Effects of Chemicals in Thermal Effluent on *Homarus americanus* Maintained in Aquaculture Systems[1]

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ABSTRACT

The effects of chemicals in thermal effluent on American lobsters, *Homarus americanus*, maintained in aquaculture systems were evaluated. Atomic absorption analysis of intake and effluent water samples from three fossil-fuel generating stations in southern California indicated that their chemical additions did not affect the concentrations of Cu, Zn, Cd, Co, Cr, Pb, and As in the thermal effluent. Concentrations of these metals in the intake and effluent water at the San Diego Gas & Electric Company's Encina Power Plant were not significantly different than their concentrations in seawater from the Scripps Institution, and were well within the reported ranges for levels of those metals in normal seawater. The amounts of heavy metal chelating agents in Encina effluent were significantly greater than in Encina intake and Scripps seawater.

No significant differences were observed in mean concentrations of Cu, Zn, Cd, Pb, Cr, and Co, in larval, juvenile and adult lobsters maintained in three water types (Encina effluent, Encina intake and Scripps). Lobsters maintained in

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Scripps seawater were dissected and the metal levels in the gills, hepatopancreas, tail muscles, claw muscles, digestive tract, and exoskeleton were analyzed by atomic absorption. The highest concentrations of Cu, Zn, Co, and Cr were found in the gills, while Cd was found in equally high concentrations in the gills and hepatopancreas. The lowest concentrations of Cu, Zn, Cd, and Cr were found in the exoskeleton, while Pb and Co were found in the lowest concentrations in the tail muscle. Regressions of metal levels in tissues of the lobsters on carapace length indicated that H. americanus does not accumulate metals in the tissues, but rather regulates the levels.

The 24, 48, 72, and 96 hour median lethal limits for Cu^{++}, Zn^{++}, Co^{+++}, Cd^{++}, Pb^{++}, Cr^{6+}, Pb^{++}, Cl^{-}, and acids were established for lobster larvae held in static systems. Median effective times also were determined. Median lethal limits for these eight chemicals were all far above levels of the chemicals encountered in the generating station effluents. In most cases they were at least an order of magnitude higher. Long-term bioassays in static systems indicated that growth and survival of larval and juvenile lobsters were similar in Encina effluent, Encina intake, and Scripps seawater at constant temperature.

All of these studies indicate that the thermal effluent from typical generating stations in southern California provides an essentially pollution-free heated water source for the culture of H. americanus.

INTRODUCTION

Aquaculture research must consider both the potential adverse and beneficial effects of many artificial culture conditions on economically important aquatic organisms. Taylor (1950) and Hughes et al., (1972) have recognized the positive effects of using warm water in culturing the American lobster, Homarus americanus. The results of earlier work done at San Diego State University indicate that growth of H. americanus can be significantly increased in thermal effluent (Ford et al., 1975).

However, there are specific problems involved with the use of thermal effluent in aquaculture systems. Becker and Thatcher (1973) have described a large number of chemicals found in the effluents of generating stations and their possible harmful effects on aquatic life. Levels of any toxic substance above its normal concentration in natural seawater can cause chronic, sublethal effects, such as a reduction in growth and fecundity, as well as acute lethal effects (Daudoroff and Katz, 1953; Bowen, 1966; Spraque,1969).

Based on information from the review by Becker and Thatcher (1973) and preliminary analyses of water chemistry, the research described here was conducted to evaluate the effects of copper, zinc, cadmium, cobalt, chlorine, chromium, lead, arsenic, and acids on representative life history stages of H. americanus reared in aquaculture systems. The study was conducted primarily in the San Diego State University Aquaculture Laboratory at the San Diego Gas & Electric Company's Encina Power Plant, Carlsbad, California.

Three basic approaches were used to evaluate the effects of these chemicals on H. americanus in a comprehensive manner. These were: 1) analyses of water quality characteristics in laboratory water sources at the Encina Power Plant and at the Scripps Institution of Oceanography; 2) analyses of metal accumulations in lobster tissues; and 3) tolerance experiments to determine acute and chronic biological effects of the chemicals at various concentrations.

The results of this research should have considerable practical value for agencies such as the U.S. Food and Drug Administration, and for commerical mariculturists who plan to use thermal effluent as an inexpensive source of warm seawater.

MATERIALS AND METHODS

Water Sample Collection

Water samples were collected in 2 liter polypropylene containers over a six month period, on a biweekly schedule, from September, 1974, through February, 1975. These samples were taken by hand from three water sources used in San Diego State University lobster culture systems: 1) water in the Scripps Institution of Oceanography seawater system pumped from the end of SIO pier, La Jolla, California; 2) seawater from the surface of the outer Agua Hedionda Lagoon, which is the cooling water source for the Encina Power Plant in Carlsbad, California; and 3) thermal effluent from the surface of the Encina Power Plant effluent cooling pond (Ford et al., 1975). Such 2 liter samples also were taken from the intake and effluent waters of the Southern California Edison Company's large generating stations at Redondo Beach and Ormond Beach in southern California. All samples were treated carefully to prevent contanination, and handling was reduced to a minimum.

Determination of Chemical Water Quality and Heavy Metal Concentrations in the Water Samples

In addition to temperature, the pH, dissolved oxygen, total salinity, and suspended solids levels of each water sample were determined by standard techniques. A method of metal ion chelation and organic extraction, similar to that used by Brooks et al.,(1967), was employed as a multi-element preparation for atomic absorption analysis of the heavy metal concentrations in the water samples. Particulate fractions (particles size >0.45 μ) of each sample were prepared with HCl digestion in an organic matrix. Water samples and standard solutions were aspirated directly into an air-acetylene flame of a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer, and analyzed against blanks in the absorption mode. The concentrations of copper, cobalt, chromium, arsenic, lead, cadmium, and zinc in the water samples were determined, using the routine procedure for atomic absorption analysis (Perkin-Elmer, 1971).

A method utilized by Kunkel and Manahan (1973) was employed to prepare the seawater samples for analysis of strong heavy metal chelating agents, by atomic absorption. The amount of heavy metal chelating capacity in each sample was expressed as the mg per liter copper equivalent chelating capacity.

Collection and Preparation of Tissue Samples for Atomic Absorption Analysis

American lobsters (Homarus americanus) were removed from different open flow culturing systems in the San Diego State University laboratories as soon as possible after their natural death. These lobsters were immediately labelled, sealed in plastic bags, and placed in a freezer at -20°C. The approximate age, and the water source in which each animal was raised for known lengths of time were determined from the experimental records. Individual lobsters were weighed, measured and placed in a drying oven at 80 C for 24 hours. Lobsters having dry weights of less than 0.200 g were grouped with other animals of the same carapace length and culture history in a given water source to attain a pooled aggregate dry weight of at least 0.200 g.

Seven organs and tissues were carefully dissected from lobsters of 10 mm size classes within the range 4 to 60 mm in carapace length. The organs and tissues dissected from each lobster were the hepatopancreas, the gills, the digestive tract, the muscles in the claws, the muscles in the tail, and the exoskeleton. After dry weight determinations had been made, all of the tissue samples were prepared using the same HCl digestion procedure. Concentrations of Cu, Zn, Cd, Co, Pb, and Cr in the digested samples were determined by atomic absorption spectrophotometry.

Methods Used in Evaluating Acute and Chronic Toxic Effects on Homarus americanus of Chemicals which May be Associated with Power Plant Effluents

Static bioassays were employed to determine the individual acute lethal effects of acids, Cl^{-}, Cu^{++}, Zn^{++}, Cd^{++}, Cr^{6+}, Co^{+++}, and Pb^{++} on Homarus americanus larvae. Rectangular plastic freezer containers of 0.75 liter were employed. One newly hatched, first stage lobster larvae was placed in each container. Twenty larvae were tested at each concentration of each chemical. Series of 4-10 concentrations for each chemical were evaluated to determine acute toxicity. All containers were maintained in a single water bath at 22±1 C.

Data concerning observed mortalities were plotted on log-probit paper and the LD_{50}'s (Median lethal limits) for each chemical at exposures of 24, 48, 72, and 96 hours duration were calculated. The series of LD_{50}'s obtained at different exposure times was used to construct toxicity curves from which ET_{50}'s (median effective times) could be derived (Sprague, 1969). Median effective time is defined as the time within which 50% of the lobsters died at a given concentration. Confidence limits of the LD_{50} and the slope function of the log-probit line were calculated, using the nomographic method (Litchfield and Wilcoxon, 1949).

Long-term static bioassay experiments were used for comparison of growth and mortality of H. americanus larvae and juveniles held in intake and effluent waters of the Encina and Redondo generating stations and in Scripps water at the same constant temperature. Three groups of 30 lobster larvae less than 24 hour post-hatching (Larval Stage I) were held individually in 0.75 liter plastic freezer containers. Each group of thirty containers was supplied with water from one of the following sources: 1) Encina thermal effluent; 2) Encina intake seawater; and 3) Scripps Institution seawater. The latter two water types were employed as controls. All experimental water was treated similarly prior to introduction to the cups. The water was passed through a 5 μ cellulose filter and an ultra-violet sterilizer prior to introduction to the cups. The entire experiment was conducted at the Encina laboratory in a single water bath at 22±1C. Thus, all experimental subjects were exposed to the same holding conditions, including feeding, light, and constant temperature regimes.

There was no supplemental aeration in the containers during the experiment. The surface area of the rectangular cups was sufficient to maintain a normal dissolved oxygen concentration above 6.0 ppm. Approximately one-quarter of the water in each cup was removed daily by siphoning it to remove detritus, feces, and excess food. After siphoning, the cups were refilled to their original volume with replacement water from aerated holding tanks. The animals were fed a slight excess of live brine shrimp daily during the larval stages (about two weeks), and every other day for the duration of the experiment.

Mortality data for the animals were recorded daily throughout the experiment. The carapace lengths of all the animals were measured each time it was observed that 50% of the animals in the three water types had molted to a new stage. The duration of the experiment was five months, from June 5, 1974 through November 3, 1974. During this period the lobsters attained Juvenile Stages XI and XII.

A second identical experiment was initiated on July 16, 1974, and run for three and one-half months through November 3, 1974. All conditions for the two runs were similar.

A similar experiment was initiated on January 24, 1975, incorporating water from the Redondo Beach Generating Station. In this experiment the newly hatched Stage I lobsters were reared only through the larval phase to Stage IV in one of three water types: 1) Redondo ambient intake seawater; 2) Redondo thermal effluent; and 3) Scripps seawater. Ninety larvae, thirty in each water type, were reared individually in 0.75 liter plastic freezer containers at 22±1C in a single water bath at the Scripps laboratory. All conditions of maintenance and sampling were the same as those employed in the two experiments at Encina.

RESULTS AND DISCUSSION

Heavy Metal Concentrations in the Water Sources

Table 1 summarizes data on concentrations of Cu, Zn, Cd, Pb, Co, Cr, and As in the soluble and particulate fractions of seawater from the Encina intake, Encina effluent, and Scripps Institution sources, obtained in twelve biweekly water samples during the period September 12, 1974, through February 12, 1975. Separate parametric one-way analyses of variance revealed no significant differences (p values >0.05) between the three sources in the mean concentrations of Cu and Zn in both the soluble and particulate fractions. Concentrations of particulate Pb and As, and soluble As in all samples for the three sources were below the detection limits of atomic absorption, using the method of sample preparation chosen. A majority of the samples contained concentrations of Cd, Pb, Co, and Cr in both particulate and soluble fractions which were below the detection limits of atomic absorption.

Variations between samples for each metal within a water source during the sampling period are revealed by the ranges and standard deviations in Table 1. Although there is no objective way to compare such fluctuations, it appeared that the metal concentrations obtained on a given sampling date at the three sites were very similar. Sample plots of this kind for Cu are shown in Figure 1. Concentrations of all elements in both fractions from each of the three water sources, with the possible exception of particulate Zn (Figure 2) appeared to parallel each other closely in their variation between sampling dates. The concentration of particulate Zn in the Scripps water remained fairly stable among all the samples, while it fluctuated somewhat in the early samples of both Encina intake and effluent water.

Table 2 summarizes concentrations of the seven heavy metals in the intake and effluent sources of the Southern California Edison Company's Redondo Beach and Ormond Beach generating stations. These were based on two replicate analyses made for water samples obtained at each location during February and March, 1975. The mean concentrations of the elements found in the twelve sets of samples taken at Encina and Scripps also are included in Table 2. Comparison of mean concentrations of the seven heavy metals in samples from the three Southern California generating stations indicated that there were no evident difference in the levels of these metals in the effluent and intake waters at any of the generating stations.

The chemical composition of the thermal effluent probably is unique to each generating station and depends on such factors as intake water quality, additives for pre-operation cleaning, and additives used to control corrosion and biological growth (Becker and Thatcher, 1973) These chemical additives are usually introduced on a periodic schedule, on a schedule which is based on demand and is more sporadic, or continuously at a lower residual level.

Information from the San Diego Gas & Electric Company indicated that the schedule of chemical additions at the Encina Power Plant was rather sporadic. Chemicals used for specific purposes were added to the cooling water whenever they were considered necessary. It appears that treatments involving additions of the chemicals being evaluated in this study occur about 10-20 times per year. Details of the amounts of each chemical added during a given treatment were not available. However, reports of water quality monitoring at Encina for 1973 indicated the total tonage of each chemical added annually (San Diego Gas & Electric Co., 1973).

The flow rate of the cooling water through the Encina Power Plant (86.5 million liters per hr) and the relatively small size of the effluent pond ($\approx$2100 m^3) diluted the amounts of each chemical added to the effluent pond at any given time to a very small amount. Approximate estimates of average residual concentrations of all seven metals added to the cooling waters through chemical treatments, based on the total annual additions, were less than 0.01 μg per liter per hour of each metal. The similarities in the concentrations of the seven metals in the samples from the intake and effluent sources at Encina seem to confirm this.

Periodic applications of any chemical additive, even in large quantities, would be difficult to detect in the effluent pond at Encina. Because the quantity of water being pumped through the power plant is large, and the effluent pond is small in size, the turnover rate is very great. A continuous flow monitoring system coupled to a pump cutoff circuit, as well as specific information from the operators of the generating station concerning the time and nature of any chemical additions, would be necessary to detect and avoid possible harmful levels of chemicals, which could occur almost instantaneously. Unfortunately, little is known about the effects of periodic additions of chemicals in above normal levels. However, it appears that most aquatic organisms are able to survive relatively short exposures to what would be lethal levels of heavy metals if exposure extended over a longer period of time (Bowen, 1966).

Reported values for concentrations of these heavy metal microconstituents of normal seawater vary somewhat from study to study. Discrepancies in reported values occur because of differences in sampling procedure, sample preparation, and sample analysis. Robertson (1972) has established the most widely accepted values for minimal concentrations of most heavy metals in seawater, utilizing coprecipitation and neutron activation. However, ranges such as those established by Parker (1971) are probably more representative of normal seawater concentrations over wide geographical and depth ranges. Concentrations of the seven heavy metals in the three aquaculture water sources (Encina effluent, Encina intake, and Scripps as a control) are well within the reported ranges for the concentrations of these elements in normal seawater (Table 3). The levels of soluble and particulate Cu, As, Cd, and Co in the intake and effluent waters of the Redondo Beach and Ormond Beach generating stations are similar to the levels of these same metals in Scripps Institution seawater, and they are also well within the reported ranges for normal seawater (Table 3).

Levels of soluble Zn and particulate Cr at Ormond Beach, and soluble Zn and Pb at Redondo Beach, were considerably higher than concentrations of the same metals at Scripps and Encina. However, they were very similar within the intake and effluent samples at both plants, indicating that the high levels were not a consequence of generating station operations. It is likely that the high levels of these metals are the result of automobile exhaust (Daines et al., 1970), and municipal sewage discharges.

LaRoche et al., (1973) have reported concentrations of copper as high as 100 µg per liter in thermal effluent from a power plant in Florida. Recent abalone mortalities in central California have been attributed to copper poisoning resulting from start-up operations of the Pacific Gas & Electric Company's new Diablo Canyon nuclear power plant. It is known that $CuSO_4$ is a widely used algacide for the prevention of fouling in the condensers of fossil fuel generating stations. Also, at the Encina Power Plant, the condenser tubing itself is a copper alloy. Therefore, it is especially important and encouraging for the culture of H. americanus and other species in the thermal effluent at Encina that copper concentrations and concentrations of the other six metals were within normal ranges.

Heavy Metal Chelating Capacity of the Water Sources

Chelating agents such as NTA, EDTA, humic acid, and citric acid have been used to reduce copper toxicity in seawater (Sprague, 1968; Nishikawa and Tabata, 1969; Wildish et al., 1971). Nielson and Wium Andersen (1970) have shown that ionic copper at normal seawater concentrations is extremely detrimental to photosynthesis and growth of unicellular algae. However, copper in complexes with organic materials is not toxic to algae, indicating that Cu is not normally present in ionic form but is complexed with such things as polypeptides.

Parametric one-way analysis of variance indicated that there was a significant difference ($p < 0.05$) in the strong heavy metal chelating capacity of the Encina intake water, the Encina effluent water, and the Scripps Institution water. Data summarized in Figure 2 show that the chelating capacity of the Encina effluent was consistently greater than the chelating capacity of the other two sources throughout the sampling period. Because strong heavy metal chelating agents have the capacity to detoxify heavy metals, it is encouraging that the thermal effluent at Encina is more enriched with these ligands and particles than the intake water and normal nearshore water at Scripps.

In view of the possible detoxification effects of chelating agents, it appears that the total concentrations of each toxic metal in seawater in a summation of all forms is somewhat misleading. The amount of each element not chelated is probably a better indication of the amount of an element that can exert a toxic influence on lobsters in culture systems.

Concentrations of Heavy Metals in the Tissues of *Homarus americanus*

Having established that the total metal levels in the thermal effluent at Encina were not affected by the passage of the cooling water through the power plant, it was necessary to determine whether even small levels of the trace metals in the aquaculture water systems were accumulated in lobster tissues. Also, it was important to determine whether the metal ions were taken up differentially by the lobsters in the thermal effluent because of differences in the chemical forms of the metals in the water, which were indicated by the high chelating capacity of the effluent.

A total of 345 lobsters was removed soon after natural death from the culture systems at the Encina and Scripps laboratories, and utilized in analysis of metal concentrations in all body tissues combined. This is referred to as the whole body concentration in subsequent descriptions. There were 184 animals which had been held in Scripps water, 93 animals which had been held in Encina intake water, and 68 animals which had been held in Encina effluent water. Those animals represented a wide range of carapace lengths, from 4 to 114 mm,with a mean carapace length of 16.5±31.0 mm, a mean dry weight of 5.022±6.806 g, and a mean wet weight of 16.852±24.505 g. Multiple variance analysis indicated that there were no significant differences (p values >0.05) in the means of the carapace lengths, dry weights, wet weights, and ages of the lobsters utilized in the samples, between the three water sources in which they were held.

Mean concentrations, ranges, and standard deviations of Cu, Zn, Cd, Pb, Cr, and Co in the digested whole-body samples of H. americanus are given in Table 4 for all sizes of lobsters combined. A series of one way analyses of variance indicated that there were no significant differences (p values >0.05) in the mean concentrations of any of the six metals among the three water types in which the animals were held: 1)Encina effluent, 2. Encina intake, and 3) Scripps Institution. Coupled with the six month data on the levels of these metals in the water sources, the similarities of tissue metal levels indicates that any chemical additions to the cooling water within the station were not being observed at the point of discharge. The lack of any difference in tissue metal levels between the three sources tends to negate the possibility that periodic chemical additions, which may have been missed entirely in the water sampling, were significantly affecting the lobsters in the culture systems.

Concentrations of the six metals in five different organs and tissues of

115 juvenile and adult H. americanus, representing a size range of 11 to 114 mm in carapace lengths, are presented in Table 5. Copper, zinc, lead, cobalt and chromium were found in the gills, while cadmium was found at equally high levels in the gills and hepatopancreas. Copper, zinc, cadmium, and chromium were found in the lowest concentrations in the exoskeleton, while lead and cobalt were found in the lowest concentrations in the tail muscle.

The mean concentrations of the six metals in the whole-body and tissue samples of larval, juvenile, and adult H. americanus are similar to concentrations of the same metals found in decapod crustaceans by Bryan (1968), Windom (1972), Gale (1973), and Eisler et al., (1972). It has been shown that the gills of decapod crustaceans are the main site for absorption and loss of zinc and other heavy metals across the body surfaces. Bryan (1968) observed very little uptake through the exoskeleton, except when the lobsters were starved in water with an abnormally high Zn content. He also observed that Homarus gammarus, the European lobster, probably obtains much more Cu and Zn than is required, but that temporary storage by the hepatopancreas and removal across the gills and in the urine is so efficient that the lobsters can withstand above normal concentrations in seawater. The regulatory efficiency of H. gammarus also probably is manifested in H. americanus. High levels of six heavy metals in the gills and hepatopancreas, and correspondingly low levels in the exoskeleton, indicate that H. americanus also accumulates heavy metals from the water primarily across the gill filaments. The presence of high concentrations of the six metals in the hepatopancreas also indicates the storage function of this organ in H. americanus. The ability to regulate the concentrations of the heavy metals internally may give H. gammarus and H. americanus a degree of protection in regions where metal pollution is found.

The levels of Cu, Zn, Cd, Pb, Co, and Cr in the edible portions of the lobster (claw and tail muscle) were relatively low when compared to metal levels in vertebrates

(Vallee,1959). The levels of all six metals in whole-body samples, and in the edible tissues, are well below the United States Public Health Service maximal limits for these metals in foodstuffs. It is encouraging for the future aquaculture potential of H. americanus that the species appears able to regulate efficiently any above normal concentrations of toxic metals introduced from the water without any appreciable harmful effects. It is especially important to note that accumulations of the non-essential metals, cadmium and lead, seem to be regulated similarly to the metals which have some metabolic function.

It is also encouraging that the tissue levels of the six metals in the lobsters held in effluent from the Encina Power Plant were similar to levels in lobsters maintained in Scripps control water. Since it appears that H. americanus is an effective regulator of metals, it seems likely that high concentration chemical treatments in the cooling water within the power plant would have little effect on the tissue levels of the lobsters.

The degree of bioaccumulation of heavy metals varies widely depending on the particular species studied. Least-squares regressions of the six metals in the whole-body and tissue samples on carapace lengths provided information about the accumulation of these toxic metals with age in larval and juvenile H. americanus. Of the 38 regressions, 70 percent revealed no significant correlations (p values >0.05) of the tissue metal levels on carapace lengths, the latter used as an indication of age. Tables 6 to 11 show the mean concentration of each metal in the whole-body and tissue samples by 10 mm size classes, from 1 to 60 mm, for each water source in which the lobsters were held. These tables show a tendency of decreasing metal concentration with increasing carapace length, indicating a general trend of regulation and no accumulation in H. americanus. There are several plausible explanations for the apparent regulation of metals with age in H. americanus, including: 1) the importance of metal ions in early growth metabolism; 2) differential mechanisms which affect uptake and elimination of metals; and 3) inhibition of the enzymes regulating the carrier system in active transport of metal ions out of the cells as the lobster grows.

Acute Tolerance Levels of *H. americanus* larvae to Potentially Toxic Chemicals Associated with Thermal Effluent

Little is known about the effects of toxic chemicals on macro-crustaceans. Most of the work has been done in connection with the development of antifouling compounds for the protection of ships and pilings (Clarke, 1947; Barnes and Stanbury, 1948). The 24, 48, 72, and 96 hour LD_{50}'s (Median lethal limits) for Cu^{++}, Zn^{++}, Cd^{++}, Pb^{++}, Cl^{-}, Co^{+++}, Cr^{6+}, and acids established by conducting static bioassays for *H. americanus* larvae are presented in Table 6. Unfortunately, it is not possible to compare these tolerance limits to any observed by other authors for *H. americanus* larvae, because none have been reported. The LD_{50}'s established for *H. americanus* larvae for all eight chemicals are probably good estimates of the minimum tolerance limits for lobsters of all life history stages, because the larvae seemingly are more sensitive than juveniles and adults (McLeese, 1974; Hubschman, 1967).

Although "safe" levels can only be established by long-term chronic bioassays, some indication of the levels of a chemical which can be tolerated by a lobster can be drawn from the median effective limit (ED_{50}) estimates for the eight chemicals. Comparing these estimates to the concentrations of heavy metals found in the effluents at Encina, Redondo Beach, and Ormond Beach, it appears that the concentrations of all of the eight chemicals are at least an order of magnitude lower than the median effective limit estimates for the chemicals.

Evaluation of Growth and Mortality of *H. americanus* in Power Plant Intake and Effluent Water at Constant Temperature

Long-term static evaluations of the generating station cooling waters were used to determine the possible sub-lethal and lethal effects of the chemicals in the thermal effluents, which might occur because of synergism, antagonism, or metal species differences. The results of chi-square tests indicated that there were no significant differences (p values >0.05) in survival among Stage IV through XII for lobsters maintained in Encina intake, Encina effluent, and Scripps seawater. An examination of the growth regression lines for the animals reared in the three water types indicated that the slopes of the three curves were equivalent. Similarly, the results of a parametric one-way analysis of variance indicated that there was no significant difference (p values >0.05) in the carapace lengths attained at Stages I to III for larvae reared in Redondo Beach intake, Redondo Beach effluent, and Scripps Institution seawater.

There was no evidence to suggest that any deaths or sub-lethal harmful effects had been caused by toxic concentrations of metals in the thermal effluent at Encina. In fact, the water analyses, tissue accumulations, and acute toxicities of the metals all seem to indicate that the thermal effluent from both the Encina and Redondo generating stations provide an essentially pollution-free, heated water source for the culture of *H. americanus*.

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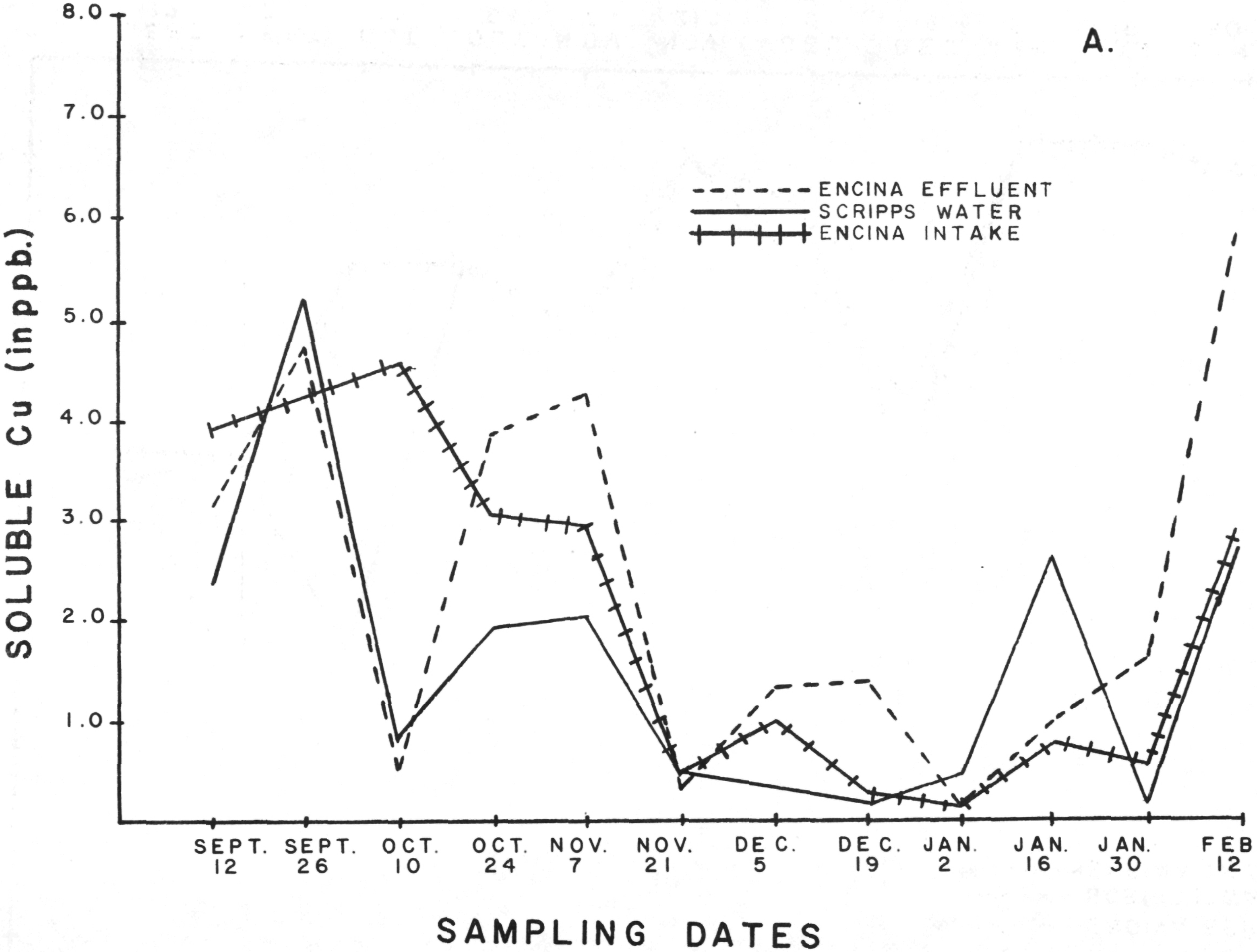
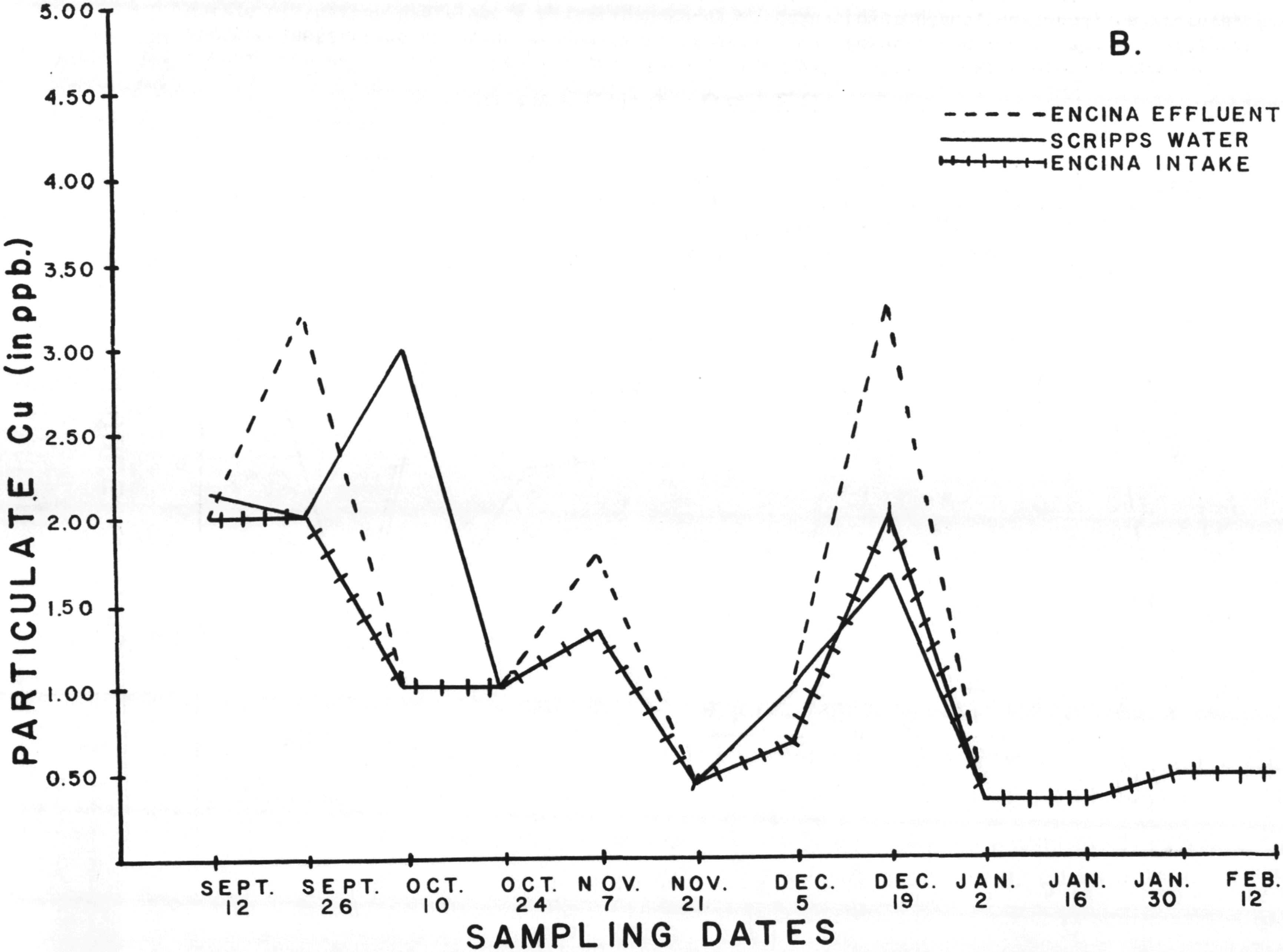
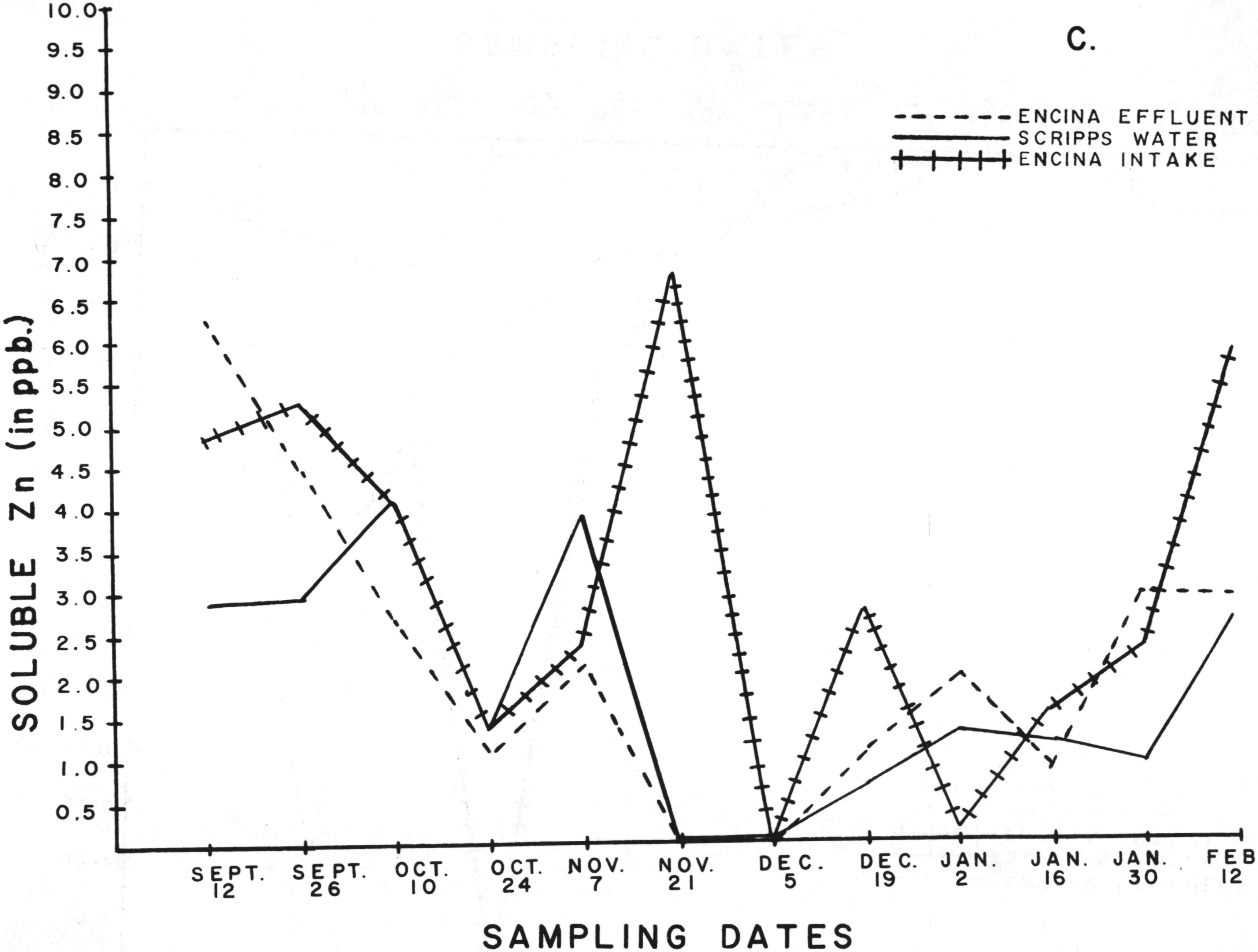
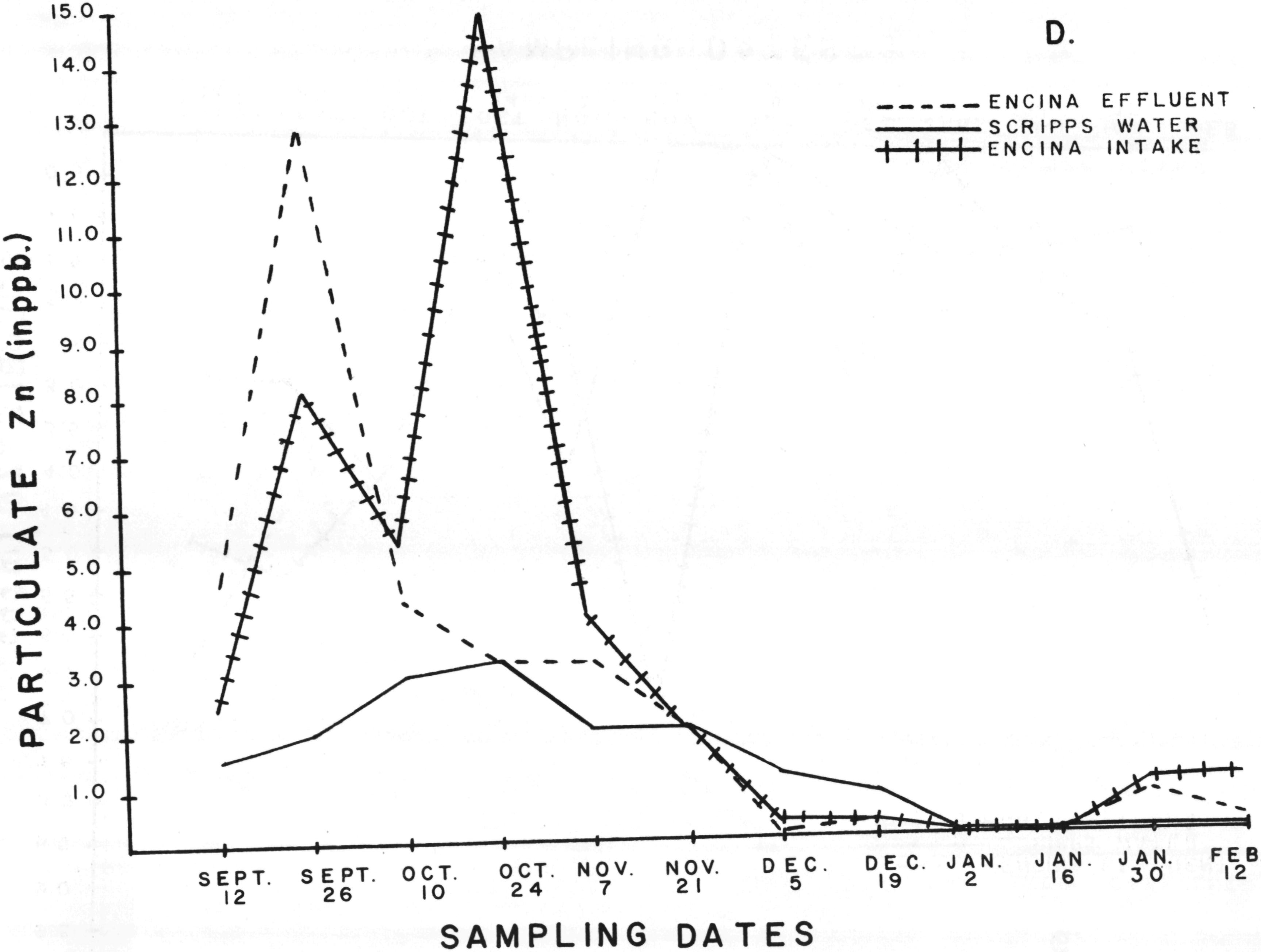
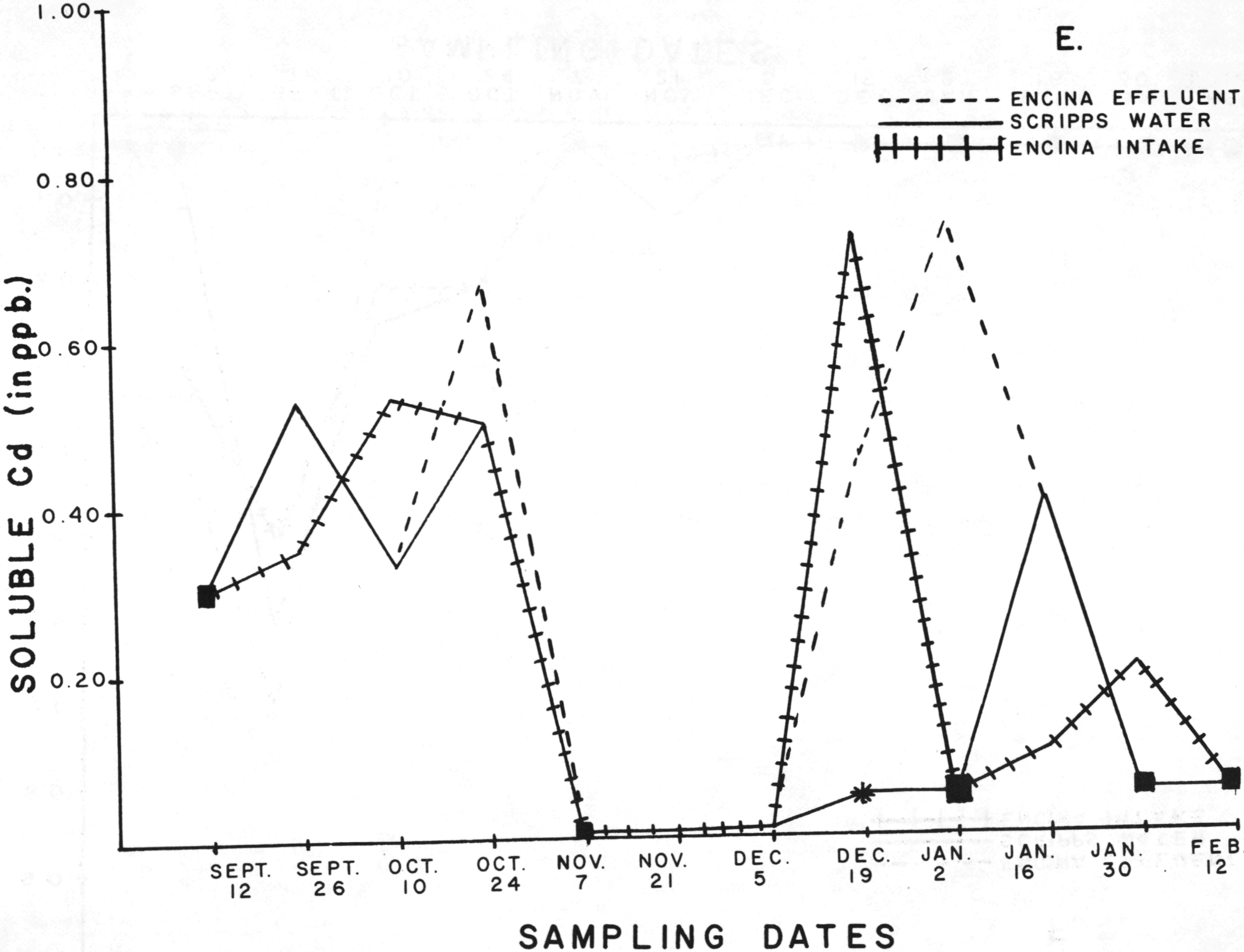


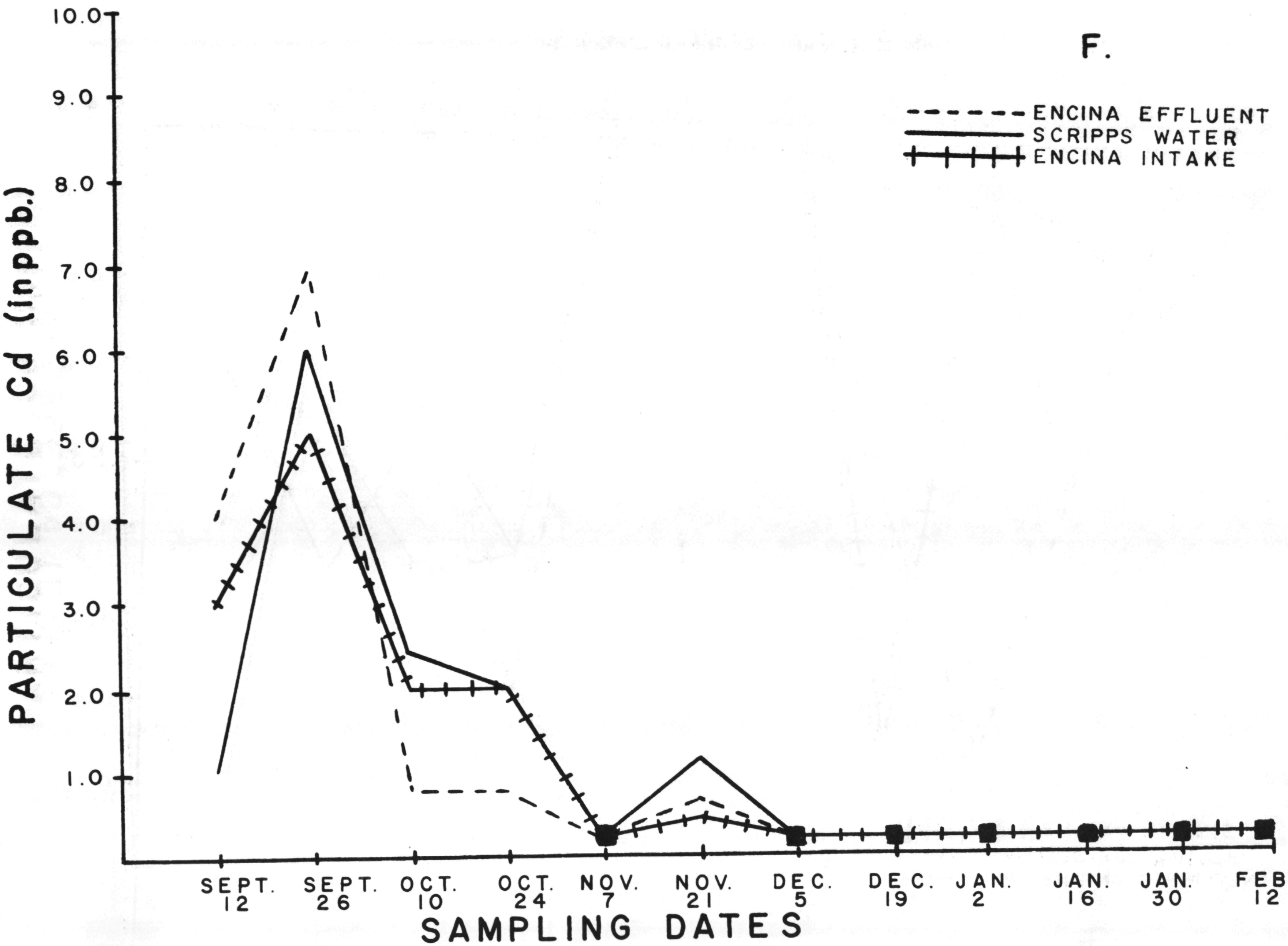
FIGURE 1 : Average concentrations of heavy metals in each biweekly-replicate water sample from the Scripps Institution of Oceanography pier, La Jolla, California, and the intake and effluent waters of the San Diego Gas & Electric Company's Encina Power Plant, Carlsbad, California. Data points for each water sample are joined to indicate trends and fluctuations. The symbols; ■ for Scripps, □ for Encina intake, and ○ for Encina effluent indicate the detection limits for a given element which the concentration is below, for that sample.

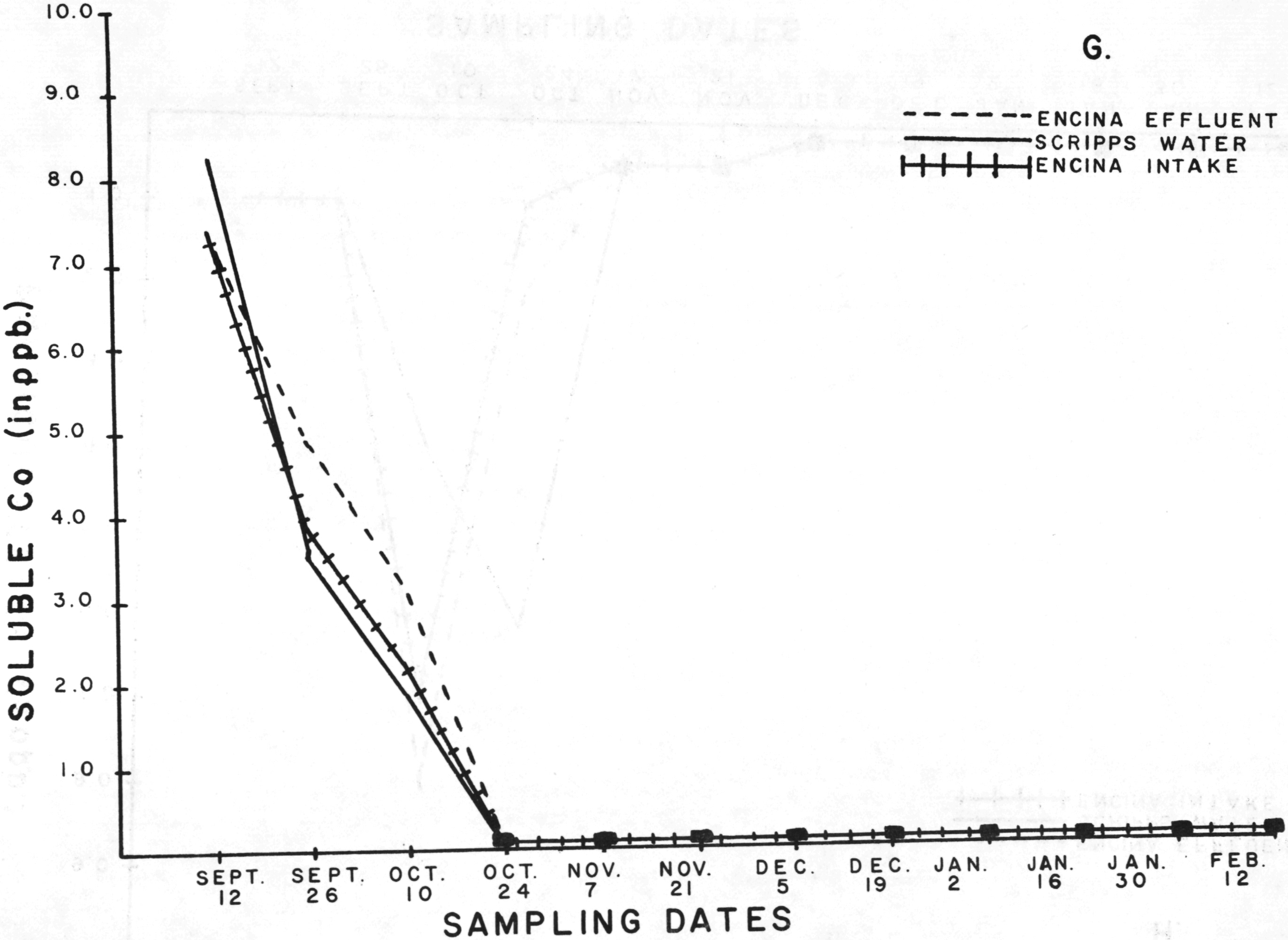

B.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Cu (in ppb.)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES


C.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
SOLUBLE Zn (in ppb.)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES

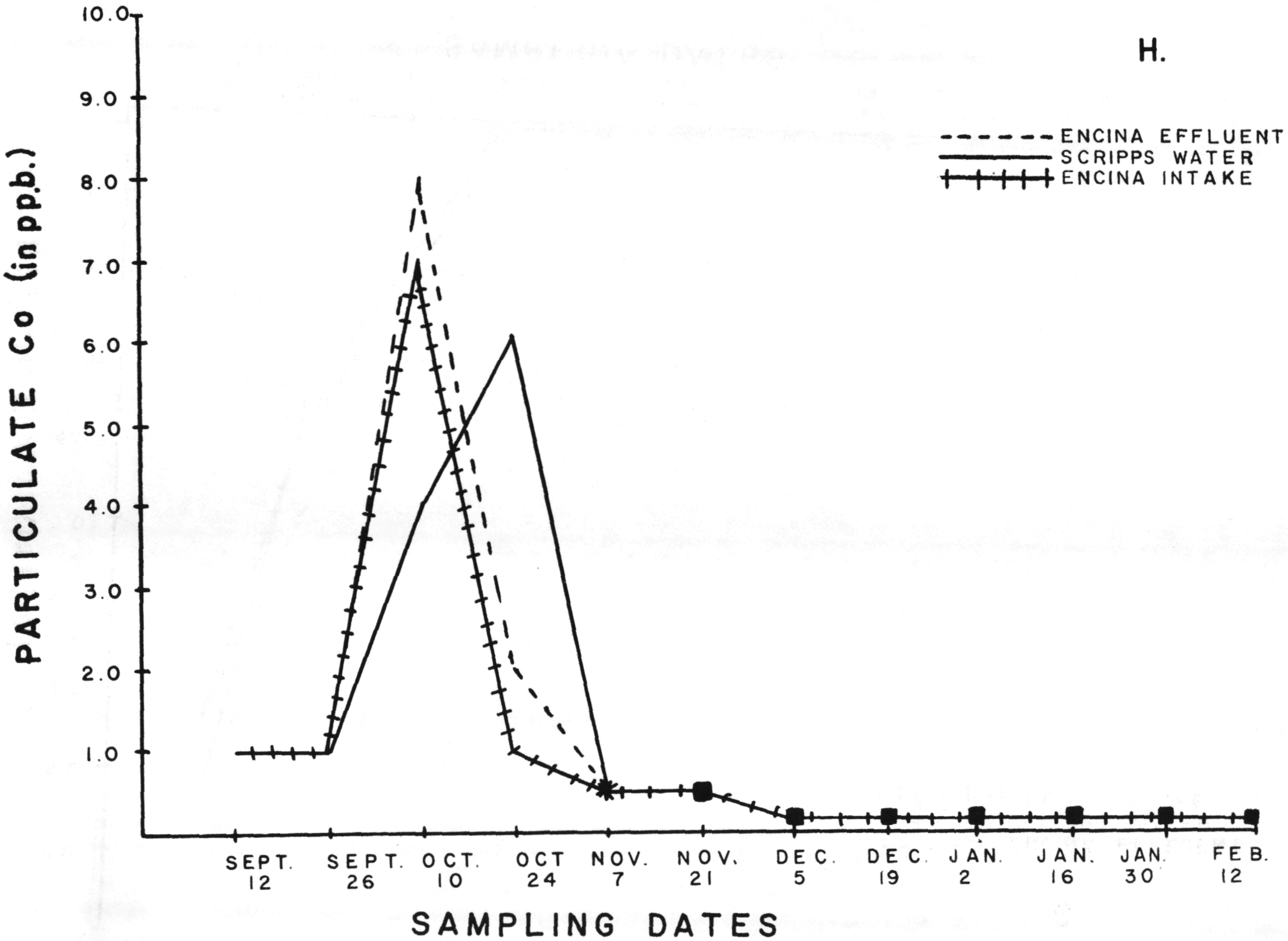

D.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Zn (in ppb.)
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES


E.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
SOLUBLE Cd (in ppb.)
1.00
0.80
0.60
0.40
0.20
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES

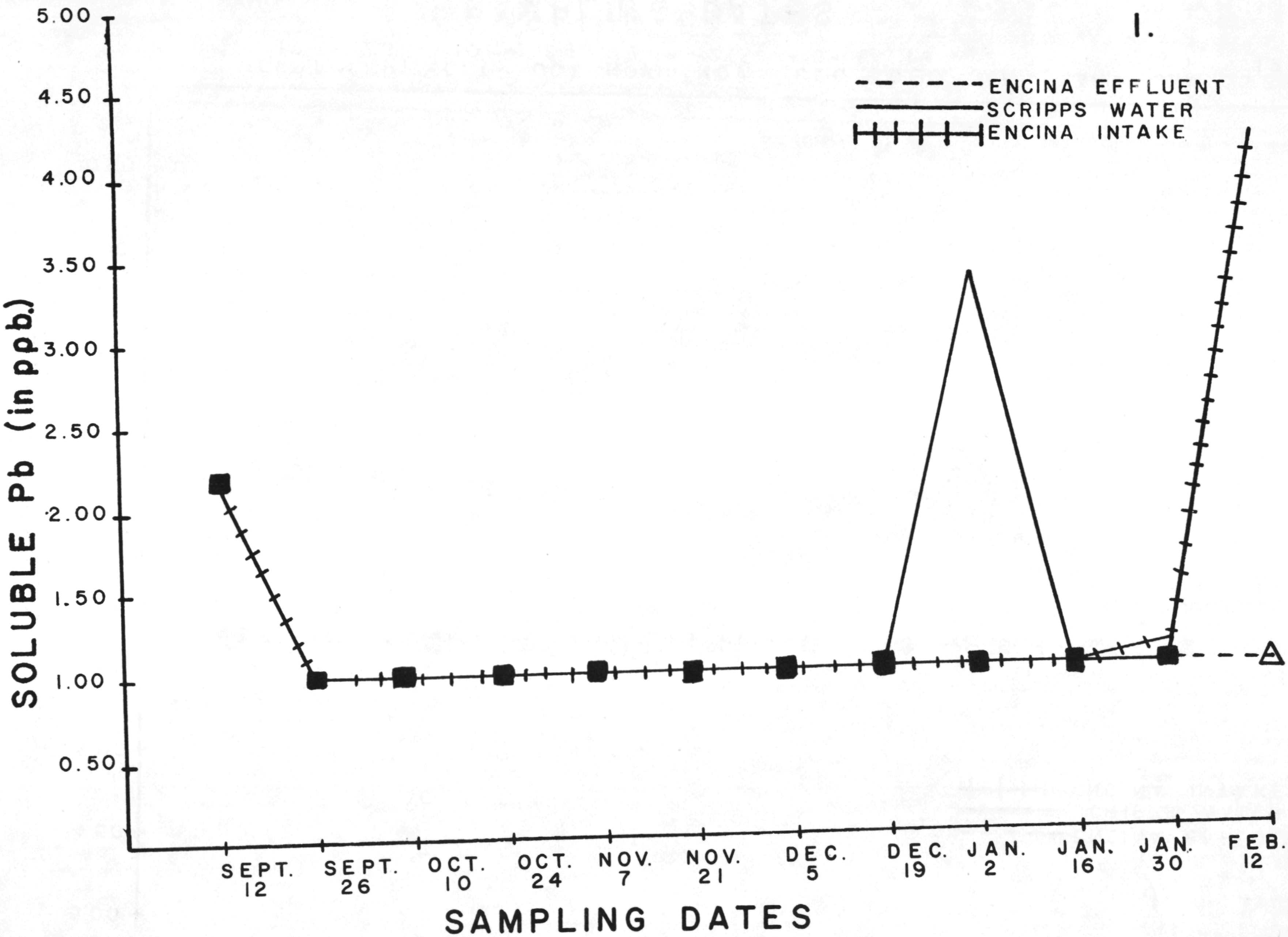

F.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Cd (In ppb.)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES



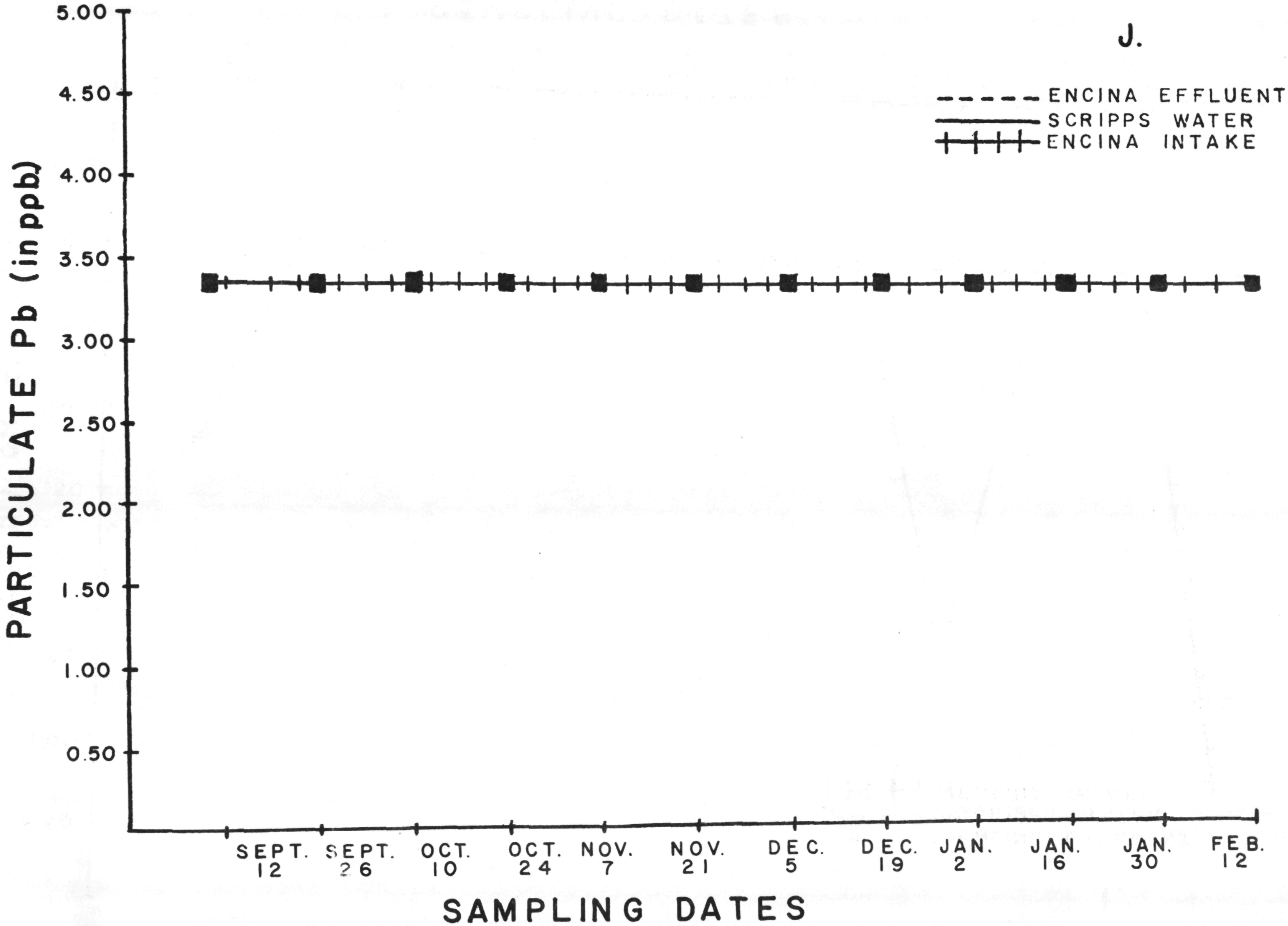
G.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SOLUBLE Co (in ppb.)
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES

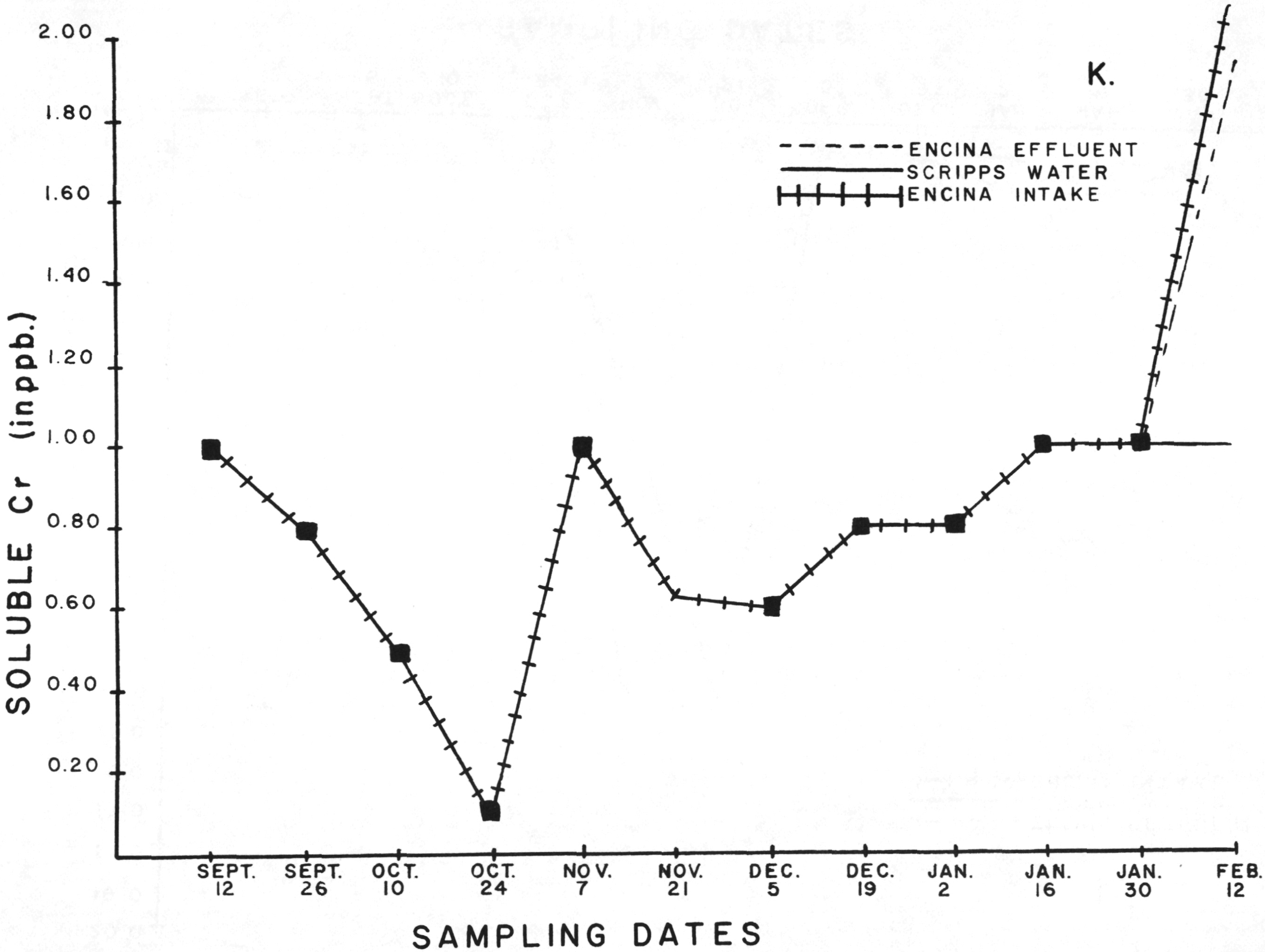


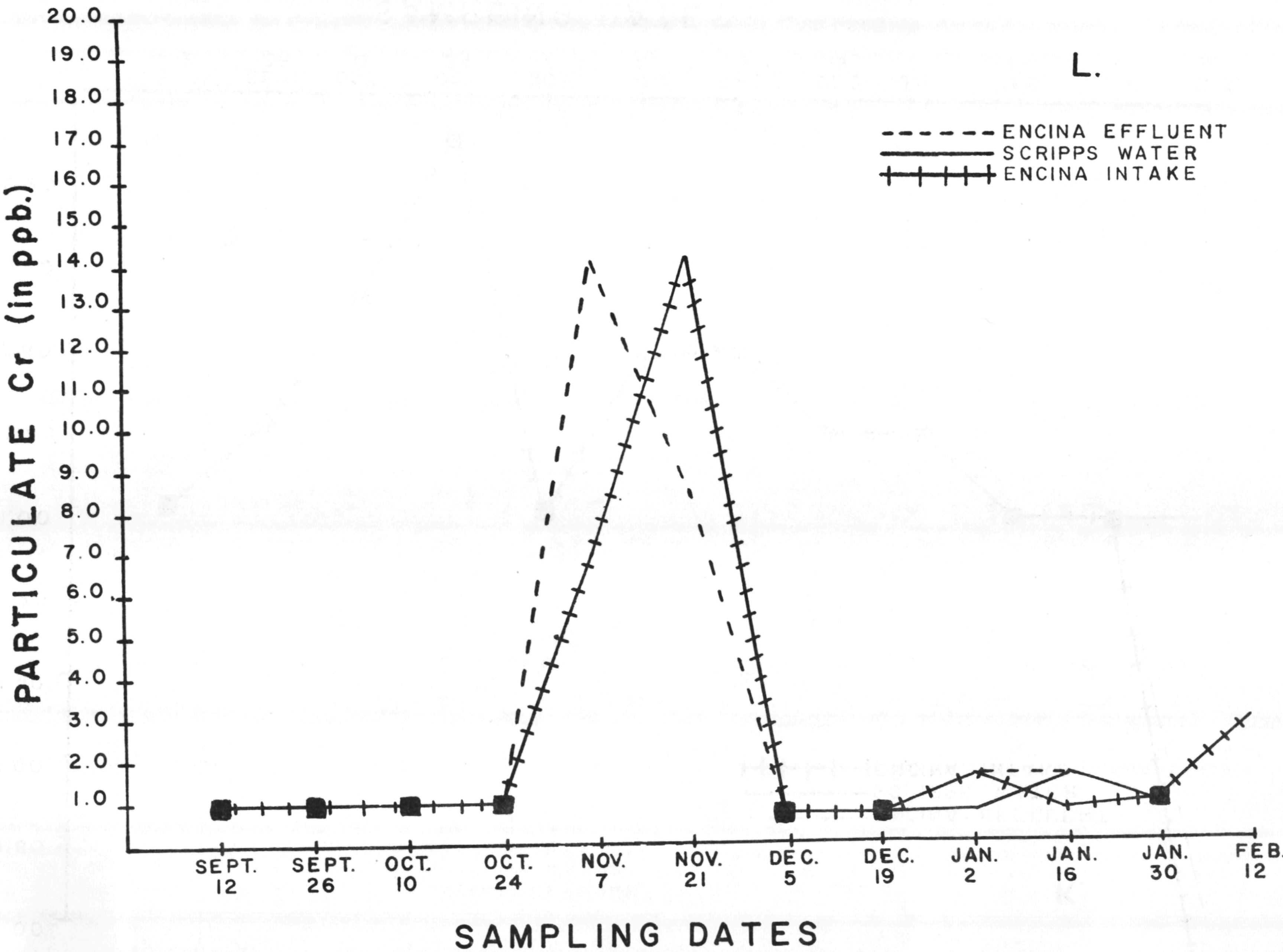
H.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Co (in p.p.b.)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SEPT. 12
SEPT. 26
OCT. 10
OCT 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES



1.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
SOLUBLE Pb (in ppb.)
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES


J.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Pb (in ppb)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES


K.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
SOLUBLE Cr (in ppb.)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES


L.
ENCINA EFFLUENT
SCRIPPS WATER
ENCINA INTAKE
PARTICULATE Cr (in ppb.)
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
SEPT. 12
SEPT. 26
OCT. 10
OCT. 24
NOV. 7
NOV. 21
DEC. 5
DEC. 19
JAN. 2
JAN. 16
JAN. 30
FEB. 12
SAMPLING DATES

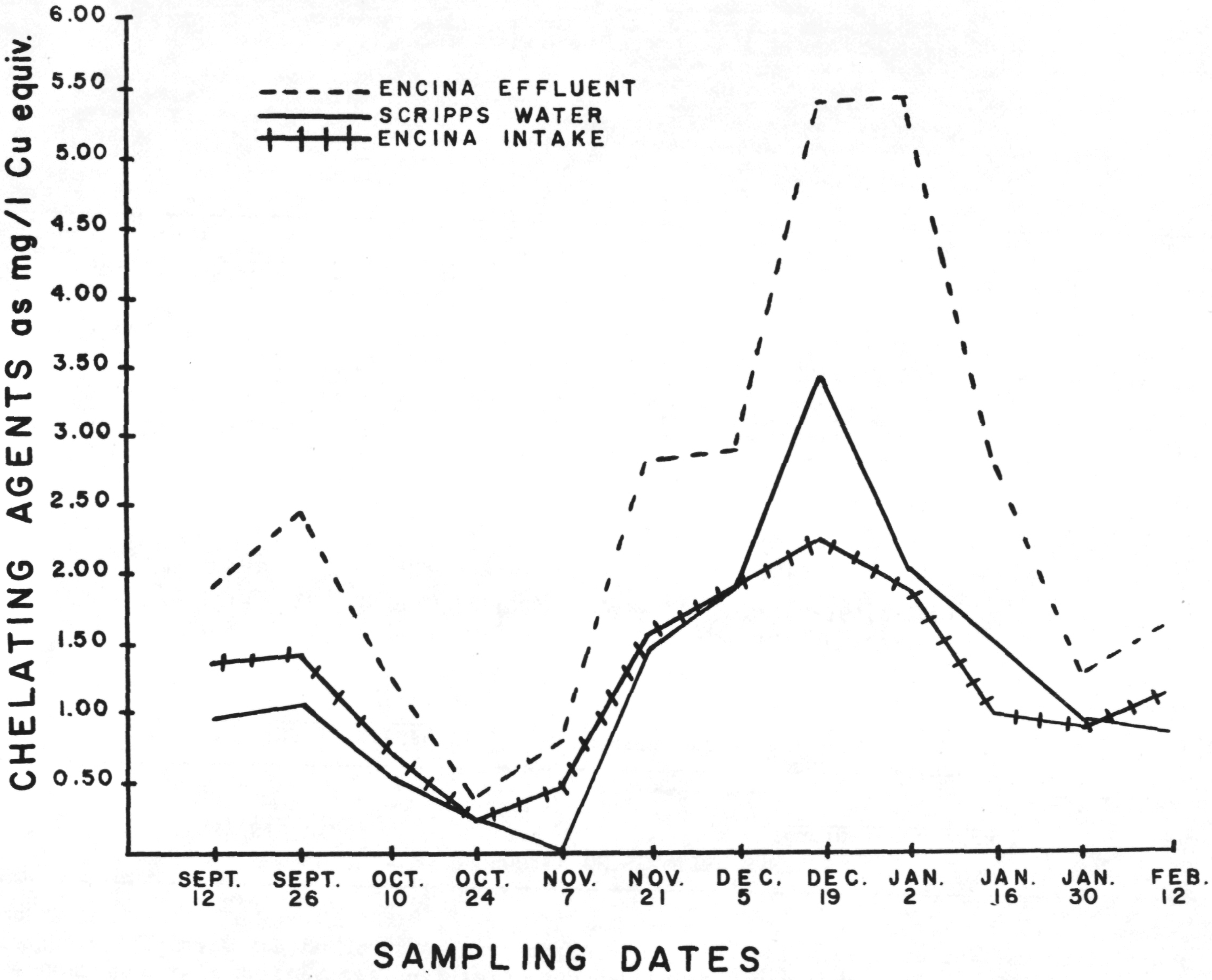


FIGURE 2 : Levels of strong heavy metal chelating agents at each sampling date in water from Scripps Institution pier, and from the intake and effluent waters of the SDG&E Encina Power Plant. Data points for each water source are joined to show trends and fluctuations.

Table 1.--Concentrations of heavy metals in seawater samples from the Scripps Institution of Oceanography pier, La Jolla, California; and from the intake and effluent waters at the San Diego Gas & Electric Company's Encina Power Plant, Carlsbad, California. Separate determinations were made of the soluble fraction (>0.45) of each sample. Data are based on 12 biweekly samples obtained during the period from September 1974 through February 1975. Concentrations are expressed as g/liter. Values in parenthesis indicate the percentages of samples with levels greater than the detection limit.

		SOURCE OF SEAWATER SAMPLES					
		SIO Pier		Encina Power Plant			
				Intake Water		Thermal Effluent	
METAL		MEAN STD.±DEV.	RANGE	MEAN±STD. DEV.	RANGE	MEAN±STD. DEV.	RANGE
Copper	Sol.	1.61±1.49	0.15-5.18(100)	2.34±1.93	0.15-5.85(100)	2.09±1.68	0.15-4.60(100)
	Part.	1.08±0.86	0.33-3.00(100)	0.91±0.57	0.33-2.00(100)	0.93±1.10	0.33-3.24(100)
Zinc	Sol.	1.84±0.38	0.01-4.14(100)	3.11±2.23	0.01-6.34(100)	1.73±1.39	0.01-6.79(100)
	Part.	1.15±1.26	0.04-3.28(100)	2.78±4.86	0.04-16.42(100)	2.53±3.93	0.04-13.40(100)
Cadmium	Sol.	<0.10	<0.10-0.53(50)	<0.10	<0.01-0.73(67)	<0.10	<0.10-0.73(75)
	Part.	<1.00	<0.23-6.00(42)	<1.00	<0.20-4.60(42)	<1.00	<0.20-7.00(42)
Lead	Sol.	<0.80	<0.80-3.33(17)	<0.80	<0.80-4.16(17)	<0.80	<0.80-2.20(0)
	Part.	<3.33	<3.33(0)	<3.33	<3.33(0)	<3.33	<3.33(0)
Chromium	Sol.	<0.80	<0.50-1.00(17)	<0.80	<0.10-2.00(17)	<0.80	<0.10-2.00(17)
	Part.	<1.00	<0.69-14.28(42)	<1.00	<0.69-14.28(42)	<1.00	<0.69-14.28(42)
Cobalt	Sol.	<0.10	<0.05-8.31(25)	<0.10	<0.05-7.42(25)	<0.10	<0.05-7.42(25)
	Part.	<0.20	<0.15-6.00(17)	<0.20	<0.15-3.00(8)	<0.20	<0.15-8.00(17)
Arsenic	Sol.	<1.00	<1.00(0)	<1.00	<1.00(0)	<1.00	<1.00(0)
	Part.	<1.00	<1.00(0)	<1.00	<1.00(0)	<1.00	<1.00(0)

Table 2.--Mean concentrations of heavy metals in seawater samples from the Scripps Institution of Oceanography pier and from intake and effluent sources at three fossil fuel generating stations in Southern California: 1) the SDG&E Encina Power Plant in Carlsbad; 2) Units 1-6 of the SCE Redondo Generating Station in Redondo Beach; and 3) the SCE Ormond Beach Generating Station. Separate determinations were made of the soluble fraction (<0.45 μ) and the particulate fraction of each sample (>0.45 μ). Means for SIO and Encina are based on 12 biweekly samples obtained during the period September 1974 - February 1975; those for the Redondo Beach and Ormond Beach plants are based on two samples taken during March and April, 1975. Concentrations are expressed as μg/liter (ppb).

METAL		SOURCE OF SEAWATER SAMPLES						
		Scripps Institution	Encina P. P.		Redondo G. S.		Ormond Beach G. S.	
			Intake	Effluent	Intake	Effluent	Intake	Effluent
Copper	Sol.	1.61	2.34	2.09	4.80	4.23	2.93	2.59
	Part.	1.08	0.91	0.93	1.45	1.32	1.75	1.64
Zinc	Sol.	1.84	3.11	1.73	34.70	30.30	18.56	21.73
	Part.	1.15	2.78	2.53	1.10	1.01	0.94	1.02
Cadmium	Sol.	<0.10	<0.10	<0.10	<0.08	<0.08	<0.08	<0.08
	Part.	<1.00	<1.00	<1.00	1.80	1.72	1.65	2.02
Lead	Sol.	<0.80	<0.80	<0.80	2.08	2.57	<1.00	<1.00
	Part.	<5.00	<5.00	<5.00	<5.00	6.18	<0.10	<0.10
Chromium	Sol.	<0.80	<0.80	<0.80	<1.00	<1.00	<1.00	<1.00
	Part.	<1.00	<1.00	<1.00	<0.01	<0.01	2.05	2.05
Cobalt	Sol.	<0.10	<0.10	<0.10	<0.01	<0.01	<0.01	<0.01
	Part.	<0.20	<0.20	<0.20	3.33	2.92	1.75	1.68
Arsenic	Sol.	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
	Part.	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00

Table 3.--Values for normal concentrations of heavy metals in seawater reported in the literature. A. From Goldberg (1962) for normal concentrations in ocean water. B. From Parker (1972) for ranges of minor constituents for seawater. C. From Brooks et al. (1967) for soluble (a) and particulate (b) fractions taken from the Southern California coast, 38 miles SW of San Pedro. D. From Preston (1973) for British Coastal Waters. E. From Robertson (1972) for open ocean.

METAL (all concentrations in ppb)	SOURCE A	B	C a	C b	D	E
Copper	3.00	1 - 25	1.7	0.5	0.66	2
Zinc	10.00	7 - 21	3.4	0.5	4.2	3
Cadmium	0.11	0.03 - 0.06	-	-	0.04	0.02
Cobalt	-	0.05 - 0.70	0.1	0.1	-	0.03
Lead	0.03	4	0.2	0.4	0.11	0.02
Chromium	-	0.04 - 2.50	-	-	-	0.3
Iron	10.00	0 - 60	0.6	1.3	-	5
Nickel	2.00	0.1 - 2.6	1.7	0.1	-	2
Arsenic	3.00	3	-	-	-	2
Silver	0.30	0.3	-	-	-	0.01

Table 4.--Whole-body metal levels of the six heavy metals in lobsters, *Homarus americanus*, maintained in Scripps Institute seawater, and the intake and effluent waters of the Encina Power Plant. All metal levels are expressed as μg/g dry weight.

			METAL			
	Copper	Zinc	Cadmium	Lead	Cobalt	Chromium
SIO Lobsters						
mean±	27.64±	36.65±	5.27±	23.11±	28.27±	28.25±
std. dev.	22.40	25.85	5.26	15.92	22.73	63.56
range	3.21-	1.04-	<0.37-	<2.00-	<0.75-	<3.21-
	134.67	314.85	21.44	38.67	130.43	525.00
% above detection limit	100	100	95	86	58	90
N	80	80	80	80	80	80
Encina Intake Lobsters						
mean±	25.92±	33.62±	4.50±	16.75±	26.17±	53.13±
std. dev.	20.86	21.23	5.48	13.18	21.22	67.13
range	4.55-	1.00-	0.23-	<4.17-	<5.93-	<2.19-
	162.71	121.72	22.22	57.14	100.00	236.84
% above detection limit	90	95	100	54	83	90
N	41	41	40	41	40	41
Encina Effluent Lobsters						
mean±	26.63±	27.03±	2.04±	19.12±	37.32±	29.69±
std. dev.	22.88	15.43	2.22	10.00	21.32	55.73
range	5.19-	14.70-	0.28-	<4.35-	1.14-	1.85-
	145.45	86.38	9.90	57.34	80.00	197.37
% above detection limit	100	100	100	91	100	100
N	36	36	35	35	35	35

Table 5.--Tissue concentrations of the six metals in lobsters, H. americanus, maintained in Scripps Institution seawater. All metal levels are express as μg/g dry weight.

TISSUE	Copper	Zinc	Cadmium	Lead	Cobalt	Chromium
Hepatopancreas						
mean±	72.04±	29.29±	12.33±	12.33±	34.57±	45.78±
std. dev.	82.27	13.11	11.33	5.25	60.94	58.33
% above detection	100	100	80	30	95	95
N=20						
Gills						
mean±	135.03±	76.99±	11.20±	192.27±	233.59±	315.38±
std. dev.	129.16	72.64	14.95	173.29	139.53	315.55
% above detection	100	100	47	47	32	89
N=19						
Claw Muscle						
mean±	60.85±	68.77±	3.70±	19.99±	18.16±	80.70±
std. dev.	61.13	27.42	4.79	15.49	10.11	65.57
% above detection	100	100	45	50	55	86
N=22						
Tail Muscle						
mean±	17.24±	30.17±	1.86±	8.27±	12.03±	37.54±
std. dev.	10.87	16.42	2.49	3.39	8.37	53.30
% above detection	95	100	71	38	57	95
N=21						
Exoskeleton						
mean±	13.99±	10.79±	2.67±	17.91±	48.15±	18.54±
std. dev.	10.37	7.79	2.65	11.77	30.86	18.54
% above detection	100	100	87	70	91	96
N=23						
Digestive Tract						
mean±	64.69±	28.85±	6.53±	13.47±	39.63±	181.50±
std. dev.	47.18	17.55	3.52	4.47	26.79	347.41
% above detection	100	100	53	20	60	87
N=15						

Table 6. Median lethal limits (LD50) for American lobster larvae subjected to chemicals associated with thermal effluent for intervals of 24, 48, 72, and 96 hours. The 95% confidence interval and the slope of the toxicity curve are given for each LD50.

	Time Intervals (hrs)			
	24	48	72	96
Copper (μg/l)				
LD50	165	75	60	60
95% conf. inter.	105-215	50-85	45-70	45-70
Slope	0.80	0.94	1.05	1.05
Cobalt (μg/l)				
LD50	1090	1090	810	810
95% conf. inter.	890-1180	890-1180	750-865	750-865
Slope	1.02	1.02	1.15	1.15
Cadmium (μg/l)				
LD50	900	430	220	180
95% conf. inter.	820-980	370-490	180-260	140-220
Slope	2.76	3.02	2.64	2.35
Chromium (μg/l)				
LD50	1500	700	700	700
95% conf. inter.	1100-4900	600-800	600-800	600-800
Slope	1.45	2.06	2.06	2.06
Lead (μg/l)				
LD50	4100	2600	2100	1000
95% conf. inter.	3050-4900	2000-3200	1600-2600	850-1150
Slope	1.21	1.69	1.22	1.18
Zinc (μg/l)				
LD50	2500	1700	1700	1700
95% conf. inter.	2050-2900	1300-2100	1300-2100	1300-2100
Slope	1.32	1.45	1.45	1.45
Acids (pH units)				
LD50	4.7	6.1	6.3	6.5
95% conf. inter.	4.3-5.1	5.6-6.6	6.1-6.5	6.3-6.7
Slope	1.43	1.67	1.06	1.00
Chlorine (ml/l)				
LD50	0.31	0.25	0.25	0.22
95% conf. inter.	0.29-0.33	0.22-0.28	0.22-0.28	0.20-0.24
Slope	1.90	1.38	1.38	1.75