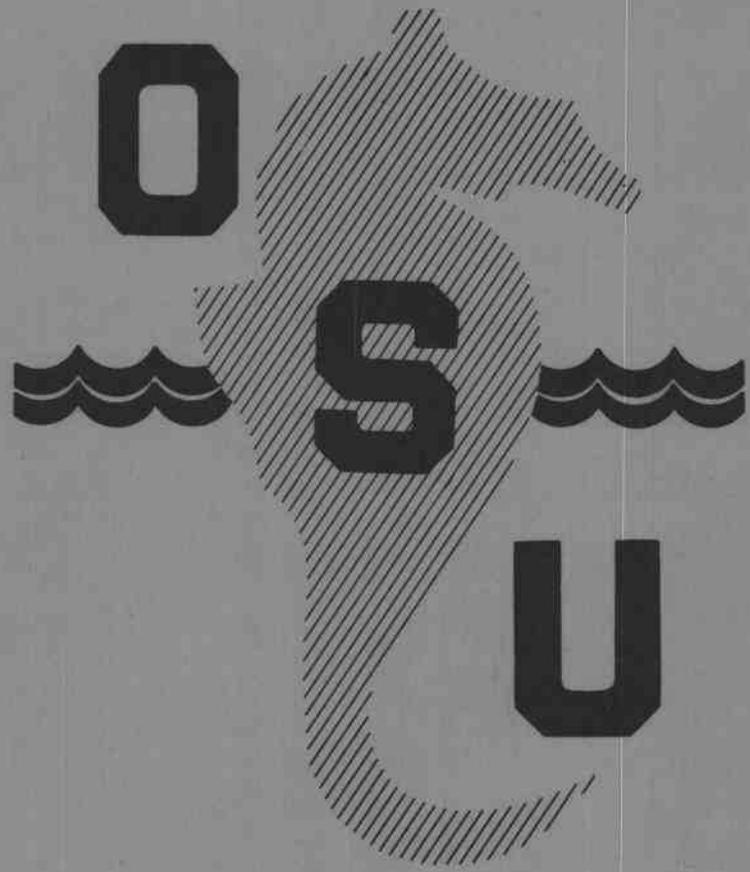


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# OCEANOGRAPHY



OREGON STATE UNIVERSITY

FINAL REPORT

**Biological Effects Data:  
Fluoride and Sulfur Dioxide**

by

Robert L. Holton  
Richard J. Ulbricht  
and  
John B. Morgan

Submitted to  
Alumax Pacific Aluminum Corporation

Contract Period  
1 November 1973 through 30 April 1975

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April 1975

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NOTICE

Much of the narrative in this report is preliminary and is based upon incomplete analysis of portions of the data. Consequently the reader is cautioned that conclusions presented are tentative and are subject to change when the complete data base has been more thoroughly digested.

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## INTRODUCTION

The Alumax Pacific Aluminum Corporation has proposed construction of an aluminum reduction facility near Youngs Bay at Warrenton, Oregon. This report comprises one part of the final report to Alumax on a research project entitled, "Physical, Chemical and Biological Studies of Youngs Bay." It presents data pertaining to the potential biological effects of fluoride and sulfur dioxide, two potentially hazardous plant-stack emissions, on selected aquatic species of the area. Companion volumes provide a description of the physical characteristics (Boley *et al.*, 1975), the geochemistry, (Johnson and Cutshall, 1975), and the aquatic animals present in Youngs Bay and adjacent ecosystems (Higley and Holton, 1975). An introductory volume provides general information and maps of the area, and summarizes the conclusions of all four studies (Slotta, Cutshall, and Holton, 1975).

The primary objective of the biological effects study was to determine the effects of fluoride on selected estuarine organisms, including

- 1) determination of the levels of fluoride in the marine ecosystem that will prove toxic to important sport and commercial species or important species in their food chains;
- 2) examination of sublethal effects that fluorides added to the marine ecosystem will have on sport, commercial or food-chain species;
- 3) determination of the effect of fluorides on the rate of carbon fixation in phytoplankton.  
(Slotta *et al.* Research Proposal Addendum, 1973)

Although fluoride is not the only potentially harmful substance to be released by the proposed plant (Cramer and Bowers, 1974), it has been the major cause for public concern. Although there is a dearth of information available on the effects of fluoride on estuarine biota, it has

been demonstrated that at certain concentrations free fluoride (F<sup>-</sup>) is harmful to certain aquatic species (Neuhold and Sigler, 1960; Sigler and Neuhold, 1972).

Sulfur dioxide is a second airborne substance that would be emitted from the Warrenton plant (Cramer and Bowers, 1974). The effects of this gas as sulfite in water have also been shown to pose a potential danger to specific aquatic species (Idler, 1969; Marier, 1972).

The data from the two phases of the experimental program are included in this report: (1) lethal studies on the effects of selected levels of fluoride and sulfur dioxide on the survival rate of eleven Youngs Bay faunal species from four phyla, and (2) sublethal studies on the effects of fluoride and sulfur dioxide on the rate of primary production by phytoplankton.

Lethal studies on the interaction of the two pollutants and sublethal studies using selected animal species were begun in 1974 and are being completed in 1975. The data from these studies will be reported in a supplementary report.

The laboratory facility used to conduct this work is located in the Oregon State University Marine Science Center on Yaquina Bay at Newport, Oregon. These experiments were conducted in a 400-square foot wet laboratory, containing twelve water tables for flowing seawater which is pumped directly from the bay.

## LETHAL EFFECTS: FAUNAL SURVIVAL RATES

### LITERATURE SURVEY

A literature review was undertaken to determine the present status of knowledge on fluorides in estuarine and marine systems. The studies summarized below indicate that the focus has been on the uptake and toxicity of fluorides in several species of animals. Little effort has been directed toward the more subtle, long term sublethal effects. Since these effects are potentially as significant in an ecological context as acute toxicity, it seems imperative that studies of such effects now be pursued. A more limited review of sulfur dioxide effects literature was also undertaken.

### *Fluoride Effects*

Only a few types of animals--humans, livestock, and rodents--are extensively represented in the fluoride effects literature (see Smith, 1966; National Academy of Sciences, 1971). Fluoride effects in intact fish have been reported during the past 15 years by de Ross, 1957; Holland *et al.*, 1960; Neuhold and Sigler, 1960, 1962; Angelovic, Sigler and Neuhold, 1961a, b; Herbert and Shurben, 1964; Vallin, 1968; Sigler and Neuhold, 1972. Fluoride effects in intact marine and estuarine invertebrates have been reported more recently (Stewart and Cornick, 1964; Moore, 1969, 1971). Fluoride effects in several estuarine invertebrates and fish species have been reported by Hemens and Warwick (1972), Hemens, Warwick and Oliff (1974) and Wright and Davison (1974).

Symptoms of acute fluoride intoxication in carp and rainbow trout have been described (Roos, 1957; Neuhold and Sigler, 1960, Angelovic, Sigler, and Neuhold, 1961a). The sequence begins with a general lethargy, followed by violent, erratic movements. Death follows with the muscles in a partially or completely contracted state. Neuhold and Sigler (1960) reported that epithelial mucous cells in the head and gills produce excessive quantities of mucous and are presumably important in fluoride excretion. Another defense against fluoride intox-

ication is the formation of a stable mineral complex in the skeleton (Roos, 1957; Neuhold and Sigler, 1962; Hemens, Warwick and Oliff, 1974; Wright and Davison, 1974). The trout were much more sensitive to fluoride intoxication than were the carp.

Fluoride toxicity in fish is affected by a variety of factors. Hard water lessens the response of salmon and trout to fluoride poisoning (Neuhold and Sigler, 1960; Herbert and Shurben, 1964; Vallin, 1968).

Neuhold and Sigler (1960, 1962) noted that fluoride toxicity in rainbow trout is dependent upon the chloride concentration in the water. Fish tempered in NaCl (34 ppm) for 48 hours had decreased responses to the fluoride concentrations--LC<sub>50</sub> values of 6 and 22 ppm for the nontempered and tempered fish, respectively. Chloride secretory and fluoride excretory systems may be associated with each other in the same system (Neuhold and Sigler, 1962).

Fluoride intoxication in fish is also influenced by temperature (Neuhold and Sigler, 1960; Angelovic, Sigler and Neuhold, 1961a, b). Fluoride concentrations were 0, 2, 4, 7, 13, and 25 ppm. Increases in temperature decreased the LC<sub>50</sub> value and response sensitivity of rainbow trout (Angelovic, Sigler and Neuhold, 1961a). Fluoride levels did in fact influence the times to initial and final mortality, rate of mortality, and duration of the mortality period for an experimental group of animals. However, the effects of the interaction between fluoride and temperature were highly significant (99% confidence level) for the times to initial and final mortality, but were not significant for the rate of mortality or the duration of the mortality period. Temperature increases are thought to increase the toxicity of fluorides by increasing the rate of metabolism. Fluorides affect various metabolic pathways through their actions as "enzymatic poisons" (Smith, 1966; National Academy of Sciences, 1971). The increasing metabolic rate which results from increasing temperature would result in a more rapid poisoning of the enzyme(s) in question.

Stewart and Cornick (1964) exposed 500-gram lobsters (*Homarus americanus*) to NaF concentrations (0.0, 0.9, 2.25 and 4.5 ppm) for 10 days at 2°C. No deaths resulted from exposure to any of these fluoride levels.

Fluoride was accumulated by the oyster (*Crassostrea virginica*) when exposed to 8 ppm, but not when exposed to 1.5 ppm (Moore, 1969). Moore (1969) suggested that fluoride concentrations of 52 ppm would kill populations of oysters. Fluoride was readily released when the oysters were placed in fluoride-free water.

Moore (1971) reported that the growth of *Callinectes sapidus*, the blue crab, decreased 4.7% per molt when the crabs were exposed to 20 ppm fluoride. If this decrease in the growth increment occurs at each molt and the crabs molt 20 times after reaching the first-crab stage, one might expect more than a 50% reduction in the final average size.

Fluoride accumulation in the tissues of *C. sapidus* was also studied (Moore, 1971). Thirty-day uptake rates were determined at 0.5, 2, 8, 32 and 128 ppm fluoride in the water. Significant increases in uptake occurred in exoskeleton, gill, hepatopancreas, and muscle tissues at 8, 32 and 128 ppm fluoride. Fluoride levels in these four tissues returned to near normal when the crabs were placed in fluoride-free water for 20 days. Approximately 50 ppm fluoride (dry weight) accumulated in the muscle tissue when the crabs were exposed to 20 ppm for 90 days. If the wet weight fluoride level in crab muscle is 12.5 ppm, one can expect 5.7 mg of fluoride per pound of crab meat. Fluoride levels in muscle tissues exposed to 100, 200 and 400 ppm were much higher, reaching 600 ppm fluoride when exposed to water containing 400 ppm.

Short and long-term effects of soluble fluoride on a number of estuarine species were reported by Hemens and Warwick (1972). Three fish species (*Mugil cephalus*, *Ambassis bafgha* and *Therapon jarbua*) and two species of prawns (*Penaeus indicus* and *P. monodon*) tolerated fluoride levels up to 100 ppm for 96 hours. However, evidence of fluoride intoxication of *Perna perna*, the brown mussel, was observed after exposure to 1.4-7.2 ppm fluoride for five days. The nutritional state of the mussels may not have been adequate for a toxicity study and interpretations limited to fluoride alone do not seem warranted.

Hemens and Warwick (1972) also conducted a long-term effects study (72 days) with two artificial communities. Both communities included eel grass (*Zostera capensis*) and associated epiphytes, mud, juvenile prawns and shrimps (*Penaeus indicus* and *Palaemon pacificus*), small mud crabs (*Tylosinoplax belphariskios*) and juvenile mullet (*Mugil cephalus*). Only one community was dosed with fluoride at 55 ppm F<sup>-</sup>. Increased mortality and a deteriorating physical condition characterized the *M. cephalus* and *T. belphariskios* in the test community. Fluoride may have had some harmful effect(s) on the reproduction of *P. pacificus*. Fluoride levels in the tissues of all four species were much higher in the test community than were those in the control group--e.g., *M. cephalus* values of 7743 and 148.1 µg F<sup>-</sup>/g ash, respectively.

Fluoride levels in *Z. capensis* and other plants did not change appreciably following the addition of the NaF. Presumably, the high levels of fluoride in the animal tissues of the test group reflect uptake from the water and through the food web.

#### Sulfur Dioxide Effects

The toxicity of sulfur dioxide (SO<sub>2</sub>) to aquatic organisms may be assessed from sulfite studies. The concentration of sulfite (SO<sub>3</sub><sup>=</sup>), an ion resulting from the combination of SO<sub>2</sub> and water, is proportional to the SO<sub>2</sub> level. In most experiments with aquatic organisms sulfite is administered as waste liquors from pulp mills. These liquors contain many known and unknown substances in varying concentrations. However, in a study on the effects of sulfur dioxide *per se*, Idler (1969) reported that the survival time for 50% of a trout population exposed to 1 ppm SO<sub>2</sub> was 15 hours.

Marier (1972) has discussed the potential interactions between soluble fluoride and sulfur dioxide.

#### METHODS AND MATERIALS

##### Species Selection

The major criteria used in selecting species were (1) species abundance, (2) species biomass, (3) sport and commercial value to the area, and (4) position in the food web. Another important factor was the ability to capture, transport and maintain a species in the laboratory. The selection process was aided by several publications: Haertel and Osterberg, 1967; Haertel *et al.*, 1969; Haertel, 1970) and conversations with biologists familiar with the Columbia River estuary (Kujala, personal communication; Durkin, personal communication).

Initially five species were selected: the calanoid copepod, *Eurytemora hirundooides*; a tube-dwelling amphipod, *Corophium salmonis*; the Dungeness crab, *Cancer magister*; threespine stickleback, *Gasterosteus aculeatus*; starry flounder, *Platichthys stellatus*.

Because Youngs Bay water was characteristically low in salinity throughout the first half of 1974 (Higley and Holton, 1975), freshwater cladocera, *Daphnia longispina* and *Euryceerus lamellatus*, were included. Other species, opossum shrimp *Neomysis mercedis*, and sand shrimp, *Crangon franciscorum*, were added because of their availability and food-web position. A small clam, *Macoma inconspicua*, and a burrowing worm, *Neanthes diversicolor*, were added because of their availability, and to provide phylogenetic and habitat diversity in the studies. Food-web relationships involving some of the above species are indicated in Haertel and Osterberg (1967).

A brief checklist of species used is provided in Data Section I. A more detailed list is given in Higley and Holton (1975).

#### *Animal Collection*

Live animals were collected with five different kinds of gear: box trawl, meter net, Smith-McIntyre grab, plankton sled and beach seine. Except for the plankton sled, all gear used for live collections is described by Higley and Holton (1975). The plankton sled consists of a one-half meter net mounted on runners which collects animals in the water 15-70 centimeters above the bottom. A breakdown of animal species caught with each gear type is shown in Table 1. Station locations are shown on Figure 1.

All live animals were collected on the last day of each sampling trip, and transported in covered styrofoam chests or 5-gallon plastic buckets filled with surface water from the collection site. Each container was aerated with a Sears Live Bait Aerator (Model 39241). The salinity of the water in which these animals were transported was not always the same as the salinity at which they were collected because salinities recorded at collection sites on the bottom of Youngs Bay were often higher than those on the surface several feet above (Higley and Holton, 1975).

Samples were transported to Corvallis and placed in a cold room overnight. When possible, the animals were put into fresh Youngs Bay water and aerator batteries were changed before the samples were taken to the Marine Science Center the following morning. Once in the lab, all animals were acclimated to test salinities similar to those found in their natural environments.

Limited collections of *Corophium salmonis* and *Cancer magister* were also made in the Yaquina Bay area. The same methods of collection were used. Experiments using these animals are clearly identified in the data. Unless otherwise indicated, all animals were collected in the Youngs Bay area.

#### *Laboratory Acclimation and Maintenance*

*Acclimation.* Prior to exposure to a toxicant animals were acclimated to laboratory conditions at salinities and temperatures approximating readings taken at time of capture. Water at specific salinities was prepared by diluting seawater from Yaquina Bay (pumped directly into the laboratory) with distilled water. Salinity was measured with a specific gravity hydrometer in a one-liter graduated cylinder. Hydrometer readings were then corrected for temperature and converted to parts per thousand (‰) with the use of a U.S. Coast and Geodetic Survey Conversion table. Large quantities of water were prepared in an 18-liter graduated polyethylene carboy.

Most invertebrate species were acclimated for several days at 1 to 25‰, however, *Cancer magister*

was acclimated for six days at 15‰. Both fish species, *Gasterosteus aculeatus* and *Platichthys stellatus*, received a prophylactic treatment of formalin in water (1:4000) for two hours. This minimized problems associated with skin flukes (*Gyrodactylus* sp.), white spot (*Ichthyophthirius multifiliis*), and other possible parasites. The fish were subsequently acclimated in the laboratory for a minimum of six weeks prior to fluoride exposure.

*Culturing and Feeding.* All acclimations and experiments were conducted in static containers, ranging in size from 25-milliliter (ml) test tubes for *Eurytemora hironoides* up to 25-liter glass aquaria for the larger test species. A summary of procedural variations followed in culturing and feeding animals prior to and during lethal studies is found in Table 2. Those animals not fed by hand probably obtained food by feeding on plankton and detritus in their diluted bay water medium.

Aquaria cultures were cleaned daily with dip nets and were aerated with a small compressor (Silent Giant, Aquarium Pump Supply, Inc., Prescott, Arizona, Model 120). During fluoride lethal experiments the medium in each container was changed every one to four days. However, during sulfur dioxide lethal experiments the medium in each container was changed daily due to the rapid oxidation of sulfite to sulfate. The metabolic wastes (primarily ammonia) released by most of these animals was not monitored, since their water was changed frequently and no signs of poisoning from their own waste products were apparent.

*Temperature and Photoperiod Control.* The aquarium water medium in which the fish species (*Platichthys stellatus* and *Gasterosteus aculeatus*) were cultured was thermoregulated with a water bath in one of the water tables. The water bath temperature was maintained between 11.8° and 13.0°C by a heating unit, consisting of an immersion heating coil, thermostat (Versa-Therm Model 2149-2) and thermoregulator (Bronwill, Jumbo), in conjunction with a submerged cooling coil (Bronwill, Model CTC-25). Two stirrers (Talboys Engineering Corp., Model 109) circulated the water uniformly around the half submerged aquaria.

The rest of the experiments were conducted in containers partially submerged in water tables which were continuously fed by Yaquina Bay water. These animals were exposed to seasonal fluctuations in temperature, ranging from 8.4°C to 17.0°C with a mean of approximately 12.0°C.

Photoperiod was not controlled, but in general the animals received approximately 9 hours of light per day.

#### *Dosing Procedure*

A series of experiments were conducted on individual species using either fluoride or sulfur dioxide. A standard procedure was followed for each experiment and provided continuity from experiment to experiment. Animals were exposed to pollutants in the same type of container in which they

Table 1. Live animal collection: Summary of gear used and collection sites. Station locations are shown on Figure 1.

| GEAR:                         | Box Trawl | Smith-McIntyre Grab | Meter Net | Plankton Sled | Beach Seine |
|-------------------------------|-----------|---------------------|-----------|---------------|-------------|
| STATION(S):                   | PW        | Ch 8, P3 FLG:3      | CW-Ch 4   | PW, CWRR      | P3          |
| SPECIES                       |           |                     |           |               |             |
| <i>Neanthes diversicolor</i>  |           | X                   |           |               |             |
| <i>Eurytemora hirundoides</i> |           |                     | X         | X             |             |
| <i>Daphnia longispina</i>     |           |                     | X         |               |             |
| <i>Eurycercus lamellatus</i>  |           |                     | X         |               |             |
| <i>Corophium salmonis</i>     |           | X                   |           |               |             |
| <i>Neomysis mercedis</i>      |           |                     | X         | X             |             |
| <i>Crangon franciscorum</i>   | X         |                     |           |               |             |
| <i>Cancer magister</i>        | X         |                     |           |               |             |
| <i>Macoma inconspicua</i>     |           | X                   |           |               |             |
| <i>Gasterosteus aculeatus</i> | X         |                     | X         |               | X           |
| <i>Platichthys stellatus</i>  | X         |                     |           |               |             |

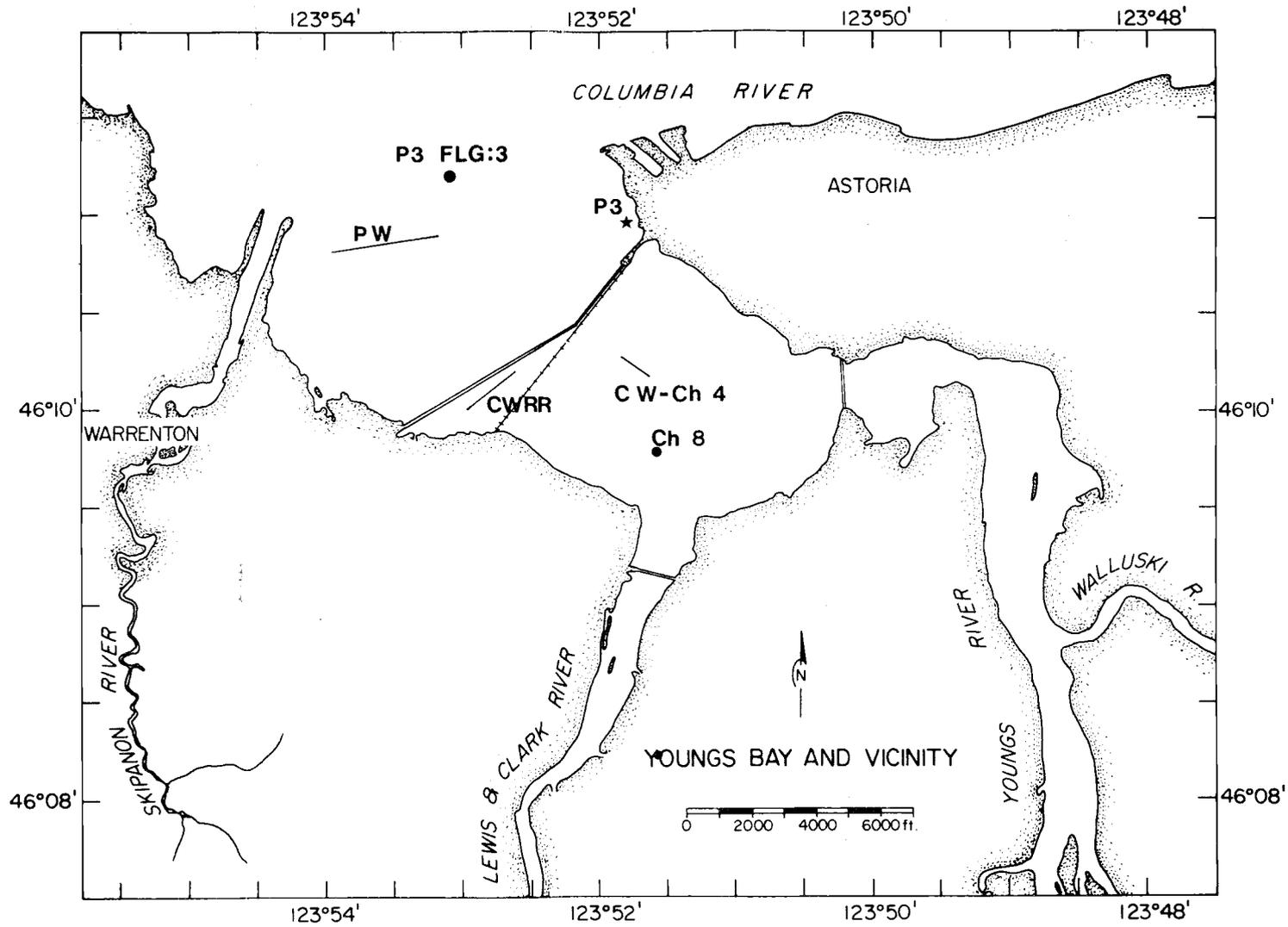


Figure 1. Location of stations at which most of the live animal collections were made. Gear used and animals collected at each station is noted on Table 1.

Table 2. Variations in procedure for culturing and feeding animals prior to and during lethal studies.

| <u>Species</u>                 | <u>Container</u>  | <u>Food</u>                    | <u>Salinity (‰)</u> |
|--------------------------------|---|--------------------------------|---------------------|
| <i>Neanthes diversicolor</i>   | 250-ml culture dishes   | not fed                        | 5                   |
| <i>Eurytemora hirundooides</i> | 25-ml test tubes  | <i>Isochrysis</i> sp.          | 5                   |
| <i>Daphnia longispina</i>      | 250-ml culture dishes   | not fed                        | 1                   |
| <i>Eurycercus lamellatus</i>   | 250-ml culture dishes   | not fed                        | 1                   |
| <i>Corophium salmonis</i>      | 250-ml culture dishes<br>(capillary tubing for<br>artificial burrows) | not fed                        | 1, 10, 11, 25       |
| <i>Neomysis mercedis</i>       | 1000-ml culture dishes  | not fed                        | 5                   |
| <i>Crangon franciscorum</i>    | 250-ml and 1000-ml culture dishes                                     | sections of <i>Mytilus</i> sp. | 1, 10               |
| <i>Cancer magister</i>         | 25-liter aquaria  | sections of <i>Mytilus</i> sp. | 15                  |
| <i>Macoma inconspicua</i>      | 250-ml culture dishes   | not fed                        | 5                   |
| <i>Gasterosteus aculeatus</i>  | 25-liter aquaria  | sections of <i>Mytilus</i> sp. | 1                   |
| <i>Platichthys stellatus</i>   | 25-liter aquaria  | sections of <i>Mytilus</i> sp. | 1                   |

were acclimated (Table 2). Experiments were planned to run for 30 days, but were terminated sooner whenever 50% of animals at the lowest toxicant level died. Results of experiments were interpreted to reflect the influence of a toxicant only when 80% or more of the control group survived.

**Fluoride Measurements.** Soluble fluoride (F<sup>-</sup>) was administered as sodium fluoride (NaF) at concentrations ranging from 0.18 to 1810 parts per million (ppm) F<sup>-</sup> in various salinities (0 to 25‰). Fluoride solutions were usually made in the same containers in which the animals had been acclimated.

Soluble fluoride (F<sup>-</sup>) levels in water samples from animal containers were determined with a fluoride electrode (Orion model 94-09) in conjunction with a specific ion meter (Orion model 407A). Levels of soluble fluoride in water were determined from calibration curves. Marier (1972) and Johnson and Cutshall (1975) note that the level of soluble fluoride, that is fluoride in a form available for uptake, is a function of salinity. Therefore, calibration curves of electrode potential versus logarithm of fluoride concentration were prepared at several salinities.

Instrumentation was not available for routine analysis during the period when most of the lethal toxicity experiments were performed. However, some fluoride measurements were made of representative experimental solutions. This served to demonstrate the relationship between amounts added to test containers and levels measured in water samples taken from the containers 2 to 24 hours later.

Water samples were usually collected and analyzed for soluble fluoride after reaching room temperature. If this was not possible, samples were stored at 5°C for subsequent fluoride analysis. A reasonably good agreement between expected and measured values was obtained for the lower fluoride levels (Table 3). However, for experimental solutions at 181 ppm or higher and at a salinity of 15‰, significantly less was measured than originally added. This tends to agree with observations of Johnson and Cutshall (1975) showing that precipitation of fluoride occurs at concentrations of 50 ppm or higher in seawater of 32‰. Although Yaquina Bay water was diluted, naturally occurring fluoride probably contributed to the fluoride detected in the control samples (Table 3).

Table 3. Levels of soluble fluoride (F<sup>-</sup>) in lethal-study test media determined with a fluoride electrode. Exposure time was 24 hours at 5‰ and 2 hours at 15‰ salinity

| Salinity (‰) | Expected F <sup>-</sup> (ppm) | Measured F <sup>-</sup> ppm ± SE (n) |
|--------------|-------------------------------|--------------------------------------|
| 5            | 0                             | 0.254 ± 0.0165 (2)                   |
| 5            | 20                            | 20.0 ± 1.98 (3)                      |
| 15           | 0                             | 0.3                                  |
| 15           | 1.81                          | 1.3                                  |
| 15           | 18.1                          | 16.6                                 |
| 15           | 181                           | 93.1                                 |

**Sulfur Dioxide Measurements.** Sulfur dioxide was administered as H<sub>2</sub>SO<sub>3</sub> or NaS<sub>2</sub>O<sub>5</sub>. Solutions at concentrations ranging from 0.5 to 75 ppm SO<sub>2</sub> in salinities of 0, 1, and 5‰ of sulfur dioxide were sealed with Parafilm® or glass lids and stopcock grease in order to minimize the oxidation of sulfite to sulfate. The levels of sulfur dioxide in container water were not measured. They were determined only by weighing the amount added to the culture medium. However, a decline in these values appears likely because sulfite (SO<sub>3</sub><sup>=</sup>) is readily oxidized to sulfate (SO<sub>4</sub><sup>=</sup>), which is not considered hazardous.

#### Data Processing

**LC<sub>50</sub> Values.** The unit used to express the lethality of a pollutant is called the LC<sub>50</sub> value. This is the theoretical lethal concentration at which 50% of the experimental population dies during a specified interval of exposure. Sprague (1969) recommended using the incipient LC<sub>50</sub> as a generally preferable criterion of lethality. The incipient LC<sub>50</sub> value is the concentration of the pollutant at which 50% of the sample would be able to live for an extended period of time. The four-day LC<sub>50</sub> has been suggested by Sprague (1970) as an approximation that may be substituted for the incipient LC<sub>50</sub>. This asymptotic value was sought in the experiments reported herein.

LC<sub>50</sub> values are used as an end product of this study because they have been widely used in similar work (see Table 4). However, the reader must be cautioned that particular values obtained are not absolute. On the contrary, they are dynamic values which should be expected to vary along with environmental conditions. The LC<sub>50</sub> values obtained in this study dramatically illustrate the effect of exposure time on the LC<sub>50</sub> value. In addition, however, we would expect a variety of other factors, such as temperature, salinity, physiological state of the animal, and the availability of food, to effect the LC<sub>50</sub> values calculated for any given experiment.

Determination of LC<sub>50</sub> values has been described by Doudoroff *et al.* (1951). As used in the present study, linear-regression analysis yields a regression line:

$$y = a + bx \quad (1)$$

where y = survivor percentage; a = y-intercept; b = slope; and x = log toxicant concentration. Interpolation in the present study involves solving for the antilogarithm of x (the LC<sub>50</sub>) when y = 50. A sample calculation follows. The data deals with the effect of soluble fluoride on the survival of *Daphnia longispina* for one day at approximately 14.5°C and 1‰ salinity.

Table 4. Fluoride toxicity studies from selected sources.

| <u>Taxon/Preparation</u>  | <u>LC50 (ppm F<sup>-</sup>)</u>       | <u>Exposure Period</u>        | <u>Temperature (°C)</u>     | <u>Salinity (‰)</u> | <u>Source</u>                             |
|---|---------------------------------------|-------------------------------|-----------------------------|---------------------|---|
| 4-14 inch carp<br>( <i>Cyprinus carpio</i> )  | 75-91*                                | -                             | 18.3-23.9                   | ~0                  | Neuhold and Sigler (1960)                 |
| 4-8 inch rainbow trout<br>( <i>Salmo gairdneri</i> )  | 2.7-4.7*                              | 20 days                       | 12.8                        |                     |   |
| rainbow-trout eggs  | 222-273*<br>242-261*<br>237-281*      | 424 hrs<br>214 hrs<br>167 hrs | 7.8<br>12.8<br>15.6         |                     |   |
| rainbow-trout embryos and<br>alevin   | 61-85.3*                              | 825 hrs                       | 15.6                        |                     |   |
| 3-5 inch rainbow trout  | 5.9-7.5<br>2.6-6.0<br>2.3-7.3<br><2.0 | 10 days                       | 7.2<br>12.8<br>18.3<br>23.9 | ~0                  | Angelovic, Sigler, and<br>Neuhold (1961a) |
| 4-7 inch rainbow trout<br>tempered in 34 ppm NaCl for<br>48 hr<br>nontempered   | 22<br>6                               | 5 days                        | 7                           | ~ 0                 | Neuhold and Sigler (1962)                 |
| rainbow trout   | 8.5                                   | 21 days                       | -                           | -                   | Herbert and Shurben (1964)                |
| lobster ( <i>Homarus americanus</i> )   | >4.5                                  | 10 days                       | 2                           | "sea water"         | Stewart and Cornick (1964)                |
| juvenile mullet ( <i>Mugil cephalus</i> )<br>small fish ( <i>Ambassis safgha</i> )<br>small fish ( <i>Therapon jarbua</i> )<br>prawn ( <i>Penaeus indicus</i> )<br>prawn ( <i>Penaeus monodon</i> ) | >100                                  | 4 days                        | 20.5 ± 0.5                  | 10, 20, 28          | Hemens and Warwick (1972)                 |
| juvenile mullet ( <i>P. indicus</i> )<br>mud crab<br>( <i>Tyloiplax blephariskios</i> )   | >5.5                                  | 113 days                      | 23.5-27.0                   | 20                  | Hemens, Warwick, and<br>Oliff (1974)      |

\* 95% confidence level.

| <u>Identification</u> | <u>Replication</u> | <u>F<sup>-</sup> concentration (ppm)</u> | <u>x value</u> | <u>Survivor percentage (y value)</u> |
|-----------------------|--------------------|--|----------------|--------------------------------------|
| 1                     | A                  | 1810                                     | 3.258          | 0                                    |
| 1                     | B                  | 1810                                     | 3.258          | 0                                    |
| 2                     | A                  | 181                                      | 2.258          | 100                                  |
| 2                     | B                  | 181                                      | 2.258          | 100                                  |

If the y distribution about the regression line  $y = a + bx$  is normal with a mean of zero and a variance independent of x, then

$$b = \frac{\Sigma(x - \bar{x})(y_2 - \bar{y})}{\Sigma(x - \bar{x})^2} \quad , \quad (2)$$

and

$$a = \bar{y} - b\bar{x} \quad , \quad (3)$$

in which  $\bar{y}$  = y-value mean; and  $\bar{x}$  = x-value mean.

A computer program facilitated completion of repetitious calculations. Data were entered into the in pairs (x,y). The program produced the slope, y-intercept, correlation coefficient, and LC<sub>50</sub> value for each set of circumstances including the period of toxicant exposure.

From the above data

slope (b) = -100.0,  
y-intercept (a) = 325.8,  
correlation coefficient (r) = -1.00, and  
one-day LC<sub>50</sub> = 572.4 ppm F<sup>-</sup>

## RESULTS

The data from most of the lethal experiments is presented in Data Tables I-1 through I-28 in the form of daily summaries. The slope and y-intercept values on these tables are the constants in Equation 1. The correlation coefficient, r, is a measure of the goodness of fit of the data to the assumption of a linear relationship between the survival rate and the log of the toxicant concentration. The fit is perfect when the absolute value of r equals unity and random when r equals zero. A table of r values was used to test the fit of the line to the experimental data. Most of this data has also been plotted on graphs to illustrate the relationship between the LC<sub>50</sub> and exposure time (Data Figures I-1 through I-10).

### *Pollutant Concentration Versus Exposure Period*

Both pollutant concentration and exposure period are critical variables in tolerance studies

(Sprague, 1969). Therefore, experiments were run for 30 days, whenever possible, in order to determine changes in LC<sub>50</sub> values during various exposure periods. The LC<sub>50</sub> also illustrates the relative sensitivity of each species to each pollutant. A summary of the LC<sub>50</sub> values determined in these experiments is presented in Table 5. As illustrated on Table 3, whenever salinity varies, rigorous comparisons cannot be made.

Sprague (1969) and Warren (1971) observed that acute lethal effects generally occur within four days. Therefore, four-day LC<sub>50</sub> values probably reflect the short-term action of each toxicant.

*Fluoride.* The zooplankton species were most sensitive to soluble fluoride at four days (Table 5). Four-day LC<sub>50</sub> values were 29 ppm F<sup>-</sup> for ovigerous *Eurytemora lamellatus* and 33 ppm F<sup>-</sup> for *Eurytemora hirundooides*. The lowest seven-day LC<sub>50</sub> for soluble

Table 5. LC<sub>50</sub> values (ppm) at selected exposure times.

| Species                                       | Salinity (‰) | 4 Day | 7 Day | 10 Day | Maximum Exposure Time (days) |      |
|---|--------------|-------|-------|--------|------------------------------|------|
| <i>SOLUBLE FLUORIDE</i>                       |              |       |       |        |                              |      |
| <i>Neanthes diversicolor</i>                  | 5            | >180  | 76    | 33     | <1.8                         | (30) |
| <i>Eurytemora hirundoides</i>                 | 5            | 33    |       |        | 29                           | (5)  |
| <i>Daphnia longispina</i>                     | 1            | 120   | 22    |        | 22                           | (7)  |
| <i>Eurycerus lamellatus</i><br>(ovigerous)    | 1            | 29    |       |        | 6.2                          | (5)  |
| <i>Eurycerus lamellatus</i><br>(nonovigerous) | 1            |       |       |        | 69                           | (3)  |
| <i>Corophium salmonis</i> *<br>(juveniles)    | 11           |       |       |        | 9.8                          | (2)  |
| <i>Corophium salmonis</i>                     | 1            |       |       |        | 300                          | (1)  |
| <i>Corophium salmonis</i> *                   | 11           | >180  | >180  | >180   | 22                           | (18) |
| <i>Corophium salmonis</i>                     | 1            | 71    |       |        | 39                           | (5)  |
| <i>Corophium salmonis</i>                     | 10           | >180  |       |        | 92                           | (5)  |
| <i>Corophium salmonis</i>                     | 25           | 100   |       |        | 21                           | (5)  |
| <i>Neomysis mercedis</i>                      | 5            | <90   |       |        | <90                          | (5)  |
| <i>Crangon franciscorum</i>                   | 1            | 57    | 57    | 57     | 57                           | (10) |
| <i>Crangon franciscorum</i>                   | 1            | 54    | 32    | 27     | 27                           | (12) |
| <i>Crangon franciscorum</i>                   | 10           | 570   | 57    |        | 57                           | (9)  |
| <i>Cancer magister</i> *                      | 15           | >180  | >180  |        | >180                         | (9)  |
| <i>Macoma inconspicua</i>                     | 5            | >100  | >100  | 73     | 73                           | (12) |
| <i>Macoma inconspicua</i>                     | 5            | 120   | 100   | 93     | 81                           | (30) |
| <i>Gasterosteus aculeatus</i>                 | 1            | >180  | >180  |        | >180                         | (8)  |
| <i>Platichthys stellatus</i>                  | 1            | >180  | >180  | >180   | >180                         | (13) |
| <i>SULFUR DIOXIDE</i>                         |              |       |       |        |                              |      |
| <i>Neanthes diversicolor</i>                  | 5            | >10   | >10   | >10    | >10                          | (26) |
| <i>Eurytemora hirundoides</i>                 | 5            | >15   |       |        | >15                          | (6)  |
| <i>Corophium salmonis</i>                     | 1            | 1.3   | 0.20  |        | 0.20                         | (7)  |
| <i>Corophium salmonis</i>                     | 1            | 1.7   | 1.2   | 0.90   | 0.56                         | (15) |
| <i>Crangon franciscorum</i>                   | 1            | >11   |       |        | >11                          | (5)  |
| <i>Crangon franciscorum</i>                   | 1            |       |       |        | 22                           | (2)  |
| <i>Macoma inconspicua</i>                     | 5            | >10   | >10   | >10    | >10                          | (26) |
| <i>Macoma inconspicua</i>                     | 5            | >75   | >75   | 75     | 49                           | (30) |

\* *Corophium salmonis* collected from Pooles Slough (Yaquina Bay); *Cancer magister* collected from Yaquina Bay.

fluoride occurred with *Daphnia longispina* (22 ppm F<sup>-</sup>), although *Crangon franciscorum* did not appear much more resistant (32 ppm F<sup>-</sup>). The lowest 10-day LC<sub>50</sub> values for species in which 80% of the controls survived for 10 days were 27 ppm F<sup>-</sup> (*C. franciscorum*) and 33 ppm F<sup>-</sup> (*Neanthes diversicolor*). *N. diversicolor* had the lowest LC<sub>50</sub>, less than 1.8 ppm F<sup>-</sup> after 30 days. The most resistant species were Dungeness crabs, threespine sticklebacks, and starry flounders.

**Sulfur Dioxide.** The amphipods, *Corophium salmonis*, were most sensitive to sulfur dioxide at 4, 7, 10, and 15 days. The 15-day LC<sub>50</sub> of these animals was 0.56 ppm SO<sub>2</sub>. All of the other species had LC<sub>50</sub> values at least an order of magnitude greater than those of *C. salmonis*.

**Other Variables.** Several other trends are apparent in the experiments with single toxicants. In certain experiments, size, salinity and toxicant seemed to be important variables.

Juvenile and adult *C. salmonis* populations from Pooles Slough (Yaquina Bay) were not equally sensitive to soluble fluoride. Two-day LC<sub>50</sub> values were 9.8 ppm F<sup>-</sup> for juveniles (2 mm total length) and 180 ppm F<sup>-</sup> for adults (Data Tables I-6 and I-8 and Figure I-4).

Sensitivity to soluble fluoride also appears to be a function of salinity. Five-day LC<sub>50</sub> values for *C. salmonis* were 39, 92, and 21 ppm F<sup>-</sup> for 1, 10, and 25‰ salinity, respectively (Data Tables I-9, I-10, and I-11, and Figure I-5). Nine-day LC<sub>50</sub> values for *Crangon franciscorum* were 32 ppm F<sup>-</sup> at 1‰ and 57 ppm F<sup>-</sup> at 10‰ salinity (Data Tables I-14 and I-15, and Figure I-6).

Two crustaceans, *Crangon franciscorum* and *C. salmonis*, appear to be more sensitive to sulfur dioxide than to equal levels of soluble fluoride. For example, five-day LC<sub>50</sub> values for *Crangon franciscorum* were 38 ppm for soluble fluoride and 4.2 ppm for sulfur dioxide at a salinity of 1‰ (Data Tables I-14 and I-25 and Figures I-6 and I-9). The same values were 39 ppm F<sup>-</sup> and 1.4 ppm SO<sub>2</sub> and *Corophium salmonis* (Data Tables I-9 and I-24 Figures I-4 and I-8).

## DISCUSSION AND CONCLUSIONS

Juvenile *C. salmonis* amphipods were more sensitive to soluble fluoride than were the adults. The reason for this is not clear, but it may involve size, ontogeny or differences in the uptake rate of F<sup>-</sup> between adults and juvenile animals.

Sensitivity to soluble fluoride appears to be a function of salinity for certain crustaceans. Johnson and Cutshall (1975) noted that soluble fluoride apparently forms complexes with certain divalent cations in seawater. As a result, less fluoride is presumably available for uptake at increasing salinities. What appears to have been an increasing sensitivity to soluble fluoride for *C. salmonis* at 25‰ may really reflect an osmotic stress to these amphipods which were collected at 0-1‰ salinity. In an ancillary study, *C. salmonis*

from Pooles Slough were held at seven different salinities for 30 days. The greatest numbers of survivors occurred at salinities close to the salinity at the collection site.

*Crangon franciscorum* and *Corophium salmonis* appeared to be more sensitive, with respect to survival, to sulfur dioxide than to soluble fluoride. Both of these crustaceans are important in food chains (Haertel and Osterberg, 1967). Higley and Holton (1975) reported observing *C. salmonis* in the digestive tracts of twelve species of fish from Youngs Bay and *C. franciscorum* in the digestive tracts of four species. The influence of sulfur dioxide and soluble fluoride on the other species from Youngs Bay merits further study. Possible sublethal effects of these two toxicants on *C. salmonis*, *C. franciscorum*, and other species may be more difficult to assess, but they may also be more meaningful in terms of estimating long-range survival.

The species which appeared most sensitive to soluble fluoride were some of the Youngs Bay zooplankton (see Table 5). This may reflect the possible influence of size mentioned above. Small poikilotherms generally have greater metabolic rates than do larger forms (Zeuthen, 1953). The uptake rate of soluble fluoride may be greater with the zooplankton than with any other species studied due to the greater metabolic rate of the zooplankton.

Soluble fluoride did not appear to affect the survival of Dungeness crabs, threespine sticklebacks, and starry flounders, at the salinities used in these studies. However, these experiments should be regarded as exploratory due to the small numbers of animals in each.

Two generalizations follow from a comparison of literature values and results of the present study. LC<sub>50</sub> values (ppm F<sup>-</sup>) for rainbow trout in the literature (Table 4) are much lower than the values for the two fish species included in the present study (Table 5). These differences probably reflect differences in species, salinity, temperature, and perhaps even in collection and handling prior to exposure.

LC<sub>50</sub> values (ppm F<sup>-</sup>) for the invertebrate species in Table 4 are imprecise and limited to crustaceans. LC<sub>50</sub> values in the present study are precise and generally lower than those in the literature (e.g., 26-day LC<sub>50</sub> of 5.0 ppm F<sup>-</sup> for *Neanthes diversicolor*, see Appendix Table I-1). Invertebrates from several phyla; Annelida, Mollusca, and Arthropoda, in the present study represent diverse taxa.

The species which appeared most sensitive to sulfur dioxide is *Corophium salmonis* (see Table 5). The critical position in the Youngs Bay food web held by this species and the 15-day LC<sub>50</sub> of 0.56 ppm SO<sub>2</sub> appear sufficient to warrant additional experiments with *C. salmonis* and SO<sub>2</sub>.

## SUBLETHAL EFFECTS: PHYTOPLANKTON PRIMARY PRODUCTION

### INTRODUCTION

Fluoride and sulfur dioxide are harmful at certain levels to several types of terrestrial plants (Boertitz, Siegfried, and Ranft, 1972; Daessler, Ranft, and Rehn, 1972; Ballantyne, 1973; Facteau, Wang, and Rowe, 1973; LeBlanc and Rao, 1973). Ishio and Nakagawa (1971) noted that atmospheric levels of hydrogen fluoride greater than 1.8 ppm were lethal to the alga *Porphyra tenera*. Since phytoplankton populations are the principal primary producers of estuaries, changes in the biology of the phytoplankton probably do influence other inhabitants of the estuarine community. The affect of soluble fluoride and sulfur dioxide on primary production in Youngs Bay was the prime concern in the experiments described below.

### METHODS AND MATERIALS

Rates of primary production were determined with Youngs Bay phytoplankton *in situ* in June and August 1974 and with Yaquina Bay phytoplankton at the Marine Science Center laboratory (Newport, Oregon). The laboratory studies generally preceded those in Youngs Bay and provided a basis for the field studies.

#### *Laboratory Measurements*

Surface water was collected from the main channel of Yaquina Bay and taken to the Marine Science Center in a 5-gallon polyethylene carboy. Unless otherwise stated, the rate of primary production was determined from the uptake of radioactive carbon as specified in Strickland and Parsons (1972). Narrow-mouthed bottles (250 milliliter capacity) were used to incubate the phytoplankton with radioactive carbon ( $^{14}\text{C}$ ). One-fourth of the bottles were painted black and wrapped with overlapping strips of black tape (hereinafter called "dark" bottles in contrast to the remaining "light" bottles).

Each 250-milliliter (ml) bottle was completely filled. After a one-hour equilibration period, two ml of  $\text{NaH}^{14}\text{CO}_3$  (approximately 2 microcuries) were added to each bottle. The  $^{14}\text{C}$ -incubation period and the illumination were constant within any single experiment. The incubation period ranged from two to three hours for all experiments, and the illumination ranged from 10,760-15,064 lux. Both equilibration and incubation occurred at 12°C. Incubation for the initial experiments was terminated upon the addition of one ml of borax-buffered formalin. Incubation for all subsequent experiments was terminated by transferring the bottles to an ice bath in darkness.

#### *In-Situ Measurements*

Rates of primary production were determined for Youngs Bay phytoplankton by the same method described above. Bottles were stacked in clear plexiglass cylinders (3 cm diameter, 37 cm long) for *in situ* incubation (see Figure 2). Swivel eyes attached to both ends of each holder permitted attachment to a mooring line. The bottles and holders were held in a vertical position. Bottles were retained in holders by noncorroding wire at the lower end and latex tubing at the upper end.

Incubation for the June study was terminated upon the addition of one ml of borax-buffered formalin as the bottles were pulled from the water. Incubation for the August study was terminated by transferring the bottles to an ice bath in darkness. One ml of borax-buffered formalin was added to each of these latter bottles within three hours of being pulled from the water.

#### *Carbon-14 Counting*

Phytoplankton was collected on 0.8  $\mu\text{m}$  AAWP Millipore filters which were then added to liquid scintillation vials containing ten ml of Aquasol® (New England Nuclear). Radioactivity was counted

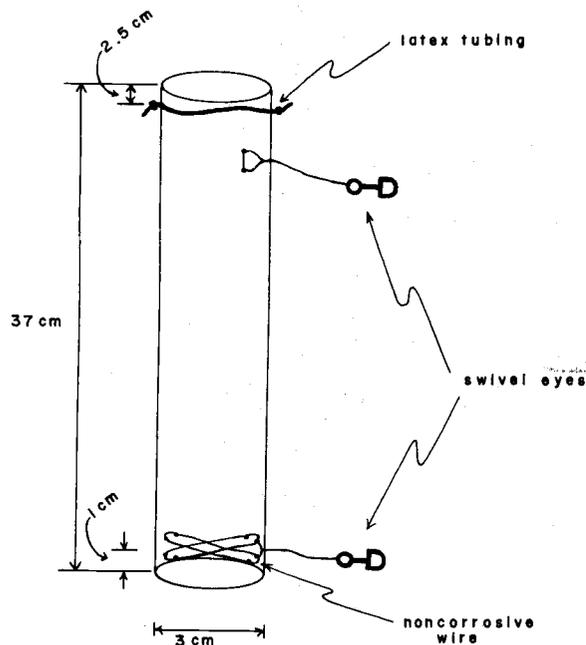


Figure 2. *In situ* bottle holder.

with a Packard Tri-Carb liquid scintillation spectrometer (model 3375).

## RESULTS

The photosynthetic rate is routinely expressed as milligrams carbon per meter cubed ( $\text{mg C/m}^3$ ) per hour (Strickland and Parsons, 1972). However, the influence of a pollutant upon this rate within a single experiment may be apparent from a comparison of the counts per minute (assuming that chemical, color, dilution, or other changes do not occur in the Aquasol® mixture for each experiment). The following results are all expressed as counts per minute (a relative rate of photosynthesis).

Pollutant levels to which the phytoplankton was exposed are expressed as logarithmic intervals.

### Laboratory Studies

The effect of soluble fluoride (added as NaF) on phytoplankton primary production is depicted in Data Figure II-1. Each of the values is the difference between the mean of two or three light bottles and a single dark bottle. Values for Figure II-1 are found in Data Table II-1.

Experiments A-C were conducted with Yaquina Bay phytoplankton at 34‰ salinity, whereas experiments D and E were conducted with Yaquina Bay phytoplankton from Mill Creek at 0-1‰ salinity (Figure II-1). Unusually high levels of soluble fluoride had a deleterious effect on primary production in experiment A.

Water from the sample used in experiment A was used in experiment B, but B followed A after 48 hours of mild aeration at ambient temperatures.

The possible influence of a 24-hour exposure period prior to incubation was tested in experiments C-E. Phytoplankton in experiments A and B was not exposed to soluble fluoride prior to the incubation period.

Extremely low salinities were characteristic of Youngs Bay throughout the first half of 1974 (Higley and Holton, 1975). Therefore, Yaquina Bay phytoplankton from low-salinity waters was used in experiments D and E. Experiment E was undertaken due to some doubt about how well the phytoplankton was distributed among the bottles in experiment D.

The effect of sulfur dioxide (added as  $\text{H}_2\text{SO}_3$ ) on phytoplankton primary production is depicted in Data Figure II-2 (Values in Data Table II-2). Experiments A and B were conducted with Mill Creek phytoplankton at low salinities. Levels close to 1 ppm  $\text{SO}_2$  and above led to negligible photosynthetic rates. Experiment A was repeated (as experiment B) due to multiple errors in handling the light bottles of the control group (in experiment A).

The effect of the interaction of soluble fluoride and sulfur dioxide on phytoplankton primary production is depicted in Data Figure II-3 (values given in Data Table II-3). Not all of the possible combinations of pollutant concentrations were utilized, but a trend was observed in which pollutant antagonism appeared proportional to pollutant concentration. In other words, primary production rose with increases in the paired pollutant levels. Experiment B was undertaken to check for recurring antagonism.

### Youngs Bay Studies

The effect of soluble fluoride (added as NaF) on the total uptake of  $^{14}\text{C}$  by plankton at various locations and depths in the Columbia River estuary was studied in June 1974 (Data Figure II-5, values given in Data Table II-4). Reduced uptake was observed at three points in Youngs Bay (B-D). However, equivocal changes in uptake occurred at two points in Youngs Bay (A and E), and paradoxical changes occurred at both locations along the Skipanon Waterway (F and G).

The influence of the interaction of soluble fluoride and sulfur dioxide on phytoplankton primary production differed from what might have been expected from experiments using these toxicants singly. Exposure to soluble fluoride began 24 hours prior to  $^{14}\text{C}$  incubation, whereas exposure to sulfur dioxide began with the incubation period. Groups A-C (Data Figure II-6, values given in Table II-5) depict the effect of soluble fluoride at unchanging levels of sulfur dioxide per level of  $\text{SO}_2$ . Groups D-F (Data Figure II-6) depict the effect of sulfur dioxide at unchanging levels of soluble fluoride per level of F-. The addition of a single pollutant resulted in decreasing rates of photosynthesis in five of six possibilities. However, pollutant antagonism

occurred in 15 of 18 possibilities and generally was proportional to pollutant concentration. The interaction study (Data Figure II-6) was conducted in August 1974.

#### DISCUSSION AND CONCLUSIONS

Some tenuous trends may be suggested, but these are far from conclusive, due to the limited amounts of data collected.

Soluble fluoride may have contributed to a decrease in the primary production of Yaquina Bay and Mill Creek waters (experiments A, C, and E of Figure II-1). Failure to inhibit photosynthesis (experiments B and D) may have resulted from using a phytoplankton sample that was not freshly collected. The same may have resulted from unequal amounts of phytoplankton in each of the  $^{14}\text{C}$ -incubation bottles. Soluble fluoride significantly decreased the total uptake of  $^{14}\text{C}$  at several points (A, E, F, and G) as seen in Data Figure II-5. In fact, the rate of total  $^{14}\text{C}$  uptake increased with several levels of soluble fluoride at two points (F and G) along the nearby Skipanon Waterway.

Sulfur dioxide had a deleterious effect on the photosynthetic rate of the phytoplankton from the Yaquina Bay estuary (see Data Figure II-2). Concentrations approaching 1 ppm  $\text{SO}_2$  virtually eliminated all photosynthesis.

Either soluble fluoride or sulfur dioxide may lead to decreases in the rate of photosynthesis when present as the only pollutant. However, when combined, these pollutants may lead to antagonistic changes in the rate of photosynthesis. These trends seem to have occurred in both the laboratory (Data Figure II-3) and Youngs Bay (Data Figure II-6).

DATA

I

LETHAL EFFECTS: FAUNAL SURVIVAL RATES

Figures and Tables in this section are arranged first by pollutant (fluoride, sulfur dioxide) and second by species name, listed in the phylogenetic order provided below.

| <u>Species</u>                | <u>Family</u>  | <u>Common or Descriptive Name</u> |
|-------------------------------|----------------|-----------------------------------|
| <i>Neanthes diversicolor</i>  | Nereidae       | a burrowing worm                  |
| <i>Eurytemora hirundoides</i> | Temoridae      | a calanoid copepod                |
| <i>Daphnia longispina</i>     | Daphnidae      | a freshwater cladoceran           |
| <i>Eurycercus lamellatus</i>  | Chydoridae     | a freshwater cladoceran           |
| <i>Corophium salmonis</i>     | Corophiidae    | a tube-dwelling amphipod          |
| <i>Neomysis mercedis</i>      | Mysidae        | an opossum shrimp                 |
| <i>Crangon franciscorum</i>   | Natantia       | a sand shrimp                     |
| <i>Cancer magister</i>        | Brachyura      | a Dungeness crab                  |
| <i>Macoma inconspicua</i>     | Tellenidae     | a small clam                      |
| <i>Gasterosteus aculeatus</i> | Gasterosteidae | a threespine stickleback          |
| <i>Platichthys stellatus</i>  | Pleuronectidae | a starry flounder                 |

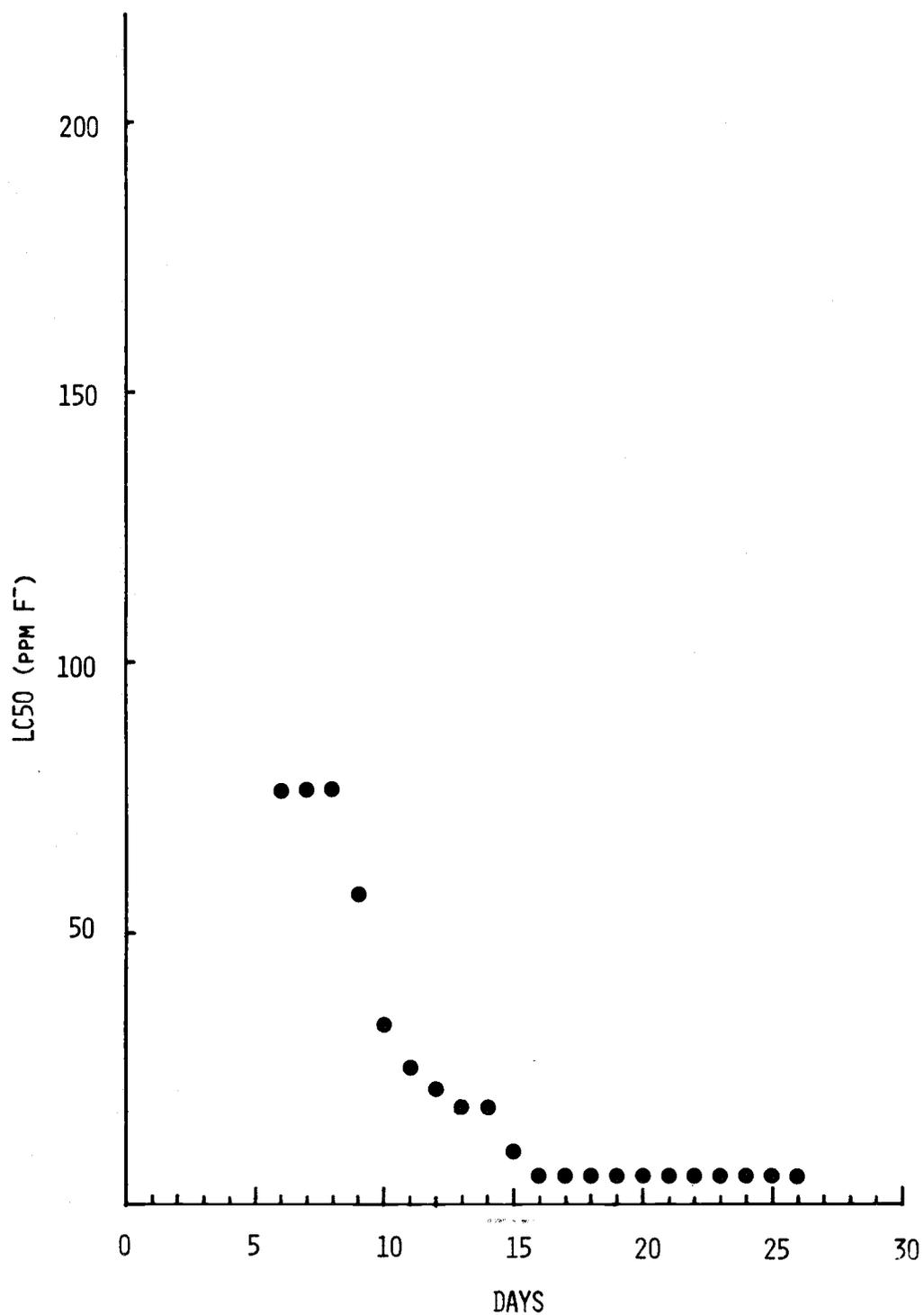


Figure I-1. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Neanthes diversicolor* at 5‰ salinity (From Data Table I-1).

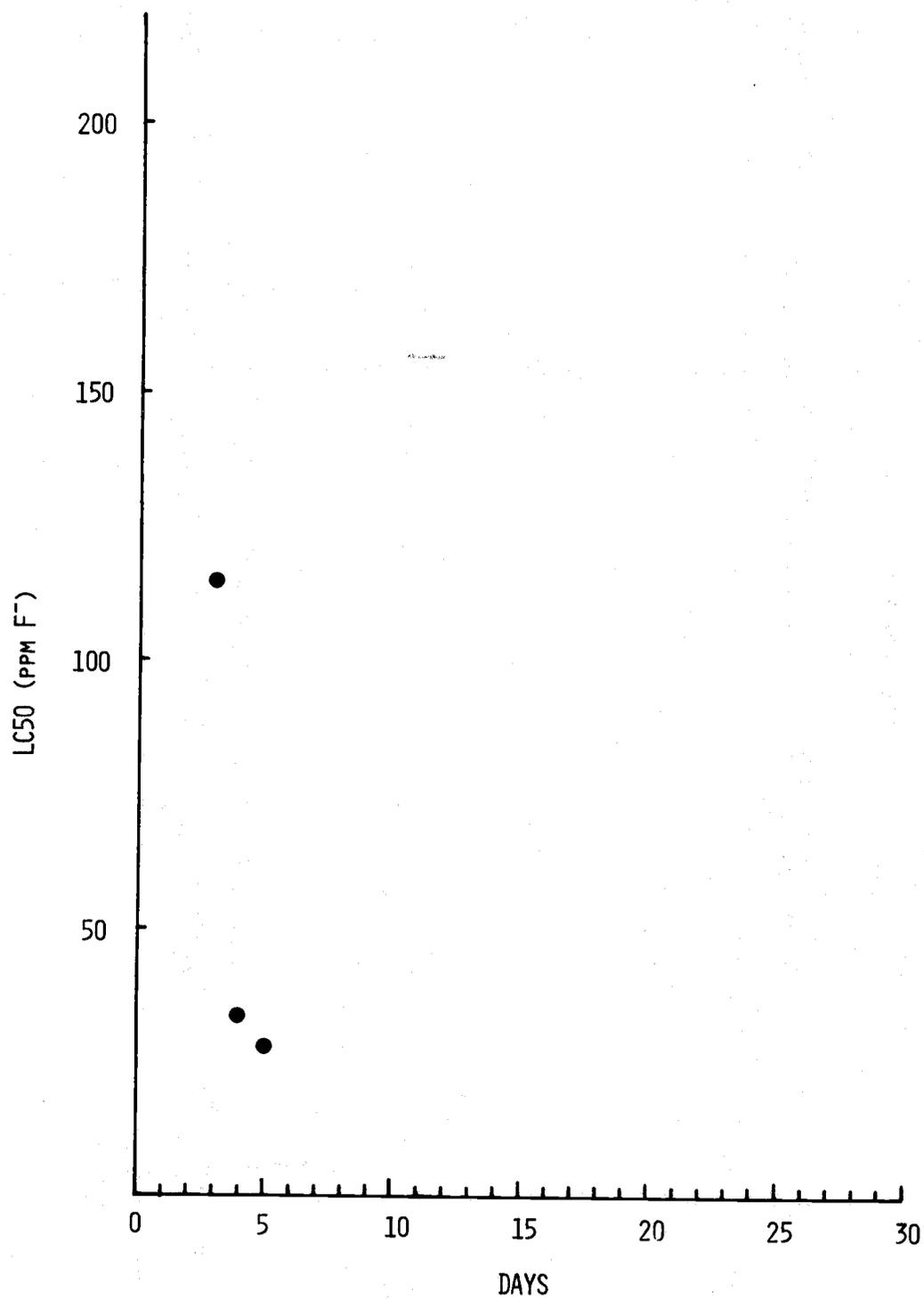


Figure I-2. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Eurytemora hirundooides* at 5‰ salinity (From Data Table I-2).

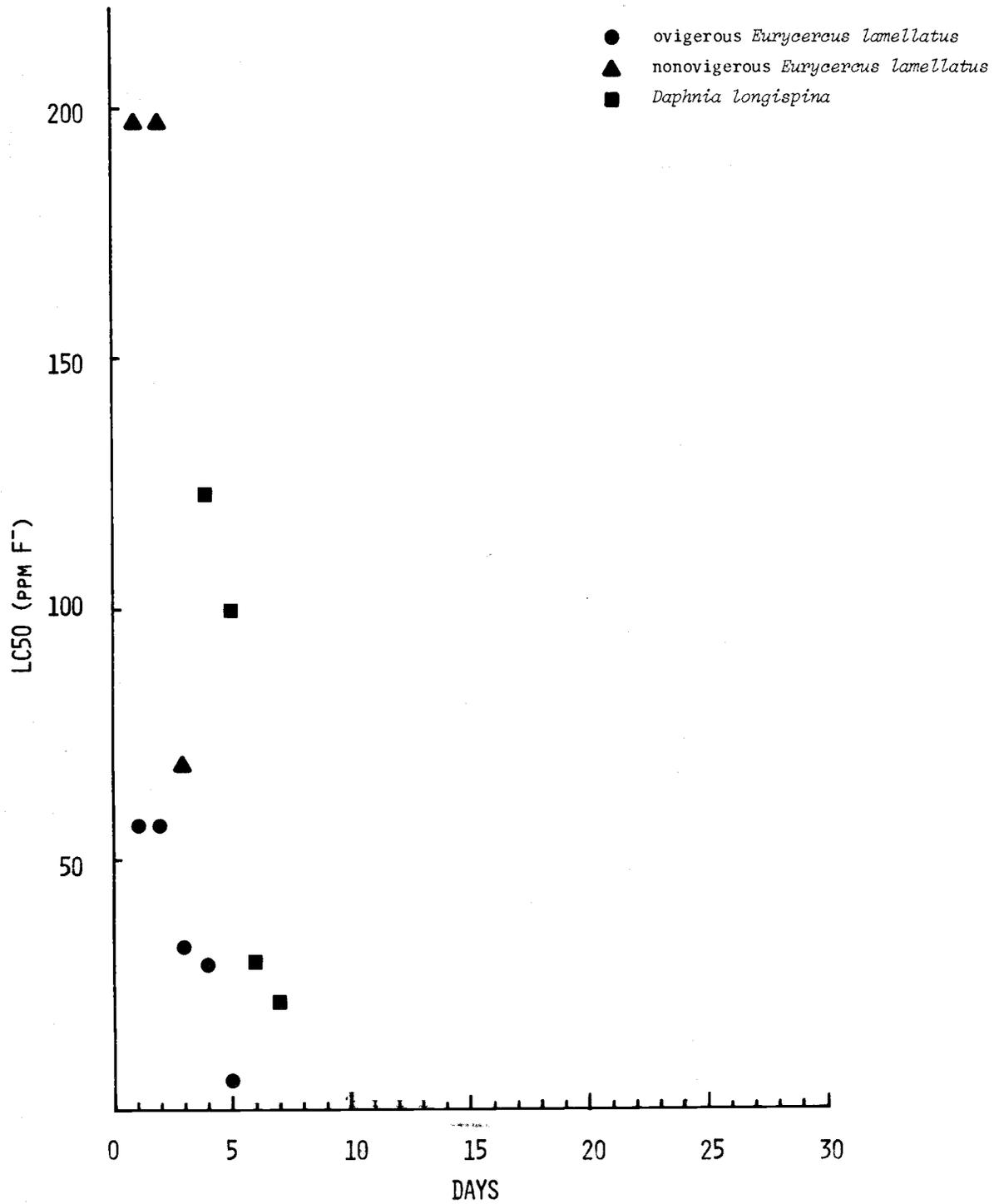


Figure I-3. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Cladocerans* at 1‰ salinity (From Data Tables I-3, I-4 and I-5).

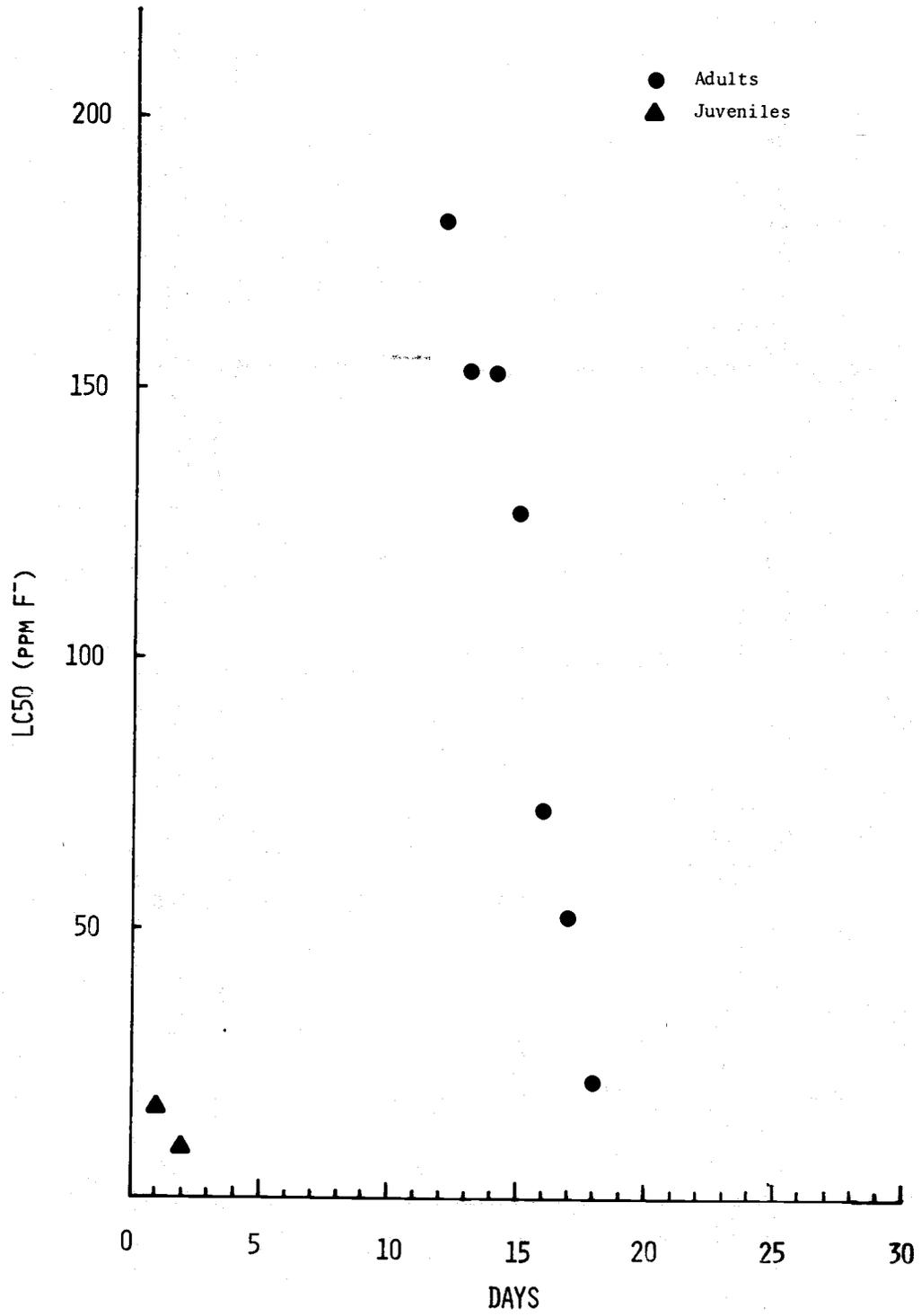


Figure I-4. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Corophium salmonis* at 11‰ salinity (From Data Tables I-6 and I-8).

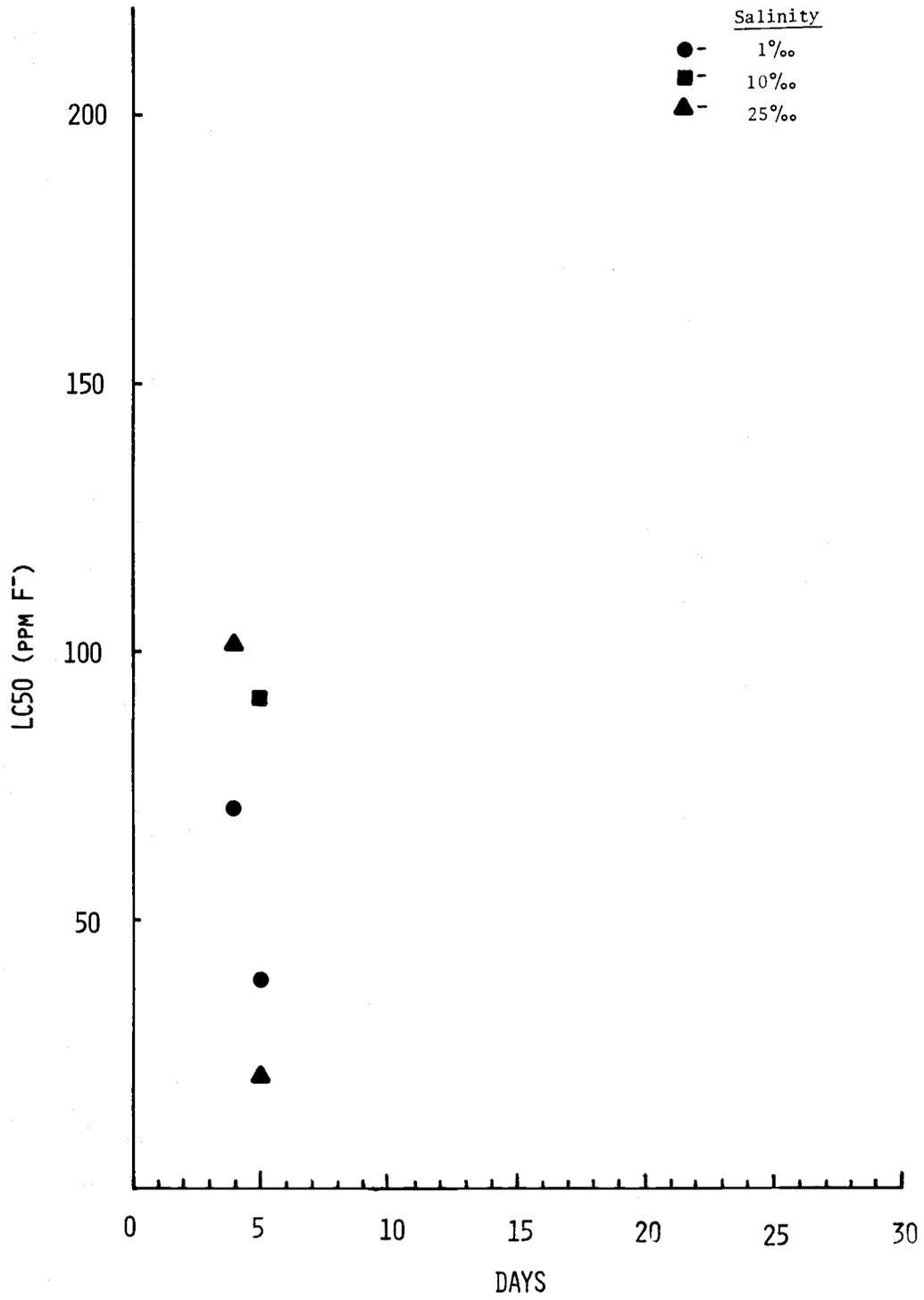


Figure I-5. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Corophium salmonis* at 1, 10 and 25‰ salinity (From Data Tables I-9, I-10, and I-11).

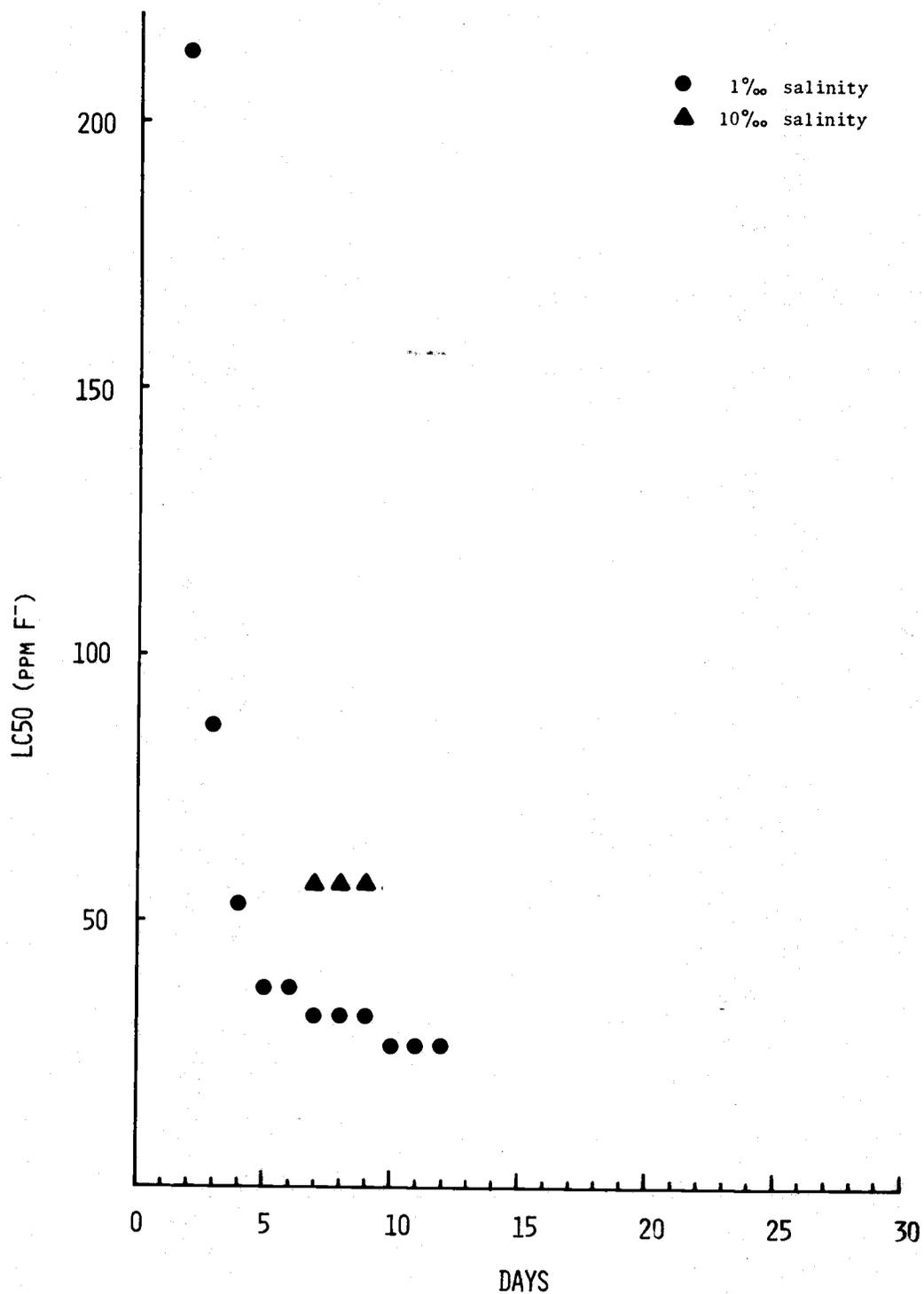


Figure I-6. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Crangon franciscorum* at 1 and 10‰ salinity (From Data Tables I-14 and I-15).

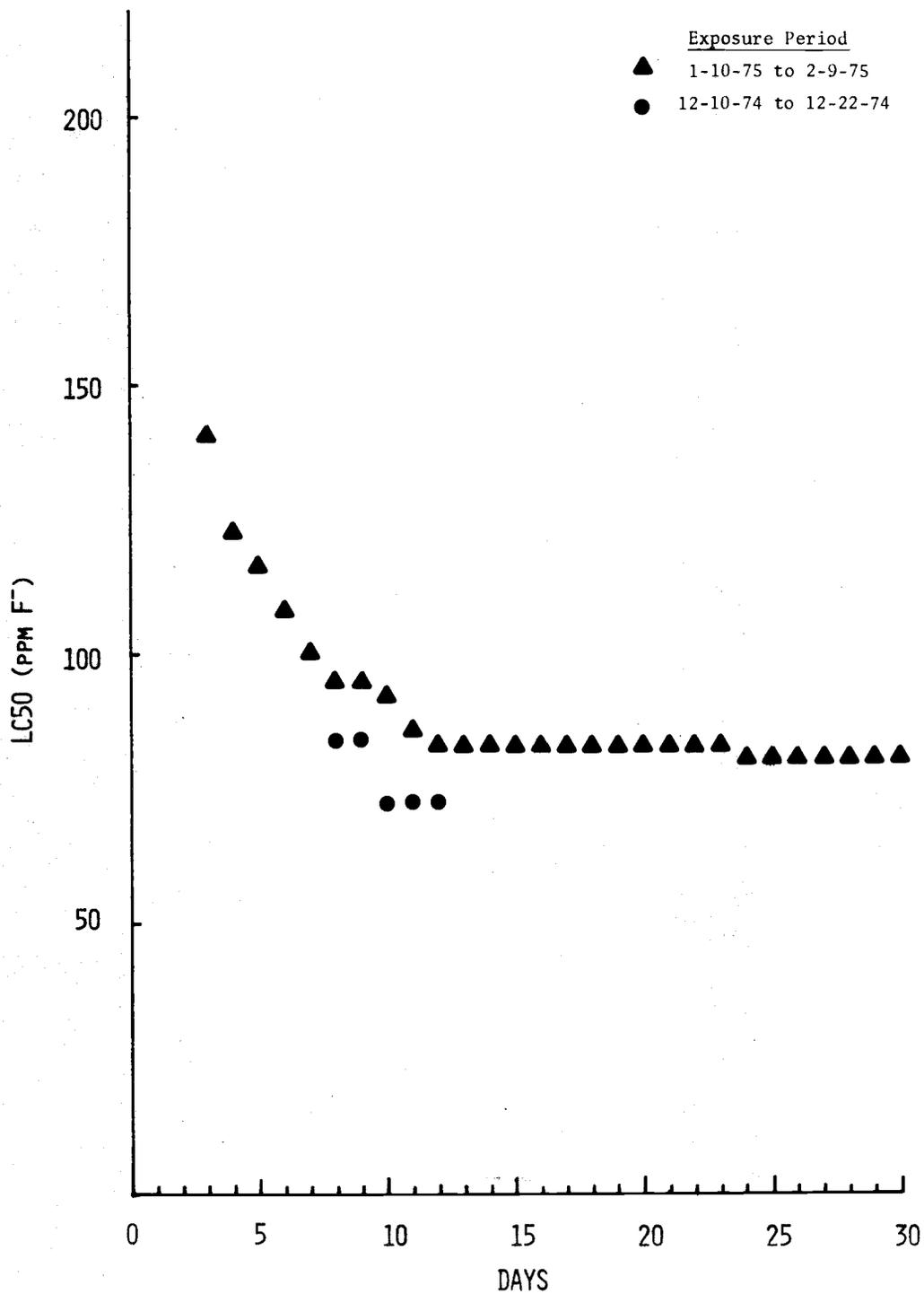


Figure I-7. Soluble fluoride LC<sub>50</sub> values (ppm) versus exposure time (days) for *Macoma inconspicua* at 5‰ salinity (From Data Tables I-17 and I-18).

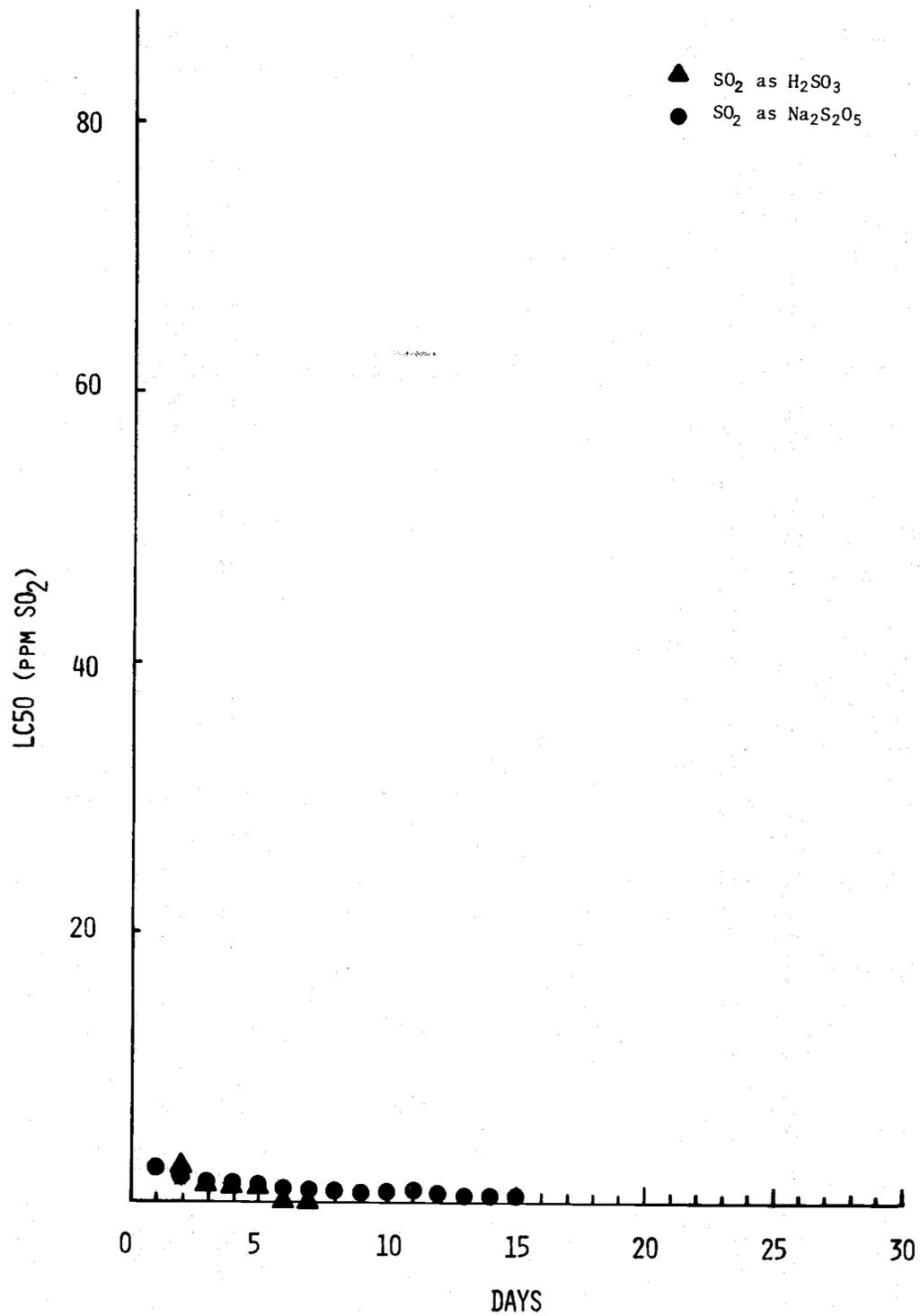


Figure I-8. Sulfur dioxide  $\text{LC}_{50}$  values (ppm) versus exposure time (days) for *Corophium salmonis* at 1‰ salinity (From Data Tables I-23 and I-24).

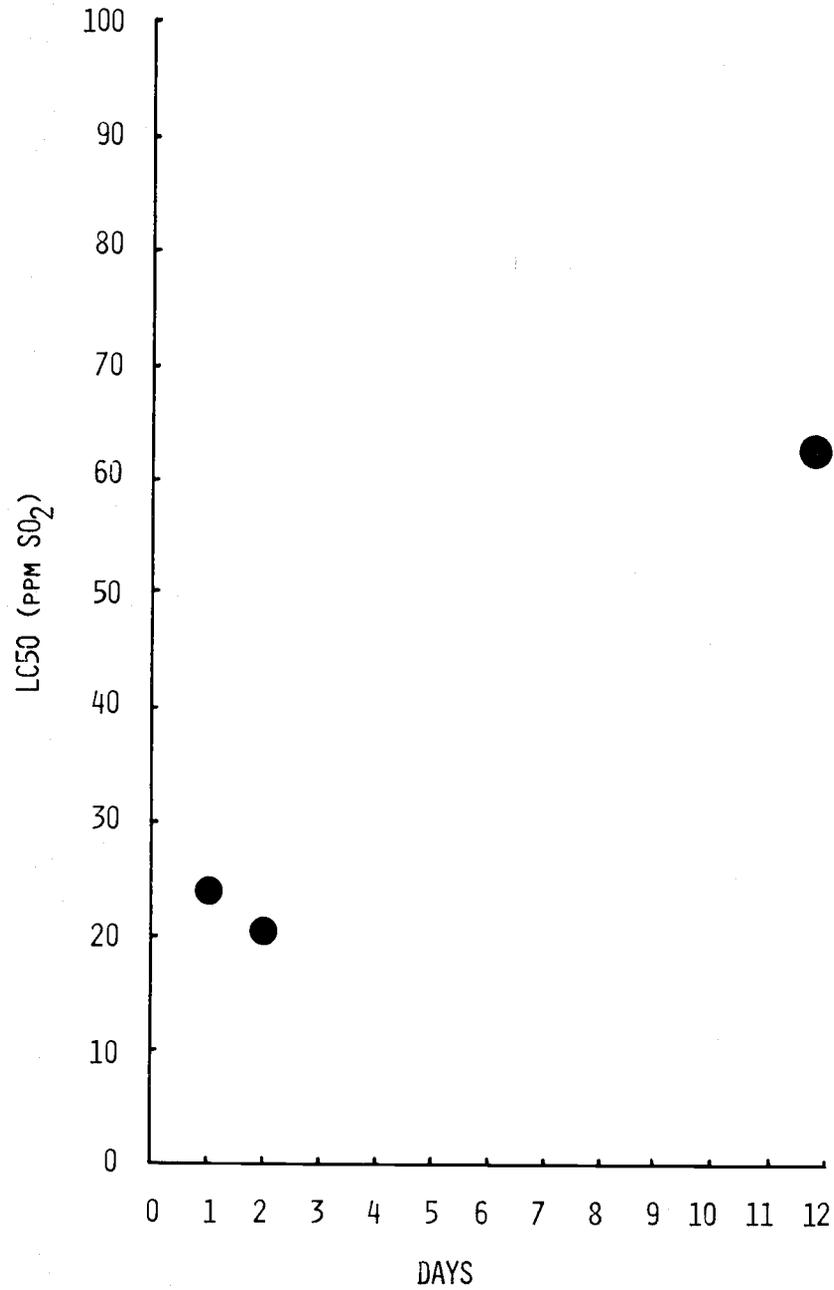


Figure I-9. Sulfur dioxide LC<sub>50</sub> values (ppm) versus exposure time (days) for *Crangon franciscorum* at 1‰ salinity (From Data Table I-26).

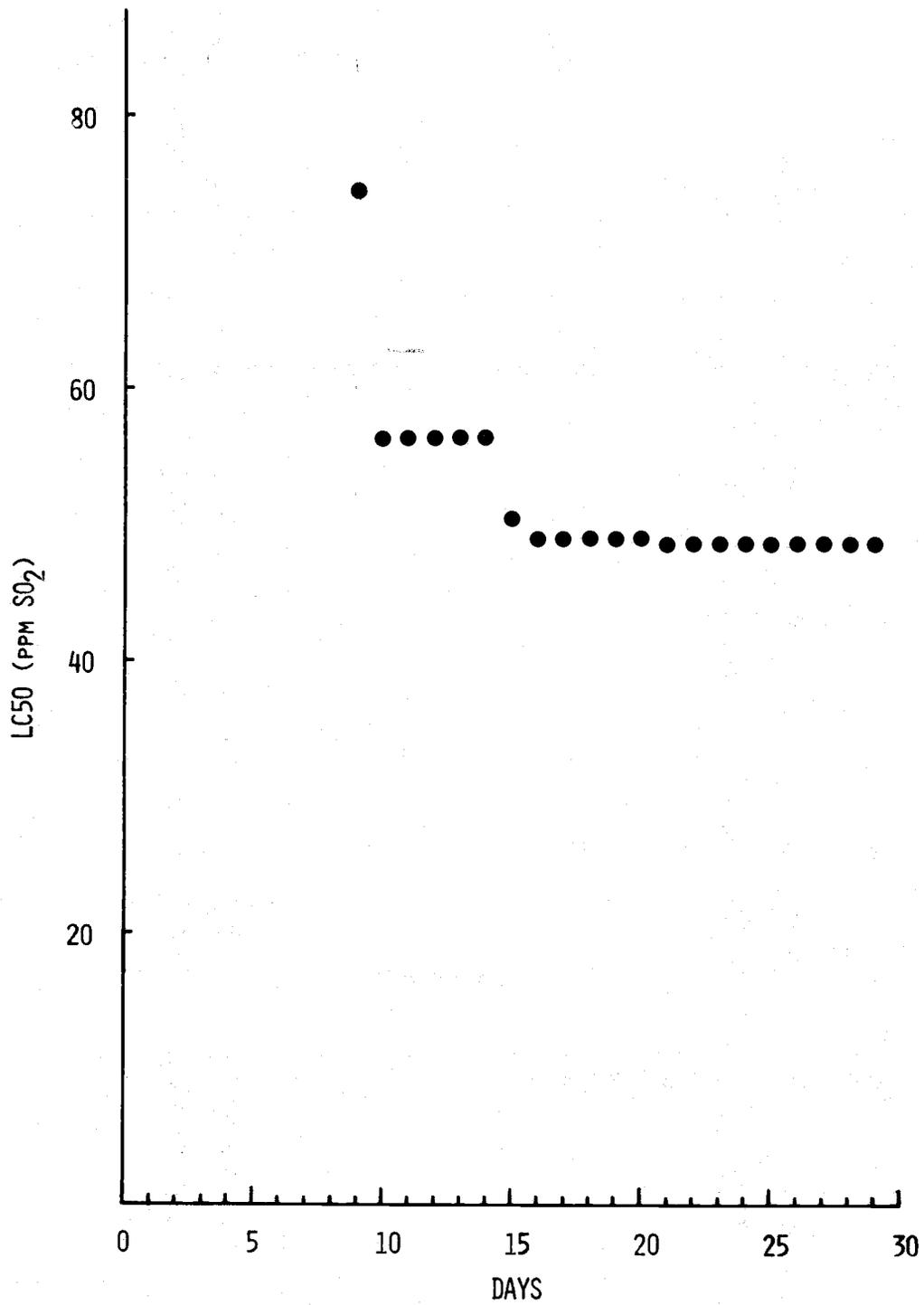


Figure I-10. Sulfur dioxide LC<sub>50</sub> values (ppm) versus exposure time (days) for *Macoma inconspicua* at 5‰ salinity (From Data Table I-28).

Table I-Ia.  $F^-$  LC50 Values (ppm) at 5‰ Salinity  
for Neanthes diversicolor from YOUNGS Bay

Animals per level of toxicant 10  
Replications per level of toxicant 2  
Toxicant levels of 1.81, 18.1, 181 (ppm)

| DATE<br>1975 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $F^-$ ) |
|--------------|------|-------------|-------|-------------|-------------|----------------------|
| 1-11         | 1    | 10.8        |       |             |             | > 180                |
| 1-12         | 2    | 11.5        |       |             |             | > 180                |
| 1-13         | 3    | 11.9        |       |             |             | > 180                |
| 1-14         | 4    | 10.8        |       |             |             | > 180                |
| 1-15         | 5    | 12.9        | -10   | 113         | -0.58       | > 180                |
| 1-16         | 6    | 10.8        | -80   | 201         | -0.94*      | 76                   |
| 1-17         | 7    | 12.0        | -80   | 201         | -0.94*      | 76                   |
| 1-18         | 8    | 12.3        | -80   | 201         | -0.94*      | 76                   |
| 1-19         | 9    | 12.0        | -100  | 226         | -1.00**     | 57                   |
| 1-20         | 10   | 11.7        | -50   | 126         | -0.90*      | 33                   |
| 1-21         | 11   | 11.0        | -45   | 113         | -0.90*      | 25                   |
| 1-22         | 12   | 11.4        | -45   | 110         | -0.93**     | 21                   |
| 1-23         | 13   | 12.9        | -40   | 100         | -0.86*      | 18                   |
| 1-24         | 14   | 11.3        | -40   | 100         | -0.86*      | 18                   |
| 1-25         | 15   | 10.5        | -40   | 90          | -0.78       | 10                   |
| 1-26         | 16   | 9.8         | -30   | 71          | -0.72       | 5.0                  |
| 1-27         | 17   | 9.9         | -30   | 71          | -0.72       | 5.0                  |
| 1-28         | 18   | 11.3        | -30   | 71          | -0.72       | 5.0                  |
| 1-29         | 19   | 9.9         | -30   | 71          | -0.72       | 5.0                  |
| 1-30         | 20   | 8.9         | -30   | 71          | -0.72       | 5.0                  |
| 1-31         | 21   | 9.0         | -30   | 71          | -0.72       | 5.0                  |

\*Significant at the 5% level

\*\*Significant at the 1% level















Table I-8.  $F^-$  LC50 Values (ppm) at 11‰ Salinity  
for Corophium salmonis from Yaquina Bay

Animals per level of toxicant 30  
Replications per level of toxicant 3  
Toxicant levels of 0.18, 1.8, 18.1, 181 (ppm)

| DATE<br>1974 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $F^-$ ) |
|--------------|------|-------------|-------|-------------|-------------|----------------------|
| 6-14         | 1    | 11.0        | -1    | 98          | -0.26       | >180                 |
| 6-15         | 2    | 12.0        | -3    | 98          | -0.52       | >180                 |
| 6-16         | 3    | 11.5        | -6    | 94          | -0.68*      | >180                 |
| 6-17         | 4    | 13.5        | -6    | 92          | -0.65*      | >180                 |
| 6-18         | 5    | 13.5        | -8    | 90          | -0.67*      | >180                 |
| 6-19         | 6    | 13.0        | -7    | 86          | -0.54       | >180                 |
| 6-20         | 7    | 12.0        | -9    | 85          | -0.64*      | >180                 |
| 6-21         | 8    | 12.0        | -9    | 80          | -0.61*      | >180                 |
| 6-22         | 9    | 11.5        | -9    | 80          | -0.61*      | >180                 |
| 6-23         | 10   | 10.5        | -7    | 76          | -0.50       | >180                 |
| 6-24         | 11   | 10.5        | -8    | 75          | -0.50       | >180                 |
| 6-25         | 12   | 10.0        | -10   | 73          | -0.58*      | 180                  |
| 6-26         | 13   | 9.0         | -9    | 70          | -0.55       | 150                  |
| 6-27         | 14   | 10.0        | -9    | 70          | -0.55       | 150                  |
| 6-28         | 15   | 11.0        | -9    | 68          | -0.55       | 130                  |
| 6-29         | 16   | 12.0        | -8    | 65          | -0.50       | 72                   |
| 6-30         | 17   | 13.5        | -9    | 65          | -0.51       | 52                   |
| 7-1          | 18   | 14.0        | -9    | 62          | -0.51       | 22                   |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |

\*Significant at the 5% level

\*\*Significant at the 1% level











Table I-14.  $F^-$  LC50 Values (ppm) at 1 ‰ Salinity  
for CRANGON FRANCISCORUM from YOUNGS Bay

Animals per level of toxicant 15  
Replications per level of toxicant 3  
Toxicant levels of 18.1, 181, 1810 (ppm)

| DATE<br>1974 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $F^-$ ) |
|--------------|------|-------------|-------|-------------|-------------|----------------------|
| 8-13         | 1    | 11.0        | -47   | 165         | -0.88**     | 300                  |
| 8-14         | 2    | 11.0        | -47   | 159         | -0.86**     | 210                  |
| 8-15         | 3    | 12.0        | -43   | 134         | -0.95**     | 87                   |
| 8-16         | 4    | 11.0        | -40   | 119         | -0.86**     | 54                   |
| 8-17         | 5    | 11.0        | -73   | 166         | -0.94**     | 38                   |
| 8-18         | 6    | 11.0        | -73   | 166         | -0.94**     | 38                   |
| 8-19         | 7    | 12.0        | -67   | 151         | -0.98**     | 32                   |
| 8-20         | 8    | 12.0        | -67   | 151         | -0.98**     | 32                   |
| 8-21         | 9    | 12.5        | -67   | 151         | -0.98**     | 32                   |
| 8-22         | 10   |             | -60   | 135         | -0.93**     | 27                   |
| 8-23         | 11   | 13.5        | -60   | 135         | -0.93**     | 27                   |
| 8-24         | 12   | 14.0        | -60   | 135         | -0.93**     | 27                   |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |
|              |      |             |       |             |             |                      |

\*Significant at the 5% level

\*\*Significant at the 1% level







Table I-18a.  $F^-$  LC50 Values (ppm) at 5 ‰ Salinity  
for MACOMA INCONSPICUA from YOUNGS Bay

Animals per level of toxicant 18  
Replications per level of toxicant 3  
Toxicant levels of 50, 100, 200 (ppm)

| DATE<br>1975 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $F^-$ ) |
|--------------|------|-------------|-------|-------------|-------------|----------------------|
| 1-11         | 1    |             |       |             |             | > 200                |
| 1-12         | 2    | 11.5        | -1666 | 432         | -0.83*      | > 200                |
| 1-13         | 3    | 11.5        | -332  | 764         | -1.00**     | 140                  |
| 1-14         | 4    | 12.0        | -1666 | 397         | -0.89**     | 120                  |
| 1-15         | 5    | 12.9        | -1666 | 393         | -0.92**     | 120                  |
| 1-16         | 6    | 11.1        | -1666 | 388         | -0.96**     | 110                  |
| 1-17         | 7    | 12.1        | -1666 | 382         | -0.95**     | 100                  |
| 1-18         | 8    | 12.3        | -1666 | 378         | -0.95**     | 95                   |
| 1-19         | 9    | 12.0        | -1666 | 378         | -0.95**     | 95                   |
| 1-20         | 10   | 12.3        | -1666 | 377         | -0.96**     | 93                   |
| 1-21         | 11   | 12.5        | -1666 | 371         | -0.92**     | 86                   |
| 1-22         | 12   | 11.0        | -1666 | 369         | -0.89**     | 84                   |
| 1-23         | 13   | 12.0        | -1666 | 369         | -0.89**     | 84                   |
| 1-24         | 14   | 11.3        | -1666 | 369         | -0.89**     | 84                   |
| 1-25         | 15   | 10.5        | -1666 | 369         | -0.89**     | 84                   |
| 1-26         | 16   | 9.8         | -1666 | 369         | -0.89**     | 84                   |
| 1-27         | 17   | 9.9         | -1666 | 369         | -0.89**     | 84                   |
| 1-28         | 18   | 11.3        | -1666 | 369         | -0.89**     | 84                   |
| 1-29         | 19   | 9.9         | -1666 | 369         | -0.89**     | 84                   |
| 1-30         | 20   | 8.9         | -1666 | 369         | -0.89**     | 84                   |
| 1-31         | 21   | 9.0         | -1666 | 369         | -0.89**     | 84                   |

\*Significant at the 5% level

\*\*Significant at the 1% level













Table I-24.  $SO_2$  LC50 Values (ppm) at 1 ‰ Salinity  
for Corophium salmonis from Youngs Bay

Animals per level of toxicant 30

Replications per level of toxicant 3

Toxicant levels of 0.011, 0.11, 1.1, 11.2 (ppm)

| DATE<br>1974 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $SO_2$ ) |
|--------------|------|-------------|-------|-------------|-------------|-----------------------|
| 7-25         | 1    | 14.5        | -47   | 70          | -0.88**     | 2.7                   |
| 7-26         | 2    |             | -50   | 66          | -0.89**     | 2.1                   |
| 7-27         | 3    | 11.0        | -50   | 65          | -0.91**     | 2.0                   |
| 7-28         | 4    | 11.0        | -29   | 57          | -0.80**     | 1.7                   |
| 7-29         | 5    | 11.0        | -30   | 55          | -0.83**     | 1.4                   |
| 7-30         | 6    | 12.5        | -28   | 54          | -0.79**     | 1.4                   |
| 7-31         | 7    | 13.0        | -25   | 52          | -0.75**     | 1.2                   |
| 8-1          | 8    | 11.0        | -25   | 52          | -0.75**     | 1.2                   |
| 8-2          | 9    | 11.0        | -24   | 50          | -0.75**     | 0.99                  |
| 8-3          | 10   | 10.5        | -25   | 49          | -0.76**     | <0.011                |
| 8-4          | 11   | 10.0        | -25   | 49          | -0.76**     | <0.011                |
| 8-5          | 12   |             | -25   | 48          | -0.77**     | <0.011                |
| 8-6          | 13   |             | -22   | 46          | -0.70*      | <0.011                |
| 8-7          | 14   |             | -21   | 45          | -0.68*      | <0.011                |
| 8-8          | 15   |             | -21   | 45          | -0.68*      | <0.011                |
|              |      |             |       |             |             |                       |
|              |      |             |       |             |             |                       |
|              |      |             |       |             |             |                       |
|              |      |             |       |             |             |                       |
|              |      |             |       |             |             |                       |
|              |      |             |       |             |             |                       |

\*Significant at the 5% level

\*\*Significant at the 1% level







Table I-28a.  $SO_2$  LC50 Values (ppm) at 5 ‰ Salinity  
for Macoma inconspicua from Youngs Bay

Animals per level of toxicant 10  
Replications per level of toxicant 3  
Toxicant levels of 25, 50, 75 (ppm)

| DATE<br>1975 | DAYS | TEMP.<br>°C | SLOPE | Y-INTERCEPT | COR. COEFF. | LC50<br>(ppm $SO_2$ ) |
|--------------|------|-------------|-------|-------------|-------------|-----------------------|
| 1-11         | 1    |             |       |             |             | >75                   |
| 1-12         | 2    | 11.5        | -12   | 116         | -0.45       | >75                   |
| 1-13         | 3    | 11.5        | -81   | 213         | -0.84**     | >75                   |
| 1-14         | 4    | 12.0        | -81   | 213         | -0.84**     | >75                   |
| 1-15         | 5    | 12.9        | -81   | 213         | -0.84**     | >75                   |
| 1-16         | 6    | 11.1        | -81   | 213         | -0.84**     | >75                   |
| 1-17         | 7    | 12.1        | -81   | 213         | -0.84**     | >75                   |
| 1-18         | 8    | 12.3        | -81   | 213         | -0.84**     | >75                   |
| 1-19         | 9    | 12.0        |       |             |             |                       |
| 1-20         | 10   | 12.3        | -112  | 259         | -0.84**     | 75                    |
| 1-21         | 11   | 10.5        | -155  | 322         | -0.86**     | 57                    |
| 1-22         | 12   | 11.0        | -155  | 322         | -0.86**     | 57                    |
| 1-23         | 13   | 12.0        | -155  | 322         | -0.86**     | 57                    |
| 1-24         | 14   | 11.3        | -155  | 322         | -0.86**     | 57                    |
| 1-25         | 15   | 10.5        | -155  | 322         | -0.86**     | 57                    |
| 1-26         | 16   | 9.8         | -187  | 368         | -0.90**     | 51                    |
| 1-27         | 17   | 9.9         | -197  | 384         | -0.90**     | 50                    |
| 1-28         | 18   | 11.3        | -197  | 384         | -0.90**     | 50                    |
| 1-29         | 19   | 9.9         | -197  | 384         | -0.90**     | 50                    |
| 1-30         | 20   | 8.9         | -197  | 384         | -0.90**     | 50                    |
| 1-31         | 21   | 9.0         | -197  | 384         | -0.90**     | 50                    |

\*Significant at the 5% level

\*\*Significant at the 1% level



II

SUBLETHAL EFFECTS:  
PHYTOPLANKTON PRIMARY PRODUCTION

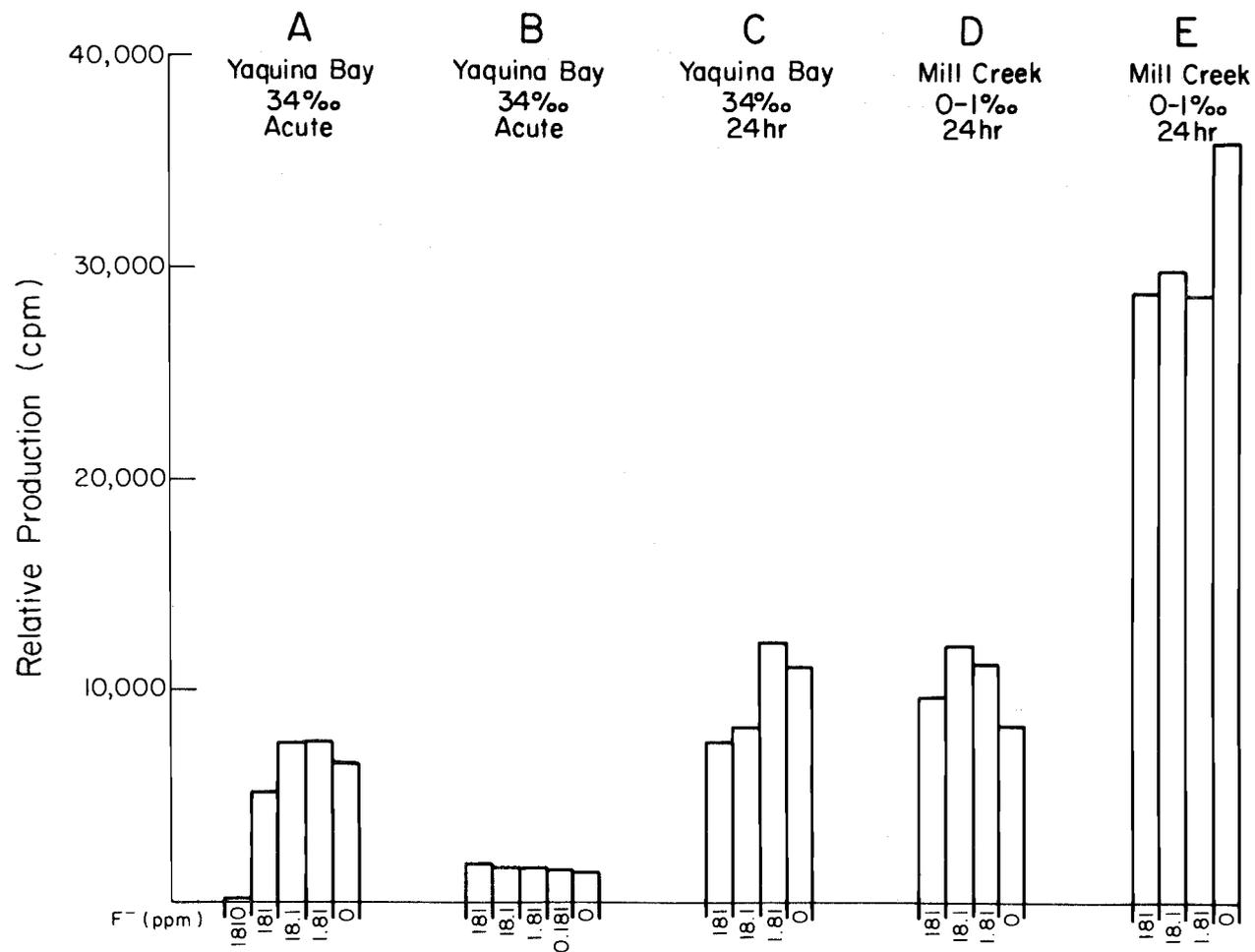


Figure II-1. Effect of soluble fluoride (added as NaF) on the relative rate of photosynthesis by Yaquina Bay phytoplankton measured at the Marine Science Center. Values are given in Table II-1. Mill Creek is in upper Yaquina Bay.

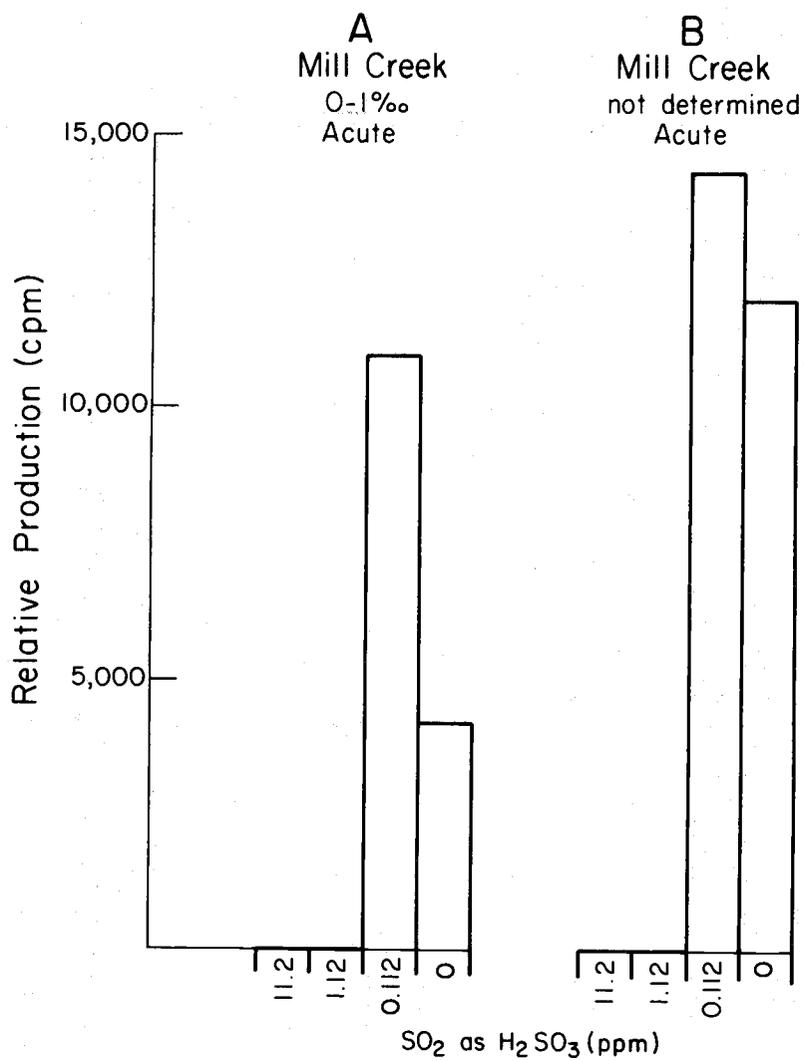


Figure II-2. Effect of sulfur dioxide (added as H<sub>2</sub>SO<sub>3</sub>) on the relative rate of photosynthesis by upper Yaquina Bay (Mill Creek) phytoplankton measured at the Marine Science Center. Values are given in Table II-2.

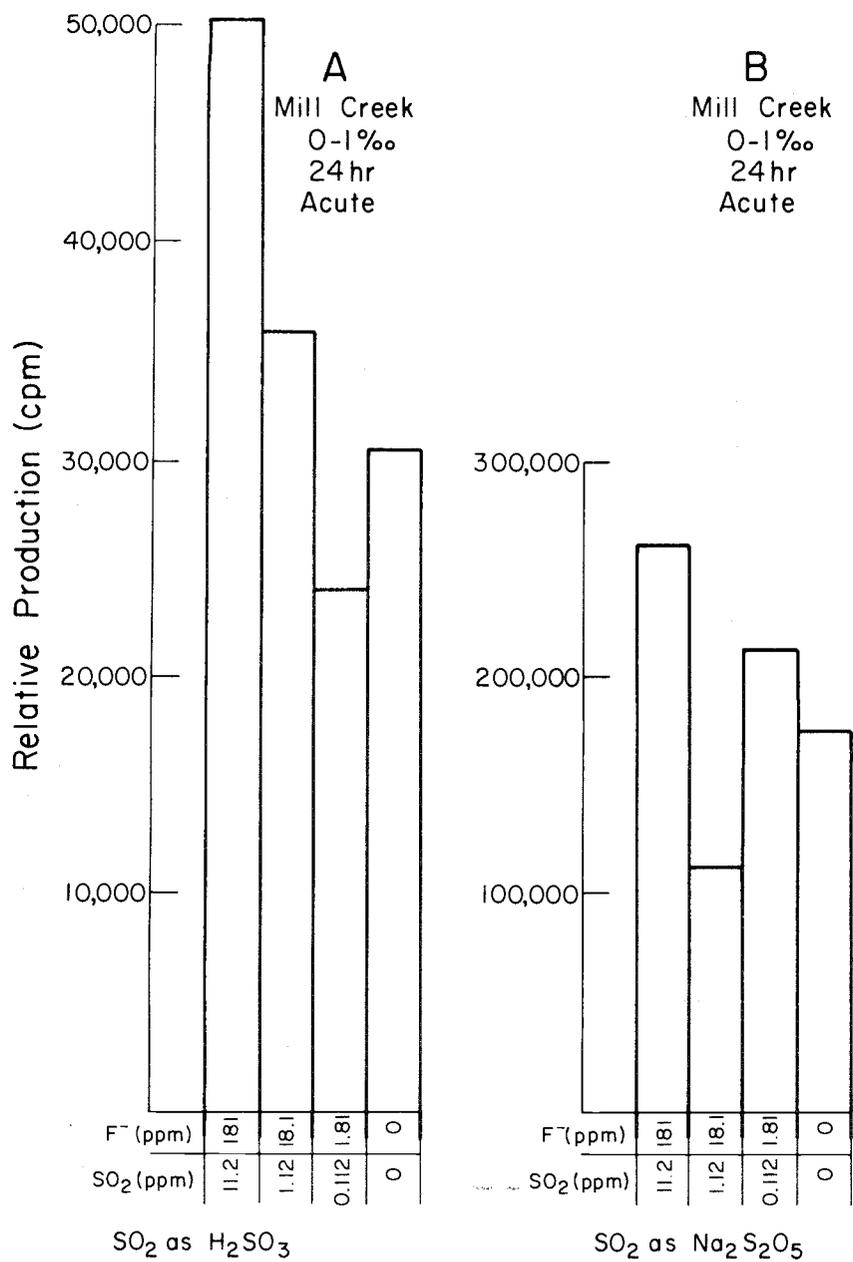


Figure II-3. Effect of the interaction of soluble fluoride and sulfur dioxide on the relative rate of photosynthesis by upper Yaquina Bay (Mill Creek) phytoplankton measured at the Marine Science Center. Values are given in Table II-3.

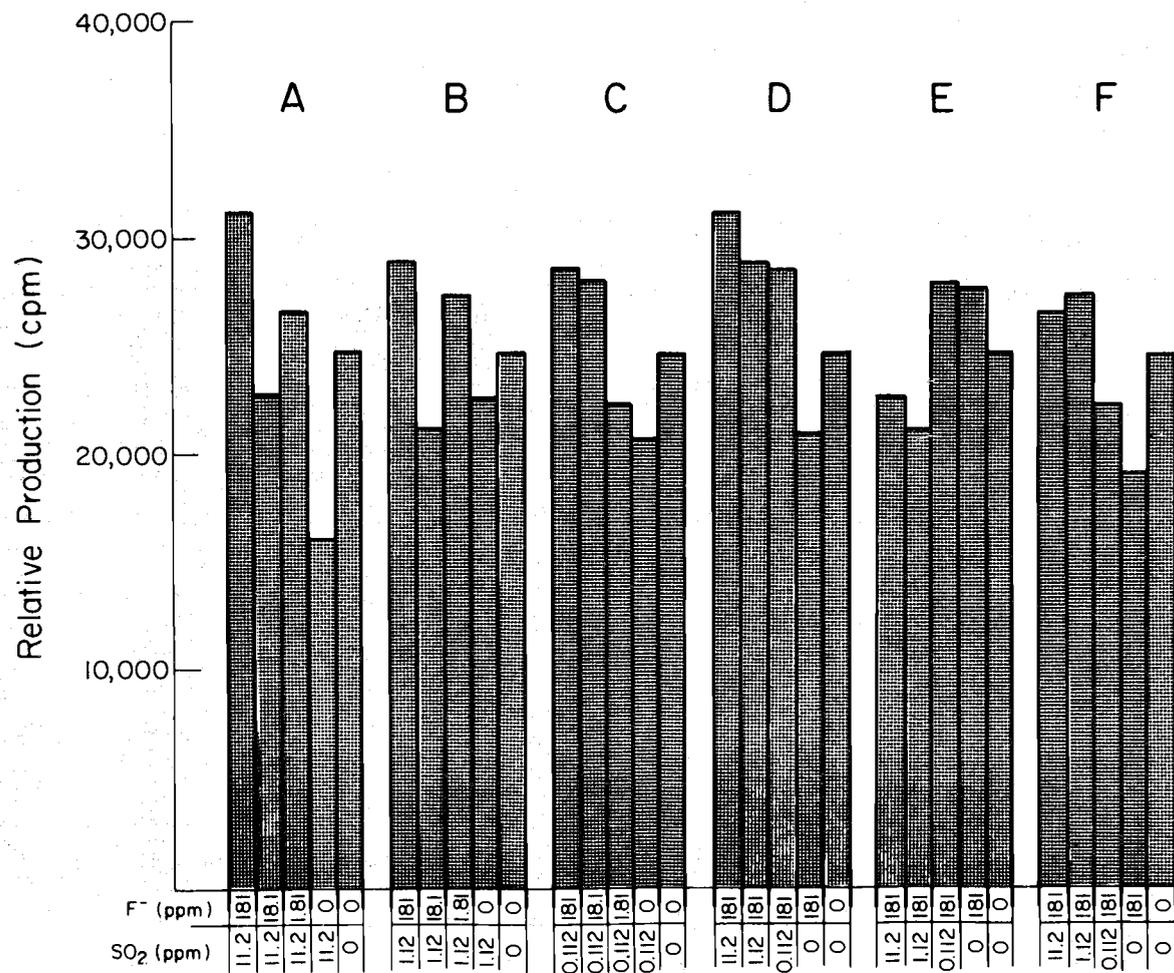


Figure II-6. Effect of the interaction of soluble fluoride and sulfur dioxide interaction on the relative rate of photosynthesis by phytoplankton in the Youngs Bay area. Values are given in Table II-5.

Table II-1. Effect of soluble fluoride (added as NaF) on the relative rate of photosynthesis by Yaquina Bay phytoplankton at the Marine Science Center. All values are counts per minute.

| Experiment | Identification<br>(ppm F <sup>-</sup> ) | $\bar{X}_{light}$ | N | Upper limit<br>( $\alpha = 0.05$ ) | Lower limit<br>( $\alpha = 0.05$ ) | $X_{dark}$ | $X_{net}$ |
|------------|---|-------------------|---|------------------------------------|------------------------------------|------------|-----------|
| A          | 1810                                    | 568               | 2 | 734                                | 400                                | 470        | 98        |
|            | 181                                     | 5340              | 2 | 5854                               | 4827                               | 108        | 5232      |
|            | 18.1                                    | 7546              | 2 | 7872                               | 7218                               | 36         | 7510      |
|            | 1.81                                    | 7617              | 2 | 8441                               | 6793                               | 75         | 7542      |
|            | 0.0                                     | 6732              | 2 | 8834                               | 4628                               | 79         | 6653      |
| B          | 181                                     | 2188              | 2 | 2451                               | 1926                               | 426        | 1762      |
|            | 18.1                                    | 2036              | 2 | 2798                               | 1273                               | 454        | 1582      |
|            | 1.81                                    | 1922              | 2 | 2108                               | 1735                               | 367        | 1555      |
|            | 0.181                                   | 1919              | 1 | ----                               | ----                               | 508        | 1411      |
|            | 0.0                                     | 1754              | 2 | 1892                               | 1615                               | 415        | 1339      |
| C          | 181                                     | 8223              | 3 | 11478                              | 4969                               | 612        | 7611      |
|            | 18.1                                    | 9287              | 3 | 11794                              | 6780                               | 1046       | 8241      |
|            | 1.81                                    | 12845             | 2 | 12895                              | 12796                              | 470        | 12375     |
|            | 0.0                                     | 12179             | 3 | 13020                              | 11338                              | 855        | 11324     |
| D          | 181                                     | 10135             | 3 | 12120                              | 8150                               | 523        | 9612      |
|            | 18.1                                    | 12669             | 3 | 13566                              | 11771                              | 578        | 12091     |
|            | 1.81                                    | 12589             | 3 | 14007                              | 11171                              | 656        | 11933     |
|            | 0.0                                     | 9101              | 3 | 12376                              | 5826                               | 772        | 8329      |
| E          | 181                                     | 29785             | 3 | 34011                              | 25558                              | 986        | 28799     |
|            | 18.1                                    | 30637             | 3 | 43057                              | 18216                              | 774        | 29863     |
|            | 1.81                                    | 29660             | 3 | 36544                              | 22776                              | 1060       | 28600     |
|            | 0.0                                     | 36595             | 3 | 42657                              | 30533                              | 706        | 35889     |

Table II-2. Effect of sulfur dioxide (added as H<sub>2</sub>SO<sub>3</sub>) on the relative rate of photosynthesis by Yaquina Bay phytoplankton at the Marine Science Center. All values are counts per minute.

| Experiment | Identification<br>(ppm SO <sub>2</sub> ) | $\bar{X}_{light}$ | N | Upper limit<br>( $\alpha = 0.05$ ) | Lower limit<br>( $\alpha = 0.05$ ) | $X_{dark}$ | $X_{net}$ |
|------------|--|-------------------|---|------------------------------------|------------------------------------|------------|-----------|
| A          | 11.2                                     | 379               | 3 | 402                                | 357                                | 431        | -52       |
|            | 1.12                                     | 412               | 3 | 431                                | 394                                | 407        | 5         |
|            | 0.112                                    | 12008             | 3 | 15873                              | 8144                               | 1068       | 10940     |
|            | 0.0                                      | 4423              | 1 | ----                               | ----                               | 232        | 4191      |
| B          | 11.2                                     | 416               | 3 | 432                                | 400                                | 413        | 3         |
|            | 1.12                                     | 426               | 3 | 435                                | 417                                | 424        | 2         |
|            | 0.112                                    | 14849             | 3 | 17347                              | 12351                              | 549        | 14300     |
|            | 0.0                                      | 13154             | 3 | 17185                              | 9123                               | 683        | 12471     |

Table II-3. Effect of the interaction of soluble fluoride and sulfur dioxide on the relative rate of photosynthesis by Yaquina Bay phytoplankton at the Marine Science Center. All values are counts per minute.

| Experiment  | Identification              |  | $\bar{X}_{light}$ | N | Upper limit<br>( $\alpha = 0.05$ ) | Lower limit<br>( $\alpha = 0.05$ ) | $X_{dark}$ | $X_{net}$ |
|---|-----------------------------|--|-------------------|---|------------------------------------|------------------------------------|------------|-----------|
|   | ppm F <sup>-</sup><br>(NaF) | ppm SO <sub>2</sub><br>(H <sub>2</sub> SO <sub>3</sub> or<br>Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> ) |                   |   |                                    |                                    |            |           |
| A<br>(H <sub>2</sub> SO <sub>3</sub> )                | 181                         | 11.2   | 51125             | 3 | 65635                              | 36616                              | 800        | 50325     |
|   | 18.1                        | 1.12   | 37042             | 3 | 38234                              | 35850                              | 1186       | 35856     |
|   | 1.81                        | 0.112  | 25568             | 3 | 28232                              | 22903                              | 1545       | 24023     |
|   | 0.0                         | 0.0  | 31250             | 3 | 32771                              | 29729                              | 717        | 30533     |
| B<br>(Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> ) | 181                         | 11.2   | 261189            | 3 | 339117                             | 183261                             | 1368       | 259821    |
|   | 18.1                        | 1.12   | 113208            | 3 | 149532                             | 76883                              | 828        | 112380    |
|   | 1.81                        | 0.112  | 213643            | 3 | 254446                             | 172840                             | 1175       | 212468    |
|   | 0.0                         | 0.0  | 176771            | 3 | 214719                             | 138824                             | 2850       | 173921    |

Table II-4. Effect of soluble fluoride (added as NaF) on the *in situ* <sup>14</sup>C-uptake rate by phytoplankton in the Youngs Bay area. All values are counts per minute. Station locations shown on Figure II-4.

| Location | Depth (m) | Identification        |  | $\bar{X}_{light}$ | N | Upper limit<br>( $\alpha = 0.05$ ) | Lower limit<br>( $\alpha = 0.05$ ) | $X_{dark}$ | $X_{net}$ |
|----------|-----------|-----------------------|--|-------------------|---|------------------------------------|------------------------------------|------------|-----------|
|          |           | (ppm F <sup>-</sup> ) |  |                   |   |                                    |                                    |            |           |
| I        | 0.5       | 181                   |  | 11572             | 3 | 13070                              | 10073                              | 851        | 10721     |
|          |           | 18.1                  |  | 11049             | 3 | 13033                              | 9065                               | 778        | 10271     |
|          |           | 1.81                  |  | 11280             | 2 | 12398                              | 10160                              | 803        | 10477     |
|          |           | 0.0                   |  | 13002             | 3 | 14240                              | 11764                              | ---        | -----     |
| II       | 0.5       | 181                   |  | 3441              | 3 | 3755                               | 3127                               | 652        | 2789      |
|          |           | 18.1                  |  | 3741              | 3 | 4626                               | 2855                               | 609        | 3132      |
|          |           | 1.81                  |  | 3564              | 3 | 4116                               | 3012                               | 497        | 3067      |
|          |           | 0.0                   |  | 10859             | 3 | 11093                              | 10626                              | ---        | -----     |
| II       | 3.0       | 181                   |  | 1562              | 3 | 1947                               | 1176                               | 734        | 828       |
|          |           | 18.1                  |  | 1655              | 3 | 1787                               | 1522                               | 532        | 1123      |
|          |           | 1.81                  |  | 1546              | 3 | 1761                               | 1331                               | 538        | 1008      |
|          |           | 0.0                   |  | 24579             | 3 | 26096                              | 23063                              | ---        | -----     |
| III      | 0.5       | 181                   |  | 10438             | 3 | 11025                              | 9851                               | 1109       | 9329      |
|          |           | 18.1                  |  | 11299             | 3 | 12286                              | 10312                              | 769        | 10530     |
|          |           | 1.81                  |  | 10905             | 3 | 12456                              | 9354                               | 682        | 10223     |
|          |           | 0.0                   |  | 46434             | 3 | 60356                              | 32511                              | ---        | -----     |
| III      | 3.0       | 181                   |  | 1827              | 3 | 1953                               | 1701                               | 611        | 1216      |
|          |           | 18.1                  |  | 1532              | 3 | 1834                               | 1230                               | 583        | 949       |
|          |           | 1.81                  |  | 1642              | 3 | 1950                               | 1335                               | 663        | 979       |
|          |           | 0.0                   |  | 1800              | 3 | 2252                               | 1347                               | ---        | -----     |
| IV       | 0.5       | 181                   |  | 15186             | 3 | 17108                              | 13264                              | 1386       | 13800     |
|          |           | 18.1                  |  | 31733             | 3 | 34627                              | 28838                              | 863        | 30870     |
|          |           | 1.81                  |  | 26995             | 3 | 27710                              | 26279                              | 1767       | 25228     |
|          |           | 0.0                   |  | 21326             | 3 | 25657                              | 16996                              | ---        | -----     |
| V        | 0.5       | 181                   |  | 21550             | 3 | 29654                              | 13446                              | 2620       | 18930     |
|          |           | 18.1                  |  | 42303             | 3 | 61114                              | 23492                              | 1264       | 41039     |
|          |           | 1.81                  |  | 44728             | 3 | 65150                              | 24306                              | 2589       | 42139     |
|          |           | 0.0                   |  | 10252             | 3 | 10900                              | 9604                               | ---        | -----     |

Table II-5. Effect of the interaction of soluble fluoride and sulfur dioxide on the relative *in situ* rate of photosynthesis by Youngs Bay phytoplankton. All values are counts per minute.

| Identification              |   | $\bar{X}_{\text{light}}$ | N | Upper limit<br>( $\alpha = 0.05$ ) | Lower limit<br>( $\alpha = 0.05$ ) | $X_{\text{dark}}$ | $X_{\text{net}}$ |
|-----------------------------|---|--------------------------|---|------------------------------------|------------------------------------|-------------------|------------------|
| ppm F <sup>-</sup><br>(NaF) | ppm SO <sub>2</sub><br>(Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> ) |                          |   |                                    |                                    |                   |                  |
| 181                         | 0.0   | 22301                    | 3 | 25632                              | 18970                              | 1374              | 20927            |
| 18.1                        | 0.0   | 28444                    | 3 | 36159                              | 20729                              | 830               | 27614            |
| 1.81                        | 0.0   | 19861                    | 3 | 24428                              | 15294                              | 787               | 19074            |
| 0.0                         | 11.2  | 17001                    | 3 | 21928                              | 12075                              | 1025              | 15976            |
| 0.0                         | 1.12  | 23549                    | 3 | 30226                              | 16872                              | 1050              | 22499            |
| 0.0                         | 0.112   | 21629                    | 2 | 29335                              | 13923                              | 1012              | 20617            |
| 0.0                         | 0.0   | 25392                    | 6 | 28872                              | 21913                              | 771               | 24621            |
| 181                         | 11.2  | 31962                    | 2 | 31980                              | 31942                              | 798               | 31164            |
| 181                         | 1.12  | 29656                    | 3 | 35655                              | 23656                              | 895               | 28761            |
| 181                         | 0.112   | 29288                    | 2 | 35048                              | 23530                              | 827               | 28461            |
| 18.1                        | 11.2  | 23515                    | 3 | 26050                              | 20981                              | 851               | 22664            |
| 18.1                        | 1.12  | 21857                    | 3 | 27673                              | 16042                              | 839               | 21018            |
| 18.1                        | 0.112   | 28876                    | 2 | 37288                              | 20462                              | 928               | 27948            |
| 1.81                        | 11.2  | 27252                    | 3 | 31328                              | 23176                              | 774               | 26478            |
| 1.81                        | 1.12  | 28105                    | 3 | 37627                              | 18583                              | 768               | 27337            |
| 1.81                        | 0.112   | 23062                    | 3 | 25697                              | 20426                              | 684               | 22378            |

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