawater.

Table 16. Average temperature, salinity, pH, ammonia, turbidity, and total residual chlorine, in the control and test (effluent) ends of the chlorinated effluent behavior tests with shiner perch.

% v/v Chlorinated Effluent on Test Side	Temperature C°	Salinity 0/00	Dissolved Oxygen ppm	pH	Ammonia ppm	Turbidity JTU	Total Residual Chlorine ppm
0 Control	9.8	29.8	9.6	8.0	0.0	0.5	0.00
Test	9.8	29.8	9.6	8.0	0.0	0.5	0.00
1 Control	10.4	29.3	9.1	8.0	0.0	0.1	0.00
Test	10.3	29.3	9.0	8.0	0.3	0.5	0.00
5 Control	10.1	28.1	8.8	8.1	0.0	1.0	0.00
Test	10.1	28.0	8.4	8.0	1.3	4.5	0.02
10 Control	9.9	26.4	9.7	8.0	0.0	0.8	0.00
Test	9.7	26.4	9.0	7.8	2.3	9.4	0.12
15 Control	10.9	25.1	9.2	8.1	0.0	0.8	0.00
Test	10.7	25.0	8.1	7.8	3.6	15.6	0.19
20 Control	11.2	23.8	9.1	8.1	0.0	0.5	0.00
Test	10.9	23.8	7.5	7.6	4.9	24.0	0.20

of the gills and changes in blood chemistry and blood cell morphology (Buckley, et al., 1976). Other studies conducted at West Point have shown shiner perch to be attracted to heated seawater (Δt 4-8°C above ambient) chlorinated to 10 to 100 ppb total residual oxidant (Stober, et al., 1977*b*). Similarly, Sprague and Drury (1969) found that rainbow trout *(Salmo gairdneri)* were repeatedly attracted to 100 ppb free chlorine in freshwater, but avoided 1, 10, 1000, and 10,000 ppb free chlorine. However, our studies of coho salmon behavior in chlorinated seawater showed significant salmon avoidance of all chlorine concentrations tested (2, 10, 25, 50, 100, 250, and 500 ppb total residual oxidant). These studies suggest that avoidance/preference responses to chlorine may be species dependent. Each fish or invertebrate species may react differently to chlorine in an aqueous environment with different thresholds of avoidance or preference.

Chlorine may be partly responsible for the preference-avoidance response reported herein. However, behavior studies of shiner perch (or other fishes) in unchlorinated sewage effluent would be required to partition the effect of chlorine in sewage effluent dischargedto marine waters.

Miller, et al. (1977) have noted differences in frequency and relative abundance of deep water demersal fishes in the vicinity of the West Point sewage outfall which may be associated with wastewater discharge by METRO. Specifically, they reported the apparent replacement of slender sole *(Lyopsetta exilis)* by rex sole *(Glyptocephalus zachirus)*, a greater total abundance during most seasons, a greater abundance of ratfish *(Hydrolagus colliei)*, and a reduction in both diversity and species richness. These differences reported by Miller, et al, may be the result (in part, at least) of differential avoidance/preference repsonses to the chlorinated effluent by the various fishes normally associated with this environment.

6.0 ECHINODERM FERTILIZATION BIOASSAY

6.1 Introduction

It has become increasingly evident that the results of conventional 96-h flow through fish bioassays of sewage effluent are only gross indicators of the toxicity of a complex and ever-changing waste. Thus, sensitive, short-term tests are required that can bioassay a discrete, chemically defined sewage sample, the results of which can be related to the biological response observed in the bioassay. One test which has proven particularly valuable is the "success of fertilization" test described in this report. This test is a refinement of test methods devised by other researchers using fertilization and subsequent development of the eggs and larvae of various invertebrate species (Woelke, 1972; Kobayashi, 1971, 1973, 1974; Kobayashi, et al., 1972). Embryo development tests are very sensitive, but have two major drawbacks: 1) development is usually based on a time period of 24 to 48 hours, and 2) the researcher is often faced with the subjective reconciliation of mortality versus abnormal development.

The "success of fertilization" test, using sea urchin or sand dollar gametes, is uniquely different from egg development because it is a bioassay of the sperm cell and not the egg. This test is conducted in a matter of minutes instead of days or months and is more sensitive than 96-h fish bioassays. The eggs act only as indicators of sperm viability.

Muchmore and Epel (1973) conducted fertilization tests with several marine invertebrates using chlorinated and unchlorinated domestic sewage in California. They found chlorinated sewage to be a potent spermicide, active in inhibiting fertilization in calculated concentrations of available chlorine as low as 0.05 ppm. Unchlorinated sewage or chlorinated sewage dechlorinated with sodium thiosulfate was found to be less toxic to the sperm.

6.2 Materials and Methods

The fertilization bioassays were conducted by exposing sand dollar *(Dendraster excentricus)* sperm to seawater dilutions of 0.5 to 25% chlorinated or composite West Point effluent. The chlorinated effluent was collected several hundred meters downstream of the postchlorinator injection point. The composite samples were collected upstream of the postchlorinator by continuous aliquot sampling over a 24-h period and subsequently analyzed by METRO for various physical/chemical constituents and trace metals.

Subsequent to 15 min exposures to the effluent, sperm were added to cups contained eggs. The resulting fertilization success in each effluent dilution was tabulated by counting the number of eggs in each sample that had an elevated fertilization membrane. Total residual chlorine concentrations were determined by back titration with a Wallace & Tiernan A-790 amperometric titrator.

The calculated value at which successful fertilization was reduced to (SF50%) was found using the BMD03S Biological Assay Computer Program for Probit Analysis (Finney, 1952).

6.3 Results

The results of 21 sand dollar fertilization bioassays using composite effluent (unchlorinated or slightly prechlorinated at plant intake) and 15 tests using postchlorinated effluent are summarized in Table 17.

Table 17. Average 50% success of fertilization (SF50) values, standard deviation, and range for 21 composite effluent samples and 15 post-chlorianted effluent samples using sand dollar eggs and sperm.

Effluent Source	Number of Bioassays	Average Total Residual Chlorine (ppm)	Average SF50 (% Effluent)	Standard Deviation	Range
Composite Effluent	21	0.04	4.41	3.66	0.14-14.26
Post-Chlorinated Effluent	15	1.01	2.06	1.51	0.11-5.02

The SF50 is approximately twice as high for the unchlorinated (or slightly prechlorinated) composite effluent than for the postchlorinated effluent. However, the variability, as noted by the range, indicates that highly variable results can be obtained between tests. This high variability is primarily due to the ever changing toxic characteristics of the effluent and secondarily due to differences in sensitivity of different batches of of eggs and sperm.

Coefficients of correlation (r) were calculated for the 21 composite effluent SF50's versus the physical-chemical parameters measured by METRO. No significant correlation was found between the SF50's and rainfall, average flow, peak flow, temperature, pH, dissolved oxygen, BOD, COD, suspended solids, settleable solids, residual chlorine, cadmium, chromium, hexavalent chromium, copper, mercury, nickel, lead, or zinc.

6.4 Discussion

The results reported here indicate that sand dollar sperm exposed to chlorinated sewage for 15 minutes (average SF50 = 2.1 %) are approximately 5-10 times more sensitive than either shiner perch or juvenile English sole to chlorinated sewage effluent in 4-day tests (96-h LC50 = 15.4 and 16.1%, respectively). Sand dollar sperm exposed to unchlorinated (or slightly prechlorinated) composite sewage samples remained viable in up to twice the amount of sewage (average SF50 = 4.4 %). Similar but less sensitive results were obtained with shiner perch and juvenile English sole exposed to sewage which had been dechlorinated with gaseous sulfur dioxide (LC50 = 28% and 32%, respectively).

These tests suggest that the highest concentration of chlorinated West Point effluent which can be discharged to Puget Sound without causing an observable toxic response to biological systems approximates 0.2% (average SF50 for chlorinated effluent x 0.1 "safety" or "application" factor). A maximum acceptable concentration of unchlorinated or dechlorinated effluent probably approximates 0.4-0.5%. These maximum acceptable concentrations are in close agreement with our previous suggested "safe" level for discharge based on chronic bioassays with shiner perch and juvenile English sole (Stober, et al., 1977*a*). Congestion, hemorrhaging, edema, and separation of epithelium from underlying vascular tissue were found in gill tissues of fish exposed to concentrations of chlorinated West Point effluent as low as 0.5%. Buckley, et al. (1976) found that the blood chemistry and blood cell morphology of yearling coho salmon were adversely affected in concentrations of chlorinated West Point effluent of 1.1 and 3.6%. The salmon were not affected in 0.3% effluent. Buckley, et al. thus concluded that the maximum acceptable concentration for chlorinated West Point effluent was between 0.3 and 1.1%. Based on salmon blood chemistry and shiner perch gill tissue pathology, our previous conclusion was that "0.3% chlorinated West Point effluent approximates an upper limit for a maximum acceptable concentration for discharge to Puget Sound receiving waters, if certain trace metals (i.e., mercury, chromium, copper) can be controlled" (Stober. et al., 1977). Based on results of the fertilization bioassay, the maximum acceptable concentration for discharge is 0.2% chlorinated West Point effluent.

7.0 REFERENCES

7.1 Copper and Zinc Bioaccumulation

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