ZINC AND BORON POLLUTION
IN COASTAL WATERS OF BRITISH COLUMBIA
BY EFFLUENTS FROM THE PULP AND PAPER INDUSTRY

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ABSTRACT

At least part of the coastal newsprint-producing pulp and paper industry has switched or plans to switch from zinc hydrosulphite (zinc dithionite, ZnS_2O_4) to sodium hydrosulphite (produced on-site by the Borol process) as a brightening agent for groundwood(mechanical)pulp. This switchover is due to the concern about the bioaccumulation of zinc by the Pacific oyster, Crassostrea gigas, which is harvested commercially and recreationally along the British Columbia coast. A survey during 1973 of heavy metals (zinc, copper and cadmium) in oysters near B.C. coastal groundwood mills and in uncontaminated control areas, showed zinc concentrations as high as 19,400 ppm dry weight and 2,500 ppm wet weight (Powell River) with the lowest 'background" levels of 2,400 ppm dry weight and 400 ppm wet weight (Nanoose Bay).

Because production of sodium hydrosulphite by the Borol process yields sodium metaborate as a by-product, it was considered prudent to examine some of the ecological effects of boron before a full-scale conversion was made. The LC50 (concentration at which one-half the test animals die) for boron, as sodium metaborate, tested on juvenile coho salmon in fresh water was 93 ppm over 23 days exposure. The mode of toxic action of boron was slow, so that the standard 96-hour bioassay could not be applied. Tissue analysis of fish dying in the 23-day period showed substantial increases in boron levels in comparison to unexposed fish. Bioaccumulation of boron in underyearling sockeye salmon was slight over an 8-day period, concentrations of boron in tissue going from an average of 0.06 ppm before exposure, to 0.193 ppm following exposure to 10-12 ppm B above ambient. Starting at an average concentration of 3.82 ppm before exposure, oysters contained 4.00 ppm after 8 days exposure to 10-12 ppm B above "normal" sea water and 13.2 ppm after 32 days, and 10.8 ppm after 47 days, suggesting that oysters gradually achieve an equilibrium of B in tissue about equal to that in surrounding sea water. Boron averaged 3.53 ppm in surface water.

Boron has an inhibiting effect on photosynthesis in phytoplankton at concentrations exceeding 1 ppm above the "natural" B concentration. However, groundwood effluent itself may exhibit a greater inhibiting effect, since a marked decline in phytoplankton production was shown at effluent concentrations greater than 4%.

From the present preliminary experimental and survey data, there appears to be no cause for alarm in terms of ecological damage by converting from zinc hydrosulphite to sodium hydrosulphite in brightening

groundwood pulp. There should be a decline in zinc concentration in oysters. However, there will be a need for continued surveillance of ecological conditions in the vicinity of groundwood mills as they undergo conversion, particularly with respect to the lower trophic levels (phytoplankton and zooplankton) and planktonic larvae.

Zinc and Boron Pollution in Coastal Waters of British Columbia by Effluent from the Pulp and Paper Industry

INTRODUCTION

The pulp and paper industry in British Columbia normally produces newsprint by a combination of groundwood pulp*, obtained either by grinding blocks of wood with large rotating circular stones, or by refining wood with a series of counter-rotating steel discs, and chemical pulp of either the sulphite or kraft (sulphate) variety. The short groundwood pulp is strengthened by the longer fibres of the chemical pulp, and the proportion of groundwood to chemical pulp is usually about 4:1.

Although a high degree of brightness is not required for newsprint, some form of basic brightening process is necessary to produce a paper on which print will show up adequately for the ordinary needs of daily newspapers. For chemical pulp, a process, known as semi-bleach in the parlance of the trade, is used. In this scheme,only about three stages of bleaching are applied out of a total of six or seven used in a high-grade bleaching sequence, involving chlorination, caustic extraction, and chlorine dioxide, or sodium hypochlorite for bright kraft or sulphite pulps. The groundwood pulp is conventionally given a treatment with so-called zinc hydrosulphite (more correctly zinc dithionite, with the formula $Zn\ S_2\ O_4$).

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* groundwood pulp is often referred to as mechanical pulp in contemporary pulpmill terminology.

It was the latter reducing process that caused the concern leading to the present work. When the use of zinc hydrosulphite for brightening groundwood pulp in British Columbia coastal mills was first examined from the environmental point of view some two decades ago, only the acute toxicity of the zinc to marine fish and other aquatic organisms was considered. At the concentrations of zinc expected to occur in the effluent from the mills, and in the coastal receiving waters, it was not anticipated that any serious harm would be caused to the living marine resources. However, the bioaccumulative aspect of metals was not appreciated at that time. More recent studies have demonstrated the enormous capacity of some organisms, particularly the invertebrates, to concentrate metals along with other pollutants.

The concern of a particular sector of the British Columbia pulp and paper industry, operating the pulp mill at Crofton, where zinc hydrosulphite was used as a brightener for groundwood, was expressed in 1971, by contracting a study of the effect of zinc in pulpmill effluents on concentrations of this metal in receiving sea waters and in oysters. This study clearly showed that the waters of Stuart Channel, in the vicinity of the Crofton Pulp Mill, had higher-than-normal concentrations of zinc. Oysters in this area also exhibited high concentrations of zinc. But even in areas considerably removed from the pulp mills, where ambient zinc concentrations appeared to be "normal" for coastal sea water, the oysters exceeded considerably the 100 ppm* (marketed weight), given in the Canada Food and Drug

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* ppm = parts per million. For simplification, and ease of comparison of values, this unit is used throughout this report synonymously for milligrams per litre, micrograms per gram, micrograms per millilitre, and millilitres per litre. The Appendices give the more scientifically rigorous units.

Act and Regulations as the maximum permissible concentration of zinc in human food.

With this situation in mind, representatives of the pulp and paper industry in British Columbia indicated in March 1973 that they would be switching to a new bleaching compound, sodium hydrosulphite (sodium dithionite, $Na_2S_2O_4$) to overcome the zinc contamination problem. However, in doing so, they would be using the Borol process to produce sodium hydrosulphite from sodium borohydride ($NaBH_4$), with sodium metaborate ($NaBO_2$) as a by-product released to the sewer.

Although boron has a comparatively low toxicity to fish, its effect on other organisms in the sea, and in fact, on other stages in the life cycle of fishes was virtually unknown. Boron was known to have an inhibiting effect on photosynthesis in terrestrial plants at concentrations of 1 to 4 ppm. While boron is a major constituent in sea water, present at about 3.5 ppm in coastal surface waters, it could conceivably have an adverse effect on phytoplankton at only a slight increase in concentration. This could also be true with other marine organisms at the lower trophic levels, and even at the higher trophic level in the sensitive egg and larval stages.

Therefore, it was decided to conduct a collaborative study, involving Fisheries Operations and Research and Development of the Fisheries and Marine Service and the Environmental Protection Service of the Department of Environment in the Pacific Region. Cooperation was also obtained from the British Columbia Pollution Control Branch and the pulpmill companies. The study would include: (a) an

investigation of zinc and boron in waters and in selected marine organisms, adjacent to pulp mills where zinc hydrosulphite is used for bleaching; (b) the acute toxicity of boron to fish and oysters; (c) the uptake of boron by fish and oysters; (d) effects of boron on oyster larvae; and (e) the effect of boron on phytoplankton.

The present review reports progress in the study mainly to September 28, 1973, although bioaccumulation experiments are reported to November 5. There have been a number of findings which are not only new, but quite unexpected. Because of technical difficulties in rearing oyster larvae, this part of the study had to be postponed. Reports of individual investigators are given in the Appendices. They will be published eventually either in the open literature or in manuscript and technical reports of their Services.

SAMPLING, ANALYTICAL TECHNIQUES AND BIOASSAY METHODS

1. Sampling of water, sediments and biological specimens

Water, sediments and biological specimens were collected in the vicinity of five coastal pulp and paper mills producing bleached groundwood pulp, located at Powell River, Port Alberni, Crofton, Campbell River (Duncan Bay) and Ocean Falls. Control samples were collected at Gambier Island, Nanoose Bay, Lasqueti Island and Sooke Basin, during July, August and November.

Water samples were collected at surface and 5 metres depth with Van Dorn sampling bottles, passed through 0.45μ Millipore filter,

acidified with dilute nitric acid and stored in polyethylene containers. Sediment samples were collected with a Peterson dredge during May and with a Ponar dredge in August. Samples were kept frozen until analysis.

Samples of mussels, clams and oysters were collected whenever possible and put into frozen storage. Species included the edible mussel, *Mytilus edulis*, little neck clam, *Protothaca staminea*, soft-shelled clam, *Mya arenaria*, butter clam, *Saxidomus giganteus*, bent-nosed clam, *Macoma nasuta*, basket cockle, *Clinocardium nuttalli* and the Pacific oyster, *Crassostrea gigas*.

In addition to chemical analysis for metals and boron on the biological specimens, condition factor measurements were made on the oysters, to determine if there is any relationship between their metal content, particularly zinc, and their physical condition, as represented by dry weight of meat in relation to shell size.

See Appendix I for details.

2. Metal analysis

Water and sediments are still awaiting analysis and will be reported later.

Biological samples for metal analysis were dried at 110°C for 48 hours and then ground with mortar and pestle. One-gram portions of the pulverized material were digested for about 6 hours with concentrated nitric acid and then made up to 100 ml with distilled water. Analyses on resultant solutions were carried out by atomic absorption using a Jarrell Ash Atomic Absorption Spectrophotometer (cf. Appendix I).

3. Boron determination

Boron determinations in seawater samples were carried out colorimetrically by the method of Grinstead and Snider. In this method, curcumin reacts with borate ion in a strongly acidic medium to produce a deep red colour due to the protonated complexing agent, which under buffered conditions leaves only the borate complex absorbing at 550 nm.

Tissue analyses for boron are being conducted by homogenizing a sample followed by digestion in sulphuric acid and hydrogen peroxide solution. The digested sample is then made up to volume with distilled water and analyzed colorimetrically by the Uppström method, which is an adaptation of the Grinstead and Snider procedure (See Appendix II). Check samples are analysed by a modified Uppström procedure developed by R.E. Drew of the Fish Inspection Laboratory.

4. Fish bioassays

Coho alevins and fry in fresh water and sockeye fry in sea water were exposed to boron in a range of concentrations from 82 to 656 ppm, using $Na_2B_2O_4.8H_2O$, in static-type bioassays, with daily solution replacement. Time required to kill 50% of the test fish at each concentration was used to determine the LC50's; but tests were not restricted to a standard 48-hour or 96-hour period, since sodium metaborate killed fish over a much more protracted time than 96 hours (cf. Appendix III).

In preliminary experiments, only boron was tested, since there was some urgency to obtain information on the toxicity of boron compounds, in view of industry plans to make an early switchover to sodium hydrosulphite brightening.

5. Bioaccumulation experiments

Boron bioaccumulation studies were conducted in 80-litre fibreglass tanks, with continuous flow of fresh sea water uniformly dosed with stock solutions of sodium metaborate in constant-head Mariotte bottles (cf. Appendix III). In the preliminary tests, there were five tanks, including one control tank and two replicates, one with 1 ppm boron and the other with 10 ppm boron above background. Each tank contained 25 sockeye fry and 30 young oysters of similar size. The choice of boron concentrations was based on the expected boron levels (0.89 ppm) in the effluent pipeline at the Powell River mill. It was felt that if there was no bioaccumulation at 1 and 10 ppm B used in the tests, then there should be no concern about the B concentrations released from the mill, especially when the anticipated dilution due to mixing and dispersion is taken into account.

6. Phytoplankton bioassay experiments

Seawater samples were obtained with a 6-litre Van Dorn sampling bottle from 1.5 m off the Pacific Environment Institute dock. Determinations were carried out for boron, pH and alkalinity on subsamples from each sample. A phytoplankton count was made. The remaining sea water from the Van Dorn bottle was stirred and added to 130-ml incubation flasks, which were used in 13 incubation sets for each experiment. Two of the sets were controls, and the remainder were incubated with various concentrations of boron (sodium metaborate) or groundwood effluent. After boron additions, approximately 5 microcuries of carbon-14 were added to each flask, which was then stoppered, shaken and incubated at 1.5 m off the P.E.I. dock for a 5-hr period. Three boron concentrations using sodium metaborate were standardized: 0.01, 1 and 10 ppm. Fresh

refiner groundwood effluent was obtained from the Western Forest Products Laboratory, Vancouver, and was added serially in volumes of 1 to 20 ml, with or without the presence of boron.

After 5-hour incubation, individual test samples were passed through 0.45μ membrane filters. The filters were then placed in scintillation vials with fluor and counted in a Packard Tri-carb scintillation counter (cf. Appendix IV).

RESULTS

1. Metal analysis in field samples

Only zinc, copper and cadmium analyses for Pacific oysters are available at this time.

Preliminary analyses of oyster samples, taken from the vicinity of pulp mills using zinc hydrosulphite for brightening, confirm earlier reports of zinc accumulations to levels considerably above background. The maximum concentration found in May was 19,000 ppm dry weight (the ratio of dry weight to wet weight zinc concentration was approximately 6:1), which is about 15,000 ppm above the 4,200 ppm considered to be a background level for that particular location. Very high zinc concentrations were confined to the immediate outfall area within a radius of 1-2 miles, where oysters were available, but elevated levels were noted for some distance downstream in the direction of net tidal flow.

The highest zinc concentrations were found in oysters from the Powell River area at a station nearest the pulpmill discharge,

where a maximum average in 5 samples of 5 oysters each was 17,280 ppm Zn. Oysters from other stations ranged in Zn concentration from 6,680 to 10,000 ppm. For comparison, oysters from the western tip of Texada Island in 1971 gave zinc concentrations of 5,600 ppm. Control samples from Okeover Arm, near the entrance to Toba Inlet, were in the range of 3,200 to 4,800 ppm, with an average of 4,200 ppm.

Oysters from the Stuart Channel area adjacent to the Crofton mill were high in zinc concentration, but not as high as the ones from the Powell River area. This may be related partly to the total volume of zinc-containing waste released from the two mills, and partly to the general oceanographic conditions in the two bodies of water. "Background" zinc levels in Stuart Channel were 2,080 ppm, compared to 4,200 ppm on the shores of the Strait of Georgia on either side of Powell River.

The affected area in Stuart Channel is concentrated between Ladysmith Harbour and Sansum Narrows. Zinc concentrations in oysters showed: a mean of 6,960 ppm, and a range from 4,600 to 8,000 ppm, on the Shoal Islands to the northwest of Crofton; a mean of 8,060 ppm, and a range of 5,100 to 11,200 ppm, just behind the two diffusers for the pulpmill outfalls; and a mean of 6,360 ppm, with a range of 5,400 to 7,800 ppm, to the southeast on Burgoyne Bay on Saltspring Island.

The net flow of effluent from the outfall appears to be in a south-easterly direction, impinging on Booth Bay of Saltspring Island, directly across Stuart Channel. Next to the sampling station nearest the outfall, the station in Booth Bay yielded oysters with the highest zinc concentrations, with a mean of 10,020 ppm and a range from 8,700 to 11,000 ppm.

Oysters collected in the vicinity of the Elk Falls pulp mill in Duncan Bay exhibited perhaps the lowest level of zinc concentration of any collected near mills using zinc hydrosulphite. No doubt this is related to the high degree of mixing and dispersion in the tide-swept waters of Discovery Passage. However, the closest oyster sampling point was 3 miles southeast of the mill discharge and within the influence of Campbell River. These oysters gave a range of zinc values from 3,200 to 8,000 ppm, with an average of 5,360 ppm. Oyster samples from Rebecca Spit, on the east side of Quadra Island, well removed from the influence of the Elk Falls pulp mill, ranged in zinc concentration from 3,600 to 4,500 ppm, with a mean of 3,940 ppm.

Lasqueti Island oysters, taken from Boat Cove on the south shore of the island, and considered to be free of artificial contamination by zinc, yielded a range of zinc concentration from 3,600 to 6,600 ppm, with an average of 5,480 ppm. Nanoose Bay oysters were lowest in zinc of any control samples analyzed, containing an average of 3,400 ppm, with a range from 2,400 to 4,400 ppm.

Total inorganic boron concentrations in water samples collected from the surface and 5 m depth at 40 stations in areas near groundwood mills ranged from 0.22 to 4.68 ppm, with an average of 3.53 for surface water and 3.86 ppm for water from 5 m depth. Boron content is related to salinity of water, inasmuch as borate is a major constituent of sea water. Therefore, surface waters in estuarine areas always have a lower natural boron content than deeper water.

2. Fish bioassays

Static bioassays with coho salmon fry in fresh soft water indicated that boron (from sodium metaborate addition) was toxic to fish over a range of 82 to 656 ppm B and that the mode of toxic action was slow. Bioassays were carried out for up to 23 days to assure cessation of acute toxicity phenomena in test tanks. For this reason, standard 96-hour toxicity tests are not applicable for this toxicant. The 23-day LC50 for coho fry at $11 \pm 0.5^{o}C$ was 93 ppm B. The coho dying during the 23-day test were analysed for boron and exhibited appreciable increases in boron concentration compared to the control fish. Sockeye fry in sea water appeared somewhat more sensitive to B, although the 23-day LC50 has not yet been determined for this species.

3. Bioaccumulation

Bioaccumulation studies with sockeye underyearlings and young oysters have been initiated and preliminary tests completed. Groups of animals exposed to 1 and 10 ppm B (above background) were analyzed for accumulation following 8, 32 and 47 days exposure to the two concentrations of B.

Control samples of sockeye showed an average concentration of 0.06 ppm B in tissue and a range of 0.046 to 0.095 ppm. Control samples of oysters held in tanks gave a range of B values from 3.60 to 4.03 ppm. The average of 10 oysters from Piper's Lagoon, Nanaimo, presumably with no artificial boron contamination, was 3.90 ppm B.

The sockeye exposed for 8 days to 1 ppm B exhibited an average of 0.12 ppm B with a range of 0.096 to 0.144 ppm B. Those exposed for 8 days to 10-12 ppm B gave an average B concentration in their tissue of 0.193 ppm, with a range from 0.147 to 0.232 ppm.

Oysters exposed to 1 ppm of B for 8 days gave an average B concentration in tissue of 3.79 ppm and a range from 3.62 to 4.00 ppm. After 32 and 47 days exposure, they showed an average of 7.1 and 5.3 ppm, compared to 6.0 and 4.8 ppm for the controls, respectively. Those exposed to 10-12 ppm B for 8 days averaged 4.00 ppm, with a range from 3.83 to 4.21 ppm B in tissue. After 32 days, they averaged 13.2 and ranged from 12.6 to 13.8 ppm; and after 47 days they averaged 10.8 and ranged from 10.4 to 11.2 ppm. The higher values after 32 days may have been due to some inadvertent desiccation.

4. Phytoplankton bioassays

Phytoplankton studies commenced on 4 July 1973 and included 15 in situ bioassays, 9 of which involved exclusively boron additions and 6 dealt with groundwood or kraft mill effluent additions plus boron.

In all 9 experiments, boron additions at 1 and .01 ppm caused a slight inhibition of growth of phytoplankton, at 0.1 and 0.5 ppm a slight stimulation, and at 1 ppm production was not seriously affected. However, at concentrations higher than 1 ppm, growth decreased rapidly, with negligible growth at 50 ppm. In most experiments, growth of phytoplankton in the control flasks exceeded that in flasks receiving boron, suggesting that even small concentrations of boron require some algal acclimation.

Results with groundwood effluent showed an inhibition of phytoplankton growth at 0.78%, a slight inhibition at 3.84% and then a tailing off to complete growth inhibition at 7.69% and higher concentrations of groundwood effluent. Additions of 1 ppm of boron in the tests with groundwood effluent showed a depression in production,

but no significant difference in effects on phytoplankton from those with groundwood alone. However, 1 ppm B without groundwood effluent resulted in growth of phytoplankton equal to that of the control, which is indicative of a toxic effect of groundwood effluent over-riding any negative boron effects at that boron concentration.

Effects of pH on phytoplankton were examined, and it was noted that a change of 0.1 to 0.2 pH units appeared not to affect the metabolism of algae. However, a pH change of 0.5 to 1.0 pH units could lead to a serious shock response, resulting in an inhibition of photosynthesis. The range of pH change in the experiments with boron and groundwood effluent could not be considered as being responsible for the changes noted in phytoplankton production.

SUMMARY AND CONCLUSIONS

Zinc concentrations in oysters in the vicinity of pulp and paper mills, producing groundwood pulp and using zinc hydrosulphite as a brightening agent, were found to be up to 19,000 ppm dry weight or almost 5 times as high as "background" levels in the area. The lowest concentrations of zinc in oysters, well removed from pulp and paper mills, were found to be on the average 3,400 ppm dry weight or 550 ppm wet weight.

Boron in waters in the vicinity of the mills at 40 stations averaged 3.53 ppm in surface water and 3.86 ppm in water at 5 m depth.

The 23-day LC50 of sodium metaborate for coho fry at $11 \pm 0.5^{o}C$ was 93 ppm B, while that for sockeye in sea water was considered to be somewhat less. The mode of toxic action is slow, and for this reason, the standard 96-hour toxicity test is not meaningful for this substance.

Oysters show a natural concentration of boron in their tissue of about 3.8 ppm, compared to about 0.06 ppm in underyearling sockeye. With exposure to 1 and 10 ppm boron, oysters showed no increase after 8 days at 1 ppm and about 0.2 ppm or 5% increase in tissue boron at 10-12 ppm. However, after 32 and 47 days exposure to 10-12 ppm boron, the oysters exhibited 13.2 and 10.8 ppm, respectively, in their tissue boron, suggesting an equilibration of B in tissue with that in the water. The sockeye showed a bioaccumulation of 0.12 ppm, or 100% increase at 1 ppm and 0.19 ppm, or over 200% increase, at 10-12 ppm after 8 days exposure.

Phytoplankton production is inhibited at concentrations of boron greater than 1 ppm above background. It appears that 1 ppm above background (3.1 ppm at P.E.I. dock) is a critical level, and at concentrations higher than that, growth is retarded. At concentrations greater than 10 ppm boron, growth is negligible.

Groundwood effluent is toxic to phytoplankton, judging by inhibition of photosynthesis, at all concentrations tested, but most significantly at concentrations exceeding 4%. Groundwood plus 1 ppm boron did not increase the negative effect of groundwood effluent, suggesting that boron will be less of a problem to phytoplankton than the effluent itself. The pH of groundwood and KME effluent, however, seems severe enough to warrant study of its in situ effect on phytoplankton production in waters adjacent to mills. However, because of the buffering capacity of sea water, it is not anticipated that the effluent would have much pH effect beyond the immediate vicinity of the outfall.

The preliminary information presented here indicated that there is no cause for serious ecological concern arising from introduction of the new brightening agent, sodium hydrosulphite produced by the Borol process, with sodium metaborate as a waste product. However, further experimentation is required to monitor the possible ecological effects of boron in receiving waters, especially those affecting lower trophic levels, i.e. phytoplankton and marine invertebrates that possess a planktonic larval stage.

APPENDIX I

APPENDIX I

Heavy Metal Monitoring Program with Emphasis on Zinc Contamination of the Pacific Oyster, *Crassostrea gigas*

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September 26, 1973

INTRODUCTION

Recent surveys have indicated that the effluent discharges from certain pulp mills employing the zinc hydrosulphite bleaching process have resulted in a significant build-up of zinc in shellfish, particularly the Pacific oyster Crassostrea gigas. On the basis of these findings, and possibly other considerations, several of the mills have decided to change to sodium hydrosulphite for groundwood bleaching. The new bleaching compound will be produced by the Borol Process with the by-products being sodium metaborate and sodium hydrosulphite. Since zinc will be largely eliminated from the effluent discharge, the Environmental Protection Service initiated a program to determine the present zinc levels in shellfish found in the areas affected, and to monitor changes occurring owing to the change-over to the Borol Process. This was part of a joint program with the Fisheries and Marine Service (Fisheries Operations and Fisheries Research and Development), Environment Canada; the Provincial Pollution Control Branch and the various companies involved. The program includes the following activities:

1. An extensive field program for the collection of biological, sediment, and water samples from areas where groundwood bleaching is in progress.

2. Analysis of the above mentioned samples for heavy metal content with particular emphasis on zinc concentrations.

3. Condition factor measurements on C. gigas to determine if a correlation exists between the heavy metal content of an oyster and its physical condition.

METHODS

Sampling Frequency and Location

Stations were set up around five groundwood bleaching mills such that the outer perimeter of any heavy metal contamination would be defined. The mills were the MacMillan and Bloedel mills at Powell River and Port Alberni, the British Columbia Forest Products mill at Crofton and the Crown Zellerbach mills at Elk Falls near Campbell River and Ocean Falls*. These areas were sampled in May, 1973 to form the initial background data. In June 1973, Gambier Island, Nanoose Bay, Lasqueti Island, and Sooke Basin were sampled as control sites and/or for background data. In order

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* This mill has been recently acquired by the B.C. Government.

to record any seasonal fluctuation in heavy metal content of oysters, another complete sample collection was made in August 1973, and a third collection was completed in November 1973. Data from the latter sampling are not shown in this report.

Letter designations for the stations appear in Table I. On the August 1973 trip, sediment and water samples were collected from new stations situated closer to the effluent discharge than the original stations.

Field Procedures

1. Biological Samples - oysters, mussels, and clams were collected from each station when available and put in frozen storage. The species collected were C. gigas, the edible mussel Mytilus edulis, the little neck clam Protothaca staminea, soft-shelled clam Mya arenaria, butter clam Saxidomus giganteus, the bent nosed clam Macoma nasuta, and the basket cockle Clinocardium nuttalli. Oyster samples consisting of five individuals each were taken from five different locations along the middle inter-tidal zone at each station to account for any local variations.

   Those specimens of C. gigas for condition factor (C.F.) determinations, taken on the August 1973 trip, were weighed and measured on the day of collection to avoid moisture loss. The shucked oyster meat was frozen and held for dry weight measurements to be applied to the equation:

$$C.F. = \frac{\text{dry wt. of meat}}{\text{wt. of oyster - wt. of shell}} \times 1000$$

2. Water Samples - water was collected from 0 and 5 meters depths in a Van Dorn bottle, passed through a 0.45μ millipore filter to remove plankton and acidified with Transistar grade HNO_3 (10 mls/ℓ). Samples were stored in polyethylene containers.

3. Sediment Samples - sediments were collected in a Peterson dredge on the May 1973 survey and in a Ponar dredge on the August 1973 survey. The samples are stored in a freezer.

Analytical Procedures

1. Biological Samples - the analytical technique for zinc, copper, and cadmium appears below. Mercury content is determined on a separate sample since the drying step is omitted.

   Zn, Cu, Cd - sample is dried for 48 hours at 110°C then ground in a mortar and pestle
   - one gram portions are digested with 10 mls conc. HNO_3, approximately 6 hour
   - samples are made up to 100 mls with water
   - analysis is done on a Jarrell Ash Atomic Absorption (A.A.) Unit.

2. Water Samples - analysis for Zn, Cu, and Cd is done on the A.A. Unit after passing the sample through an ion exchanger to concentrate the trace metals. This technique also removes interfering ions such as sodium, potassium and calcium from the sea water.

3. Sediment Samples - sediments are digested with a strong acid and analysed for Zn, Cu and Cd on an A.A. Unit.

RESULTS

Analysis has been completed for the content of Zn, Cu and Cd in oysters collected on the May 1973 survey. These results appear in Tables III to VIII, each value representing a sample of 5 oysters. The data for mean and standard deviation of Zn levels are recorded in Table II and plotted on Figures 1, 2, and 3 to show contamination levels in relation to distance from outfall. Figures 4 to 9 are nautical chart reductions showing station locations. Figures 10 - 14 are nautical chart reductions showing the mean zinc concentration found in groups of five whole oysters at each station. Values are expressed on a dry weight basis.

Tables

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Table I

Area	Shore Stations	Water Stations	Sediment Stations	Figure
Powell River	A-14 → A-23 and A-25	A-1 → A-13 A-27, 28, 29 (Georgia Strait)	A-1, A-3 → A-8 and A-12	4
Elk Falls	B-14 → B-21	B-1 → B-13		5
Alberni Inlet	C-15 → C-18	C-5 → C-9, C-13, and C-14 C-1 → C-4 (Sechart Channel)	C-1 → C-4	6
Crofton	D-9 → D-17	D-1 → D-7 D-8 (Georgia Strait)	D-1 → D-5	7
Nanoose Bay	F-1			8
Lasqueti Is.	F-2			
Gambier Is	G-1 → G-3			9
Sooke Basin	S-1			

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Table II

Station	# of Samples	Zn ppm dry wt		Zn ppm wet wt	
		$\bar{x}$	σ	$\bar{x}$	σ
Powell River					
A-14	5	9060	712.74	1360	114.02
A-16	5	8740	1071.44	1472	240.67
A-19	5	17280	1758.42	2120	248.10
A-20	5	7720	465.84	1170	178.89
A-21	5	7260	219.09	1120	44.72
A-23	5	6680	892.75	856	124.22
A-25	5	10600	2044.51	1604	161.49
Campbell River					
B-14	5	3940	427.79	650	70.36
B-17	5	5780	506.96	956	140.10
B-18	5	4660	691.38	742	125.18
B-19	5	4800	871.78	644	129.15
B-20	5	4640	1099.09	824	257.84
B-21	5	5360	1762.95	1026	280.85
Alberni Inlet					
C-15	5	10600	2391.66	2180	491.94
Crofton					
D-9	5	6360	952.89	1066	204.16
D-10	5	10020	1005.98	1420	148.32
D-11	5	4600	927.36	768	156.11
D-12	5	4760	654.21	896	125.02
D-13	5	2080	420.71	320	115.54
D-14	5	2920	471.17	520	85.15
D-15	5	6960	1453.62	1300	300.00
D-16	5	7840	531.99	--	--
D-17	5	8060	2611.13	3670	5777.50
Nanoose Bay					
F-1	5	3440	726.64	545	92.60
Lasqueti Is.					
F-2	5	5480	1154.12	844	266.23
Gambier Is.					
G-1	5	5340	3951.96	1444	390.74
G-2	5	10880	2488.37	1668	483.86
G-3	5	9420	5136.34	1620	376.83

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TABLE III SHELLFISH HEAVY METAL MONITORING PROGRAM

POWELL RIVER - OYSTERS (results expressed in μg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
A-14	9,600	1,400	230	34	10	1
	8,600	1,400	250	42	7	1
	8,300	1,200	230	33	10	1.4
	8,800	1,300	210	31	13	2
	10,000	1,500	310	46	9	1.4
A-16	7,800	1,300	140	23	4	0.7
	10,000	1,800	130	24	8	1
	9,800	1,660	150	25	7	1
	7,900	1,300	100	17	4	0.7
	8,200	1,300	130	21	<3	<0.4
A-19	18,000	2,200	270	36	4.5	0.6
	15,000	1,900	180	23		
	16,000	1,900	220	26	4	0.5
	18,000	2,100	210	24	7	0.8
	19,400	2,500	210	25	3	0.4
A-20	7,000	950	180	25	8	1
	8,300	1,400	220	35	7	1
	7,800	1,100	230	34	10	1
	7,800	1,100	200	28	10	1
	7,700	1,300	210	35	8	1
A-21	7,400	1,100	240	35	13	2
	7,200	1,200	200	33	10	2
	6,900	1,100	200	31	8	1
	7,400	1,100	200	29	9	1
	7,400	1,100	220	33	13	2
A-23	5,900	720	230	28	14	2
	7,200	1,000	320	45	11	2
	7,700	950	240	29	19	2
	5,600	740	250	33	15	2
	7,000	870	270	34	14	2

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TABLE III (cont'd) SHELLFISH HEAVY METAL MONITORING PROGRAM

POWELL RIVER - OYSTERS (results expressed in μg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
A-25	11,000	1,720	160	25	6	0.9
	9,000	1,600	150	25	5	0.9
	14,000	1,800	240	32	8	1
	9,400	1,500	170	27	4	0.6
	9,600	1,400	170	26	3	0.5
*OKEOVER ARM	4,600	820	150	26	10	1.8
	3,200	540	93	15	11	1.8
	4,200	750	92	15	8.9	1.5
	4,800	860	120	22	15	2.7

* Samples collected by L. Melville, biologist for MacMillan Bloedel, Powell River division. Each value represents a sample containing 2 oysters.

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TABLE IV SHELLFISH HEAVY METAL MONITORING PROGRAM

CAMPBELL RIVER - OYSTER SAMPLES (results expressed in µg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
B-14	3,700	680	110	21	9	2
	4,500	750	160	26	11	2
	3,600	570	110	17	9.5	1.5
	4,300	650	130	20	9	1
	3,600	600	130	22	9.5	2
B-16	6,300	930	140	21	13	2
B-17	6,100	940	140	22	13	2
	6,000	1100	180	31	12	2
	6,300	1,100	150	25	11	2
	5,100	800	120	18	13	2
	5,400	840	160	25	13	2
B-18	4,100	650	120	20	10	2
	4,800	810	180	30	15	2.5
	3,800	570	120	19	13	2
	5,200	820	130	20	15	2
	5,400	860	170	28	10	2
B-19	5,600	680	150	22	15	2.2
	3,400	540	89	14	9.9	1.5
	5,400	480	130	18	12	1.7
	5,000	740	170	25	12	1.8
	4,600	780	110	19	9.9	1.7
B-20	6,400	1,220	220	43	11	2.2
	4,600	860	170	31	9.7	1.8
	4,200	700	160	26	8.6	1.4
	4,600	820	170	30	11	1.9
	3,400	520	120	18	8.6	1.3

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TABLE IV (cont'd)

CAMPBELL RIVER - OYSTER SAMPLES (results expressed in μg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
B-21	5,200	800	250	38	17	2.5
	5,800	1,000	310	60	11	2.1
	4,600	1,000	180	38	8.8	1.9
	3,200	830	180	47	6.5	1.7
	8,000	1,500	190	36	11	1.3

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TABLE V SHELLFISH HEAVY METAL MONITORING PROGRAM

ALBERNI INLET - OYSTER SAMPLES (results expressed in µg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
C-15	12,000	2,200	58	11	4	.75
	10,000	2,000	55	11	3	.5
	8,200	1,700	67	14	<3	<0.5
	14,000	3,000	82	18	6	1
	8,800	2,000	51	12	4	.9

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TABLE VI SHELLFISH HEAVY METAL MONITORING PROGRAM

CROFTON - OYSTER SAMPLES (results expressed in μg/gm)						
	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
D-9	5,400	870	92	15	10	2
	7,800	1,400	120	21	8	1.5
	6,400	1,000	100	16	7	1
	6,600	1,100	110	18	7	1
	5,600	960	93	16	10	2
D-10	10,000	1,600	190	30	9	1
	11,000	1,500	260	35	16	2
	11,000	1,400	250	32	11	1
	9,400	1,400	190	28	9	1
	8,700	1,200	210	28	6	0.8
D-11	3,500	600	78	13	4	0.7
	5,200	970	110	19	9	1.5
	5,100	820	93	15	7	1
	3,700	620	59	9.9	<2.5	<0.4
	5,500	830	79	12	8	1
D-12	4,400	800	84	15	4	0.8
	5,800	1,100	140	26	7	1
	4,200	840	90	18	4	0.8
	5,000	930	110	20	5	0.9
	4,400	810	84	15	5	0.85
D-13	2,800	440	83	13	6	1
	2,000	340	70	12	<3	<0.5
	1,900	370	65	12	3.5	0.7
	2,000	130	51	3.3	<2.5	<0.2
	1,700	320	34	6.3	3.5	0.7
D-14	2,500	410	46	7.5	<3	<0.5
	3,000	550	50	9.3	7	1
	3,700	640	99	17	4	0.7
	2,700	480	61	11	<3	<0.5
	2,700	520	140	27	9	2

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TABLE VI (cont'd) SHELLFISH HEAVY METAL MONITORING PROGRAM

CROFTON - OYSTER SAMPLES (results expressed in µg/gm)						
	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
D-15	7,800	1,400	240	44	7.5	1
	8,000	1,400	240	43	9	2
	4,600	800	200	35	5.5	1
	6,500	1,300	240	49	8	2
	7,900	1,600	250	50	5.5	1
D-16	7,600	1,000	80	10	4	0.5
	8,700	1,300	120	18	5.5	0.9
	7,500	1,600	130	26	<3	<0.7
	8,000	1,400	150	26	<3	<0.5
	7,400	1,300	130	22	5	0.8
D-17	11,200	14,000	120	15	11	1
	6,800	1,000	100	15	3.5	0.5
	10,400	1,400	100	14	<3	<0.4
	5,100	950	120	21	<3	<0.5
	6,800	1,000	120	19	5.2	0.8

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TABLE VII SHELLFISH HEAVY METAL MONITORING PROGRAM

NANOOSE BAY (F-1) LASQUETI IS. (F-2) - OYSTER SAMPLES (results expressed in µg/gm)						
	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
F-1	3,600	540	190	28	20	2.8
	4,400	646	190	28	14	2.1
	3,600	600	200	34	11	1.8
	2,400	400	110	19	8.9	1.5
	3,200	540	160	28	11	1.9
F-2	6,600	1,200	380	68	19	3.4
	3,600	520	190	27	18	2.5
	5,400	820	280	43	15	2.3
	6,200	1,000	380	63	19	3.1
	5,600	680	280	48	18	3.1
F-2*	5,600	1,100	300	59	19	3.8
	5,800	1,000	310	60	15	2.8
	6,800	1,100	360	62	18	3
	5,000	1,100	240	53	20	4.4
	5,800	1,000	370	67	13	2.4

* One oyster per sample

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TABLE VIII SHELLFISH HEAVY METAL MONITORING PROGRAM

GAMBIER IS. - OYSTER SAMPLES (results expressed in µg/gm)

	ZINC		COPPER		CADMIUM	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
G-1	9,600	1,700	870	160	24	4.3
	1,100	1,500	1,200	160	25	3.3
	8,000	1,200	1,000	150		
	1,200	1,900	1,300	210	25	4.0
	6,800	920	760	100	34	4.7
G-2	9,400	1,740	880	160	16	2.9
	13,000	1,700	1,100	140	17	2.2
	8,200	1,100	770	100	20	2.6
	14,000	2,400	1,200	200	15	2.4
	9,800	1,400	830	120	15	2.1
G-3	15,000	2,200	1,700	260	19	2.9
	11,000	1,700	1,300	190		
	9,000	1,200	1,100	160	27	3.7
	11,000	1,400	1,300	170	24	3.1
	1,100	1,600	1,300	190	21	3.0

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List of Figures

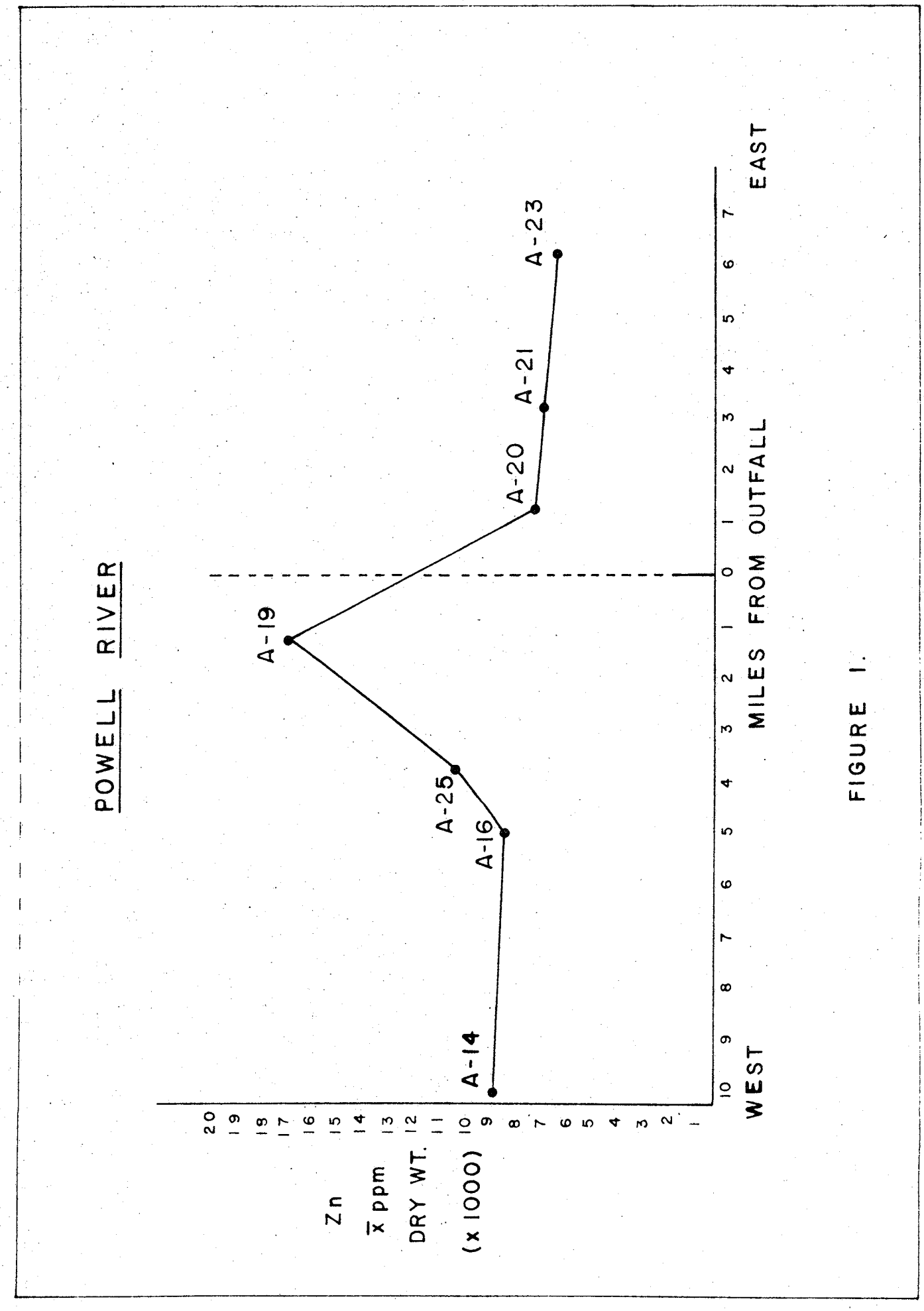
POWELL RIVER
A-19
A-25
A-16
A-14
A-20
A-21
A-23
Zn
x̄ ppm
DRY WT.
(x 1000)
WEST
MILES FROM OUTFALL
EAST

FIGURE 1.

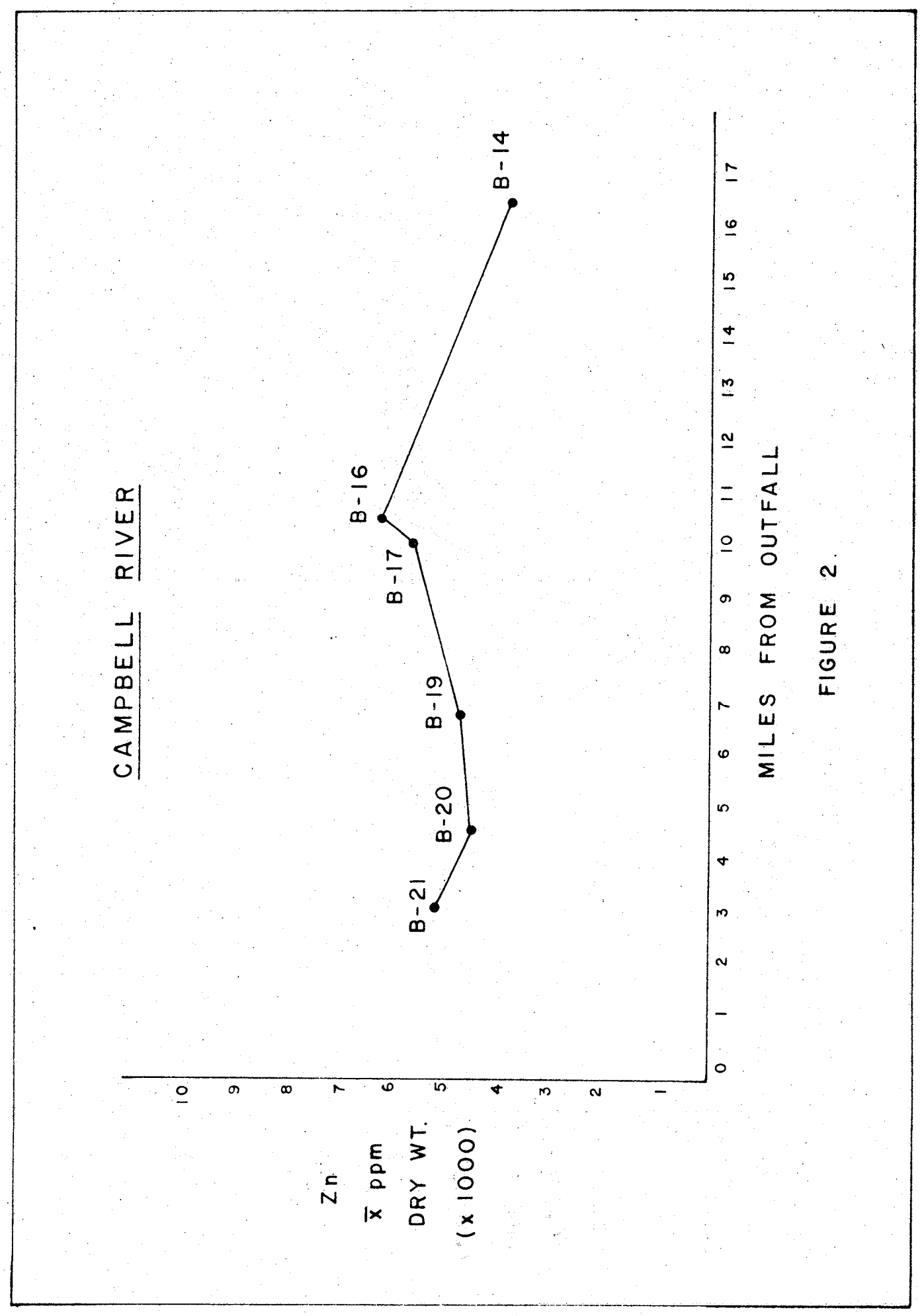
CAMPBELL RIVER
B-14
B-16
B-17
B-19
B-20
B-21
Zn
x̄ ppm
DRY WT.
(x 1000)
MILES FROM OUTFALL
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
1 2 3 4 5 6 7 8 9 10

FIGURE 2.

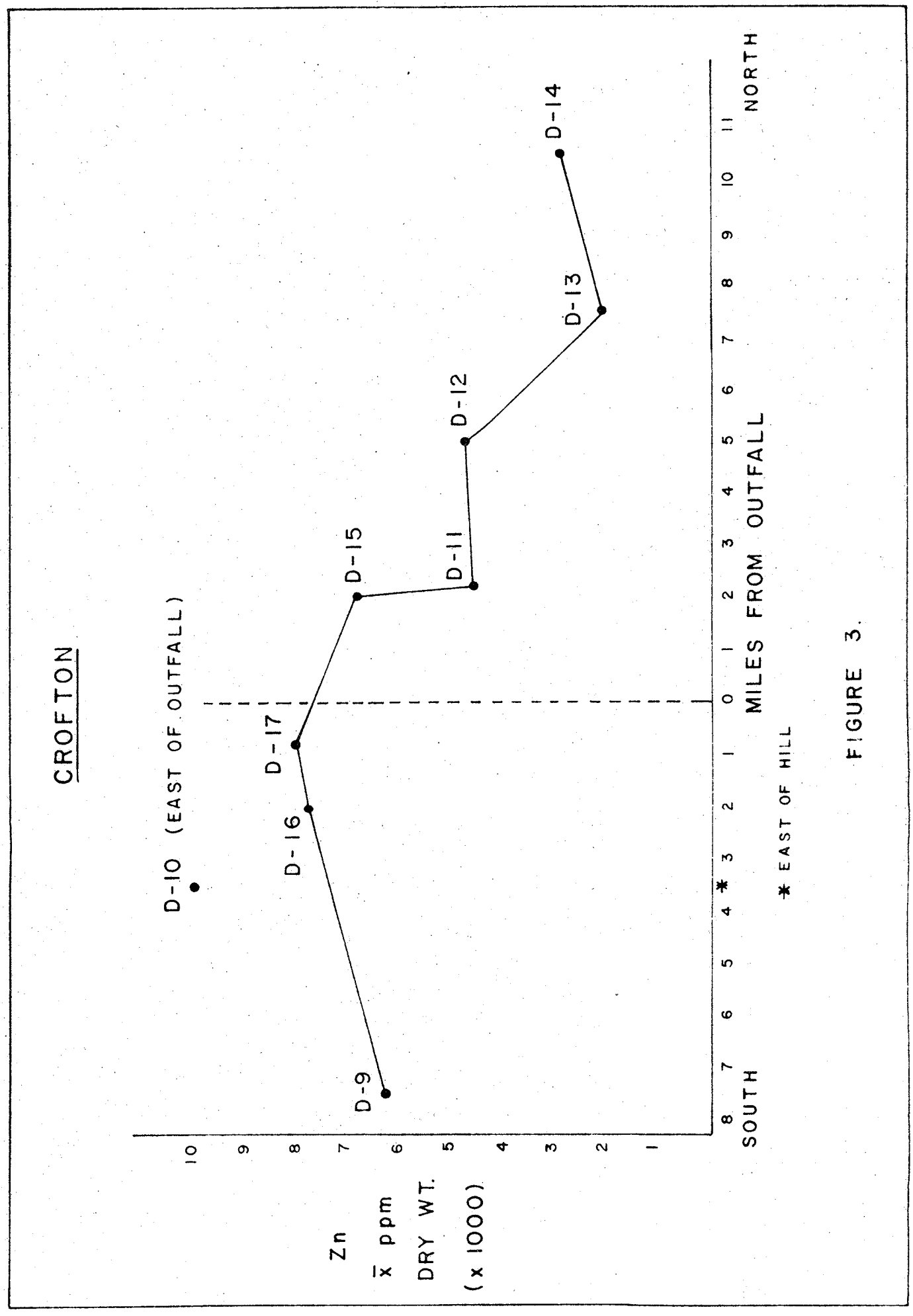
CROFTON
D-10 (EAST OF OUTFALL)
D-9
D-16
D-17
D-15
D-11
D-12
D-13
D-14
Zn
x̄ ppm
DRY WT.
(x 1000)
10
9
8
7
6
5
4
3
2
1
SOUTH
8 7 6 5 4 * 3 2 1 0 1 2 3 4 5 6 7 8 9 10 11
MILES FROM OUTFALL
NORTH
* EAST OF HILL

FIGURE 3.

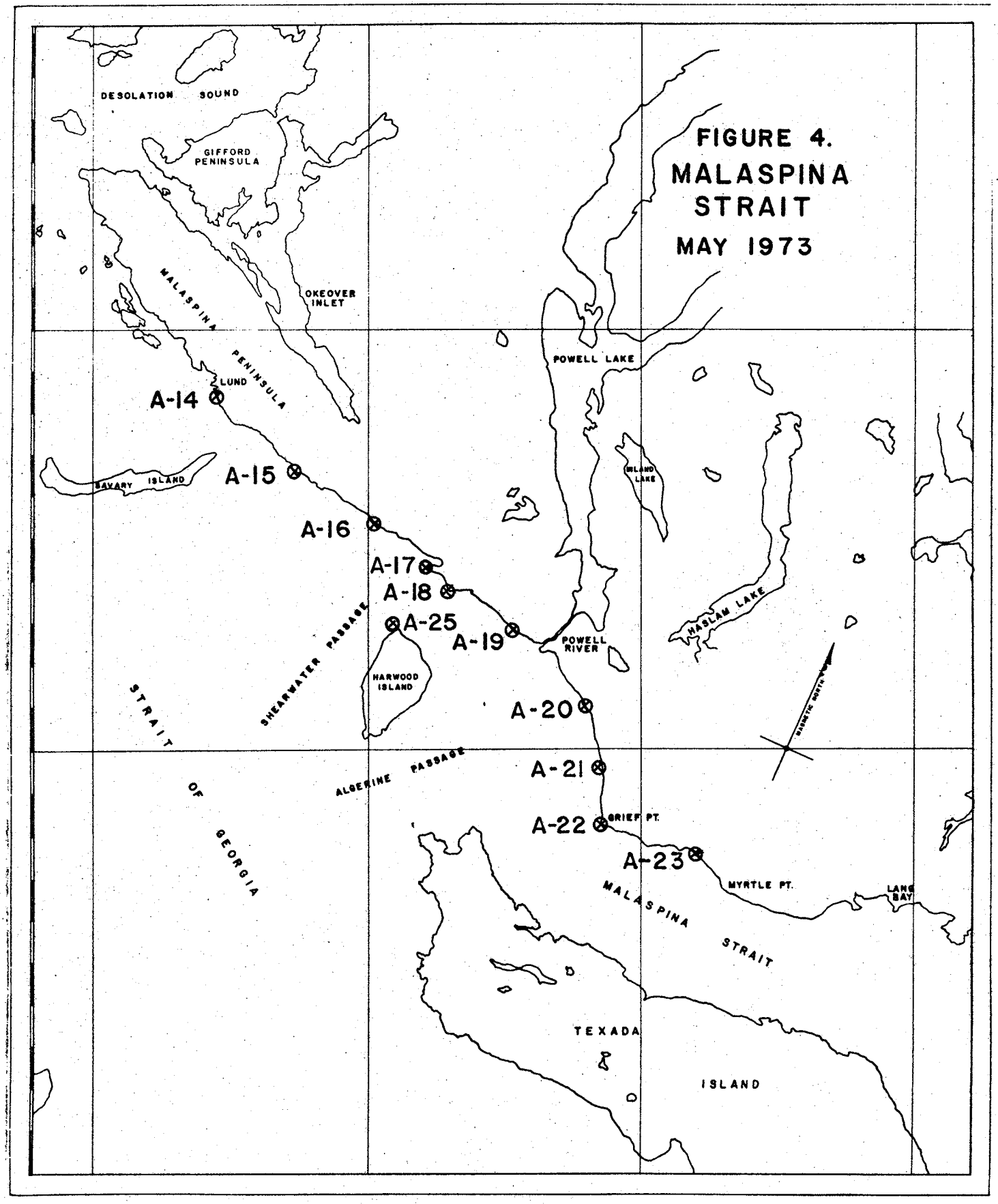

FIGURE 4.
MALASPINA STRAIT
MAY 1973

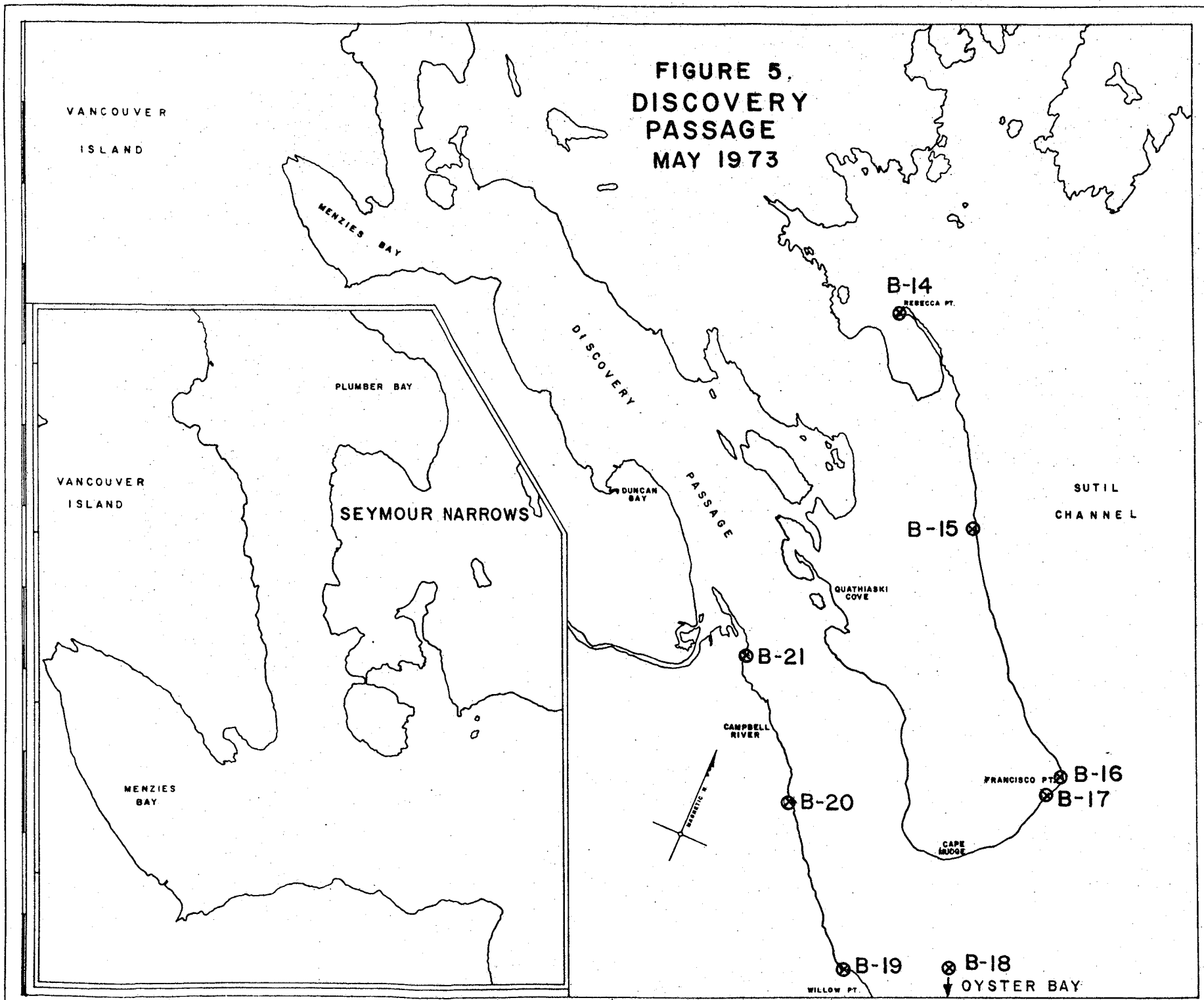
FIGURE 5.
DISCOVERY
PASSAGE
MAY 1973
VANCOUVER
ISLAND
MENZIES BAY
B-14
REBECCA PT.
DISCOVERY
PASSAGE
DUNCAN
BAY
SUTIL
CHANNEL
B-15
QUATHIASKI
COVE
B-21
CAMPBELL
RIVER
B-20
FRANCISCO PT.
B-16
B-17
CAPE
MUDGE
B-19
WILLOW PT.
B-18
OYSTER BAY
PLUMBER BAY
VANCOUVER
ISLAND
SEYMOUR NARROWS
MENZIES
BAY

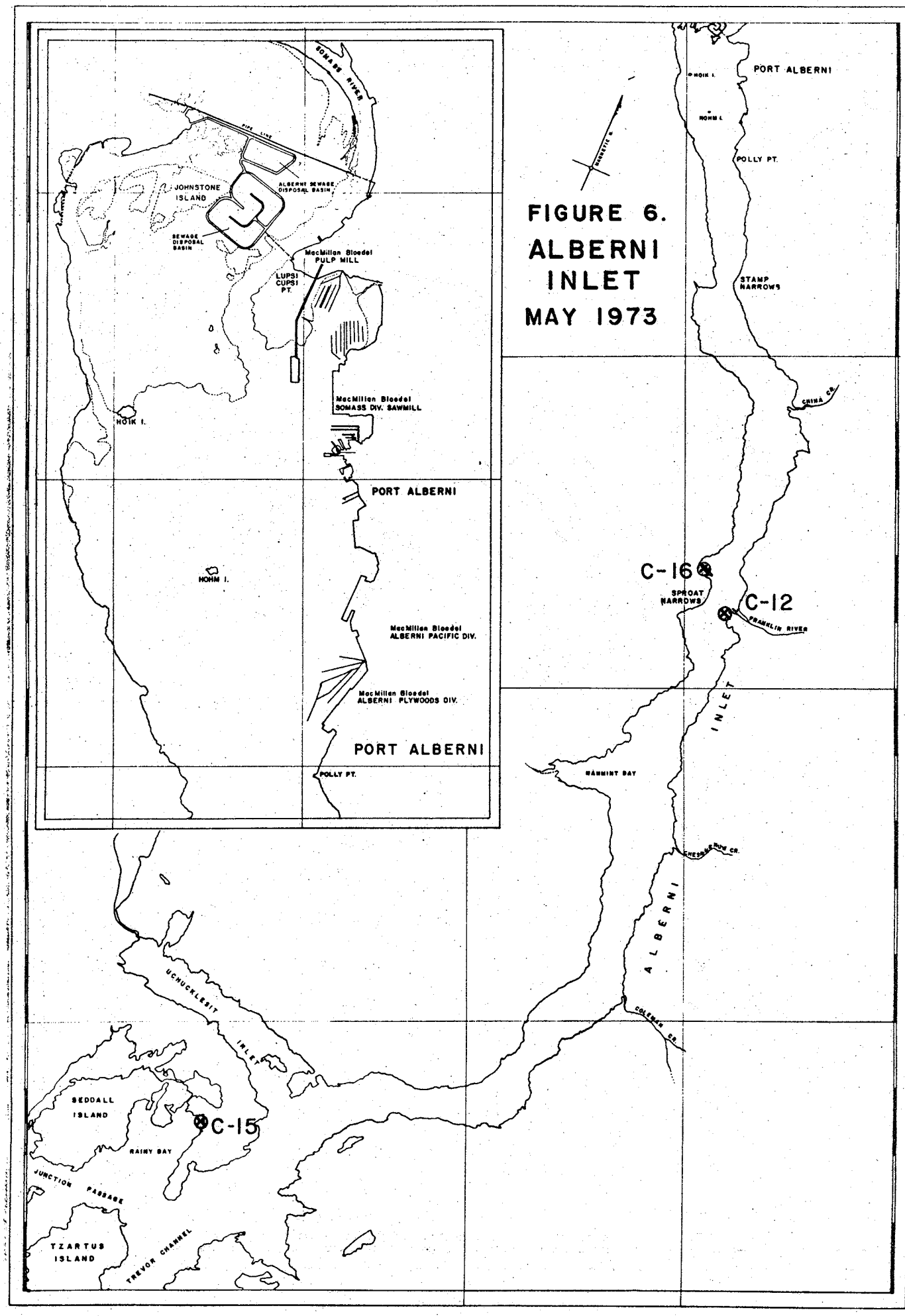

FIGURE 6. ALBERNI INLET MAY 1973

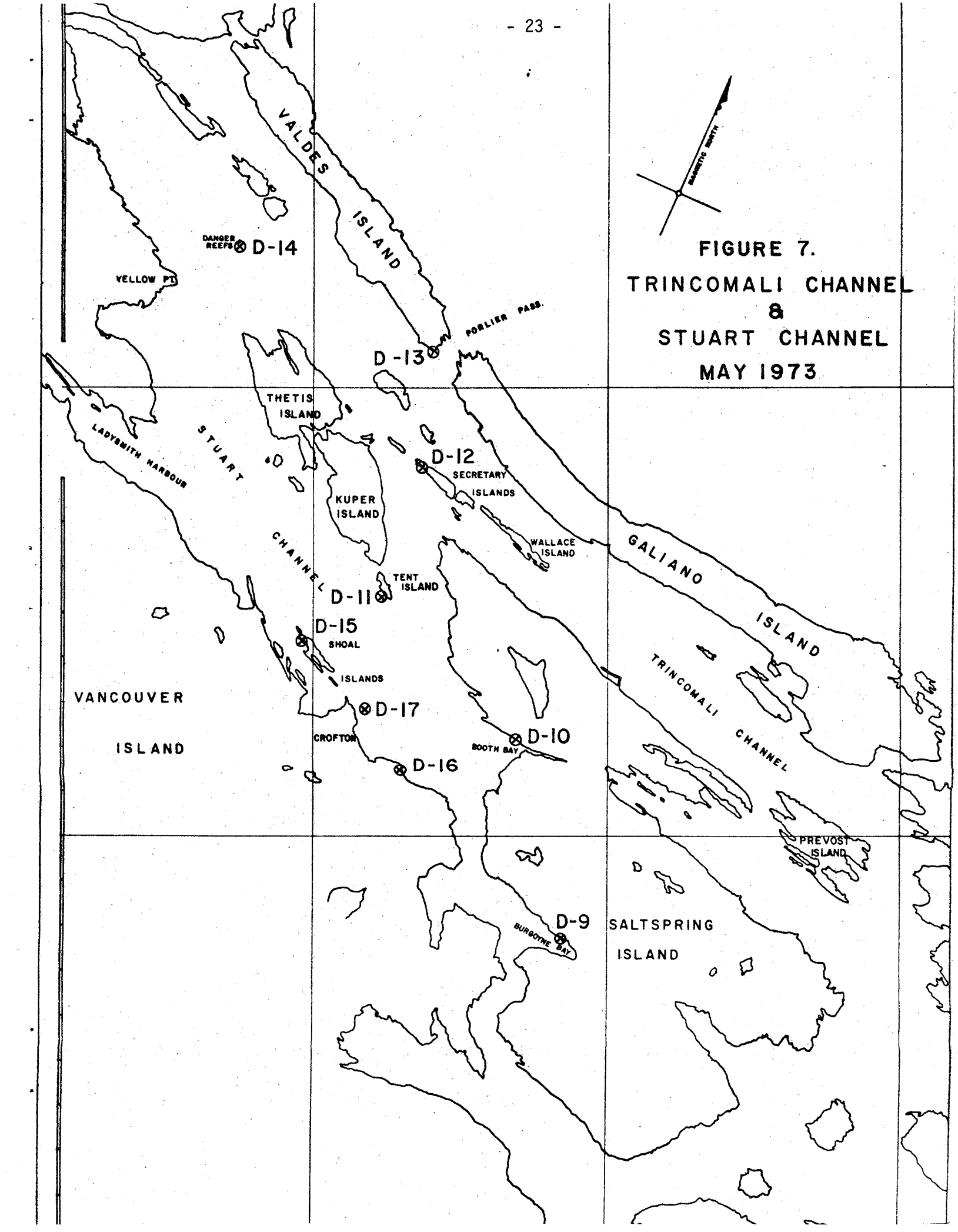

FIGURE 7.
TRINCOMALI CHANNEL
&
STUART CHANNEL
MAY 1973

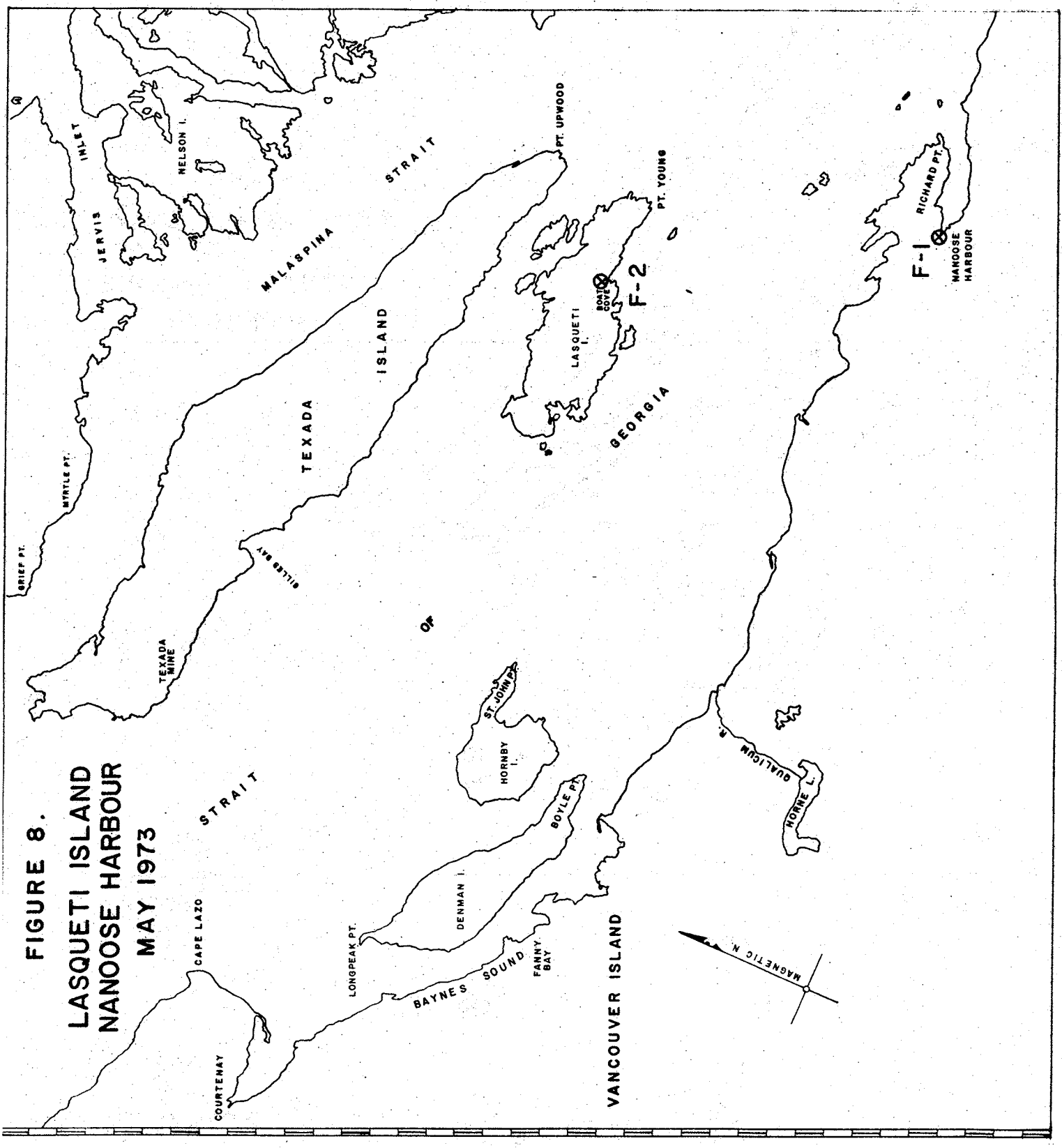

FIGURE 8.
LASQUETI ISLAND
NANOOSE HARBOUR
MAY 1973

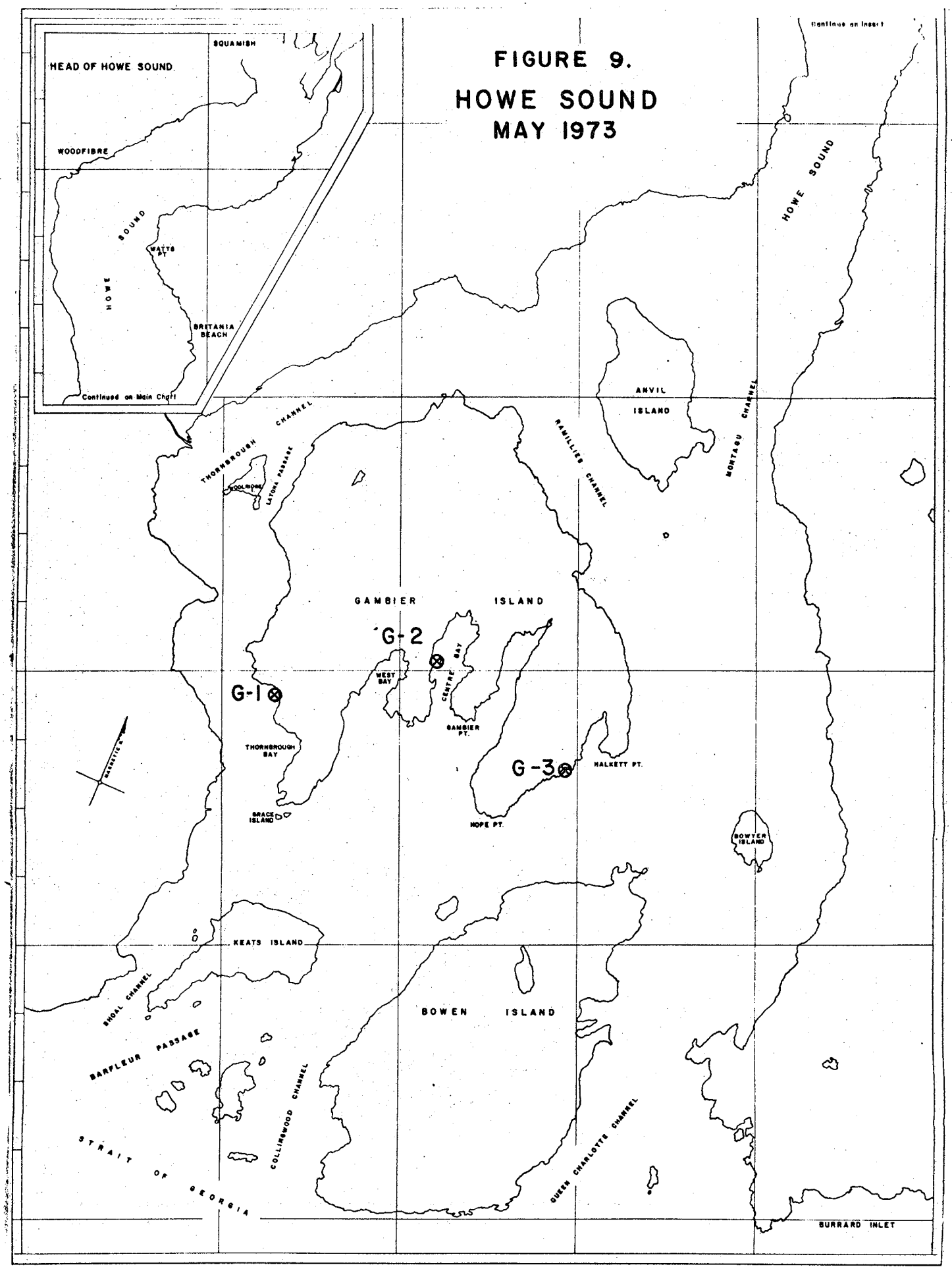
FIGURE 9.
HOWE SOUND
MAY 1973
HEAD OF HOWE SOUND
SQUAMISH
WOODFIBRE
HOWE SOUND
WATTS PT.
BRITANIA BEACH
Continued on Main Chart
Continue on Insert
HOWE SOUND
ANVIL ISLAND
MONTAGU CHANNEL
RAMILLIES CHANNEL
THORNBROUGH CHANNEL
LATONA PASSAGE
COOL RIDGE
GAMBIER ISLAND
G-1
G-2
G-3
WEST BAY
CENTRE BAY
GAMBIER PT.
THORNBROUGH BAY
HALKETT PT.
HOPE PT.
GRACE ISLAND
BOWYER ISLAND
MAGNETIC N
KEATS ISLAND
SHOAL CHANNEL
BOWEN ISLAND
BARFLEUR PASSAGE
COLLINGWOOD CHANNEL
QUEEN CHARLOTTE CHANNEL
STRAIT OF GEORGIA
BURRARD INLET

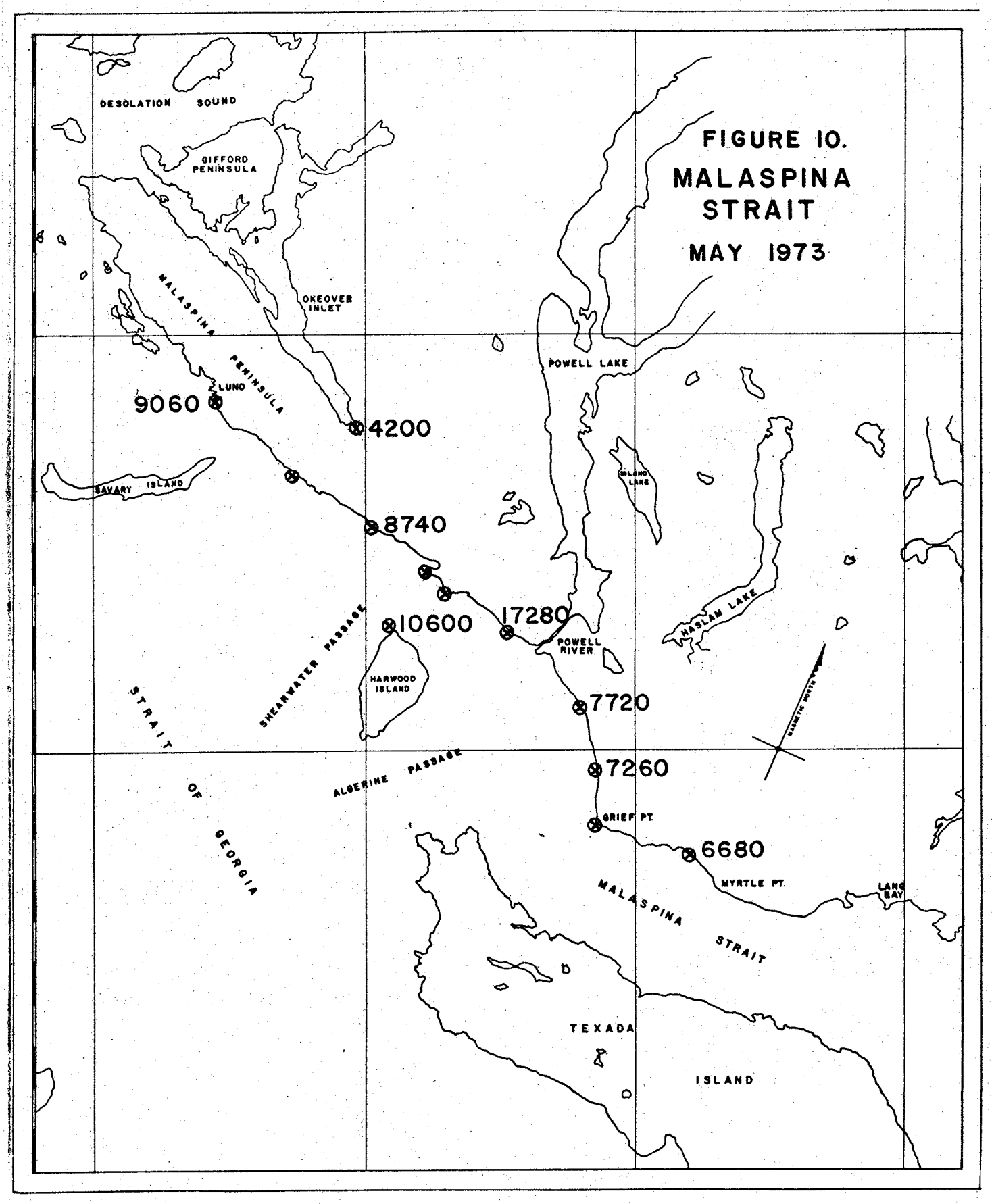

FIGURE 10.
MALASPINA STRAIT
MAY 1973

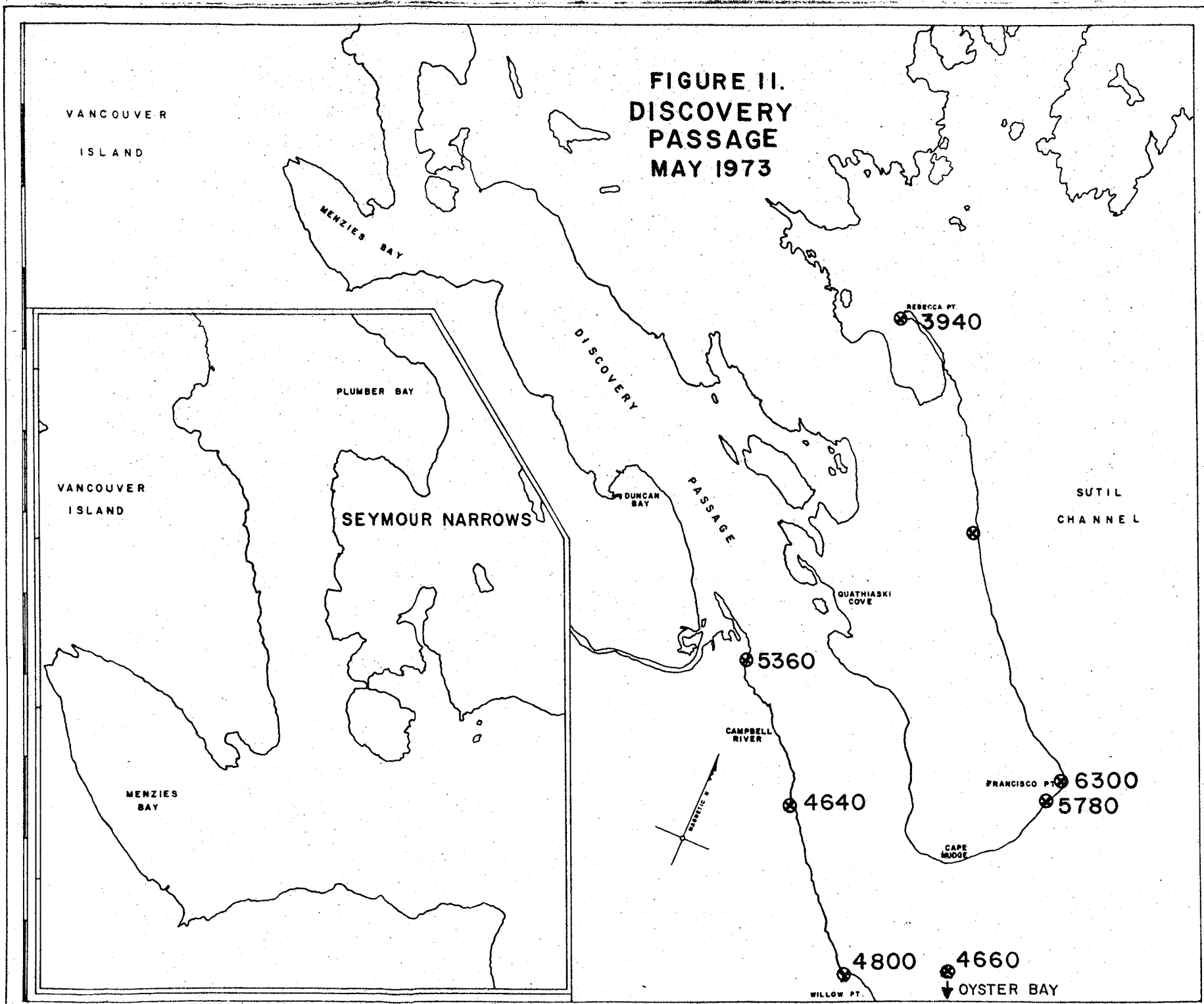
FIGURE 11.
DISCOVERY
PASSAGE
MAY 1973
VANCOUVER
ISLAND
MENZIES BAY
DISCOVERY
PASSAGE
REBECCA PT.
3940
PLUMBER BAY
VANCOUVER
ISLAND
SEYMOUR NARROWS
DUNCAN
BAY
SUTIL
CHANNEL
QUATHIASKI
COVE
5360
CAMPBELL
RIVER
MAGNETIC N
FRANCISCO PT.
6300
5780
4640
CAPE
MUDGE
MENZIES
BAY
4800
4660
WILLOW PT.
OYSTER BAY

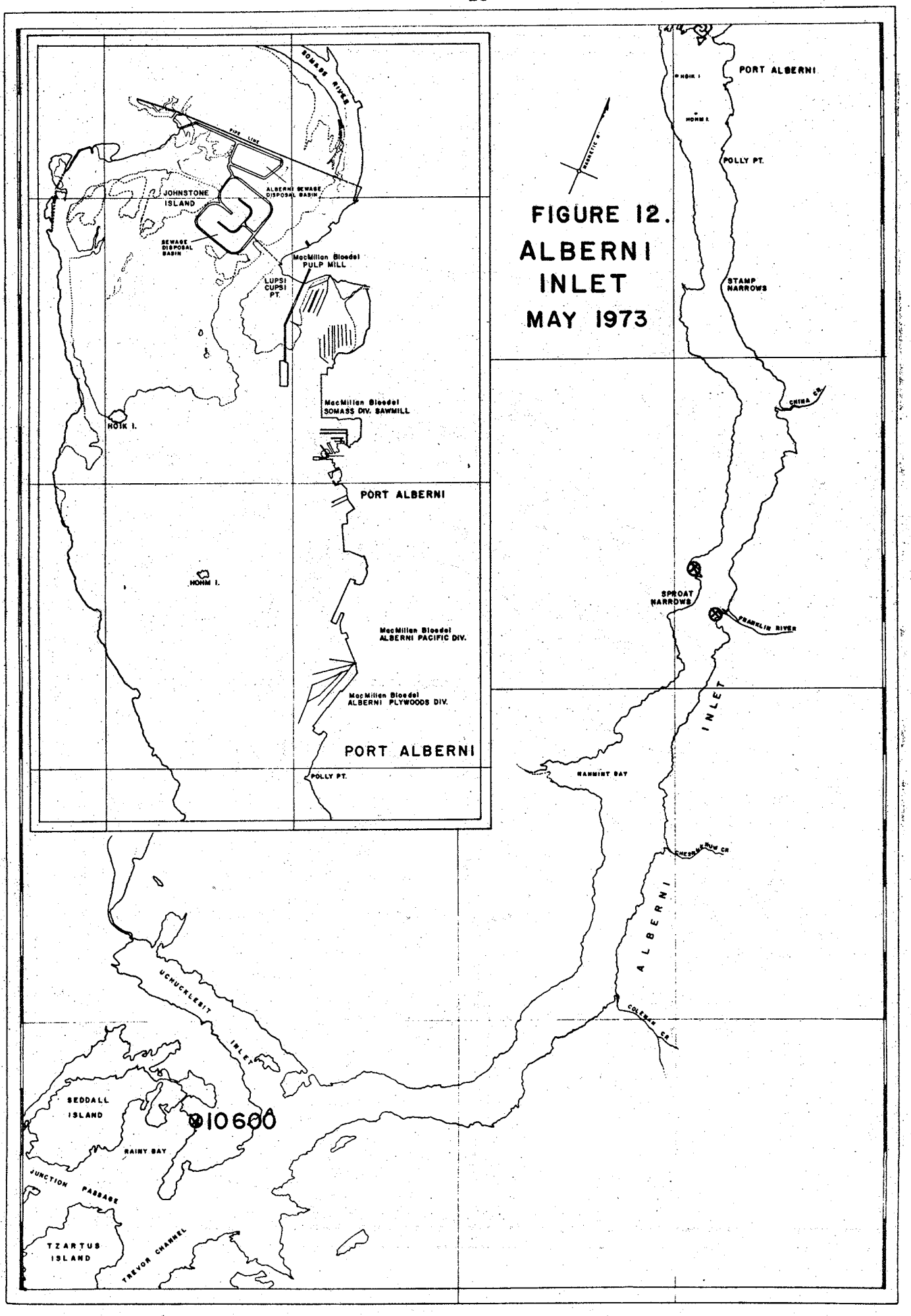

FIGURE 12.
ALBERNI INLET
MAY 1973

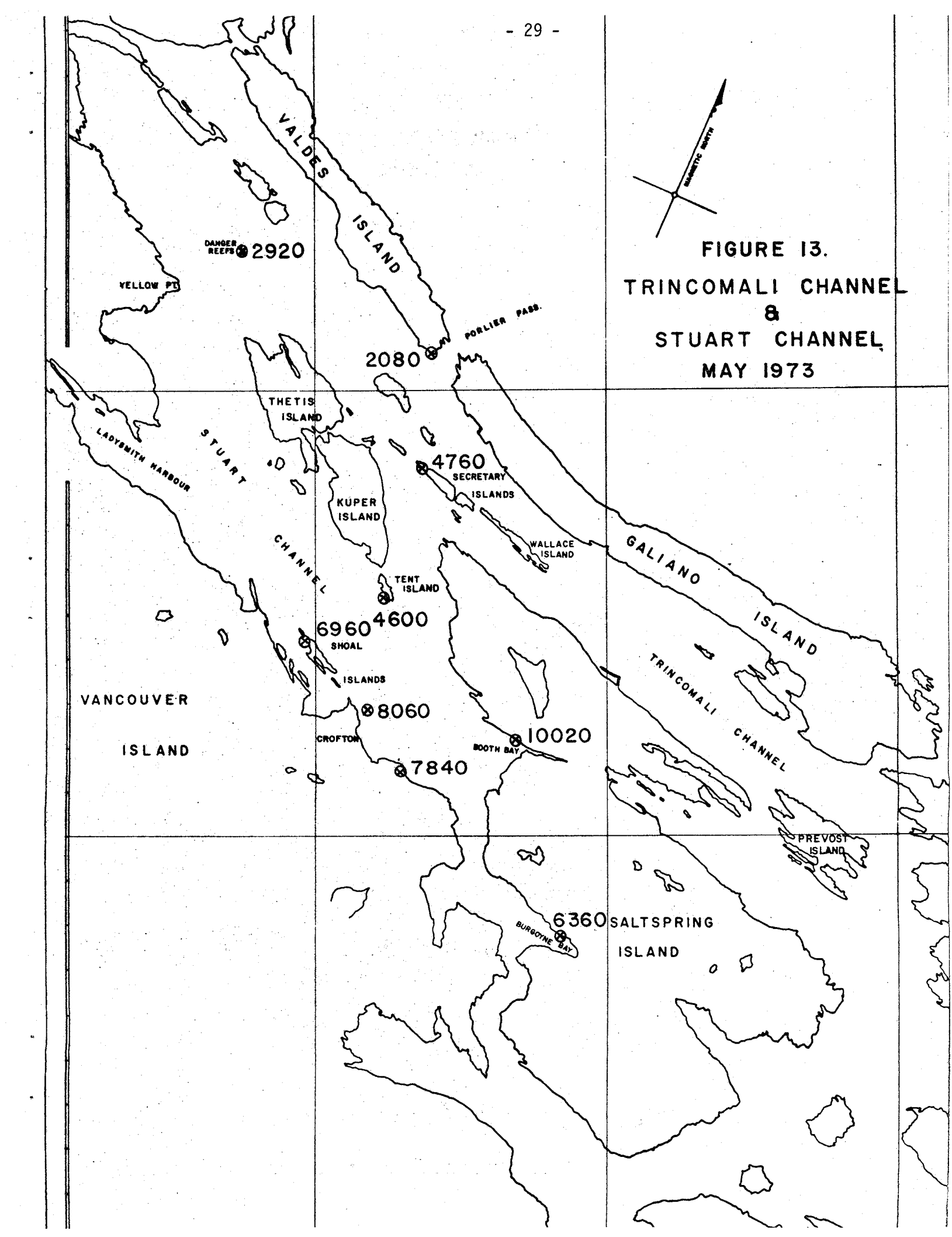

FIGURE 13.
TRINCOMALI CHANNEL
&
STUART CHANNEL
MAY 1973

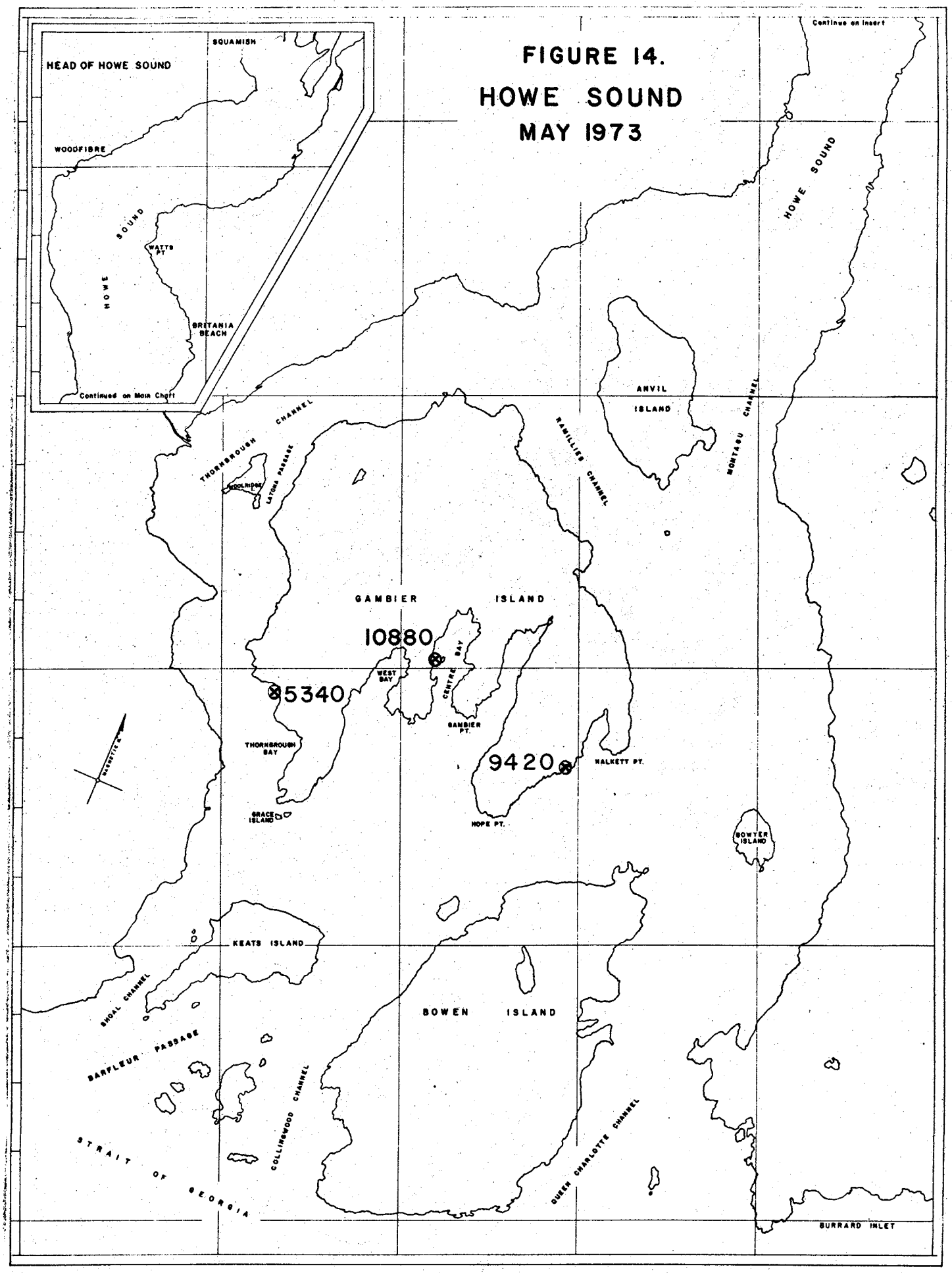
FIGURE 14.
HOWE SOUND
MAY 1973
HEAD OF HOWE SOUND
SQUAMISH
WOODFIBRE
HOWE SOUND
WATTS PT.
BRITANIA BEACH
Continued on Main Chart
Continue on Insert
HOWE SOUND
ANVIL ISLAND
MONTAGU CHANNEL
RAMILLIES CHANNEL
THORNBROUGH CHANNEL
LATONA PASSAGE
GOLDRIDGE I.
GAMBIER ISLAND
10880
WEST BAY
CENTRE BAY
GAMBIER PT.
9420
HALKETT PT.
HOPE PT.
THORNBROUGH BAY
GRACE ISLAND
BOWTER ISLAND
KEATS ISLAND
SHOAL CHANNEL
BOWEN ISLAND
BARFLEUR PASSAGE
COLLINGWOOD CHANNEL
QUEEN CHARLOTTE CHANNEL
STRAIT OF GEORGIA
BURRARD INLET

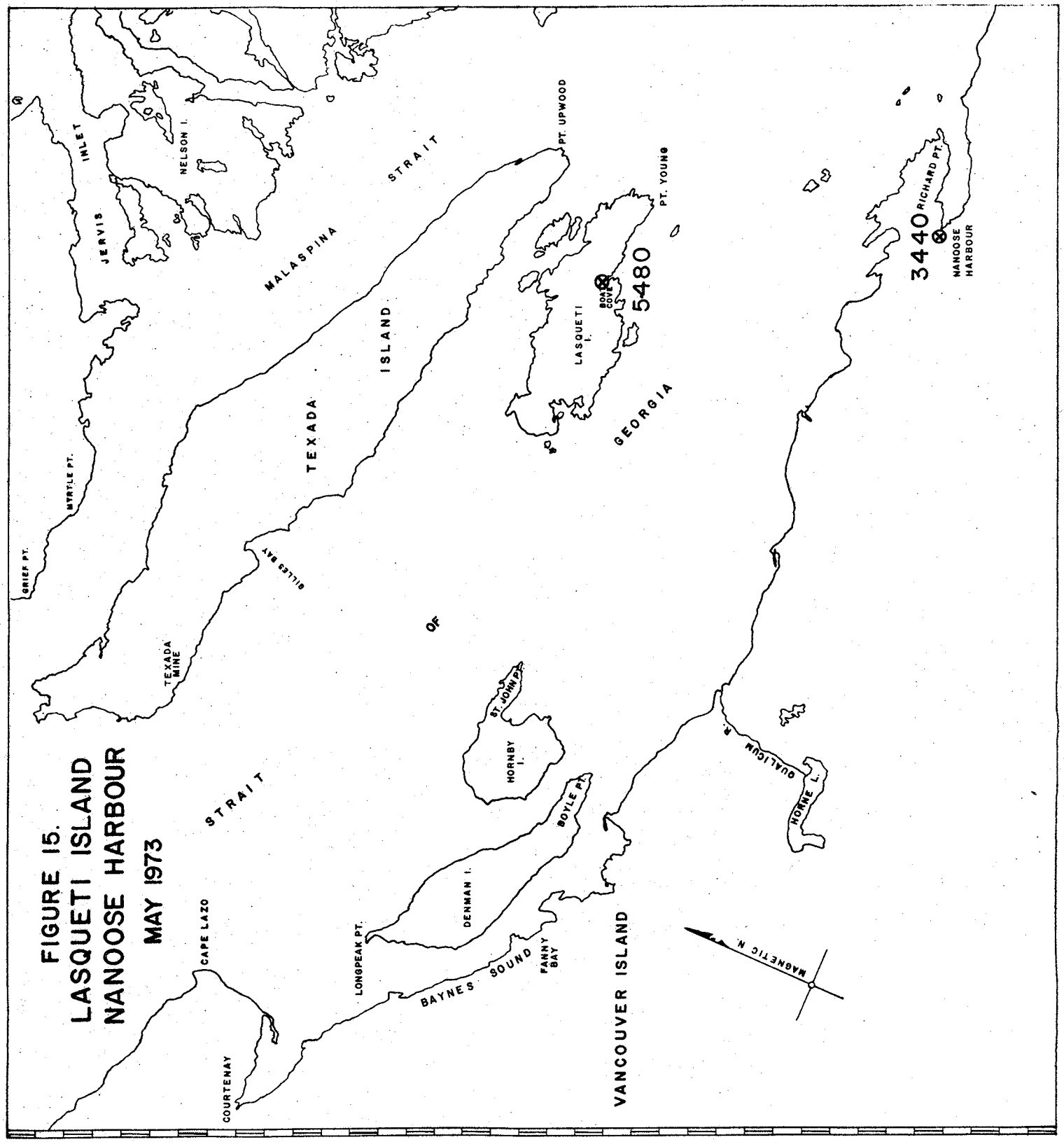

FIGURE 15.
LASQUETI ISLAND
NANOOSE HARBOUR
MAY 1973

DISCUSSION

Owing to manpower and laboratory limitations, only oyster samples from selected stations have been analyzed to date. These were selected to permit some early assessment of the area and level of zinc contamination. The water, sediment and remaining shellfish samples are presently being analyzed. Selected shellfish samples are also being analyzed to determine the mercury levels in shellfish taken from the various sampling areas.

A. Pulp Mill Areas

The preliminary results of zinc analysis conducted thus far of whole oysters from the four pulp mills employing zinc hydrosulphite bleaching confirms reports that zinc accumulation is occurring in shellfish; and in some instances, zinc levels are considerably above natural background levels. The maximum concentration found during the May, 1973, survey was in the order of 19,000 µg/gm dry weight, approximately 16,000 µg/gm above the 4,200 µg/gm considered to be background level for that particular area. As would be expected, the very large zinc concentrations in oyster tissue were confined to the immediate outfall area, a distance of about 1-2 miles. However, elevated zinc levels could be detected for considerable distances from the various newsprint effluent discharges, particularly in the direction of the net tidal movement.

The various zinc concentrations found in whole oysters also appeared to correspond closely to known or anticipated effluent or tidal flow patterns, and could serve as a means of determining the range and shape of effluent fields.

1. Powell River

Analysis of oysters from the Powell River area revealed a much higher level of contamination than the other areas tested. The maximum average zinc concentration found in five samples of 5 oysters was 17,280 µg/gm Zn dry wt. (Table II, Figure 10). These samples were obtained nearest to the mill discharge. Other stations were within the range of 6,680 to 10,000 µg/gm. Figure 10 outlines the average dry weight zinc concentrations found during the May, 1973 survey at Powell River. Patterns of zinc concentrations indicate an effluent field with a net northwesterly flow. Zinc levels were distinctly higher on the northwestern side of the Powell River mill than those to the southeast. Average zinc concentrations found at the northwestern-most station (A-14) located at Lund (approx. 10 miles) was 9,060 µg/gm, compared to 6,680 µg/gm at Myrtle Pt. (A-23) which is approximately 8 miles to the southeast. As expected, the influence

of the mill extends outward to Harwood Is. Oysters from this area showed an average zinc concentration of 10,600 µg/gm (range 9,000 to 11,000 µg/gm). For comparison, analysis of oysters from the western tip of Texada Is. in 1971 showed zinc levels of 5,600 µg/gm.

The general tendency for the mill effluent to exert a greater effect in the northwesterly direction from the mill is also confirmed by other biological and water quality studies conducted by MacMillan and Bloedel Ltd.

Control samples from Okeover Arm, which is located north of Powell River near the entrance to Toba Inlet, demonstrated an average zinc concentration of 4,200 µg/gm (range 3,200 to 4,800 µg/gm).

These oysters were collected far enough from the mill influence to be considered representative of natural background zinc levels for that geographical area.

On the basis of 4,200 µg/gm dry wt., as the average background level, the area of elevated zinc levels extends beyond the outer-most stations which were located at Myrtle Pt. and Lund.

2. Campbell River

Oyster samples collected around the Crown Zellerbach, Elk Falls mill, located at Duncan Bay, indicated that some elevation in zinc levels in whole oyster tissue was occurring. However, as would be expected owing to the extreme tidal flushing in this area, levels of contamination were below the other mill areas. Figure 11 shows the geographical distribution of zinc in oyster tissue in the vicinity of the Elk Falls pulp mill. Unfortunately, the closest point that oyster samples could be obtained was approximately 3 miles southeast of the point of discharge and within the influence of the Campbell River. The average zinc concentration in oyster tissue in this area was 5,360 µg/gm (range 3,200 to 8,000 µg/gm). Clams which were collected closer to the discharge are presently being analyzed for zinc.

Zinc concentrations in the oysters were slightly higher at Francisco Pt than those nearer the mill. This may be the result of eddy currents tending to concentrate water and associated mill effluents in this area. Water displaced southward on a flood tide from Discovery Passage, which contains mill effluent, would flow into both Sutil Channel and Discovery Passage on the ebb tide. This would tend to expose the shellfish at Cape Mudge and Francisco Pt areas more frequently to waters containing mill effluent than those along the Vancouver Island shoreline.

Samples from Rebecca Spit showed average zinc levels of 3,940 µg/gm (range 3,600-4,500 µg/gm). If this value is taken to represent the natural background level for oysters in this area,

Oyster Bay approximately 16 miles south from the mill has been slightly affected by the discharge from the Elk Falls mill, although levels are not considered to be high.

3. Crofton

As expected, results from the analysis of oysters from the Crofton area also showed a significant increase in zinc levels over background. In general, the values were lower than those found in oysters of the Powell River area, and extreme levels did not appear to range as far.

However, the tidal currents and methods of discharge differ between these two mills and undoubtedly influence the affects on the shellfish in the area. Crofton experiences a greater tidal action and diffuses the effluent, while Powell River mill discharges directly into surface waters and into Malaspina Strait, which likely has lesser tidal currents. This would lead to less dilution of effluent and a greater exposure of shellfish to zinc.

However, preliminary results indicate that oysters from the Crofton area do appear to have a lower background zinc level than those from the Powell River area, i.e. 2,080 µg/gm vs. 4,200 µg/gm, but the overall levels of contamination may be greater in some areas near Crofton.

Figure 13 shows the mean dry wt. zinc levels found in whole oysters collected in the Crofton area in May, 1973. The total area affected appears to range from somewhere between Ladysmith Harbour and the Shoal Islands (means 6,960 µg/gm, range 4,600 to 9,000) on the northwestern side of the Crofton mill to somewhere beyond Burgoyne Bay (mean 6,360 µg/gm, range 5,400 - 7,800) to the southeast. The northern extension ranges to the Secretary Islands in Trincomali Channel (means 4,760 µg/gm, range 4,200 to 5,800 µg/gm). The station (D-17) nearest the outfall was located behind the diffusers. Oysters in this area showed mean zinc concentrations of 8,060 µg/gm (range 5,110 to 11,200).

The general distribution of zinc concentrations in the oysters from the Crofton area suggests a net southeasterly flow of effluent through Stuart Channel and a tendency for effluent to concentrate in Booth Bay, which is directly across Stuart Channel from the two effluent diffusers. Booth Bay oysters showed a mean zinc level of 10,020 µg/gm and ranged from 8,700 µg/gm to 11,000 µg/gm.

4. Port Alberni

The only oysters available for the Port Alberni region were located in Barkley Sound some 23 miles from the pulp mill at the head of Alberni Inlet. Zinc levels found in oysters from this area were unexpectedly

high. A mean level of 10,600 µg/gm dry wt. zinc (range 8,200 to 14,000 µg/gm) was found in oysters taken from Rainy Bay, which is situated in Barkley Sound 24 miles from the Alberni pulp mill (Figure 12). It is conceivable that the discharge from the Port Alberni mill is contributing to these high levels. However, there is known mineralization in the area and further analysis is required to confirm any relationship to mill discharge. Clams and mussels collected along Alberni Inlet are presently being analyzed to establish this relationship.

It was noted that the oysters collected in Rainy Bay were in excellent condition with remarkably large shells filled with meat. The numbers were not great and sampling should be restricted.

5. Ocean Falls

Mussel samples along with sediment and water samples have been obtained from the area around the Crown Zellerbach*, Ocean Falls pulp mill. Analysis of these samples has not been completed.

B. Control Areas

In addition to those areas adjacent to the five pulp mills, samples were obtained jointly with Fisheries Operations from Gambier Island (entrance to Howe Sound), Lasqueti Island (Central Strait of Georgia), Boundary Bay (Southern Strait of Georgia), and Nanoose Bay (East coast Vancouver Island). Results for analyses of samples have been obtained for the May, 1973 survey in waters of Gambier Is., Lasqueti Is. and Nanoose Bay (Figure 15).

1. Gambier Is.

Oysters taken from Gambier Is., which is situated at the entrance to Howe Sound, revealed significantly high levels of both zinc and copper in their tissue. The mean zinc values found at three stations along the southern shore of Gambier Is. (Figure 14) were 5,340 µg/gm (range 1,100 to 9,600), 10,880 µg/gm (range 8,200 to 14,000) and 9,420 µg/gm (range 1,100 to 15,000). Copper levels for the three locations ranged from 760 to 1,700 µg/gm, compared to 100 to 400 µg/gm generally found in other areas. These values, particularly the copper levels, suggest that the discharge from Britannia Mine near the head of Howe Sound is affecting the oysters in this area, possibly along with some effects from the Fraser River.

2. Lasqueti Is.

Lasqueti Is. (Figure 8) also shows higher-than-anticipated zinc levels in oysters collected in Boat Cove on the south shore

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* See footnote on page 1.

of the island. The mean levels found were 5,480 µg/gm dry wt. (range 3,600 to 6,600 µg/gm). Heavy metal analyses of the oysters collected at all sample stations were performed on five separate samples taken from each station, each consisting of a group of five oysters homogenized together and digested. At Lasqueti Is., an additional sample was taken, consisting of five individual oysters from five different areas along the middle intertidal zone and analyzed for metals.

Results of analyses with one oyster per sample, as shown in Table VII for station F-2, indicate very little difference from those oysters which were analyzed in groups of five. This suggests that satisfactory results could be obtained by reducing the number of oysters to possibly 2 per sample.

3. Nanoose Bay

Oysters collected from Nanoose Bay, where numerous oyster leases exist, contained a mean zinc content of 3,400 µg/gm, and ranged from 2,400 to 4,400 µg/gm.

ACKNOWLEDGEMENTS

The assistance of Dr. J. Davis of the Pacific Environment Institute, L. Martin of the Fisheries Operations Laboratories, West Vancouver, and Environmental Protection Service technicians D. De Mill, D. Brothers, J. Landucci and D. Sullivan in this program is greatly appreciated.

APPENDIX II

APPENDIX II

PROGRESS REPORT ON THE BORON INVESTIGATION. CHEMISTRY

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SUMMARY

Seventy-one sea water samples from 40 stations in areas near groundwood pulp mills have been analysed for total inorganic boron content. Concentrations obtained colorimetrically ranged from 0.22 µg/ml to 4.68 µg/ml. A linear correlation between salinity (S) and boronity (B) was evaluated as:

$$B = 0.133\ S + 0.016$$

INTRODUCTION

Owing to the announced intentions of certain pulp and paper interests to convert to the use of the Borol process for the production of sodium dithionite in their groundwood mills, it became necessary in the spring of 1973 to launch a study into the possible effects of effluent from the process upon the aquatic ecosystem. The inorganic pollutant chemistry group undertook a program of analysis for boron in water and tissue for its part in the study. The following report describes the chemistry involved and the results of the study to date.

Nature of the Chemistry

1. The Borol Process

The Borol process can be employed on site by the mill to provide sodium dithionite (trivially named sodium hydrosulphite) to be used as the groundwood bleaching agent. Sodium borohydride, sodium hydroxide and sulfur dioxide are reacted together according to equation 1.

$$NaBH_4 + 8NaOH + 8SO_2 \rightarrow 4\ \underset{\text{sodium dithionite}}{Na_2S_2O_4} + \underset{\text{sodium metaborate}}{NaBO_2} + 6H_2O \qquad (1)$$

The intended fate of the sodium metaborate is the sewer and eventually coastal waters.

2. Aqueous Boron Chemistry

There are probably four common boron-containing compounds which find common usage commercially. These are boric acid (H_3BO_3 or $B(OH)_3$), sodium pyroborate (borax, $Na_2B_4O_7$), sodium metaborate ($NaBO_2$ or $Na_2B_2O_4$), and sodium perborate ($NaBO_3$). Borax and the perborate are used widely in cleaning agents, while boric acid has found past popularity in patent medicine. In recent years, the use of aqueous solutions of boric acid as eyewash has

been discouraged for obvious reasons. Sodium borohydride is used widely as a reducing or hydrogenating agent in research and industry.

In spite of the four oxyboron compounds mentioned above and numerous other more complex compounds found in the literature, the aqueous chemistry, from the stand-point of analysis and the nature of the systems under study, is much simpler. The two main species found in environmental waters are boric acid and the borate anion, $B(OH)_4^-$. An equilibrium relationship exists between the two species (Equation 2).

$$B(OH)_3 + 2H_2O \rightleftarrows B(OH)_4^- + H_3O^+ \qquad (2)$$

Boric acid is a weakly dissociated monobasic acid; hence the equilibrium lies to the left of the equation.

The various borate anions undergo a similar transformation in aqueous solution. In the case of the metaborate ion, conversion to $B(OH)_3$ occurs readily in dilute solution (Equation 3)

$$BO_2^- + 2H_2O \rightarrow B(OH)_4^- \qquad (3)$$

Although the description above has been simplified considerably, it does serve to illustrate the fact that although the metaborate ion may find its way into open water, it certainly does not remain as such for any time. As far as the analysis is concerned, we may use standards prepared from boric acid, since this is in fact the species being determined.

3. Analysis

A number of procedures for the analysis of boron in various matrices are to be found in the literature. There are three instrumental techniques which have found wide popularity: colorimetry, atomic absorption and spectrofluorimetry. Atomic absorption is the least desirable of these for environmental analysis, because of poor sensitivity and thus relatively high detection limits. Fluorescence spectrophotometry is the most

sensitive method, but for routine work (unless it is automated), it is probably too time-consuming. The third and most usable method of colorimetry is straight forward, moderately sensitive and inexpensive.

The most widely used colorimetric method involves curcumin as the boron-specific complexing agent. Reaction of a 0.125% solution of curcumin with borate ion in a strongly acidic medium produces a deep red colour, which is due to the protonated complexing agent. Destruction of this protonated form through buffering leaves only the borate complex which absorbs at 550 nm. Sample volumes of the order of 0.25 to 0.5 ml are generally used, inasmuch as the water matrix serves as an interferent, which is removed with strong dehydrating agents, such as sulfuric acid and acetic or propionic anhydride.

Analysis of organic matter presents special problems associated mainly with proper digestion techniques. Several workers have found that severe losses of the analyte can occur if too strong heating is applied. Thus, use of dry ashing is discouraged. A wet ashing technique employing sulfuric acid and 50% hydrogen peroxide appears to be useful. Also, if the apparatus is available, a radio-frequency low-temperature ashing system is most desirable. Once the tissue has been properly digested, the same colorimetric method as used for water analysis can be applied.

Sampling

Water samples from inlets located near groundwood pulp mills were obtained in May, 1973, by Dr. John Davis and his group. Two samples were obtained at each station at depths of 0 and 5 metres. At time of collection, 1 ml of o.1 molar sodium azide was added to each sample (approx. 1 litre) to inhibit metabolic activity.

Analytical Procedure

Water samples were analysed for total inorganic boron (as borate) using the method of Grinstead and Snider (1967) which, in turn, is a modification of that of Hayes and Metcalfe (1962). Duplicate analyses were performed on each sample. Results are shown in Table 1.

Salinities were determined initially by Dr. Davis' assistants using hydrometers. A selected number of samples were re-analysed using an Auto-Lab 601 salinometer.

RESULTS AND DISCUSSION

The boron values reported for the seventy-one sea water samples listed in Table 1 ranged from a low of 0.72 µg/ml for station C_{14} at the surface, to a high of 4.68 µg/ml for station B_{13} at the surface. A plot of boron values (boronity) against salinity (Figure 1) shows a linear relationship. The higher salinity waters all had boron levels around the accepted deep-sea value of 4.6 µg/ml. Boronity-chlorinity ratios varied from 1.80×10^{-4} to 3.09×10^{-4}. Open ocean values have been reported at around 2.4×10^{-4} (Riley and Chester, 1971).

At most of the stations a slight increase of boron content with increased depth was noted. The most striking differences between zero and 5 metres can be seen at stations A_7 (Powell River) and C_{14} (Alberni Harbor) where the halocline was very near the surface.

The analytical method used for the water analysis was found to be good. The method, as described by Grinstead and Snider (1967), had one major fault not reported by them, but one which has been noted by Uppström (1968). We found that addition of the acetic acid-acetate buffer solution to the strongly acidic sample invariably resulted in formation of long, white crystals which conceivably were those of ammonium sulfate. To obtain solutions suitable for use in the spectrophotometer, it was first necessary to filter. This was regarded as a highly undesirable step, although no apparent interference was observed. We have now adopted the more recent procedure of Uppström (1968), in which crystal formation does not occur.

Precision of the Grinstead and Snider method was determined through analysis of eight aliquots from sample A_{10} (5m). Average content was 3.98 µg/ml. Standard deviation was 0.064 µg/ml or 2%.

Tissue Analysis

We have just recently begun analysing oyster and fish tissue as part of the uptake and retention studies being carried out by the Physiology section. One method that has been tried is that worked out by R.E. Drew of the Fish Inspection Laboratory in Vancouver. In principle, the method appears sound as it is an adaptation of the Uppström procedure. However, we have found in practice that the method is unpredictable. Destruction of excess oxidizing agents in the standards is highly critical. The slightest amount of hydrogen peroxide in the presence of sulfuric acid will destroy the complexing agent immediately. In addition, the working curve produced even with successful standard determinations is not linear. We are presently working on a procedure from which a straight line calibration will be obtained.

CONCLUSIONS

We have determined the background boron concentrations in waters from eight areas near which are located groundwood pulp mills. These data will serve as a valid means of comparison for future studies into possible effects of Borol process by-products.

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Table 1: Boron and Salinity Data for Samples from 40 Stations near Groundwood Mills.

Location	Station #	Depth (m)	Boron µgB/ml	Salinity °/oo	Chlorinity °/oo	Boron/Chloride Ratio x10^4
Malaspina Strait & Peninsula	A_1	0	3.82	29.0	16.07	2.38
		5	3.82	29.0	16.07	2.38
	A_2	0	3.85	29.3	16.23	2.37
		5	4.02	29.7	16.45	2.44
	A_3	0	3.52	28.9	16.01	2.20
		5	3.95	29.9	16.57	2.38
	A_4	0	3.72	29.4	16.29	2.28
		5	4.02	29.7	16.45	2.44
	A_5	0	3.45	28.2	15.62	2.20
		5	3.75	29.8	16.51	2.27
	A_6	0	3.66	25.0	13.85	2.64
		5	3.45	29.9	16.57	2.08
	A_7	0	2.40	19.0	10.53	2.27
		5	4.20	30.3	16.79	2.50
	A_8	0	4.15	29.9	16.57	2.50
		5	4.02	29.8	16.51	2.43
	A_9	0	3.98	29.0	16.07	2.48
		5	3.88	30.0	16.62	2.33
	A_{10}	0	3.88	28.8	15.96	2.43
		5	4.00	30.0	16.62	2.40
	A_{11}	0	3.85	28.8	15.96	2.41
		5	4.05	30.3	16.79	2.41
	A_{12}	0	3.80	29.8	16.51	2.30
		5	3.95	29.8	16.51	2.39
	A_{13}	0	3.98	29.8	16.51	2.41
		5	3.82	29.8	16.51	2.31
Algerine Passage	A_{27}	0	3.90	30.3	16.79	2.32
		5	4.10	30.0	16.62	2.46

Table 1: con't

Location	Station #	Depth (m)	Boron µgB/ml	Salinity °/oo	Chlorinity °/oo	Boron/Chloride Ratio x10⁴
Georgia Strait	A_{28}	0	4.00	29.7	16.45	2.43
		5	4.05	30.0	16.62	2.44
	A_{29}	0	4.18	29.9	16.57	2.52
		5	4.00	30.0	16.62	2.41
Quadra Island Sutil Channel	B_1	0	4.20	30.0	16.62	2.53
		5	4.38	30.2	16.73	2.62
	B_2	0	4.18	30.0	16.62	2.51
		5	3.95	30.2	16.73	2.36
	B_3	0	3.98	30.3	16.79	2.37
		5	4.10	30.4	16.84	2.43
	B_4	0	4.05	29.8	16.51	2.45
		5	4.05	30.4	16.84	2.40
Strait of Georgia	B_5	0	4.10	30.6	16.95	2.41
Discovery Passage	B_6	0	4.20	30.8	17.06	2.46
	B_7	0	4.15	30.6	16.95	2.45
	B_8	0	4.20	31.0	17.17	2.45
	B_9	0	4.25	30.6	16.95	2.51
	B_{10}	0	4.25	30.8	17.06	2.49
	B_{11}	0	4.30	30.4	16.84	2.55
	B_{12}	0	4.22	30.8	17.06	2.47
	B_{13}	0	4.68	30.0	16.62	2.82
Sechart Channel Barkley Sound	C_1	0	4.05	31.6	17.51	2.31
		5	4.40	31.9	17.67	2.49
	C_2	0	3.90	28.5	15.79	2.47
		5	3.95	31.1	17.23	2.29
	C_3	0	3.42	25.1	13.91	2.46
		5	3.78	30.7	17.01	2.22
	C_4	0	4.25	24.8	13.74	3.09
		5	4.20	31.1	17.23	2.44

Table 1: con't

Location	Station #	Depth (m)	Boron µgB/ml	Salinity °/oo	Chlorinity °/oo	Boron/Chloride Ratio x10^4
Alberni Inlet	C_5	0	3.05	23.0	12.74	2.39
		5	4.40	29.3	16.23	2.71
	C_6	0	2.50	17.3	9.58	2.61
		5	4.05	29.3	16.23	2.50
	C_7	0	2.00	16.3	9.03	2.21
		5	3.50	24.6	13.63	2.57
	C_8	0	1.40	12.0	6.65	2.11
		5	3.30	23.3	12.91	2.56
Alberni Harbor	C_9	0	0.90	6.6	3.66	2.46
		5	2.90	21.2	11.75	2.47
	C_{10} - C_{12}	Shore Stations				
	C_{13}	0	0.72	6.6	3.66	1.97
		5	1.75	12.6	6.98	2.51
	C_{14}	0	0.22	2.2	1.22	1.80
		5	3.85	27.3	15.1	2.55

Mean boron concentrations for: surface samples ($\bar{S}$ =26.1°/oo) 3.53

5m samples ($\bar{S}$ =28.8°/oo) 3.86

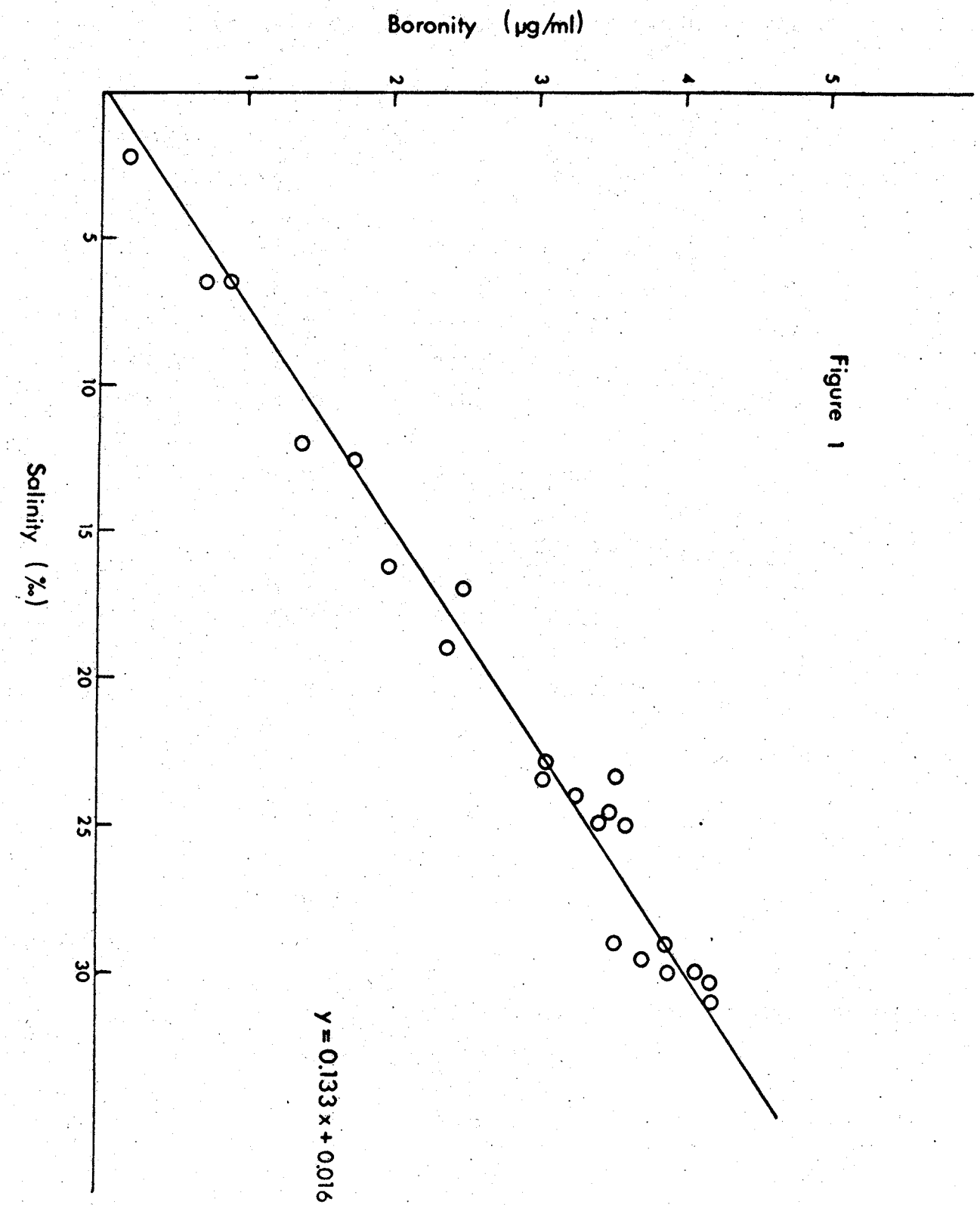

Figure 1

APPENDIX III

APPENDIX III

TOXICITY OF BORON TO PACIFIC SALMON, AND BORON UPTAKE EXPERIMENTS WITH PACIFIC SALMON AND OYSTERS

Progress Report

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SUMMARY

Boron studies with Pacific salmon have been initiated to encompass both lethal and sublethal effects and assess the potential hazard of this substance to aquatic life. Static bioassays with coho salmon fry in fresh soft water indicated that boron (from sodium metaborate addition) was toxic to fish over a range of 82 to 656 ppm B and that the mode of toxic action was slow. Bioassays were carried out for up to 23 days to assure cessation of acute toxicity phenomena in test tanks. For this reason, standard 96-hour toxicity tests are not applicable for this toxicant. The 23-day LC50 for coho fry at 11±0.5°C was 93 ppm B. Sockeye fry in sea water appeared somewhat more sensitive to B, although the 23-day LC50 has not yet been determined for this species.

Bioaccumulation studies with sockeye underyearlings and young oysters have been initiated and are not yet completed. Groups of animals are exposed to 1 and 10 ppm B (above background), and are being analysed for accumulation and clearance of B at weekly intervals. If boron proves to be bioaccumulating in fish, a series of sublethal studies to assess the effect of this buildup are planned.

A field sampling program has been set up and extensive collection of water samples and tissue samples of oysters, clams and mussels have been made in areas near groundwood pulp mills. These collections should establish boron levels in water and intertidal organisms before and after mills convert to the Borol process and provide samples for zinc analysis.

5 November 1973.

RATIONALE AND APPROACH

As the Borol process appeared with no prior warning as a potential hazard to marine systems, a crisis-type approach has been adopted in assessing the potential problem. Essentially, we decided that research into zinc bioaccumulation could wait, as the zinc problem has been with us for some time and indeed the bioaccumulative nature of zinc and other heavy metals in molluscs has been reported extensively in the literature. In addition, it was felt that the Environmental Protection Service has an extensive field program for sampling zinc levels in oysters, clams and mussels near coastal groundwood mills and a further investigation in this area would be duplicating effort. Thus, we have so far confined our activities to the following:

1) determination of acute toxicity of sodium metaborate (expressed as conc. of B) to Pacific salmon in both fresh and salt water.
2) rough estimation of presence or absence of additive (synergistic) effects of boron plus kraft mill effluent on salmon.
3) bioaccumulation studies with salmon and young oysters and boron.

It was felt that such a program would give a fairly quick answer to whether or not conversion to the Borol process would constitute a hazard to marine life.

METHODS AND RESULTS

(1) Bioassays

Bioassays using coho and sockeye salmon and boron are summarized in the accompanying figures (1, 2 & 3). Coho alevins and fry were tested in fresh soft water at 11 $\pm 0.5^{o}C$ at fish

loading densities of 0.2 - 0.7 g/l solution. Bioassays were of the static type with daily solution replacement. They were carried out using 5 to 20 fish/tank in all-glass, aerated aquaria immersed in a cooling bath. Times to death of individual fish were recorded and the time required to kill 50% of the fish at each concentration was used to determine LC50's(Fig. 1, 2). For one test, toxicity of boron to coho fry in fresh water and toxicity of a 50% by volume kraft mill effluent solution were determined separately. It was then possible to combine the two toxicants and test for synergistic effects as outlined by Sprague (1969) (Fig. 3).

Bioassays indicated that relatively large concentrations of B are toxic to salmon in a static bioassay. The LC50 for coho alevins and fry in fresh water was 93 ppm B. For sockeye fry in sea water it was lower, but has not yet been precisely determined. Further bioassays are planned using the same sockeye in fresh water to see if the apparent greater sensitivity of sockeye compared with coho (to boron) is a species difference or is related to whether the bioassay is done in fresh or salt water. In all bioassays, pH was kept at approximately 7 by addition of NaOH or H_2SO_4 to avoid pH effects. It should be noted that boron appears to kill fish slowly, and bioassays longer than the conventional 4-day type are required. There is some evidence that kraft mill effluent and high concentrations of boron are additive in toxic effect (Fig. 3).

Subsequent analysis of tissues from some of the coho that died in the higher B concentrations during the bioassay were carried out. Analyses, done on 1 g of wet tissue from the homogenate of several fish at a given concentration, were as follows:

B conc. in water (ppm)	B conc. in tissue (ppm)	Medial Survival Time of Group (hrs.)
656	354	33.4
656*	391	33.4
328	224	84.0

(* determination repeated)

These results indicate a substantial buildup of boron in the tissues during the bioassay (sockeye fry, unexposed to boron, had only 0.06 ppm B in their tissues). Furthermore, the amount of uptake appeared related to test concentration and length of survival time. Thus, boron uptake may constitute one of the modes of toxic action rather than some external influence on the gills, osmoregulatory capability or other function.

(2) Bioaccumulation Studies

Five 80-litre fibrelgas tanks, operating on a continuous-flow basis, were used for a boron bioaccumulation study. Each tank initially contained 25 sockeye fry adapted to sea water and 30 young oysters of uniform size. Dosage was accomplished by continuous flow of sea water from a constant-head device and stock solutions of sodium metaborate from constant-head Mariotte bottles. One control tank (no boron dosage) and 2 replicates of 1 ppm boron above background and 2 replicates of 10 ppm boron above background were used. Water and tissue sampling was conducted at periodic intervals after boron exposure began. Five fish and five oysters were sampled each time. Dosage was stopped after 47 days and the experiment was continued to study possible recovery of tissue B levels to pre-exposure levels.

Results, to date, of the bioaccumulation study are shown in Figure 4 for oysters only. At the start of the experiment, oysters had approximately 3.8 ppm in their tissues. After 8 days exposure there was little change, but after 32 and 47 days exposure, B levels had increased substantially in oysters exposed to 10 ppm B. Those exposed to 1 ppm B above background showed levels roughly 1 ppm above that of controls after 32 and 47 days exposure (levels reported for the 32-day exposure may be slightly high due to sample desiccation during chemical processing). The experiment is continuing to see if tissue boron concentrations in exposed oysters will decline now that B dosage has stopped.

Sockeye fry exposed to identical conditions as the oysters gave the following results after eight days of the bioaccumulation study:

Control fish had 0.06 ppm B in their tissues, while fish exposed to 1 ppm B above background had 0.12 ppm B - a 100% increase. Sockeye exposed to 10 ppm B above background had 0.19 ppm B - an increase of over 300%. Studies of the uptake of boron by fish are continuing.

The above results indicate that Pacific oysters do not bioaccumulate boron in concentrations higher than that in the water. In fact, it appears as if the oyster tissue boron concentrations may closely parallel that in the water. Boron levels in fish tissue are very low and some tendency for elevated tissue boron concentrations is evident when boron rises above background levels. It must be emphasized, however, that fish tissue boron levels in the bioaccumulation study were much lower than those in the test solution after 8 days. In addition, tissues analysed from fish which died in the bioassay showed substantial levels of tissue B; however, these levels were below that present in the water.

At this time, it appears that there is no tendency for boron to bioaccumulate in fish or oyster tissues. As B levels discharged by groundwood pulp mills on the Pacific coast are expected to be low (less than 1 ppm at the Powell River Pulp Mill), there would appear to be no bioaccumulation hazard to salmon and oysters. This is reinforced by the fact that we tested B concentrations of 10 ppm in the bioaccumulation study - a level that would be many times that occurring in the sea when dilution of a 1 ppm discharge is considered.

(3) Back-up Field Work

An extensive field sampling program was organized using facilities aboard C.F.A.V. LAYMORE over the two-week period July 23 - August 5, 1973. This was a cooperative venture between Pacific Environment Institute Tolerance Biology Staff, under Dr. J. Davis, and Environmental Protection Service personnel, under Mr. D. Goyette. The cruise involved extensive sampling of water, sediments and tissues (oysters, clams, mussels) near four local pulp mills employing groundwood pulping. Those mills were located at Powell River,

Campbell River, Port Alberni and Crofton. In addition, samples were taken at a few sites removed from pulp mills as "control" stations. E.P.S. personnel took the rest for boron analysis. It was felt that existing zinc and boron levels would then be known prior to the mills accomplishing a switch-over from use of zinc hydrosulphite to the Borol process for sodium hydrosulphite.

The Station locations (see charts in Appendix I) were as follows:

(a) Powell River
- 11 shore stations for oysters, clams, mussels along the shore on either side of the mill, extending over a total distance of 16 sea miles (A14 - A 23)
- 13 water sample stations approximately 0.5 to 1.0 sea miles offshore, roughly paralleling the shore stations (A1 - A13)
- Shore station on Harwood Is. adjacent to the mill (A25)
- 3 water samples in mid- Strait of Georgia in a line W.S.W. of mill (A27- 29).

(b) Campbell River
- 4 shore stations on the east side of Quadra Island between Rebecca Spit and Cape Mudge (B14 - B17)
- 4 water stations off the above shore stations (B1 - B4)
- shore and water station at Oyster Bay (B5 + B18)
- water stations proceeding N. through Discovery Passage to Pulp Mill (B6 - B 13) and N. to Seymour Narrows.

(c) Cape Scott (control)
- shore station

(d) Barkley Sound
- 4 water and sediment stations, Sechart Channel (C1 - C4)
- shore station in Rainy Bay (C15)

(e) Alberni

- 7 water stations proceeding down Alberni Inlet to the pulp mill (C5 - C9, C13, C14).
- 2 shore stations in Alberni Inlet, at Sproat Narrows and Franklin River (C16, C17).

(f) Crofton/Gulf Islands

- shore stations, Saltspring Island - Burgoyne Bay (D9), Booth Bay (D-10) - water stations offshore (D1, D2).
- shore stations north and south of the pulp mill - D15, D17 Shoal Island, D16 Sherard Pt.
- water stations off mill outfall (D-3) and off Ladysmith Harbour (D-4).
- shore stations at Tent Island (D-11), Mowgli Island (D-12), Valdez Island (D-13), Danger Reefs (D-14).
- water stations, Houstoun Passage (D-7), Trincomali Channel (D-6) (D-5), mid - Strait of Georgia, west of mouth of Fraser River (D-8).

The above water and tissue samples have been turned over to Dr. J. Thompson, P.E.I. Chemistry Section, for analysis. The positions of these stations are shown in the zinc-sampling station report prepared by E.P.S. for this progress report (Appendix I).

PROPOSED ACTIVITIES

On-going research is currently being done with the bio-accumulation study as previously described. The results of this study will largely determine the course of future research. If boron is shown to bioaccumulate in fish at the concentrations near those to be discharged by local mills, a program of sublethal testing will be designed. This will include respiratory and circulatory responses, blood analysis, fish performance indices and avoidance behavior tests in salmon of different sizes in sea water. If no bioaccumulation is demonstrated, sublethal work of this type would likely not be fruitful as expected boron concentrations will likely be .01 to .001 of the lethal level (to coho in fresh water) and it is unlikely that such dilutions would provoke measurable sublethal responses.

Further bioassays will be done with sockeye in salt water. Progress is necessarily slow with these tests owing to the long duration of the test, the fact that several weeks of seawater acclimation prior to testing is necessary, and the large size of test fish. As time and resources permit, bioassays will also be conducted with boron and larval oysters to assess sensitivity of larval forms.

ACKNOWLEDGEMENT

We wish to acknowledge the assistance of Mr. Ralph E. Drew of the Fish Inspection Laboratory, who carried out many of the boron analyses in oyster and fish tissues during the course of this work.

REFERENCES

Davis, J.C. and B.J. Mason. 1973. Bioassay procedures to evaluate acute toxicity of neutralized bleached kraft mill effluent to Pacific salmon. J. Fish. Res. Board Can., 30: 1565-1573.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research, 3: 793-821.

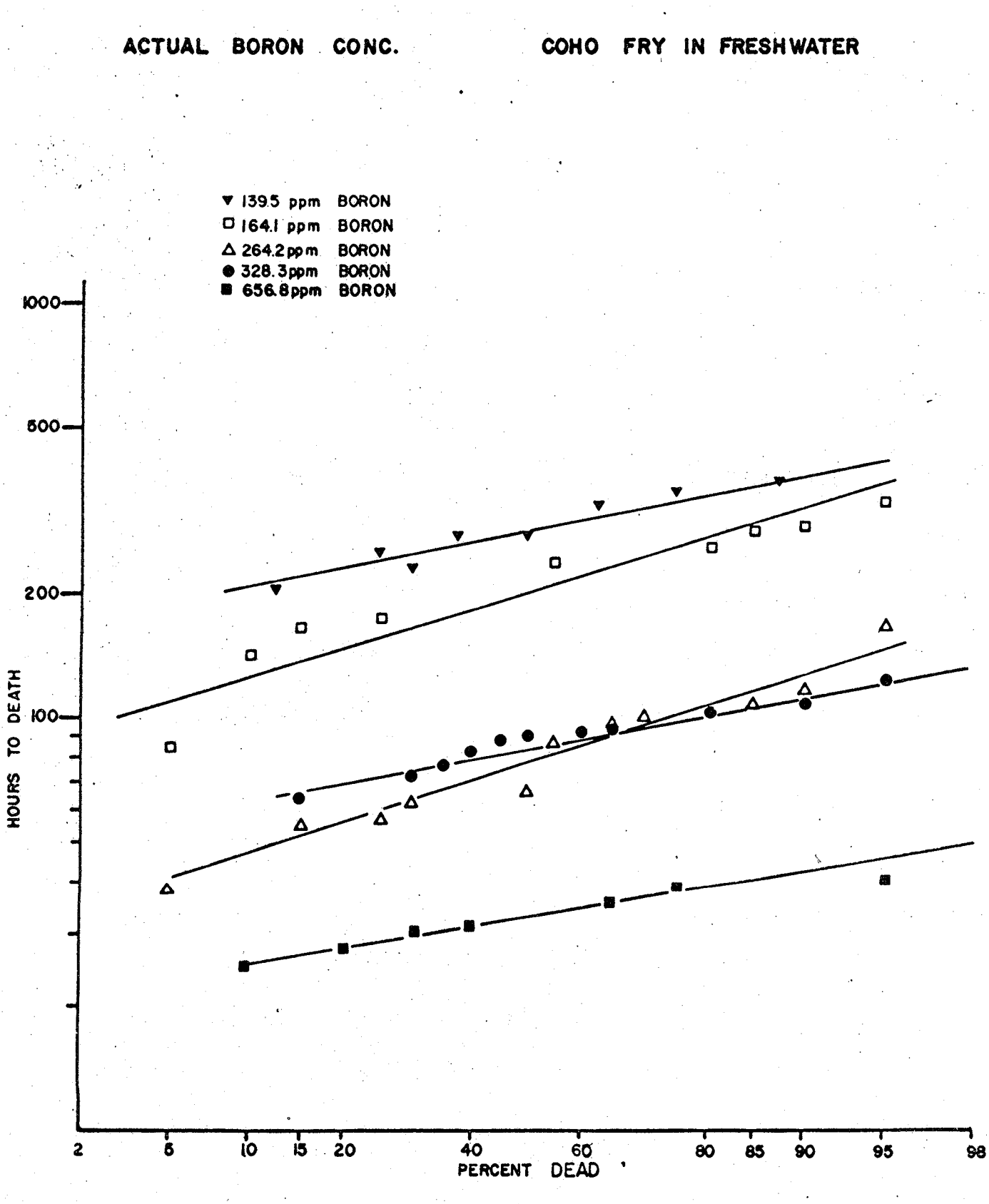

Figure 1. Mortality data from coho fry in freshwater boron solutions at 11°C. Tanks were dosed with sodium metaborate and concentrations are given as B present.

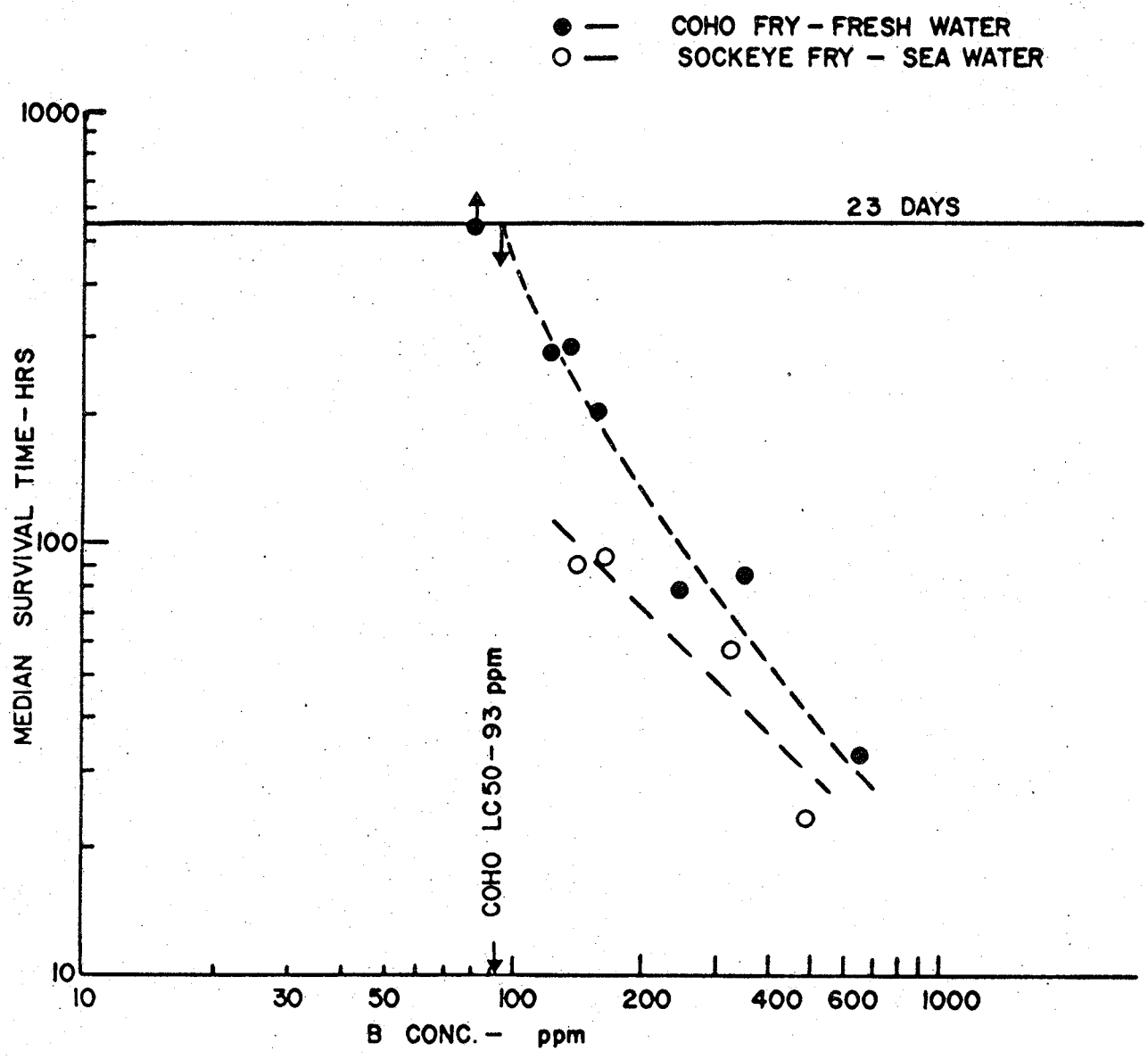

Figure 2. Calculated median survival times in boron for coho fry, using data from Figure 1 (solid circles). Data for sockeye fry at similar test concentrations and fish density are included (open circles). Median survival times for groups of test fish at each concentration are given as calculated geometric mean survival time (GMST - Davis & Mason, 1973). These values are very close to graphically estimated median survival times obtainable from plots such as Figure 1.

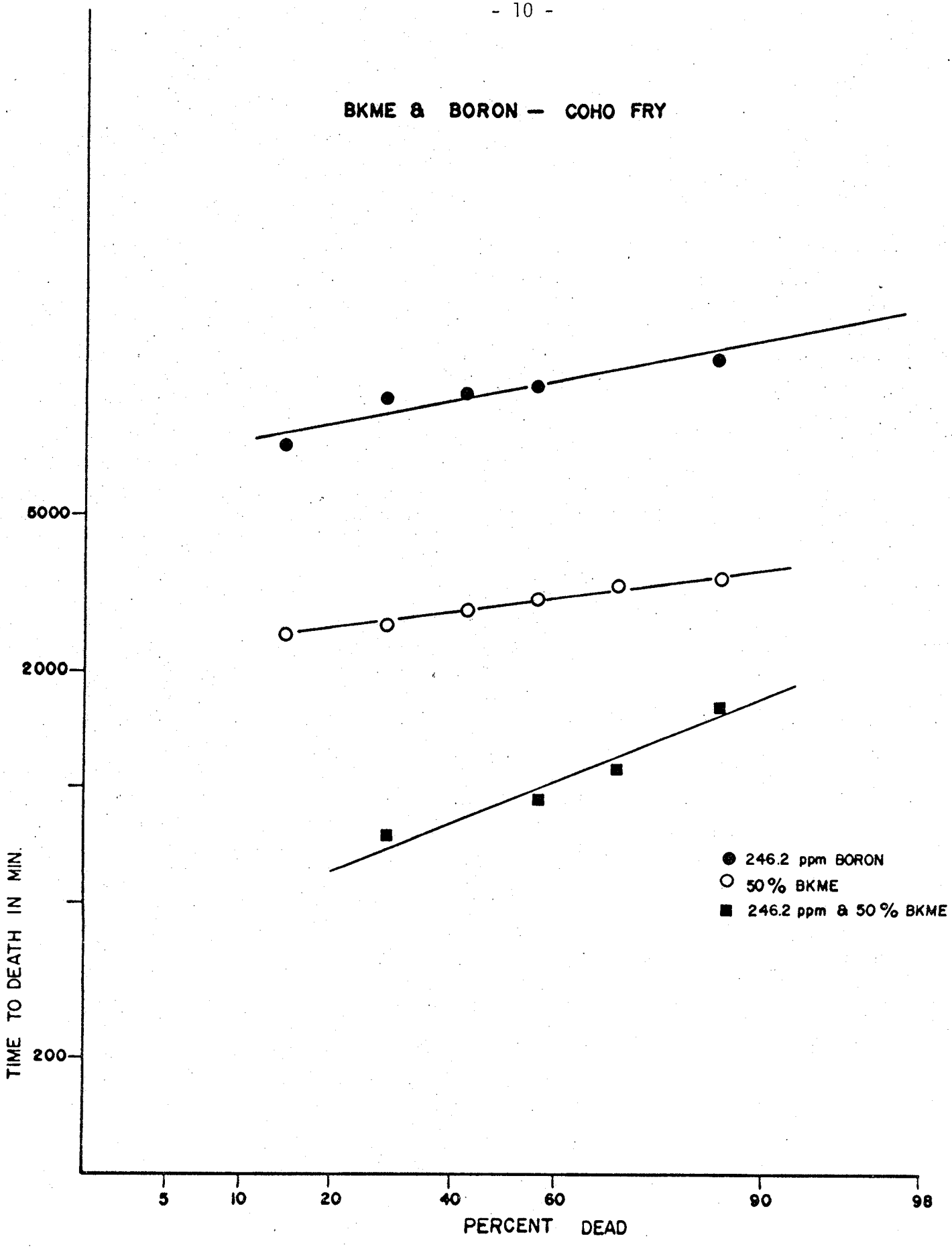

Figure 3. Median survival times of groups of coho fry tested in a toxic boron solution, a toxic BKME solution and a combination of the two solutions. Data are for tests conducted in fresh soft water at 11± 0.5°C. Mortality is expressed as a cumulative percentage dead.

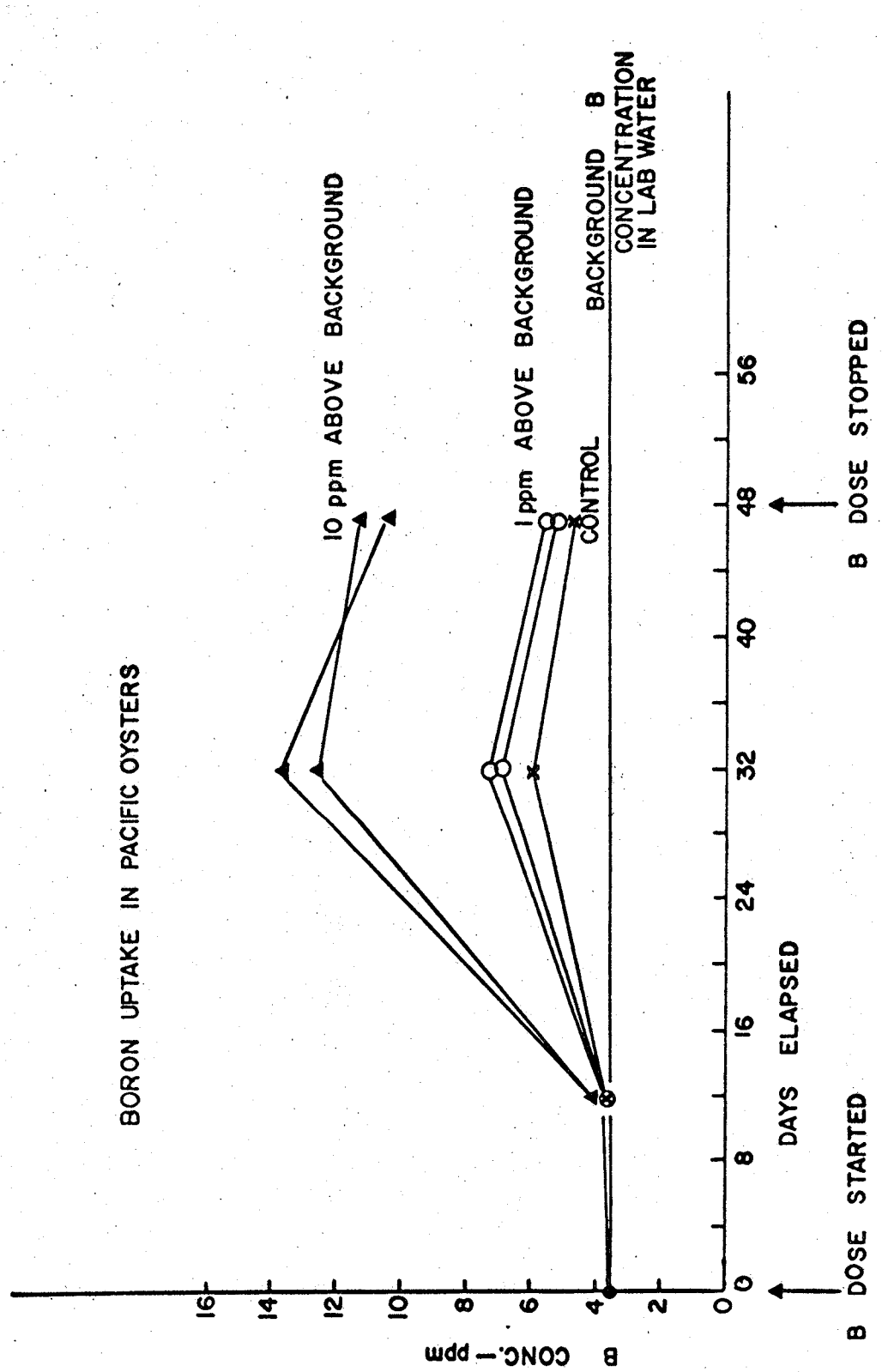

Figure 4. Results of boron uptake experiment with Pacific oysters, *Crassostrea gigas*.

APPENDIX IV

APPENDIX IV

TOXICITY OF BORON, GROUNDWOOD EFFLUENT AND KRAFT MILL EFFLUENT (KME) TO NATURAL MARINE PHYTOPLANKTON POPULATIONS

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INTRODUCTION

Rationale

Phytoplankton and attached algae are very important components of the aquatic food chain. They form the base of the food chain and, as such, support all higher trophic levels. Any substance affecting their growth will, in turn, affect the entire food chain. Phytoplankton, the prime food of zooplankton and all filter feeding invertebrates, are particularly susceptible to toxic substances as they lack suitable locomotion to avoid contact with toxicants, and their spatial distribution is accordingly strongly affected by tidal currents.

To date, there is a paucity of information regarding sublethal effects of toxicants on this primary trophic level. The work that has been done has shown conclusively that phytoplankton populations in nature or in pure culture, are sensitive indicators of effects of deleterious substances in the aquatic environment. Two bioassay procedures were developed: (a) the nutrient- or toxic substance-addition bioassay; and (b) the pure culture bioassay. In the former, nutrients and/or toxic substances are added to phytoplankton populations and incubated in situ with ^{14}C to monitor relative or absolute growth rates. In the latter, pure cultures of algae, growing in defined media, are inoculated with known concentrations of toxic substances and their growth rate is monitored over a four-day period at constant temperature and light.

Data obtained from both bioassays when married provide excellent results of the response of phytoplankton to toxic substances, and, if serial dilution encompasses a wide enough range, normally provide the investigator with a range of critical lethal concentrations. The current study has involved both bioassay procedures; however, results of in situ bioassays are the only results reported.

METHODS

Seawater samples were obtained at a depth of 1.5 meters with a 6-liter horizontal Van Dorn directly off the Pacific Environment

Institute dock. Subsamples were taken for phytoplankton counts, boron determinations, pH and alkalinity. Remaining sea water was stirred and added to 130-ml incubation flasks. There were 13 sets per experiment, each set including 2 light bottles and one dark bottle. Sets 1, 2 or 13 were control flasks, and the remaining sets were incubated with various concentrations of boron or groundwood effluent. Concentrations of boron tested during the first nine in situ bioassays appear in Table I. After boron additions, approximately 5μ Cu of ^{14}C were added, flasks stoppered, shaken, and incubated at 1.5 m for a 5-hour period off the P.E.I. dock. In this way, uniform temperature and light conditions were maintained in all sets.

Three boron standards were made from analytical grade sodium metaborate: 10 μg/ml, 1 mg and 10 mg/ml.

pH control was monitored on a digital pH meter with an accuracy of ±0.01. Light was measured on a recording solorimeter recording total incident solar radiation in g cal cm^{-2} min^{-1}. Alkalinity was determined using methods described by Strickland and Parsons (1968).

After the 5-hour incubation, separate samples were filtered on a 0.45μ millipore filter. Filters were placed in scintillation vials with fluor and counted in a Packard Tri-carb scintillation counter. Results so obtained can be expressed on a relative basis as total counts per min (TCPM), or in absolute terms of production (g C m^{-2} day^{-1}). To expedite presentation at this juncture, only TCPM are reported.

Fresh refiner groundwood effluent provided by the Western Forest Products Laboratory, Department of Environment, Vancouver, was used for testing the effects of this effluent on phytoplankton. The pH of the groundwood effluent was not neutralized prior to inoculation. Alkaline effluent from the Port Alberni kraft mill was obtained fresh, and inoculated at pH 10.0. To discern pH effect, some of the effluent was neutralized to pH 7.0 prior to inoculation.

RESULTS

Work commenced July 4, 1973, and has included to the time of writing, 15 in situ bioassays, six of which have dealt with groundwood

effluent or KME additions plus boron, and 9 dealing exclusively with boron additions. Results from all 15 bioassays will not be discussed in detail as many were repetitious, designed to determine the statistical variability of the experimental design. Three characteristic type of results will be discussed in the following pages.

Production

Production results depend largely on algal species composition, which undergoes a yearly seasonal succession, and on standing crop or biomass at time of sampling. Thus, phytoplankton assemblages dealt with in July may differ markedly from those tested in September. Therefore, comparison of absolute rates among experiments is subject to certain limitations, none of which however, invalidate conclusions drawn from comparisons among flasks in any given experiment.

Though absolute rates of production in g C m^{-2} day^{-1} have not been calculated, TCPM bears a direct relationship to these rates, and a valid comparison of TCPM among experiments can be made. For example, TCPM on July 9 in the control flask was as high as 34,000, which corresponds to a production of about 300 mg C m^{-3} day^{-1}. On a similar bright day on August 8, TCPM in the control flask was only 2,800. This tenfold difference is attributable primarily to the standing crop variation and secondarily to the physiological state of the algae. However, since the results presented are used for flask comparison on a given day, they are meaningful and valid, despite seasonal variations.

Boron Additions (Figure 1)

In all 9 experiments, there was an inhibition of growth at the 1 and 10 microgram levels, followed by a slight increase at the 100 and 500 microgram level. Additions of boron of 1 mg/l in most cases did not seriously affect production; however, at concentrations greater than 1 mg, growth decreased rapidly, with negligible growth at concentrations of 50 and 100 mg boron, respectively. It appears that 1 mg/liter above background* is a critical level and at higher

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* Mean boron concentrations off P.E.I. dock at time of experiments - 3.1 mg/liter

concentrations growth is inhibited. In most experiments, production in the control flasks exceeded growth noted in flasks receiving boron additions. This would suggest that even small concentrations of boron required some physiological adaptation or "algal acclimatization". It should be noted that the boron source for these experiments, sodium metaborate, may carry too heavy a sodium load for algae. Further experiments using boric acid as a boron source are planned.

Groundwood plus Boron Additions (Figure 2)

Additions of groundwood effluent were arranged serially from 1 to 20 milliliters. The results show a marked inhibition at the 1 ml concentration, slight inhibition at the 5 ml concentration, and tailing off to almost complete cessation of growth at higher concentrations. Additions of 1 mg/l boron at the same concentrations of groundwood effluent showed no significant difference from groundwood alone. The 1 mg/l addition of boron without groundwood effluent showed growth equal to the control, which is indicative of a toxic or strong pH effect of groundwood effluent over-riding any negative boron effects. In fact, in similar experiments (not reported here), it appears that groundwood effluent with or without boron is extremely deleterious to phytoplankton.

KME Additions (Figure 2)

Though not totally within the scope of boron work, it is informative to present the results of KME additions to natural phytoplankton populations. Additions of 5, 10 and 15 ml of alkaline effluent from the Port Alberni mill had a severe inhibitory effect on phytoplankton production. Even neutralizing the effluent to pH 7.0 did not reduce the inhibitory effects. After passing the effluent over XAD resins to remove "color and toxicants", slightly more growth was noted. However, even the smallest additions gave production only half that in the control flask. Neutralization of this XAD-resin pass to pH 7.0 had a severe inhibitory effect, negating a pH effect. Dehydroabietic acid, which is extremely toxic to salmonids, was also shown to be inhibitory to phytoplankton production.

pH Effects (Figure 3)

It was noted that additions of sodium metaborate may increase the pH in flasks, thereby affecting the physiological response of natural phytoplankton populations. To attempt to separate the effect of pH from that of the toxicity of the substance tested, pH was carefully monitored in two experiments. It can be seen that pH increased from 7.9 for sea water to about 8.1 with a 1 mg/l boron addition. Each additional milligram of sodium metaborate added resulted in an incremental change of approximately 0.16 pH units.

A change of 0.1 to 0.2 pH units should not affect the metabolism of algae. A pH change from 0.5 to 1.0 pH units could be considered as a serious shock response, and as such, inhibition of metabolism could be suggested. In this case, it appears that pH, though changing slightly, could not account solely for the results obtained. Further scrutiny of the effects of pH versus toxicants will be a part of future studies. It must be borne in mind, however, that the experimental design called for simulation of algal response to natural effluent additions to sea water. This does not mean pH neutralization to minimize pH shock to algae, for pH may in the long run be a serious by-product of effluent discharges to sea water.

CONCLUSIONS

1. Phytoplankton production is inhibited at concentrations of boron greater than 1 mg/l above background. At concentrations greater than 10 mg/l boron, growth is negligible.

2. Groundwood effluent is toxic to phytoplankton at all concentrations tested, judging by inhibition of photosynthesis, but this occurs most significantly at the higher concentrations. Because these were only preliminary experiments, with a minimal number of tests, further work is required to fully substantiate these early findings. Groundwood plus 1 mg/liter boron did not increase the negative effect of groundwood effluent, suggesting that boron will be less of a problem than the effluent itself.

3. KME with or without color removal is toxic at all concentrations to phytoplankton. Neutralization of the alkaline effluent to pH 7.0 had little positive effect. Preliminary evidence indicates that stain removal is not enough in terms of minimizing KME effect on phytoplankton populations.

4. pH changes appear to be insignificant in terms of effects of boron additions on phytoplankton populations. pH of groundwood and KME, however, seems severe enough to warrant considerable study as to the effect of effluent pH on phytoplankton production.

REFERENCES

Strickland, J.D.H., and T.R. Parsons. 1968. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Board Can., No. 167, 311 p.

Table 1. Boron concentrations added to flasks 1 through 11.

Flask No.	Standards Used	Amount Added	Final Concentration as B liter^{-1}
1	-	-	Control
2	-	-	Control
3	10 µg	0.13 ml	1 µg
4	10 µg	1.3 ml	10 µg
5	1 mg	0.013 ml	100 µg
6	1 mg	0.065 ml	500 µg
7	1 mg	0.13 ml	1 mg
8	1 mg	0.65 ml	5 mg
9	1 mg	1.3 ml	10 mg
10	10 mg	0.65 ml	50 mg
11	10 mg	1.3 ml	100 mg

FIGURE 1

Response of natural phytoplankton populations to boron additions. Boron concentrations in Flasks 1 to 9 are as follows:

Flask Number	Boron Concentration in Flasks
1	1 µg/l
2	10 µg/l
3	100 µg/l
4	500 µg/l
5	1 mg/l
6	5 mg/l
7	10 mg/l
8	50 mg/l
9	100 mg/l

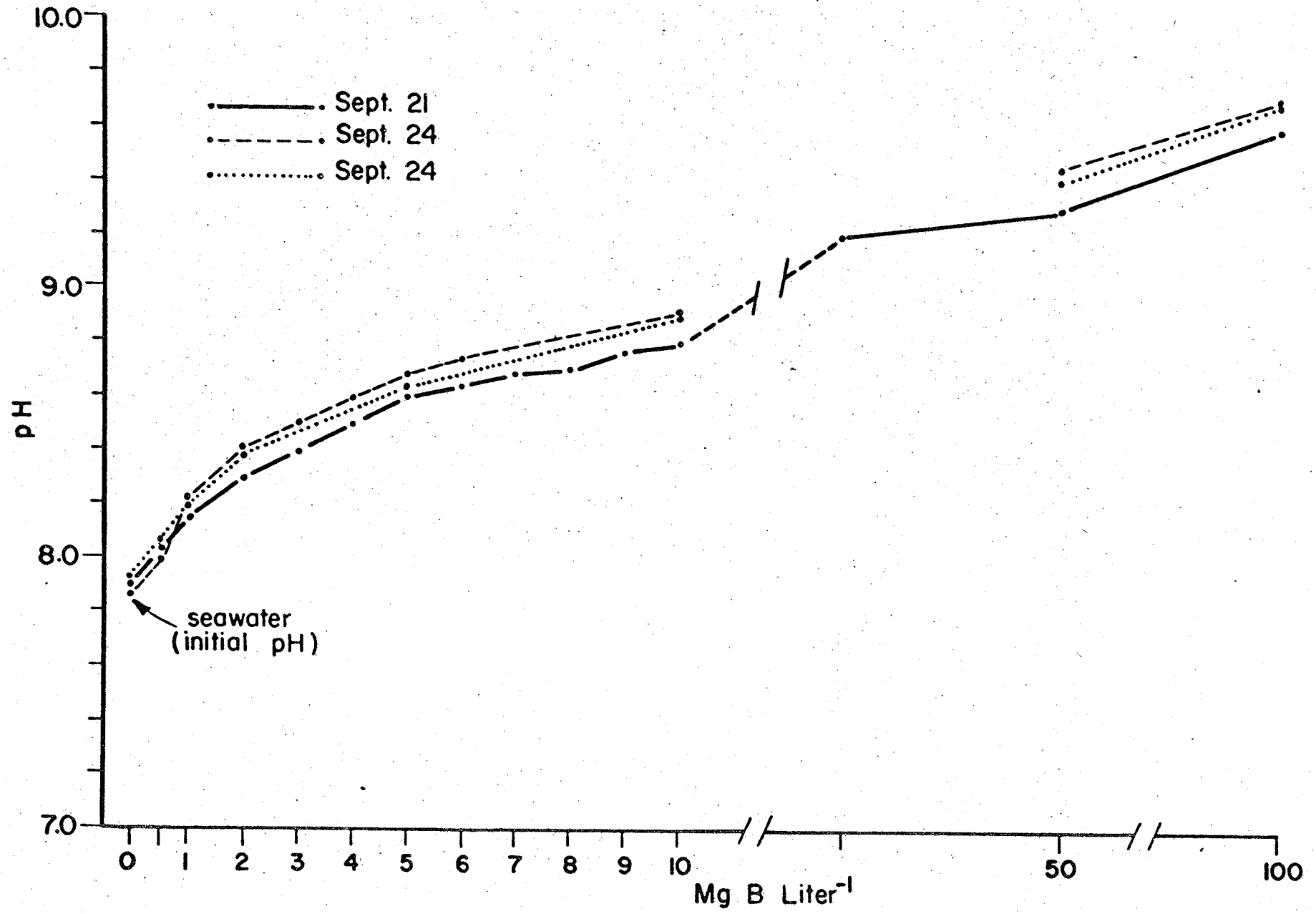
10.0
9.0
8.0
7.0
pH
Sept. 21
Sept. 24
Sept. 24
seawater
(initial pH)
0
1
2
3
4
5
6
7
8
9
10
50
100
Mg B Liter-1

FIGURE 1

FIGURE 2

The response of natural phytoplankton populations to groundwood, KME and XAD single pass. Concentrations as shown on figure.

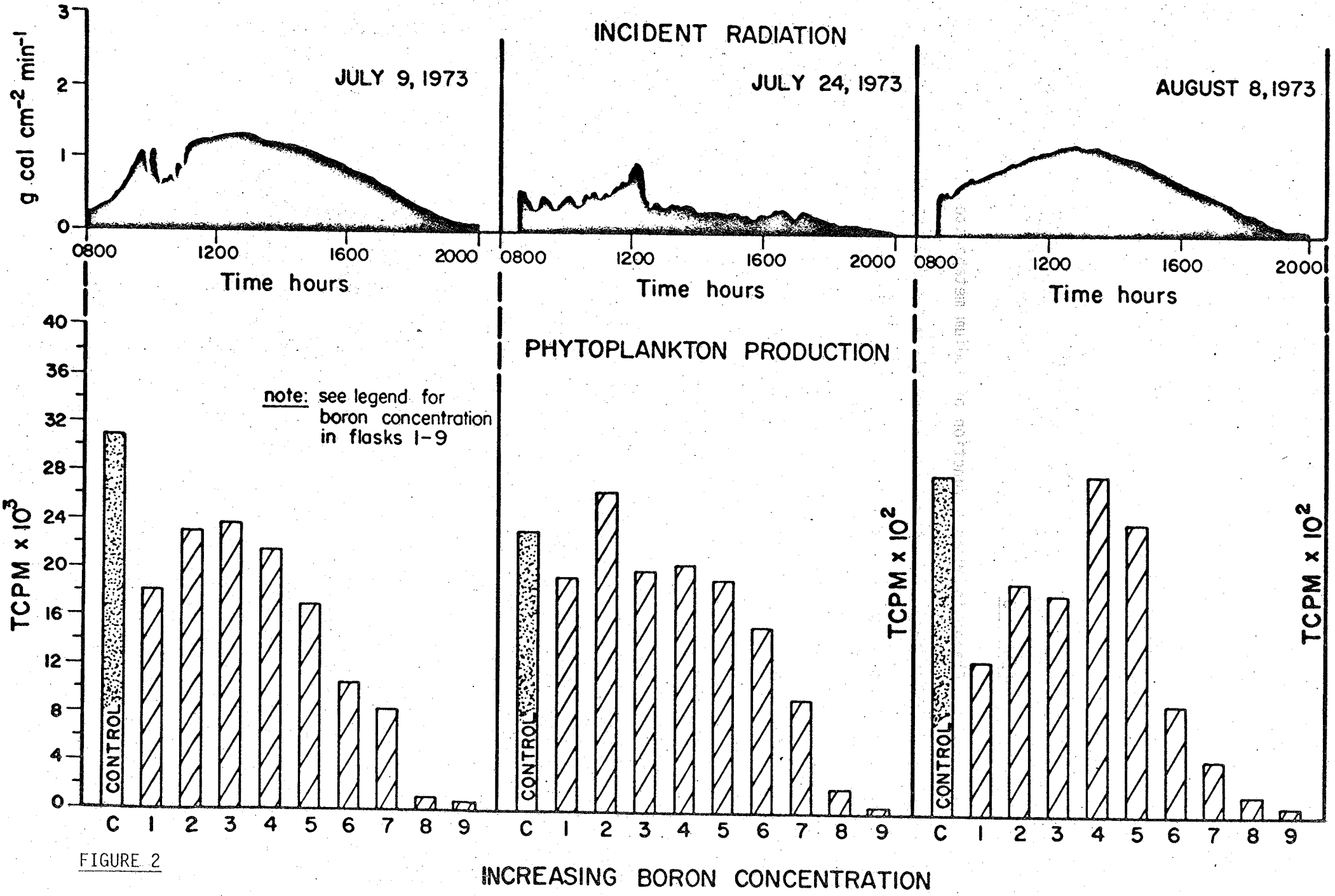
INCIDENT RADIATION
JULY 9, 1973
JULY 24, 1973
AUGUST 8, 1973
g cal cm⁻² min⁻¹
0
1
2
3
0800
1200
1600
2000
Time hours
PHYTOPLANKTON PRODUCTION
note: see legend for boron concentration in flasks 1-9
TCPM x 10³
TCPM x 10²
TCPM x 10²
0
4
8
12
16
20
24
28
32
36
40
CONTROL
C 1 2 3 4 5 6 7 8 9
INCREASING BORON CONCENTRATION

FIGURE 2

FIGURE 3

Change in pH as a function of sodium metaborate addition. Addition of metaborate expressed as milligrams of boron.

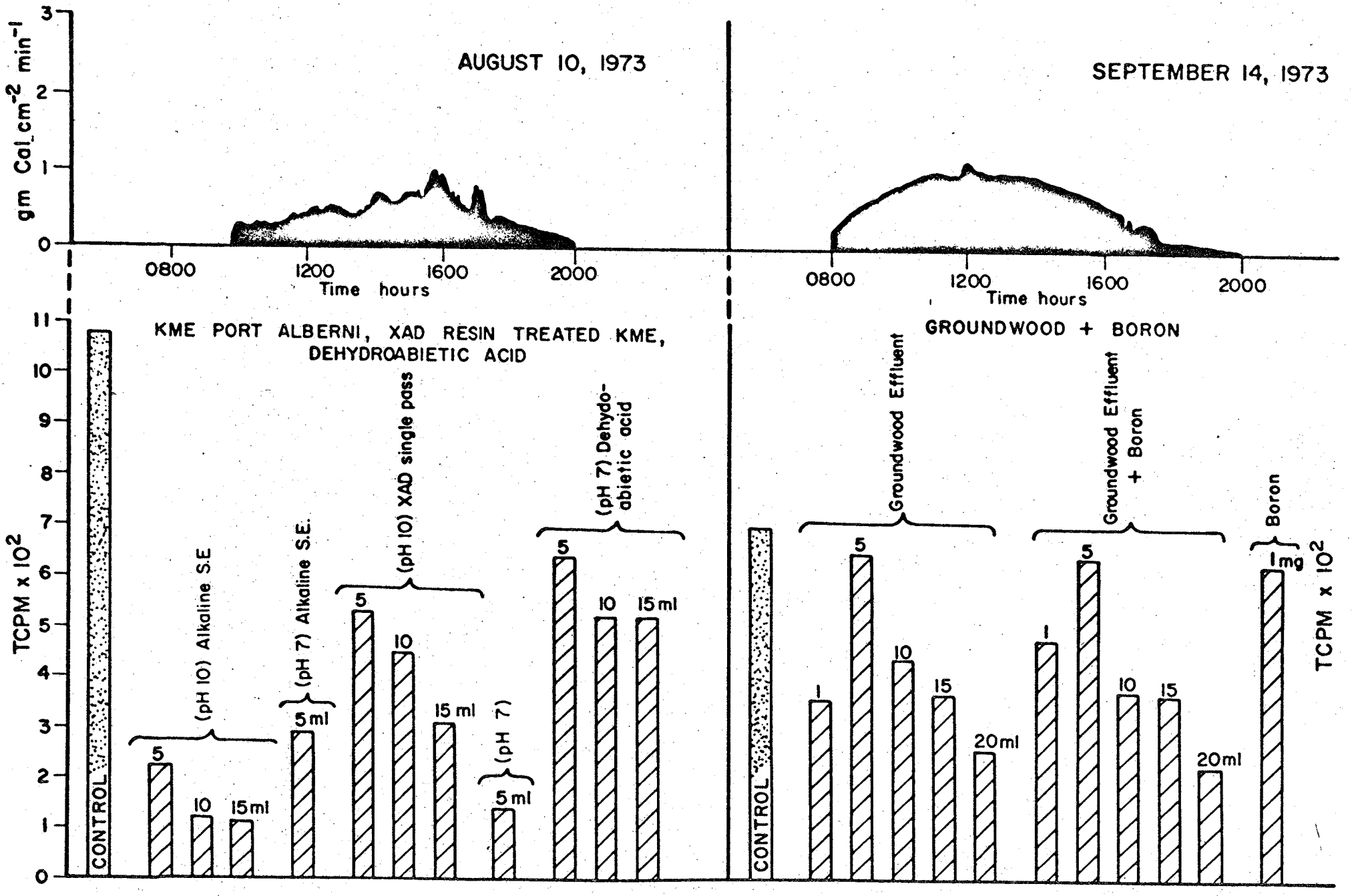
AUGUST 10, 1973
SEPTEMBER 14, 1973
gm Cal cm⁻² min⁻¹
Time hours
0800
1200
1600
2000
KME PORT ALBERNI, XAD RESIN TREATED KME, DEHYDROABIETIC ACID
GROUNDWOOD + BORON
TCPM x 10²
CONTROL
(pH 10) Alkaline S.E
(pH 7) Alkaline S.E.
(pH 10) XAD single pass
(pH 7)
(pH 7) Dehydo-abietic acid
Groundwood Effluent
Groundwood Effluent + Boron
Boron
5
10
15ml
5ml
15 ml
20ml
1
1mg

FIGURE 3