

FUCUS-EPIFAUNA AS A MARINE BIOLOGICAL MONITORING TECHNIQUE IN
ENVIRONMENTAL IMPACT AND REHABILITATION ASSESSMENTS: A
CASE STUDY WITH PRELIMINARY EXAMINATION AND DISCUSSION
OF ITS APPLICABILITY TO OTHER COASTAL REGIONS

by

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ABSTRACT

Analysis of the macroinvertebrates associated with the common 'rockweed' Fucus has been shown to provide an effective community-based biological technique for monitoring displacements in shoreline ecosystem structure and function as a result of surface imposed societal stresses.

Initially employed as one of 3 biomonitoring techniques in an environmental rehabilitation assessment (E.R.A.) at the Port Alice sulfite-based pulpmill, the method was restricted to an analysis of the amphipod associates of Fucus. Field distribution data and supportive effluent toxicity estimates clearly demonstrated environmental recovery within the inlet. Comparable physiological requirements between taxonomically similar organisms, however, was considered a possible limitation to this approach and supported a recommendation to avoid restriction of the Fucus-epifauna technique to a single group such as the amphipods. Use of the entire macroinvertebrate community was considered an appropriate alternate approach in that it incorporates organisms with differential pollutant sensitivities, and it documents structural changes due to indirect pollutant effects such as disruption of functional group dependencies.


A multivariate ordination of the epifaunal community associated with Fucus samples taken at 107 shoreline sites around Vancouver Island, and along the British Columbia mainland coast, revealed the natural variation within the 25 invertebrate species comprising this

algal habitat. Variation within the 'normal' community appears to be related to the degree to which the sampling site is exposed to wave or current action. In all cases, similarity of Fucus community structure between 'normal' sites and those sampled in the vicinity of industrial waste discharges, was directly proportional to distance from the waste source. Community responses due to changes in natural environmental parameters (e.g. salinity) were differentiated from pollutant effects by correlating species richness with a measure of numerical dominance. In regions influenced by industrial effluents, dominance increased significantly with decreased species richness, in response to the imposed waste. Natural environmental variation supported the converse relationship.

This technique was successfully tested in the Port Alice E.R.A. Fucus-epifauna samples taken both prior to and after implementation of pollution controls illustrated a significant biological recovery within the Fucus communities at sites previously affected by the discharged pulping waste. This recovery was correlated with water quality improvements observed over this period.




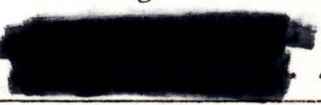
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I. INTRODUCTION

1. General

In recent decades increased industrialization and associated usage of coastal regions has stimulated a corresponding expansion in the research and development of protocols for assessing the impacts of such activities. The environmental impact assessment (E.I.A.), as defined by Munn (1979), is "an activity designed to identify and predict the impact of an action on the biogeophysical environment and on man's health and well-being, and to interpret and communicate information about the impacts". This definition suggests that an E.I.A. should encompass all relevant chemical, physical, biological, as well as economic and social factors. The study undertaken here will exclude discussion of the socio-economic component, although one should recognize that ecosystem impacts are related through complex feed-back mechanisms to social impacts and economic considerations.

Physical and chemical methodologies in E.I.A.'s are basic to estimating pollutant dispersal rates, environmental partitioning, degradation rates, transformation products, chemical persistence, etc.. Methods employed in such analyses are wide-ranging and in most cases dependent upon the type of pollutant involved. Physical tests incorporate such measurements as salinity, temperature, conductivity, color, turbidity, current flow, specific gravity, and suspended and settleable solids estimations. Chemical techniques, in contrast, include determinations of nutrient concentrations, dissolved oxygen levels, pH, and estimations of organic and inorganic constituents of

waste streams and receiving waters. Most of these physical and chemical techniques have been standardized (APHA, 1976) and are used routinely in the examination of water and wastewater.

Although physical and chemical testing provides valuable information on water quality characteristics, additional data are necessary to establish the effects of deleterious materials on the receiving aquatic ecosystem (Brungs and Mount, 1978). Thus, a complete E.I.A. protocol also incorporates biological monitoring, or biomonitoring, which can be defined as "the use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program" (Matthews et al., 1982).

A biological investigation of a polluted body of water has a number of advantages over chemical analyses. The most important of these is that biomonitoring can be less time-consuming (and thus less costly) and that a single series of samples can reveal the status of the existing biotic communities which themselves represent the results of the summation of prevailing conditions (Gaufin, 1973). Chemical testing, in contrast, is normally repeated to ensure that the data do not represent a temporary alleviation of the polluting effluent at the time of collection.

Biomonitoring methodologies can be divided into three general categories (Weber, 1973). These comprise methods for:

- (i) studying the effects of pollutants on natural populations or communities within the receiving waters;
- (ii) estimating the toxicity or other biological effects

of substances or effluents on captive organisms under controlled conditions; and

- (iii) determining the extent of bioaccumulation and thus detecting or monitoring trends in the concentrations of pollutants in surface waters.

Studies for estimating the extent of pollution effects as defined by biological responses commonly employ taxonomic, structural approaches, and thus fall under the first category above (Cummins, 1974). The methodology employed typically involves sample collection, species identification and enumeration, and biomass estimations. Data processing and evaluation usually include some form of community comparison index (e.g. Pielou, 1966; Shannon and Weaver, 1971), with major differences between sites attributed to pollutional stress. Indicator species (e.g. Phillips, 1977) or communities (e.g. Milbrink, 1973) are also used to estimate the extent of pollution in receiving waters.

Historically, approaches for studying biological responses to stress in aquatic environments have been dominated by various single species tests. These include both field distribution assessments, i.e., of indicator or keystone species, and pollutant toxicity estimates. These tests remain popular, but their value in predicting community or ecosystem responses to stress has been questioned (Cairns, 1981; NRC, 1981). Recently, biomonitoring has expanded to incorporate community level and higher tests so as to increase the accuracy of assessing the impact of pollutants on receiving systems. Research efforts presently include attempts to reduce biomonitoring data sets into useful, accurate indices of community or ecosystem responses. Ideally, indices

of this nature should be applicable to a wide geographic area, be sensitive and reproducible, suitable for long-term surveillance, and be able to distinguish seasonal and other natural changes from the introduced stress effect (Matthews et al., 1982).

2. Objectives

It is the objective of this study to investigate the resident macroinvertebrate community associated with the common intertidal alga Fucus. In particular, it attempts to test the effectiveness of this species assemblage as a marine biomonitoring technique for documenting displacements in shoreline ecosystem structure particularly as a result of surface-imposed societal stresses.

The goals of this study are achieved in two ways, each of which is presented separately in sections II and III. Section II presents the findings of a marine pollution assessment in which Fucus-epifauna is used in conjunction with two other biomonitoring techniques to document shoreline recovery processes following the implementation of pollution controls to a sulfite-process pulp mill. The second aspect of this study, presented in section III, attempts to determine the variability of this resident faunal assemblage in other coastal regions. As well as examining the structural composition of Fucus-epifaunal communities in various undisturbed environments, changes in species composition and numerical contribution are examined at a number of industrially-disturbed sites.

II. PORT ALICE - A CASE STUDY

1. INTRODUCTION

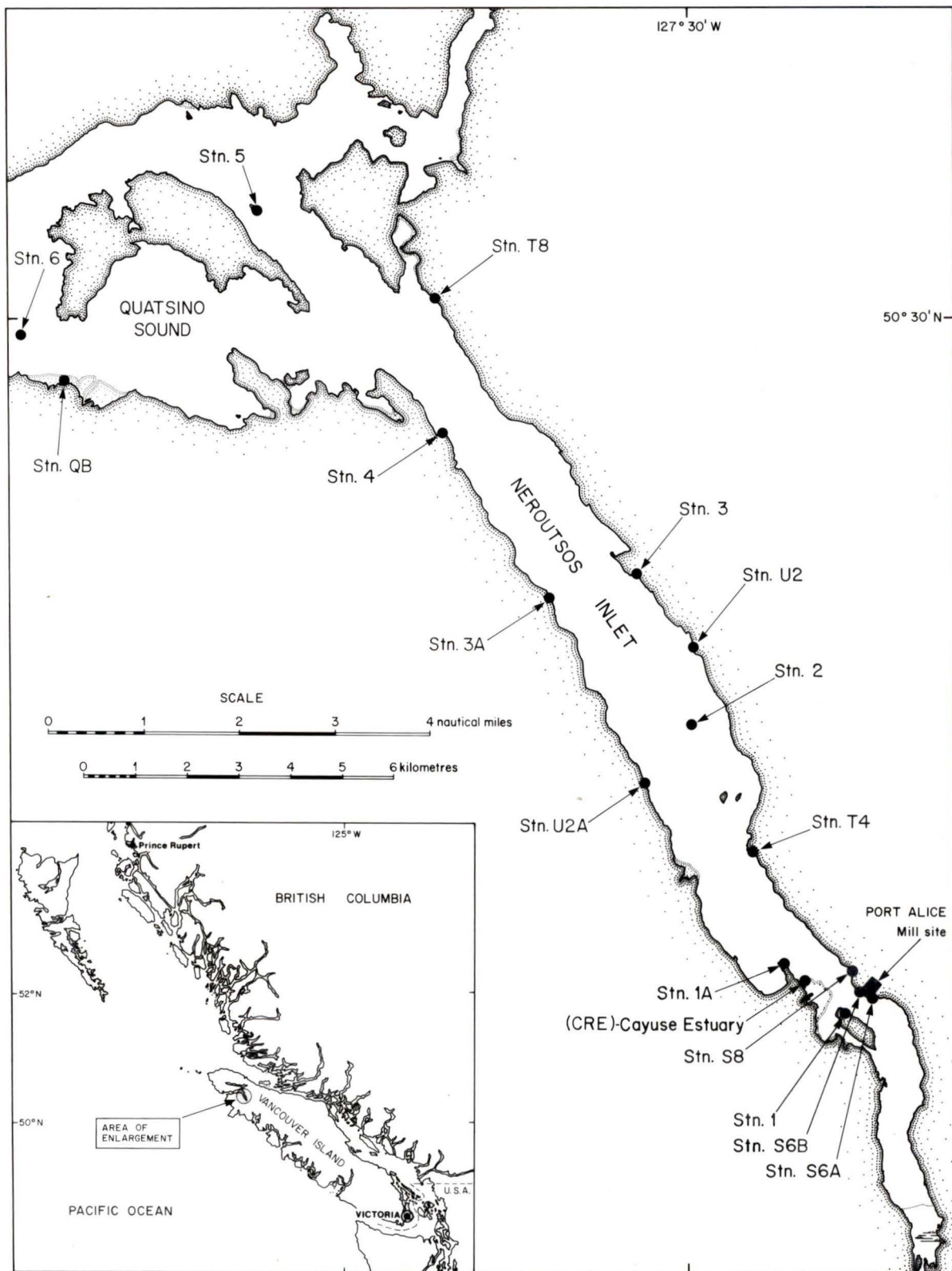
1.1 Study Area

One of the oldest pulp mills in the Province of British Columbia, and presently the only remaining sulfite pulp mill, is located at Port Alice (Plate A). The mill site is situated approximately 5 km from the head of Neroutsos Inlet, a south-extending arm of Quatsino Sound, which in turn opens into the Pacific Ocean near the northern end of Vancouver Island (Figure 1).

Neroutsos Inlet itself is 21 km long, has an average width of 1.5 km, and is U-shaped in cross section with an average centerline depth of approximately 200 m. A shallow, minor sill (45 m) starting just south of the mill site separates the head from the rest of the inlet. Depths at the mouth of the inlet and beyond reduce to about 100 m. The major freshwater source to the inlet is through the Cayeghle and Colonial Creek watershed system at the head of the inlet. These tributaries, and the resulting estuary, provide the area with the major feeding and rearing grounds for juvenile chum salmon (Oncorhynchus keta).

Most of the shoreline within Neroutsos Inlet, with the exception of broad alluvial fans produced by freshwater tributaries, is characterized by steeply rising slopes and minimal littoral formations. The intertidal zone is influenced by a mixed semi-diurnal tide with a mean tidal range of 3.0 m (Canadian Hydrographic Service, 1982).

Figure 1 - Location of sampling sites in Neroutsos Inlet and Quatsino Sound.



- Plate A - The Port Alice sodium-base acid sulfite process pulp mill. Refer to Figure 1 for location of this site on Vancouver Island, B.C.
- Plate B - South discharge sewer at the Port Alice pulp mill. Effluent discharged to the surface waters particularly affects littoral organisms with the flooding and ebbing tide. The dark bank seen at the top left of this photograph is the location of sampling station S6A.
- Plate C - Collections of Fucus, for subsequent epifaunal analysis, were sampled from the middle of this algal zone.
- Plate D - Station U2 during the summer of 1978 (one year after implementation of pollution controls). The green alga Enteromorpha is shown here to dominate the Fucus (gold/brown in color) within the littoral zone.
- Plate E - Station U2 during the summer of 1979. Fucus begins to recolonize appropriate rocky substrate within the littoral zone, displacing Enteromorpha ... shown here restricted to a small area in the foreground.
- Plate F - Station U2 in the summer of 1980. Fucus has totally recolonized the mid-littoral zone.



1.2 History of Pulp Mill

The Port Alice mill, initially designed and operated by the British Columbia Pulp and Paper Company Ltd. as a calcium-base acid sulfite process pulp mill, was originally built in 1917 and has been in continuous operation since that time. Original production has been estimated at 100 air dry tons (ADT) daily (Tollefson and Tokar, 1978). In 1942, and continuing through 1958, production reached 200 ADT which had, to that date, consisted entirely of bleached paper grades (Waldichuk, 1958). By the early 1960's, following acquisition by Alaska Pine and Cellulose (1951) and subsequent purchase by Rayonier Canada (B.C.) Ltd. (1954), mill modifications had been made so that dissolving grades could also be manufactured. Production at that time increased to slightly over 300 ADT/day and consisted of up to approximately 50% dissolving grades. In 1972 the mill was converted from a calcium to a soluble ammonia base, and since has maintained an average daily production of about 450 air dry metric tonnes of dissolving (60%) and paper (40%) pulp (Tokar and Tollefson, 1980). In 1980 I.T.T. Rayonier Canada (B.C.) Ltd. sold all of their Canadian holdings, including the Port Alice pulp mill, to a consortium of forestry companies presently operating under the title of Western Forest Products Ltd..

1.3 Historical Environmental Concerns

Neroutsos Inlet has been examined from an environmental standpoint at intervals over the past 50 years. The first study was implemented by Hutchinson and Lucas of the Canadian Federal Fisheries Department in 1927, 10 years after the mill originally commenced pulping operations (Waldichuk, 1958). Following the addition of

dissolving pulp grades to mill production in the mid 1950's, the environmental situation was studied by Waldichuk both in 1956 and then again in 1957. A degradation of water quality since 1927 was evident, and was attributed to the increased BOD of the effluent discharges associated with the added dissolving pulp grades (Waldichuk, 1958). Rayonier Canada (B.C.) Ltd. surveyed the area in 1966-1967 and in 1969, confirming the earlier reports of adverse changes in water quality due to pulping effluent. All of these studies were concerned primarily with the basic physical and chemical characteristics of the discharged effluent and the adjacent receiving waters of Neroutsos Inlet.

In 1971 the Pollution Control Board (PCB) of British Columbia issued a set of discharge guidelines to the Forest Products Industry. At that time I.T.T. Rayonier Canada (B.C.) Ltd. defined the environmental problem at Port Alice, and proposed a pollution abatement program for the mill. By 1972 the first step of this program, i.e., the conversion from calcium-base cooking to soluble ammonia-base (calcium not being amenable to the proposed pollution control system), was completed and in operation.

The second stage of the Port Alice pollution abatement program involved the installation of a recovery boiler system designed to remove and incinerate spent sulfite liquor (SSL) previously included in the effluent discharged to the adjacent inlet waters (Plate B). During the installation of this system Rayonier environmental staff collected physical, chemical, and biological data, thus forming a biological basis with which to assess the environmental benefits of the SSL furnace, once engaged (Stoll, 1975, 1976; Stoll and Vandermuelen, 1977).

1.4 Environmental Rehabilitation Assessment

The pollution abatement program initiated by Rayonier Canada (B.C.) Ltd. was intended to meet requirements outlined in a Pollution Control Permit issued to the company in 1973 by the PCB (Tollefson and Tokar, 1978). Requirements within this permit included:

- (i) maintaining a dissolved oxygen (DO) level of not less than 60% of saturation (5 ppm) at the mouth of the inlet;
- (ii) an initial reduction of effluent suspended solids to 60 lbs/ADT of pulp and of 5 day biochemical oxygen demand (BOD₅) to 500 lbs/ADT; and
- (iii) a further reduction of suspended solids to 30 lbs/ADT, and a BOD₅ to 60 lbs/ADT within 3 years.

Conditions (i) and (ii) were expected to be met by the conversion from a calcium to a soluble ammonia cooking base followed by the installation of a SSL recovery boiler system. To meet the conditions of requirement (iii), however, implementation of a secondary (undefined) treatment system would be involved.

Determining the environmental benefits sustained as a consequence of the removal of SSL from the waste stream appeared to be a logical approach prior to committing costs to developing and installing further treatment processes. In effect, an Environmental Rehabilitation Assessment (E.R.A.) would permit an evaluation of the present pollution control system and allow a decision to be made as to the need for

subsequent effluent discharge treatments. Following start-up of the recovery boiler system in the fall of 1977, I.T.T Rayonier Canada (B.C.) Ltd. initiated such a study (April, 1978). Retaining academic and private consulting agencies, as well as company personnel, the environmental investigation has since examined water quality, primary productivity, littoral invertebrate communities, salmonid migrants, and the toxicity of the discharged effluent (Corbett and Campbell, 1980; Corbett et al., 1978; Cross, 1982; Cross, In press; Cross and Ellis, 1980; Cross et al., 1980; Ellis et al., 1978; McGreer et al., 1980; Poulin and Rosberg, 1978, 1980; Vigers et al., 1978).

1.5 Study Objectives

The M.Sc. program, started in May of 1980. was initially designed to supplement on-going collections of Fucus amphipod abundance/diversity data with detailed experimental data on the tolerances of a number of these species to sulfite mill effluent. Sublethal as well as acute lethal bioassays over entire life cycles were expected to provide valuable information concerning the sensitivity of these invertebrate populations, and in turn to verify observed positional changes, species interactions, and numerical success of particular populations within the inlet since implementation of pollution controls in 1977. Due to a labour dispute extending through most of the summer in 1981, consequential shut-down of the Port Alice mill prevented all but the initial stages of this study from being completed. Thus, the direction of this project was altered from a toxicological to an ecological examination, concentrating primarily on a discussion of one of the biomonitoring techniques employed in the

E.R.A.

Process wastes from the Port Alice pulp mill are discharged to the surface of the receiving waters (Plate B). Use of intertidal communities, or of representative groups of resident invertebrates, were chosen for monitoring inlet rehabilitation due to the restriction of most invertebrate species to relatively small local habitat ranges, and since intertidal organisms would be most affected by the fluctuating surface waters and thus to the toxicity of the imposed sulfite mill effluent (SME).

A preliminary reconnaissance survey in May of 1978 suggested that the design of the assessment program could be approached in a manner comparable to that employed in a river, or stream situation, based on the premise that there exists a reduced but progressively more diverse ecosystem downstream from a discharge, the biological quality of which will improve upstream towards the discharge source as pollution controls are implemented (Hynes, 1960). Neroutsos Inlet, a narrow fjord-like inlet with net surface flow towards Quatsino Sound due to river discharges, can thus be regarded as a marine river with an analogous 'downstream' increase in biological diversity in terms of the shoreline ecosystem. Thus, it is hypothesized that as shoreline recovery progresses a shift in the species diversity and abundance will occur in the direction of the discharge source corresponding to improved water quality.

The overall objective of this study is to report the findings of the E.R.A. in terms of the littoral invertebrate communities present, paying particular attention to Fucus-epifauna as a shoreline

monitoring technique. Specifically, the study will attempt to:

- (i) illustrate the extent of littoral impact within Neroutsos Inlet in terms of the invertebrate communities present;
- (ii) document shoreline biological recovery processes using Fucus-epifaunal communities; and to
- (iii) relate temporal and spatial changes in Fucus-epifaunal communities to the reduction of discharged SSL, and subsequent water quality improvement, using bioassay techniques on selected 'sensitive' invertebrate species.

This section provides a summary of this on-going study, and emphasizes a discussion of each biomonitoring technique employed, i.e., its strengths and/or weaknesses. The bulk of raw data has been omitted from the text, but if required may be found in the documents referenced earlier.

2. FIELD METHODOLOGY

The shoreline monitoring protocol adopted for the E.R.A. consists of three biological tests:

- (i) qualitative estimations of enchytraeid/tubificid oligochaete species composition within the visually highly impacted shoreline near the discharge source;
- (ii) Fucus-epifaunal abundance and diversity measures; and
- (iii) qualitative faunistic and floristic rocky shore surveys with between-site similarity analyses.

The first two of these tests were chosen for their different sensitivities to the effluent, while the third was included to monitor the 'overall' effect of sulfite mill effluent (SME) within the inlet.

2.1 Enchytraeid/Tubificid Oligochaete Sampling

Oligochaete sampling consisted of 3 hand-driven replicate cores (inner diameter 5 cm, penetration 6 cm) taken at 6 high-tide shoreline sites with penetrable substrates (Figure 1: stations S6A, S6B, S8, CRE, U2, and QB). These substrates consisted of fine grained but often poorly sorted muds. Removal of interfering stones and rocks to permit coring prevented randomization in the sampling procedure.

Entire sediment samples were stored and transported in plastic bags on ice, and were subsequently transferred to a refrigerator (at approximately 10°C) until specimens could be separated from the sediment. Species identifications were made under light microscope

using both live specimens and specimens prepared by staining with Grenacher's alcoholic borax carmine.

2.2 Fucus-epifaunal Sampling

Sampling of Fucus was conducted monthly from May through September (1975-1981) at 8 appropriate sites (Figure 1: stations 1, 1A, 2, 3, 3A, 4, 5, and 6) extending from the mill to Quatsino Sound. The collection procedure, developed by mill environment personnel prior to 1978 (Stoll, 1975), involves 'randomly' removing 8 liters of Fucus fronds from the rocky substrate within the sampling area, and dropping it loosely into a bucket to avoid packing (Plate C). The Fucus-epifauna are then rinsed off in a dilute (approximately 4%) formalin and seawater solution. Following sorting, the organisms are removed for species identifications, and for abundance and diversity analyses.

2.3 Intertidal Rocky Shore Surveys

A series of 10 shoreline sites were used in this biological survey of the intertidal rocky habitat of Neroutsos Inlet (Figure 1: stations 1, T4, 1A, 2, U2A, 3, 3A, 4, 5, and 6). Sites were chosen such that both the west side and the east side of the inlet were represented by a set of relatively evenly distributed shoreline stations so that results of the survey could be compared and contrasted.

The survey, first conducted in August of 1979, was implemented as a single low to high tide transect encompassing a width of shore approximately 3 m across. Representative collections of organisms found within this area were retained for species identification.

2.4 Statistical Methods

Accumulated species data from the enchytraeid/tubificid sampling and the intertidal rocky shore surveys were both analysed qualitatively using a community classification (clustering) method. Samples (sites) were objectively grouped into associations dependent upon similarities of species composition (Q-analysis). A single-link, unweighted pair-group cluster analysis was preceded by the formation of a site-by-site similarity matrix using species presence-absence data. Jaccard's Coefficient of Community (Sneath and Sokal, 1973), used as the similarity index for the analysis, is as follows:

$$J_c = \frac{C}{A + B - C}$$

where: A = number of species in sample A
 B = number of species in sample B
 C = number of species in common.

The analysis of Fucus-epifauna data was accomplished primarily in a subjective manner, examining gross numerical trends of each species temporally and spatially within the inlet.

Certain morphological features of Oligochinus lighti specimens were examined for possible sublethal effluent effects. Body-length frequency data from 5 sites were compared using a one-way Analysis of Variance (ANOVA) followed by a Student Newman-Keuls multiple range test. Brood size as a function of body length was correlated over the same sites. A comparison of slope functions was performed using an Analysis of Covariance (ANCOVA). In each test above, the rejection level (alpha) was set at 0.05.

3. LABORATORY METHODOLOGY

The laboratory portion of this study consisted solely of the invertebrate bioassays using SME and two species of gammaridean amphipoda. The experiments were performed in an attempt to correlate observed distributional changes of these species with the water quality improvement noted since start-up of the recovery boiler system in 1977.

3.1 Experimental Design

A siphon-activated, continuous flow bioassay system, shown to scale in Figure 2, was designed and constructed to permit an evaluation of chronic or sublethal toxicant effects in small invertebrates, over a broad range of concentrations. The system minimizes required laboratory space by distributing the concentration test chambers over three levels, the bottom two of which are on drawer tracts to optimize accessibility during observation periods. Each of the levels was constructed of 3/4" plywood, laminated with 4 coats of Exterior Varathane, and caulked with silicone sealant.

Continual freshwater input to one side of each of the system levels was opposed by an overflow pipe on the opposite side, maintaining a constant cooling water level of 8 cm. Water temperature was held at $14^{\circ} \pm 1.5^{\circ} \text{C}$ during each of the experiments.

The siphon-activated system employed in this study was modeled after a design used by Sasnowski et. al. (1969). The glass-ware components of this system are shown (to scale) in Figure 3. Each concentration bath (40.5 cm x 30.5 cm x 13 cm) receives a constant input of test solution from the concentration reservoir

Figure 2 - Schematic presentation of the siphon-activated continuous flow bioassay system. Each level contains 3 concentration test chambers. The bottom 2 levels are mounted on drawer - tracks to optimize accessibility during observation periods.

CONTINUOUS-FLOW BIOASSAY SYSTEM

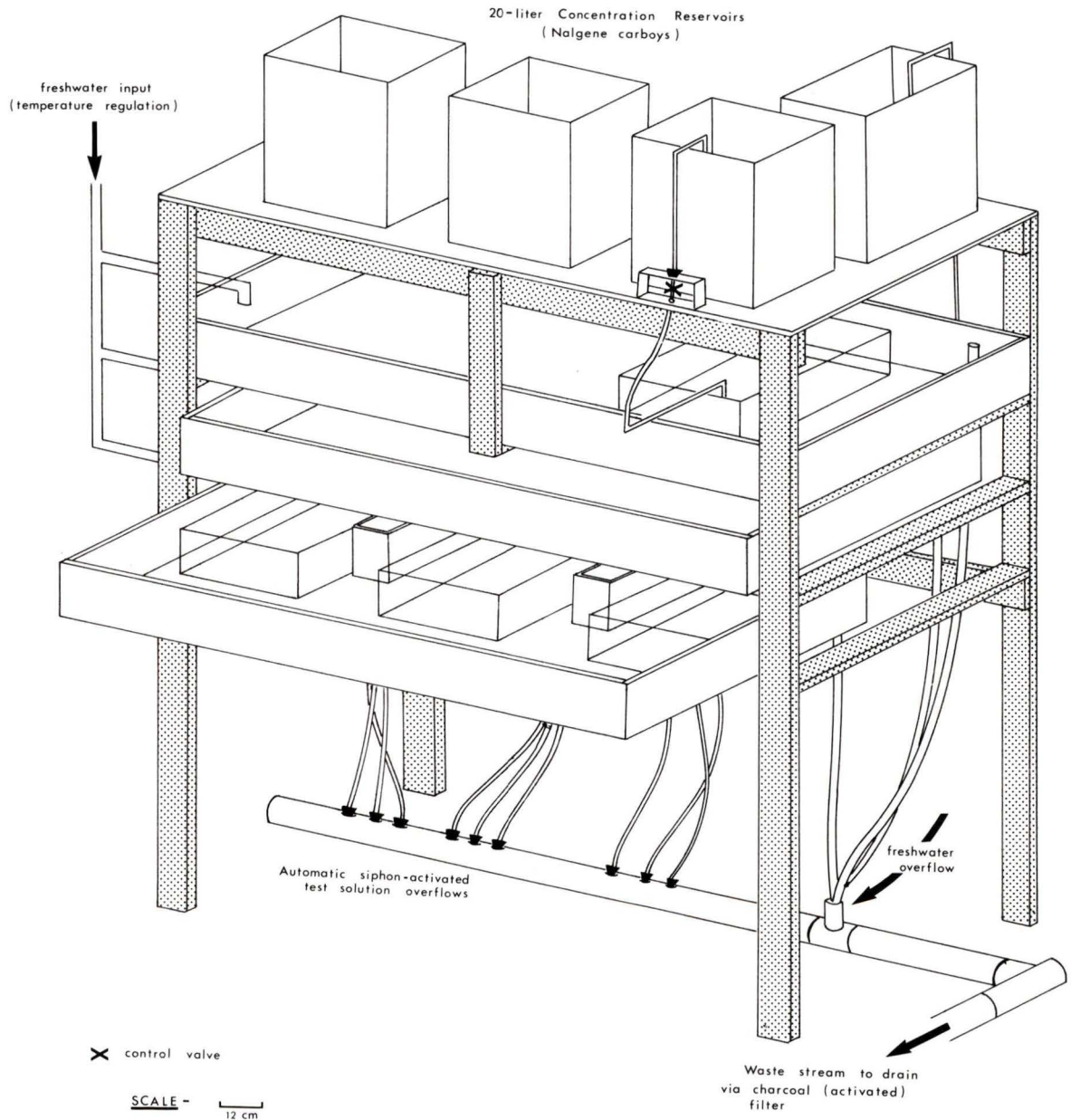
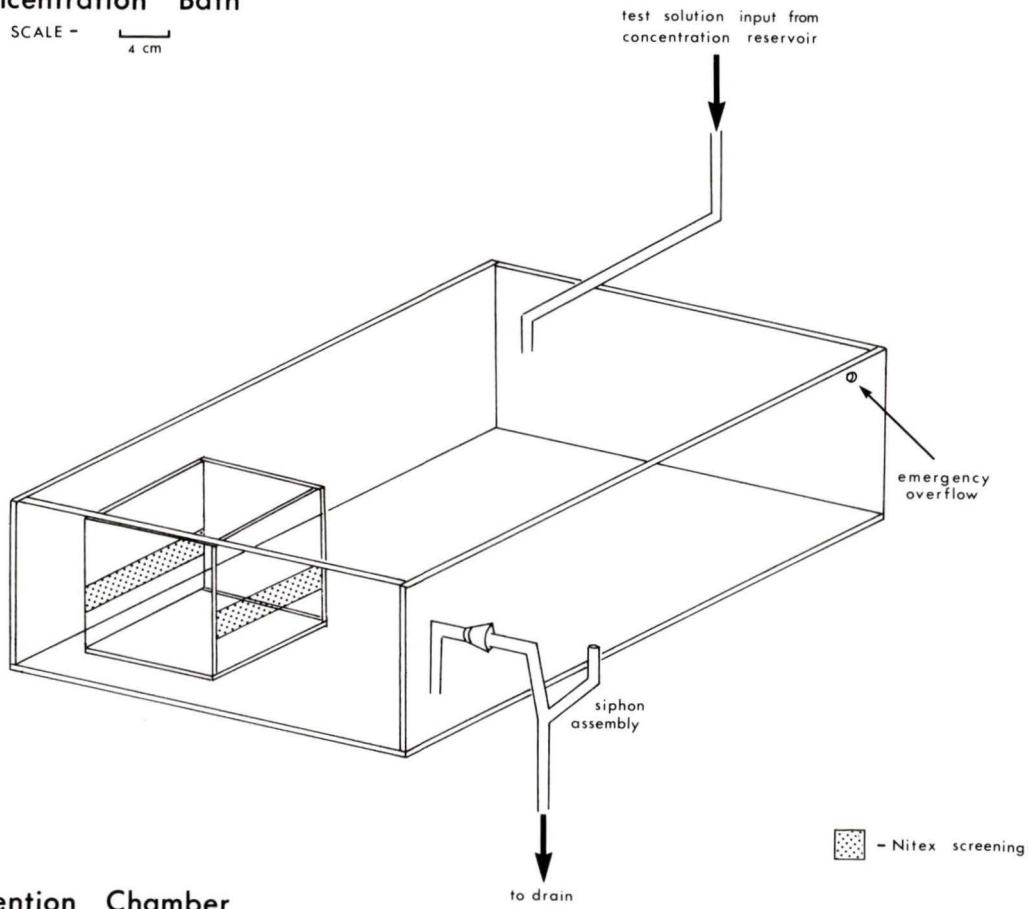


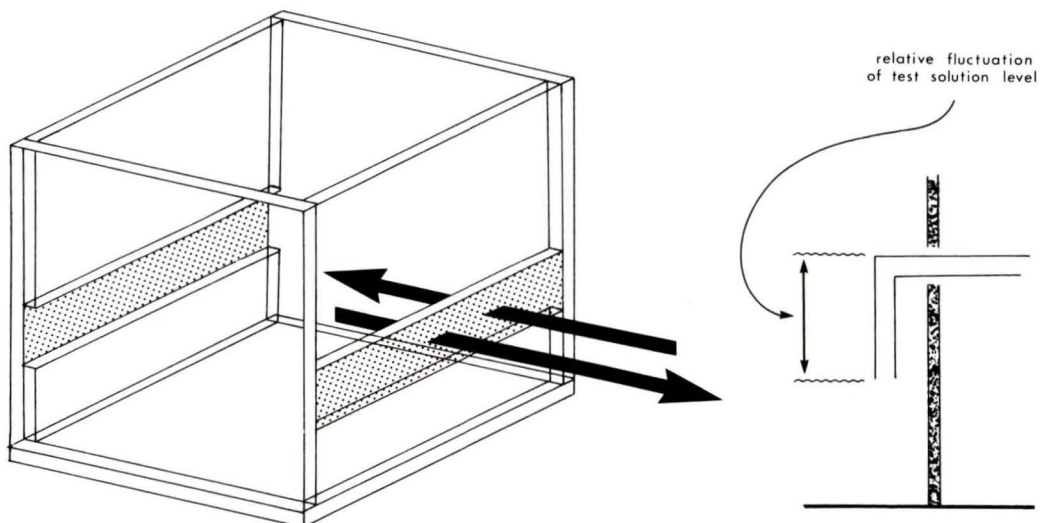
Figure 3 - Glass-ware components of the continuous flow bioassay system.

- a) Schematic of concentration bath. Each bath is constructed of 3/16" glass and is designed to hold 6 retention chambers. Siphon assembly is designed with an external Y-tube to prevent malfunction as a consequence of air bubble formation.
- b) Enlargement of a retention chamber. Constructed of 3/16" glass. A 310 um strip of Nitex screening permits solution replacement with siphon flushes:

a) Concentration Bath

SCALE -  4 cm

b) Retention Chamber

SCALE -  2 cm

positioned on the top of the bioassay system (Figure 2). When the solution level reaches 8.0 cm the siphon is automatically activated and the accumulated test solution removed until the vacuum created by the siphon action is disrupted by a solution level of 3.0 cm (bottom level of the siphon tube). The siphon assembly consists of a Y-shaped tube, one side of which acts as an air vent to prevent the blockage of the drainage line by an air bubble and the consequential malfunction of the siphon. In the event of a malfunction, however, each concentration bath is equipped with an emergency overflow port which will allow loss of waste test solution without loss of the test organisms from their respective retention chambers. Waste test solution, as well as cooling water overflow, is allowed to pass to a floor drain via Tygon tubing, PVC piping, and an activated charcoal filter.

Each concentration bath holds 6 retention chambers (Figure 3) for containment of the appropriate number of test organisms. Each chamber (10 cm x 10 cm x 10 cm) has a 2 cm strip of 310 um Nitex screening located 2.5 cm above the base, and on each of 2 opposing sides. This screening permits flow of solution between the retention chamber and the concentration bath as the automatic siphon assembly activates solution replacement within the system.

All experiments were standardized using 10 organisms in each of 3 replicate retention chambers. Test solution input was maintained at approximately 650 ml/h, allowing 3 siphon flushes and providing a 90% solution replacement in a 24 h period.

3.2 Test Procedures

Although specimens of the chosen test species could be found in the Victoria area, samples were obtained from Neroutsos Inlet so as to eliminate the possibility of differing effluent responses due to adapted physiological properties which may be inherent in the populations continually exposed to sublethal levels of SME. In situ environmental parameters were measured during collection periods using a Fisher Accumet Model 150 portable pH meter, and a YSI Model 33 S-C-T (Salinity-Conductivity-Temperature) meter to record temperature and salinity. Specimens were collected and transported to the university where they were maintained as monocultures in 10-liter holding tanks. Each tank was equipped with an air stone to maintain oxygen levels, and placed in a cooling bath to control variation in temperature ($13^{\circ}\pm 2^{\circ}\text{C}$). Approximately 1 liter of Fucus (2-3 fronds) was supplied as natural shelter and as a food resource for the amphipods. Tanks were cleaned, and Fucus replenished, monthly.

Seawater used for dilution in this study was acquired from the Victoria area, having an initial pH of about 8.0, and a recorded salinity of 30 ppt (± 2). Water was pumped through a fiber-glass Swim-Quip filter, consisting of coarse sand and ground oyster shell, and then through an Aqua-Fine UV sterilizing system. Salinity was lowered using distilled water, and pH was altered by the addition of dissolved electrolytic pellets of sodium hydroxide (NaOH) or concentrated hydrochloric acid (HCl).

Each 80 L batch of sulfite mill effluent, was obtained by mill personnel, when required, using a Sirco automatic sampler at

each of the two discharge sewers. Seventy percent of the sample originated from the south sewer 30% was taken from the north sewer, to approximate the actual discharge ratio. Samples were representative of a 24 h composite sampling design calibrated to the automatic sampler. Samples were transported to the university by air freight and courier within 24 h of collection. Once at the university samples were stored in a cold room at $3^{\circ}\pm 1^{\circ}\text{C}$ to minimize loss of toxicity with time.

Prior to each experiment a static 48h-LC50 bioassay was performed as a 'range-finding' test. Each of these tests used 5 organisms in each of 5 concentrations chosen over a logarithmic series, e.g., 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56, ...etc.. Range-finding tests were implemented in 300 ml finger bowls using 250 ml of test solution.

All glass-ware was thoroughly cleaned prior to all bioassays. Following an initial soap and water wash with a distilled water rinse, the glass-ware was washed a second time with 2 N HCl and rinsed a final time with distilled deionized water. Glass-ware was soaked in the appropriate test concentrations for 48 h prior to initiating a bioassay. This procedure reduced the possibility of loss in toxicity due to adsorption of toxic chemical constituents by the glass and/or the Nitex screening.

In addition to the SME toxicity tests, temperature and pH tolerance limits for each of the test species were also estimated. Each static bioassay (24 h solution replacement) was performed in triplicate and terminated at 96 h.

In all cases observations for mortality were taken at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, and 96 h. Organisms were observed with a Cole-Palmer illuminated magnifying glass with bottom illumination provided by light box with fluorescent light-source. Individuals were initially considered dead if no pleopodal movement was observed. Due to a tendency of these organisms to exhibit immobilization upon prolonged exposure to certain levels of toxicant, individuals presumed dead were placed in saltwater for 5 minutes. If recovery occurred within this period, the organisms were returned to their respective test solutions.

3.3 Statistical Analysis

Mortality data was statistically analyzed using the computer program PROBIT, which was modified from Davies (1971) to accommodate and utilize the mean of 'n' replicate bioassays. The program computes the probit-response / log-concentration regressions using the maximum likelihood method described by Finney (1978). In the event that mortalities were recorded in the control chambers, the number of organisms responding positively in each test case was corrected for those occurring in the controls (prior to probit transformation) using Abbott's formula:

$$P = \frac{(P' - C)}{(1 - C)}$$

where P is the corrected response, P' the response observed and C the proportion of controls responding. Output from this analysis gives the median lethal concentration (LC50) as well as the slope and the intercept of the computed regression line with appropriate descriptive

statistics.

Since a response of 0 or 100% corresponds theoretically to probits of minus and plus infinity, respectively, these observations are omitted from the PROBIT computation of the probit/log dose regression line (Davies, 1971). In order to approximate the LC50's for early observation periods during the bioassay, an expected effect value for each 0 and 100% response was estimated using the nomographic method of Litchfield and Wilcoxon (1949). Incorporating these values into a graphical approximation of the probit/dose regression line permitted line extrapolation of a LC50 solution. The 95% confidence limits for these solutions were omitted to allow differentiation between values estimated in this manner with those calculated using the computer program PROBIT.

4. RESULTS

4.1 Receiving Water Quality

In order to evaluate shoreline biological responses as a consequence of the removal of spent sulfite liquor (SSL) from the waste stream, a significant improvement in the receiving water quality should also be demonstrated. Since 1973, mill personnel at Port Alice have accumulated daily water quality data on temperature, salinity, spent sulfite (Pearl Benson Index: PBI), dissolved oxygen (DO), and pH at 5 centerline stations extending from the mill to Quatsino Sound (Corbett et al., 1978). In addition, NH_3 , color, and BOD have recently been added to this protocol.

Daily water quality data for the 4 parameters regularly recorded have been compiled from measurements taken at 4 stations regularly sampled in the inlet, omitting the fifth (control) station located in Quatsino Sound. Each value is an average of measurements taken at the surface and at a depth of 2 m.

Figure 4 reveals receiving water temperature both prior to, and after the installation of the recovery boiler system. As expected, temperature does not change significantly at any one of the stations and generally remains within the range of 14-15°C. An increase was noted, however, from the mouth of the inlet to the inlet head reflecting the degree of exposure to, and the moderating effects of the waters of Quatsino Sound and the open Pacific ocean.

Similarly, Figure 5 demonstrates the salinity stability of the inlet surface water. No apparent annual differences were observed at any one site, and generally salinities ranged from 2.7-3.0%

Figure 4 - Mean surface water temperature of 4 centerline stations of Neroutsos Inlet taken both prior to and after installation of the recovery boiler system. Each value represents an average between measurements taken at the surface and at a depth of 2 m using water quality data accumulated by mill environment staff.

TEMPERATURE

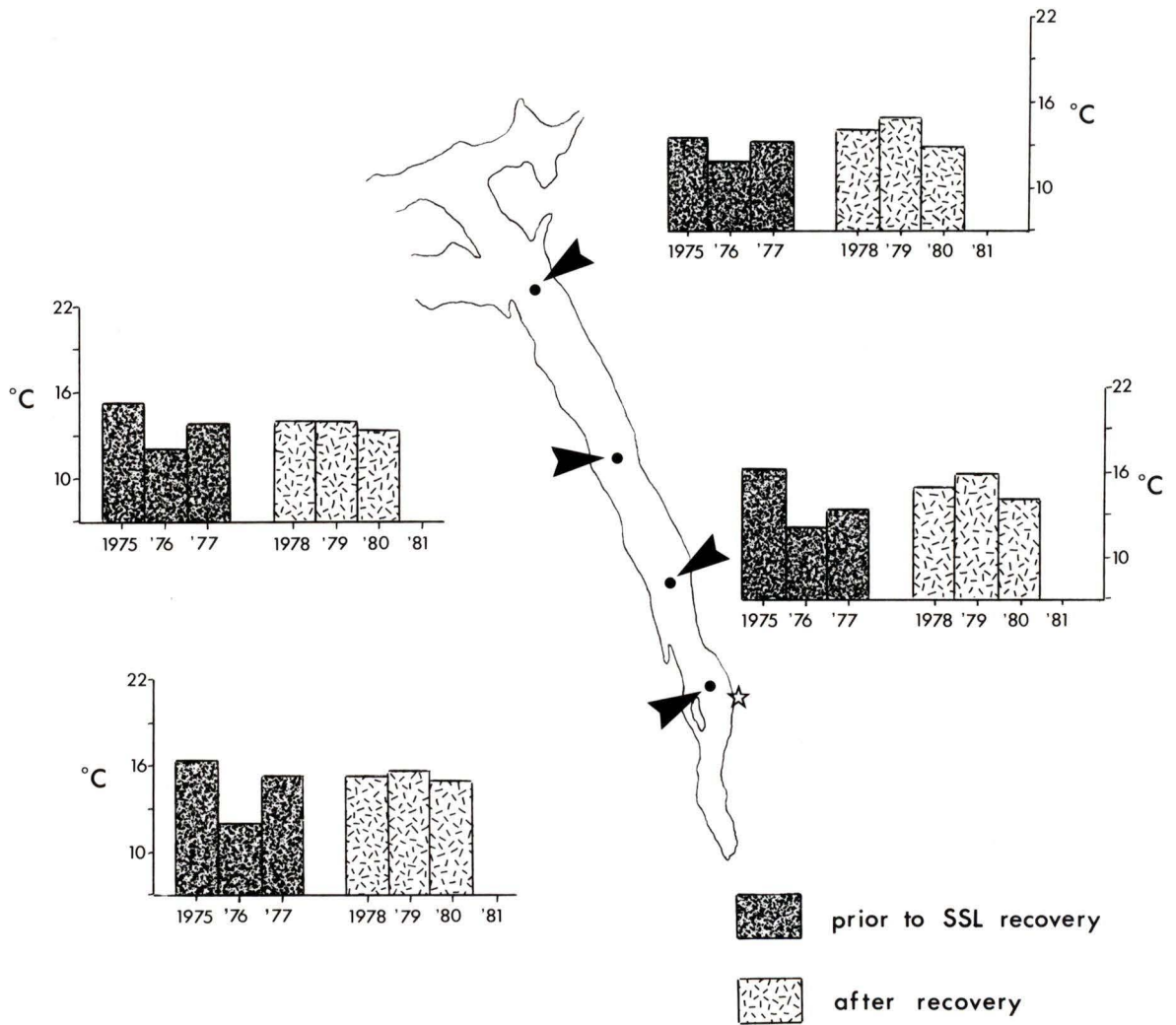
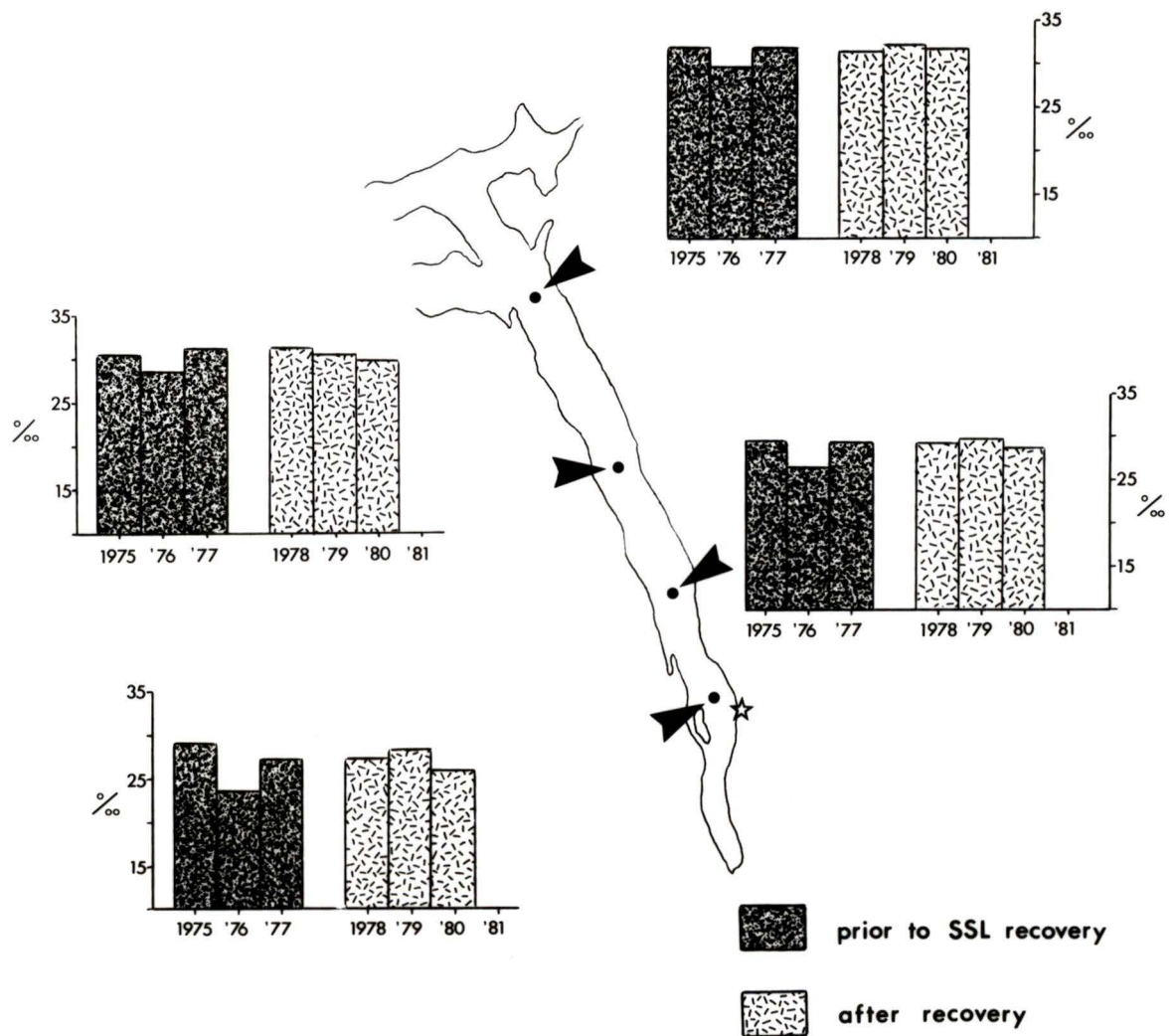


Figure 5 - Mean surface water salinity of 4 centerline stations taken both prior to and after installation of the recovery boiler system. Each value represents the average between measurements taken at the surface and at a depth of 2 m using water quality data accumulated by mill environment staff.

SALINITY



throughout the inlet as a whole. Higher surface water salinities were found near the mouth of the inlet, while slightly lower values were observed towards the inlet head, from freshwater inputs.

The result of decreased or eliminated SSL discharge is best described in terms of the concentrations of sulfite present within the receiving waters. Figure 6 illustrates temporal changes in PBI values, expressed as ppm of sulfite, with increasing distance from the mill. As expected, the concentration of sulfite within the surface waters decreases as distance from the discharge source increases. Sulfite levels at all of the 4 stations have continued to decrease since the reduction of SSL discharged. Average water sulfite levels, since initiation of SSL recovery in 1977, have been decreased by a factor of approximately 10 at each of the sampling sites.

In direct response to the substantial reduction in the high BOD fraction of effluent discharged to the inlet, Figure 7 reveals a correspondingly significant increase in the dissolved oxygen (DO) available in the water column. At the sampling sites in the mill vicinity DO remains low, but has nevertheless doubled since the implementation of SSL recovery in 1977. Average DO levels at sites as close to the mill as station 3 (Figure 7) have now risen to levels of 6 ppm, thus conforming to one of the requirements set by the PCB Pollution Control Permit of 1973.

4.2 Extent of Littoral Impact

A preliminary shoreline reconnaissance in May of 1978 revealed a highly impacted littoral area in the vicinity of the mill. Further investigation established that the macro invertebrate fauna of this

Figure 6 - Mean PBI (Pearl Benson Index) values (sulfite concentration) for surface waters at 4 centerline stations both prior to and after installation of the recovery boiler system. Each value represents an average between measurements taken at the surface and at a depth of 2 m using water quality data accumulated by mill environment staff.

PBI

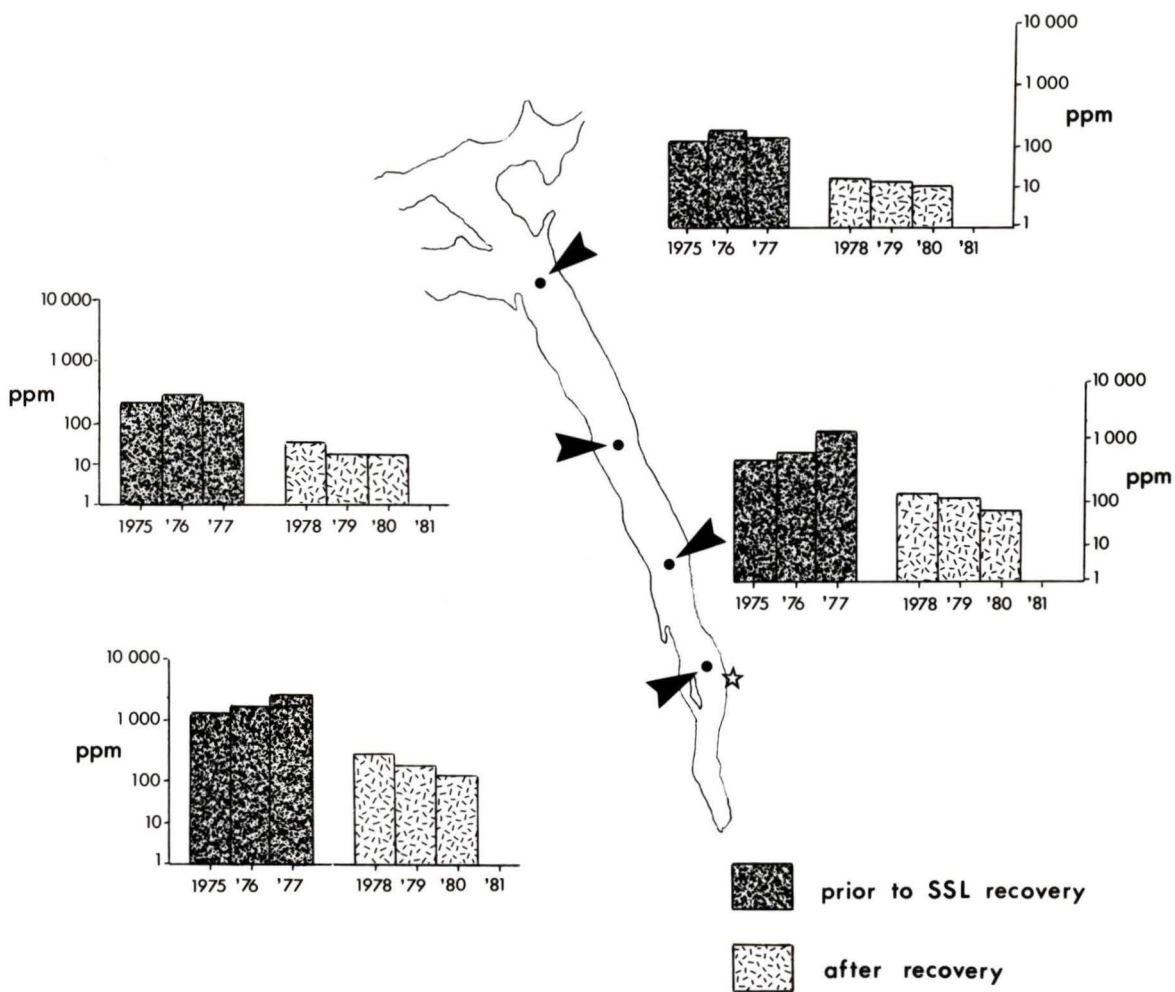
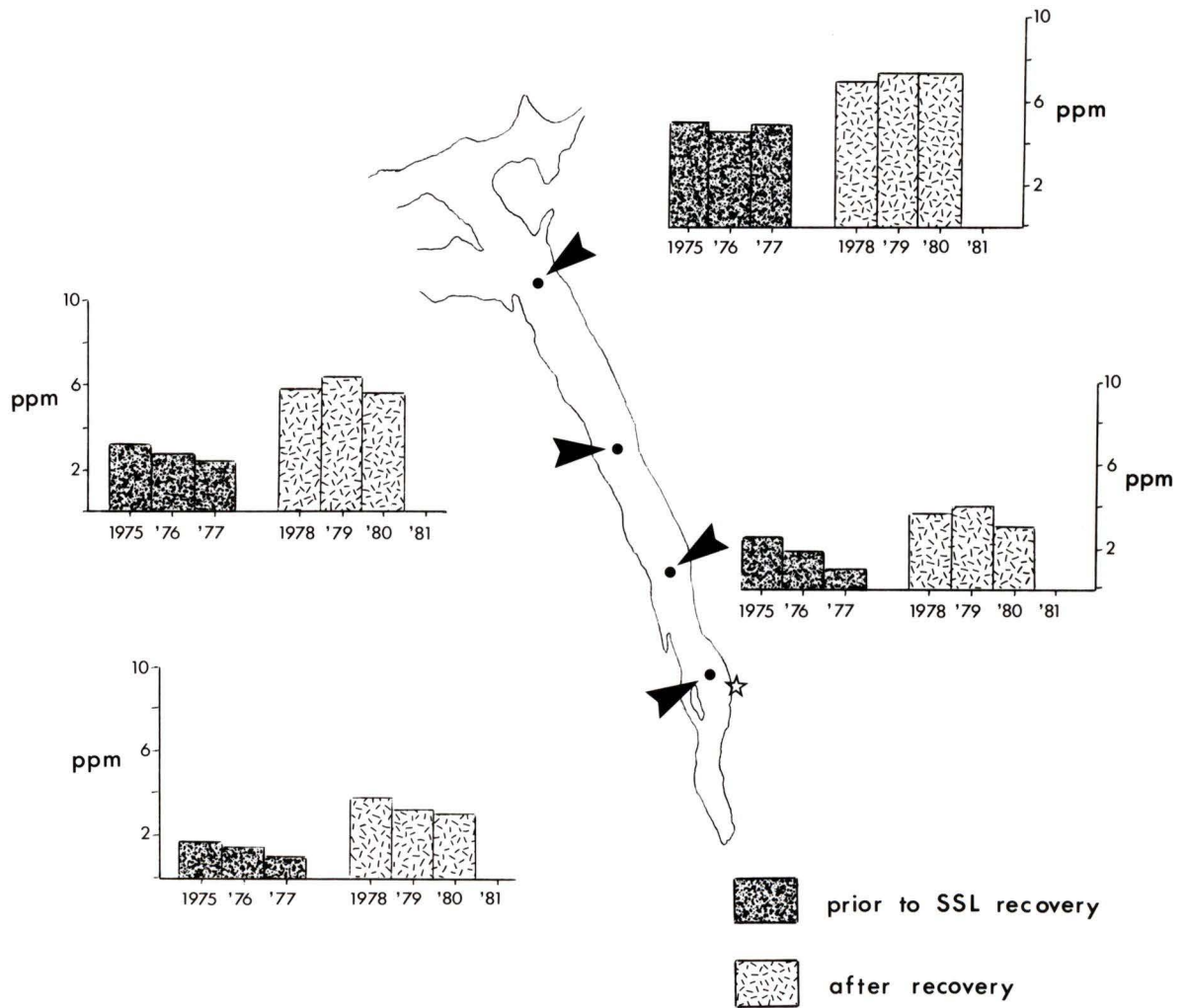


Figure 7 - Mean surface water dissolved oxygen (DO) of 4 centerline stations both prior to and after installation of the recovery boiler system. Each value represents an average between measurements taken at the surface and at a depth of 2 m using water quality data accumulated by mill environment staff.

DO



region consisted primarily of 3 species of oligochaeta, i.e., Lumbricillus lineatus, Monopylephorus cuticulatus, and M. rubroniveus.

Figure 8 summarizes the results of the community classification analysis. The high degree of similarity between sites adjacent to the receiving area (stations S6A and S6B) is clearly illustrated in the resulting dendrogram. In contrast, species composition between sites extending north from the outfall differs sufficiently to inhibit the formation of distinct clusters.

Thus, a division into two distinct zones is apparent, the first represented by stations within the gross impacted areas encompassing a region approximately 1.0 km around the discharge source, and the second by the remaining sites. The former region is characterized by Lumbricillus lineatus, an enchytraeid oligochaete recently found only in B.C. waters adjacent to 3 pulp mills (Coates and Ellis, 1980), and 2 tubificid oligochaetes, Monopylephorus cuticulatus and M. rubroniveus, often found in brackish waters and now found coexisting with L. lineatus under stressed environmental conditions. The gradual change in species composition between the more distant sites may be attributable to varying substrate type or to natural environmental changes occurring over the length of the inlet.

In addition to the analysis of Oligochaeta, and their appropriate habitats, intertidal rocky shore surveys provided valuable information as to the delimitation of invertebrate species and the extent of sulfite mill effluent impact in Neroutsos Inlet.

Diversity changes observed in large taxonomic groups (Figure

Figure 8 - Presence/absence matrix of mature marine Oligochaeta derived from 3 replicate cores taken at each station. Site by site (Q-analysis) similarity and single link cluster analyses show species associational patterns.

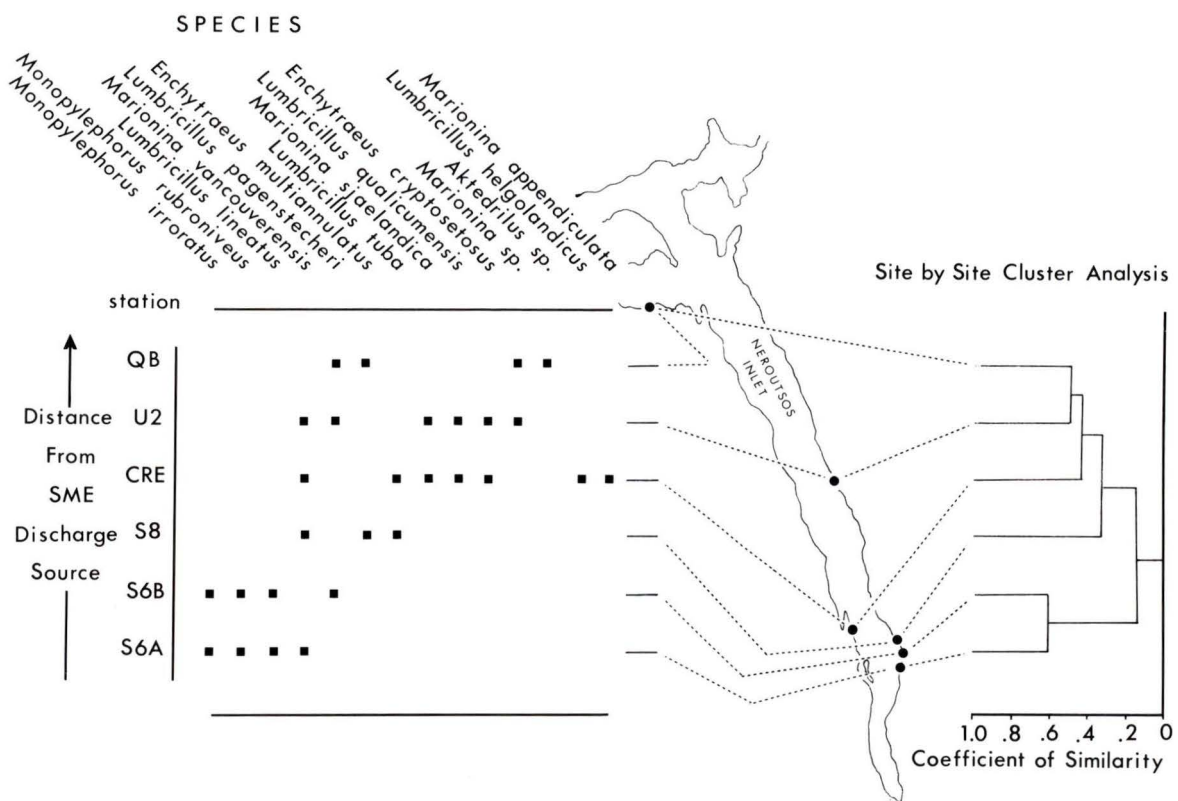
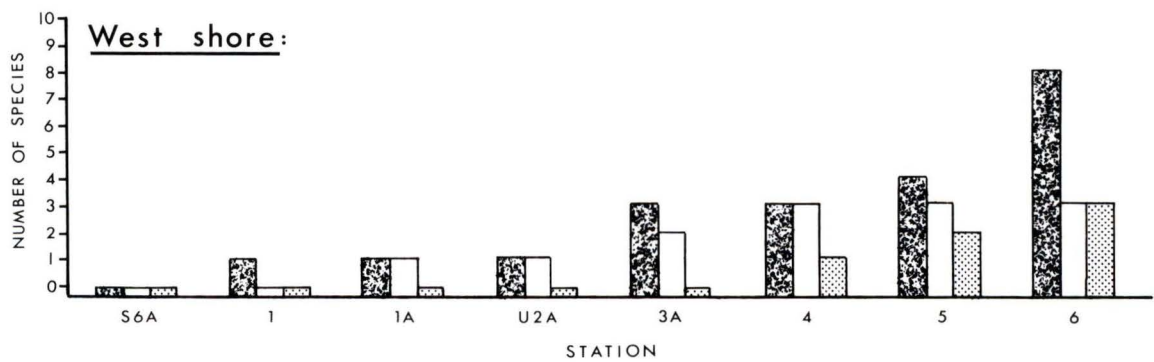
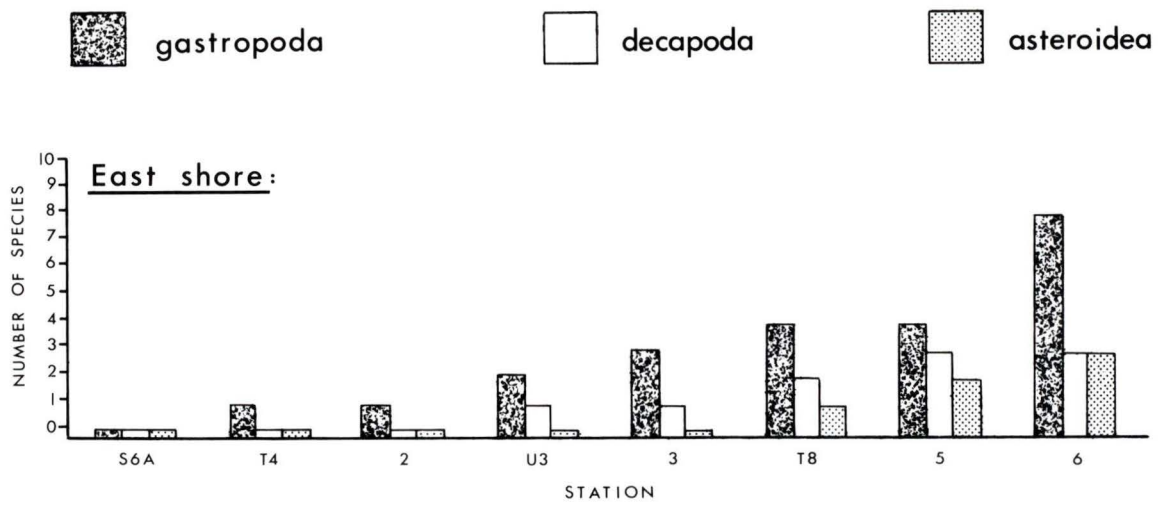


Figure 9 - Histogram plots showing intertidal rocky shore fauna diversity in 3 major taxonomic groups with respect to increasing distance (left to right) from the Port Alice pulp mill discharge source.



9) revealed a distinct increase in the number of gastropods, decapods, and asteroidean species as distance from the mill discharge increases.

Analysis of accumulated qualitative (species presence-absence) data with the similarity and single-link clustering techniques described in section 2.4, provided the basis upon which intertidal communities were compared and zones of SME influence estimated. Figures 10 and 11 summarize this analysis postulating five regions within the inlet, together representing a spectrum of responses to varying concentrations of the effluent as it is dispersed and diluted by tidal and current action.

Within Figure 11 area 'a' is characteristic of severe intertidal environmental impact and was dominated by 3 species of enchytraeid and tubificid oligochaetes (discussed above; Figure 8).

Area 'b' was recognizable by a lush growth of Enteromorpha which was present from the upper to lower-middle intertidal zones. This region supported a minimal amphipod community with moderate numbers of the species Allorchestes angusta and with incidental occurrences of Eogammarus and Anisogammarus where salinity regimes were favorable. The mussel Mytilus edulis, as well as single species of Isopoda, barnacles, and snails, were also present.

Region 'c' contained bands of the rockweed Fucus but with a depauperate associated epifaunal community. A. angusta was very abundant, and barnacle species were established, but were reduced in size as were the mussels. The shore crabs, Hemigrapsus nudus and H. oregonensis, were also present in this area.

Region 'd' regarded as the transition zone between the

Figure 10 - Dendrogram resulting from similarity and single link cluster analyses (without rotation) of Neroutsos Inlet rocky shore survey stations, based on accumulated qualitative species data. Shaded areas encompass sites of similar species composition and postulate geographical zones of SME influence within the inlet. First shaded region is based on results of the oligochaete analysis (Figure 8).

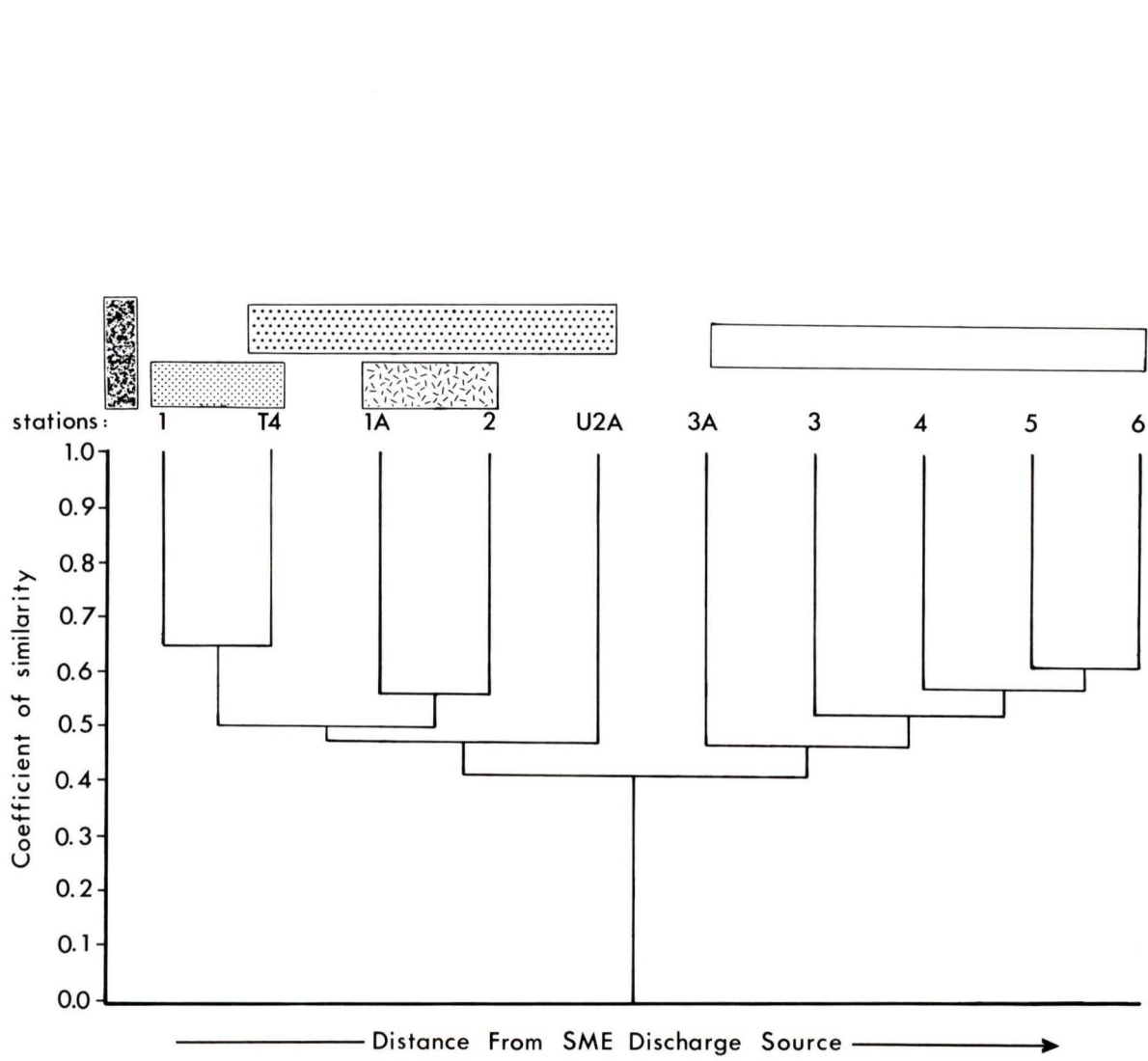
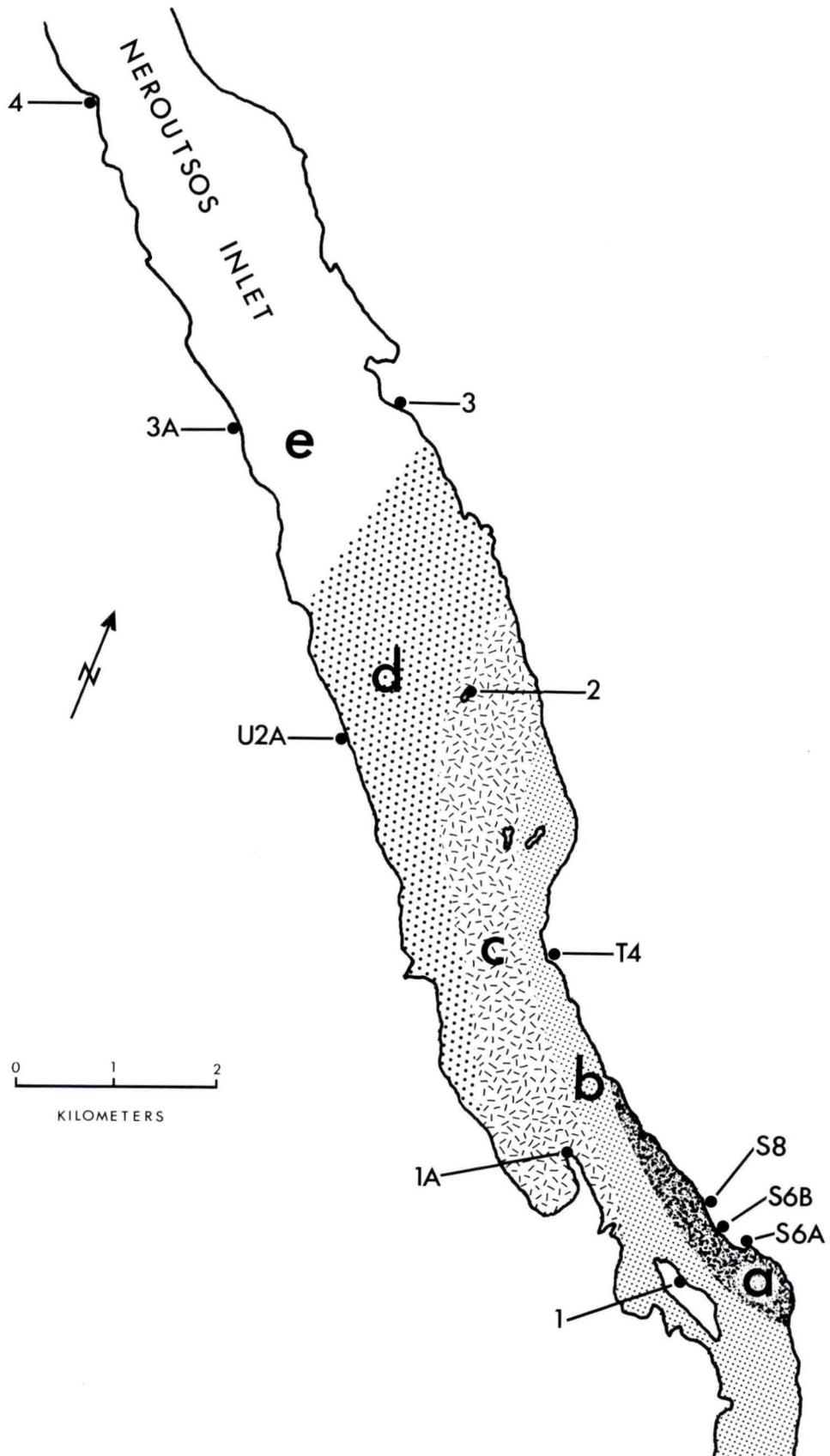


Figure 11 - Postulated zones of SME influence based on similarities between intertidal communities.



noticeably impacted shoreline stations towards the mill to the south and the "normal" intertidal shorelines extending north to Quatsino Sound, was represented by additional species of isopods, amphipods, decapods, and snails. Limpets were present, and the mussels and barnacles were not physically stunted. Enteromorpha remained within the intertidal community, but was no longer as noticeable.

A "normal" intertidal shoreline ecosystem, labelled as area 'e' in the figure, was defined as the region in which no impact could be distinguished. Additional algal species, e.g., Ulva, Cladophora, and Egregia, were present, and there was increased species diversity within each of the major groups of invertebrates.

Qualitative analysis of rocky shore intertidal communities was supplemented with regular color photographic recording for documentation of the mill impact. Since the implementation of pollution controls in 1977, this supplementary technique has documented a recolonization of Enteromorpha-dominated areas by Fucus. Plates D, E, and F exemplify this phenomenon at station U2 through the summers of 1978, 1979, and 1980, respectively. A more dramatic recolonization of Fucus was noted at station 1, where Enteromorpha totally dominated the area prior to the start-up of the SSL recovery boiler system in 1977.

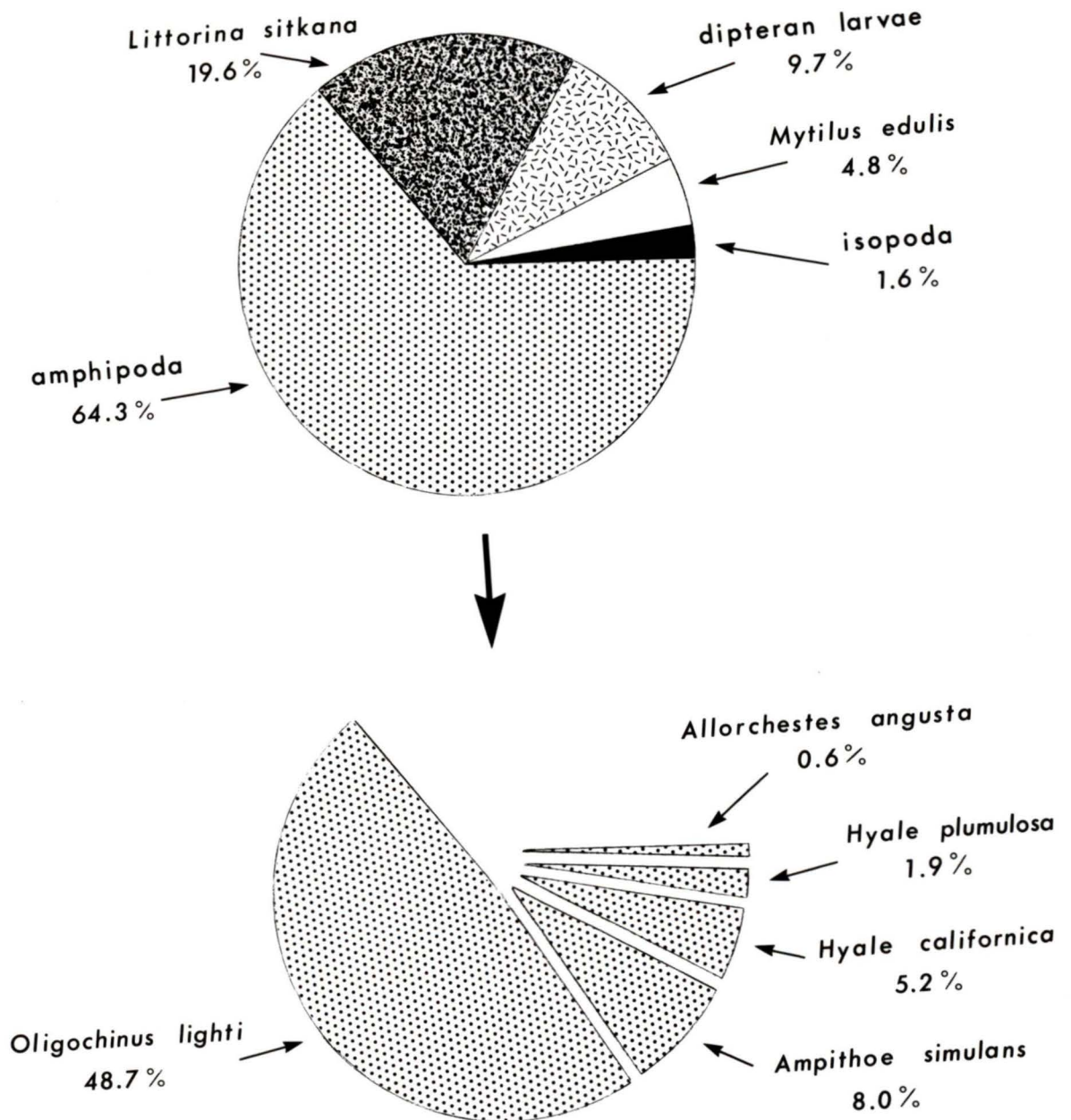
4.3 Fucus-epifauna Analysis

The analysis of Fucus-epifaunal communities as a natural substrate monitoring technique has been utilized by mill environment personnel since the pre-recovery monitoring began in 1975.

Figure 12 illustrates the numerical contributions of major

Figure 12 - Macro-epifauna of a 'control' Fucus habitat within Neroutsos Inlet; based on a summary of data collected at stations 5 and 6.

EPIFAUNA OF A 'CONTROL' FUCUS HABITAT
(contribution as % of total abundance)



macro-epifauna in a 'control' Fucus habitat within Neroutsos Inlet. Amphipods in general are shown to be the numerically dominant organisms, and were thus considered in detail for the E.R.A. shoreline monitoring program.

In 1978, routine collections and sorting of Fucus-epifauna was supplemented by species identifications of the gammarid amphipod component, previously enumerated and recorded as 'amphipods'. A summary of results from 1978-1981 are presented in Table 1.

As made evident in Figure 12 five species of gammarid amphipoda, dominated by Oligochinus lighti, were found to coexist in a 'control' Fucus habitat. However, further species associational analyses, encompassing Fucus collection sites closer to the mill, revealed an Allorchestes angusta monoculture within 9-10 km of the outfall, with replacement by the other 'control' species as distance from the SME discharge source increased.

Figure 13 shows 3 of the five Fucus-epifaunal amphipods found in Neroutsos Inlet. Oligochinus lighti and Ampithoe simulans numerically dominate the 'control' species assemblage, while Allorchestes angusta, alone, forms the amphipod component of Fucus habitats closer to the mill. Each has been drawn to a scale allowing physical size comparison between species.

Using the mean total number of amphipods found in 8-liter Fucus samples taken during June, July, and August, Table 2 illustrates numerical trends observed over the past 6 years. The extent of the A. angusta monoculture which was originally detected at stations 1A, 2, and 3 in 1978 was significantly reduced to encompass only

Table 1 - Fucus-epifauna amphipod data; 1978-1981. Averages from data collected June, July, and August of each year.

	<u>Station</u>							
	<u>1</u>	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>
<u>1978 Summary</u>								
<u>Ampithoe simulans</u>	-	0	0	0	1	80	31	3
<u>Oligochinus lighti</u>	-	0	0	0	0	5	51	70
<u>Hyale plumulosa</u>	-	0	0	0	0	0	0	20
<u>Hyale californica</u>	-	0	0	3	35	5	0	32
<u>Allorchestes angusta</u>	-	728	3541	1677	25	20	5	1
<u>1979 Summary</u>								
<u>Ampithoe simulans</u>	-	0	0	2	8	14	6	9
<u>Oligochinus lighti</u>	-	0	0	6	8	89	196	76
<u>Hyale plumulosa</u>	-	0	0	0	0	0	0	0
<u>Hyale californica</u>	-	0	0	8	40	0	1	16
<u>Allorchestes angusta</u>	-	1341	232	0	0	0	0	0
<u>1980 Summary</u>								
<u>Ampithoe simulans</u>	-	0	0	10	12	18	17	5
<u>Oligochinus lighti</u>	-	0	0	5	13	143	25	36
<u>Hyale plumulosa</u>	-	0	0	0	0	0	0	0
<u>Hyale californica</u>	-	0	0	4	12	1	1	2
<u>Allorchestes angusta</u>	-	2780	186	0	0	0	0	0
<u>1981 Summary</u>								
<u>Ampithoe simulans</u>	0	0	41	29	10	22	21	8
<u>Oligochinus lighti</u>	0	0	2	54	145	119	20	53
<u>Hyale plumulosa</u>	0	0	0	0	21	4	0	0
<u>Hyale californica</u>	0	2	126	50	74	7	1	4
<u>Allorchestes angusta</u>	2145	224	8	0	0	0	0	0

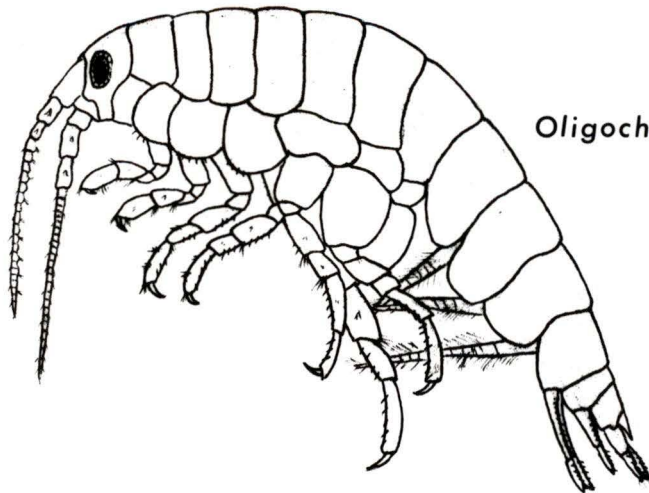
Figure 13 - Scale drawings of 3 common Fucus-epifaunal gammarid amphipoda. Oligochinus lighti is the numerically dominant form under 'normal' environmental conditions while Allorchestes angusta represents the smallest and least abundant species.

stations 1A and 2 in the following year. In 1980, maximum numbers of this species remained at station 1A, with station 2 showing a reduction to approximately 1/3 of that observed in 1979. During this period, control species of amphipods had begun to recolonize Fucus habitats closer to the mill, i.e., at station 3, and Fucus had started to displace Enteromorpha-dominated regions. By 1981 station 1, located on the northeast side of Ketchen Island, supported a substantial growth of Fucus and was consequently added as a Fucus-epifaunal sampling site. A point of maximum amphipod density, represented by a monoculture of the species Allorchestes angusta, had re-established itself at this site, and control species had moved into Fucus habitats as close to the mill as station 2.

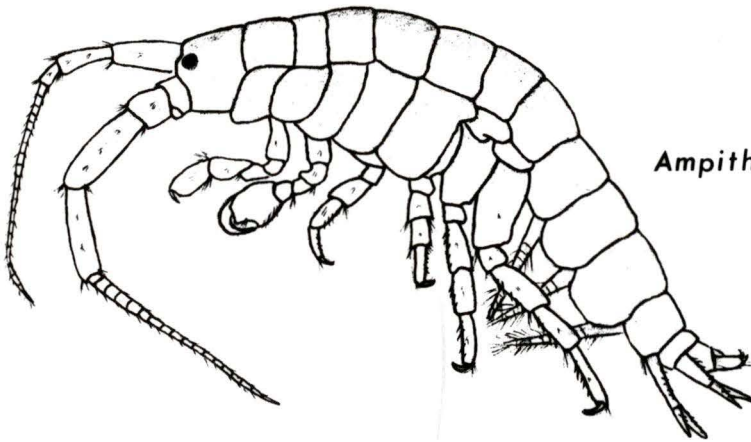
Pre-recovery Fucus-epifaunal data (Stoll, 1976; Stoll and Vandermuelen, 1977) provided a baseline index of mill impact with which results from subsequent years could be compared. The abundance of gammaridean amphipoda ('amphipods'), recorded in 1976 and 1977 were of 1-2 orders-of-magnitude greater at stations within 10 km of the mill, than at comparable control stations (Table 4). Comparison of these pre-recovery abundance data with that obtained in 1978-1981 suggests that abundant amphipods observed at stations 2 and 3 in 1976 and 1977 represented the Allorchestes angusta monoculture. Assuming that this speculation is valid, it can be further assumed that the point of maximum density of this species occurred at station 3 in both years.

In a 'control' situation, Oligochinus lighti frequently contributes 70-90% of the total number of amphipods found within the

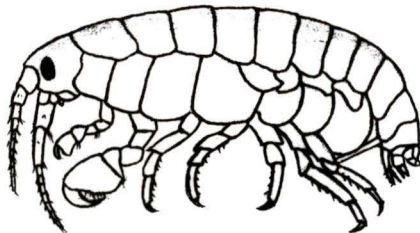
FUCUS- EPIFAUNAL AMPHIPODA

*Oligochinus lighti*

♂ 10 mm
 ♀ 8 mm

*Ampithoe simulans*

♂ 12 mm
 ♀ 9 mm

*Allorchestes angusta*

♂ 4 mm
 ♀ 3.5 mm

1 mm

Table 2 - Total number of amphipods/8 l. of Fucus. Average taken from data collected June, July and August of each year.

station	<u>year</u>					
	1976	1977	1978	1979	1980	1981
1	-	-	-	-	-	2145
1A	-	-	728	1360	2788	224
2	378	851	3541	232	186	67
3	3426	1495	1680	16	18	55
4	20	32	110	103	161	176
5	45	59	87	203	43	40
6	55	141	126	101	44	64

installation of
pollution controls

stations 1A and 2 in the following year. In 1980, maximum numbers of this species remained at station 1A, with station 2 showing a reduction to approximately 1/3 of that observed in 1979. During this period, control species of amphipods had begun to recolonize Fucus habitats closer to the mill, i.e., at station 3, and Fucus had started to displace Enteromorpha-dominated regions. By 1981 station 1, located on the northeast side of Ketchen Island, supported a substantial growth of Fucus and was consequently added as a Fucus-epifaunal sampling site. A point of maximum amphipod density, represented by a monoculture of the species Allorchestes angusta, had re-established itself at this site, and control species had moved into Fucus habitats as close to the mill as station 2.

Pre-recovery Fucus-epifaunal data (Stoll, 1976; Stoll and Vandermuelen, 1977) provided a baseline index of mill impact with which results from subsequent years could be compared. The abundance of gammaridean amphipoda ('amphipods'), recorded in 1976 and 1977 were of 1-2 orders-of-magnitude greater at stations within 10 km of the mill, than at comparable control stations (Table 4). Comparison of these pre-recovery abundance data with that obtained in 1978-1981 suggests that abundant amphipods observed at stations 2 and 3 in 1976 and 1977 represented the Allorchestes angusta monoculture. Assuming that this speculation is valid, it can be further assumed that the point of maximum density of this species occurred at station 3 in both years.

In a 'control' situation, Oligochinus lighti frequently contributes 70-90% of the total number of amphipods found within the

Fucus habitats. Since the SSL discharge reduction in 1977, a movement of this control amphipod assemblage towards the mill has been documented. The delimitation of O. lighti, and the other control species, may be related to the effluent loadings to the inlet. In addition, measureable sublethal effects of SME may be evident within these populations. A number of morphological parameters of O. lighti were thus recorded and related to distance from the effluent source.

Figure 14 gives body-length/frequency histogram plots for Oligochinus lighti specimens found at stations 3, 3A, 4, 5, and 6. Stations 5 and 6 show no apparent difference between the modal classes of individuals at these sites. Stations 3, 3A, and 4, however, reveal a decrease in body length with a shift in the modal class from 5.5 to 4.5 mm. A statistically significant difference in mean body length between sampling sites was demonstrated using a one-way ANOVA ($P < 0.05$). Subjectively, the mean body length of O. lighti individuals decreased steadily as distance between the sample site and the mill was reduced, i.e., 5.73 mm at station 6 to 3.98 mm at station 3. However, this trend is supported statistically (Student Newman-Keuls multiple range test) only between groups of sites, e.g., station 6 not significantly different from station 5, station 4 not significantly different from station 3A, but these site pairs illustrating a statistically significant difference.

Figure 15 gives the brood size of Oligochinus lighti specimens found at stations 3A, 4, and 6, as a function of body length. In each case a linear regression reveals a significant positive correlation between these variables (slopes tested with a

Figure 14 - Histograms of Oligochinus lighti frequency as a function of body length at 5 Fucus collection sites in Neroutsos Inlet. Distance from mill decreases from top to bottom. Sample size (n) for each site is as follows: $n_6=62$; $n_5=39$; $n_4=162$; $n_{3A}=294$; and $n_3=49$.

Oligochinus light

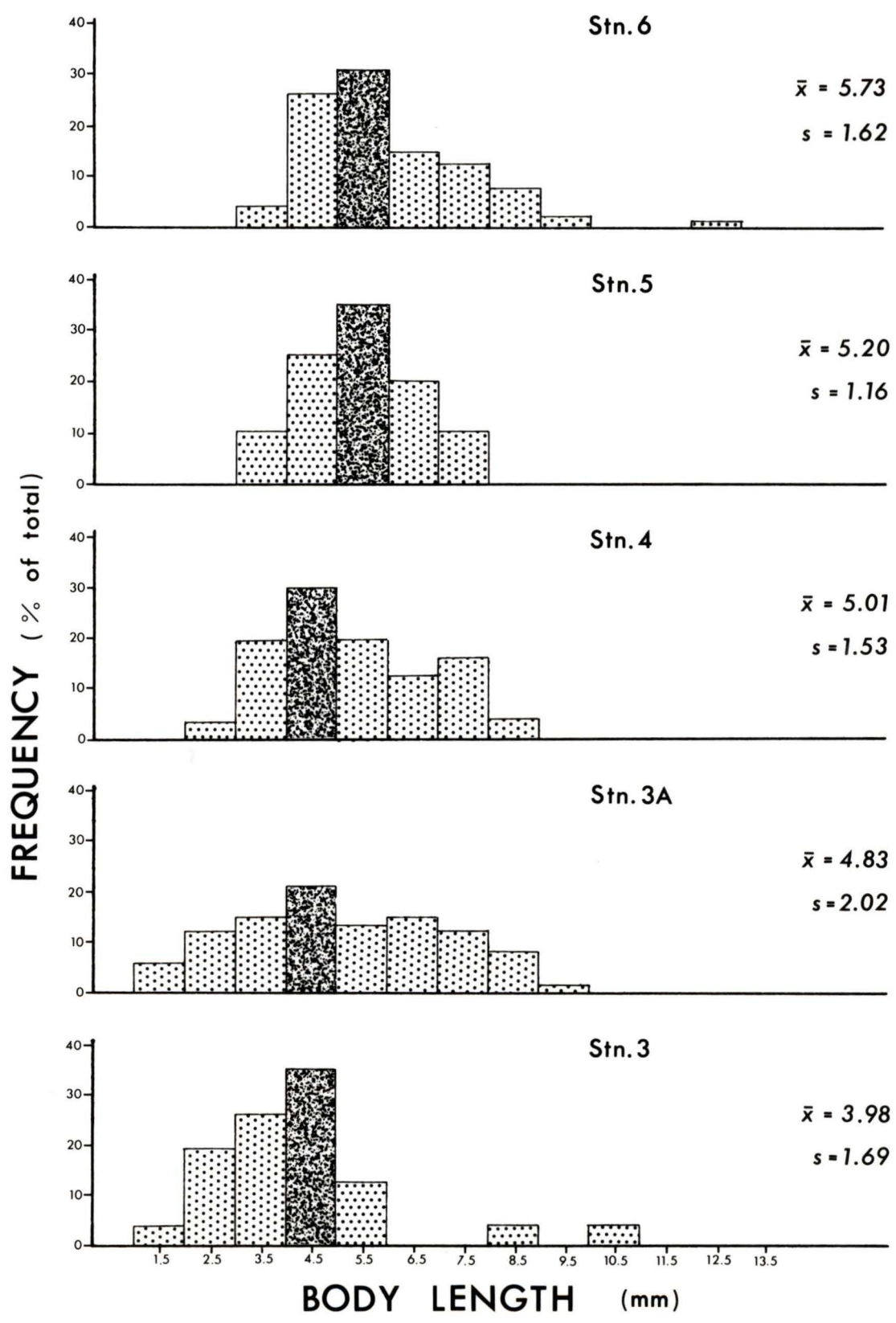
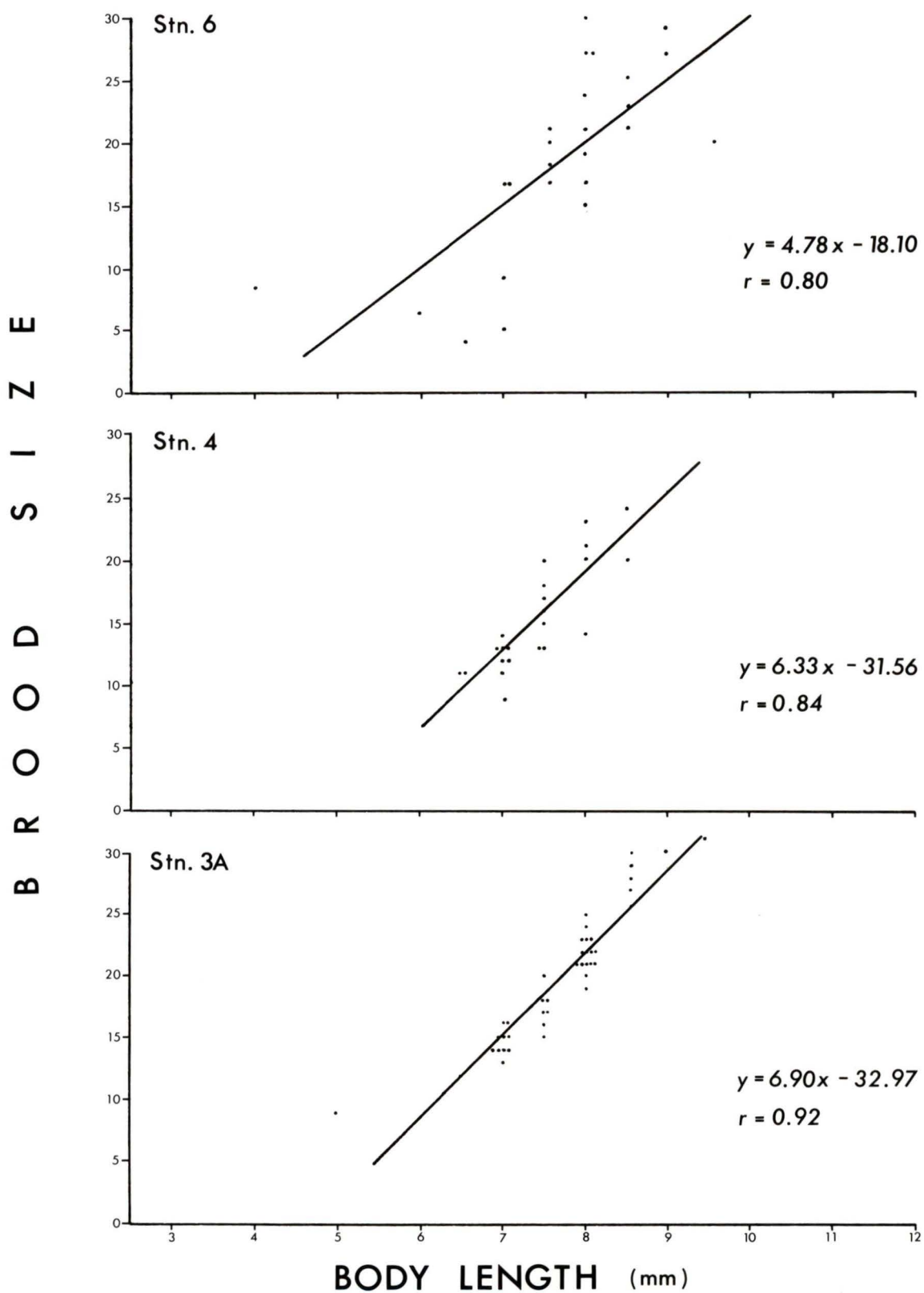


Figure 15 - Oligochinus lighti brood sizes as a function of body length at 3 Fucus collection sites in Neroutsos Inlet. Each slope function is significantly different from zero (Student's t test; $P < 0.01$). Slope functions are not significantly different between sites (ANCOVA; $P > 0.05$).

Oligochinus lighti

Student's t statistic; $P < 0.01$), but with non-significant differences found between these sites (slopes compared with an ANCOVA).

4.4 Gammarid Amphipod Bioassays

The gammarid amphipods Oligochinus lighti and Allorchestes angusta were chosen as the test organisms for the SME bioassays in an attempt to relate observed numerical and positional changes of their respective populations to the improved water quality noted since installation and implementation of the SSL recovery boiler system.

A laboratory population of A. angusta was successfully maintained, with sufficient numbers of specimens made readily available for SME toxicity tests. An effort to do the same with O. lighti, however, provided a less stable culture of animals, and consequently test specimens were acquired from the field prior to the bioassays.

The toxic response for each of the amphipod species was tested concurrently to eliminate possible variability between effluent batches, and thus allow comparison of test results. Table 5 summarizes mill operating conditions and characteristics of the SME sample used in the 96h-LC50 bioassay.

The SME toxicity curves for A. angusta and O. lighti under pH adjusted and pH unadjusted conditions are shown in Figure 16 and Figure 17, respectively. Each point on these graphs represents the LC50, with its 95% confidence limits, estimated at each observation period using accumulated mortality over the 5 test concentrations (with 3 replicates). The toxic response of each species followed an

Table 3 - Operating conditions of mill and characteristics of effluent sample used in the 96h-LC50 bioassays.

Operating Conditions:

process	Rayonex
pulp yield	433 air dry metric tonne·d ⁻¹
discharge	
flow-rate (m ³ ·d ⁻¹)	106,400(s.s.)* ; 41,500 (n.s.)*

Sample Characteristics:

sample period	0900h(16-7-81) - 0900h(17-7-81)
discharge composition	0.75s.s / 0.25n.s.
quantity	5 gallons
color	yellow/gold
odor	woody/burnt; sweet/resin
pH	2.9(s.s.); 3.1(n.s.)
TSS (mg·L ⁻¹)	167(s.s.); 161(n.s.)
conductivity (uMHOS @ 15°C)	1350
resin acid soaps (ppm)	12.73

* s.s. = south discharge sewer; n.s. = north discharge sewer.

Figure 16 - Whole sulfite mill effluent (SME) toxicity curves for Allorchestes angusta. Points represent LC50 estimates calculated at each observation period, with error bars indicating 95% confidence limits based on data accumulated from 3 replicate bioassays run concurrently. Each experiment included a series of pH unadjusted and a series of pH adjusted (to 7.8) test concentrations. All tests were terminated at 96 h.

SME TOXICITY CURVES

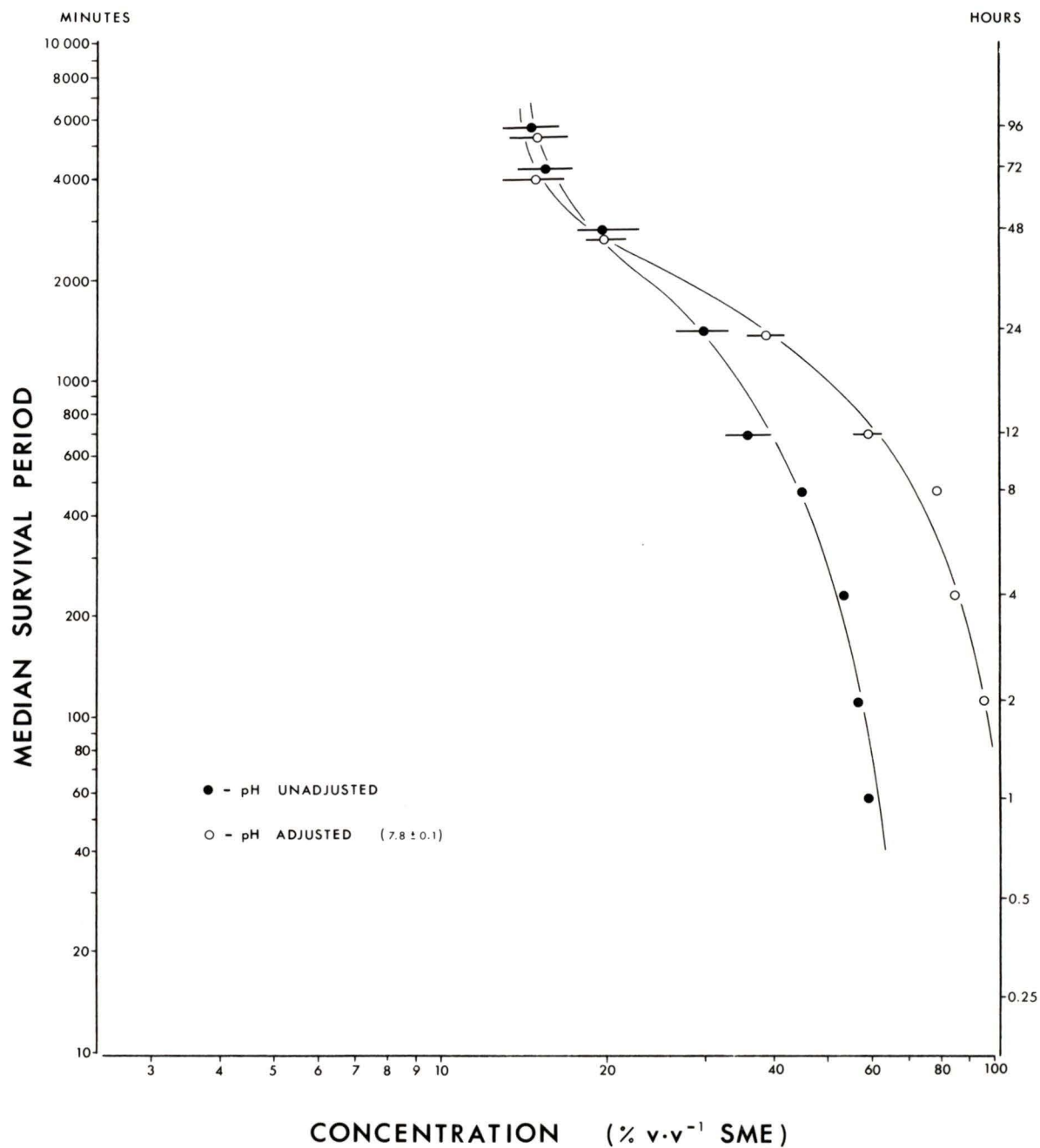
Allorchestes angusta

Figure 17 - Whole SME toxicity curves for Oligochinus lighti. Points represent LC50 estimates calculated at each observation period, with error bars indicating 95% confidence limits based on data accumulated from 3 replicate bioassays run concurrently. Each experiment included a series of pH unadjusted and a series of pH adjusted (to 7.8) test concentration. All tests were terminated at 96 h.

SME TOXICITY CURVES

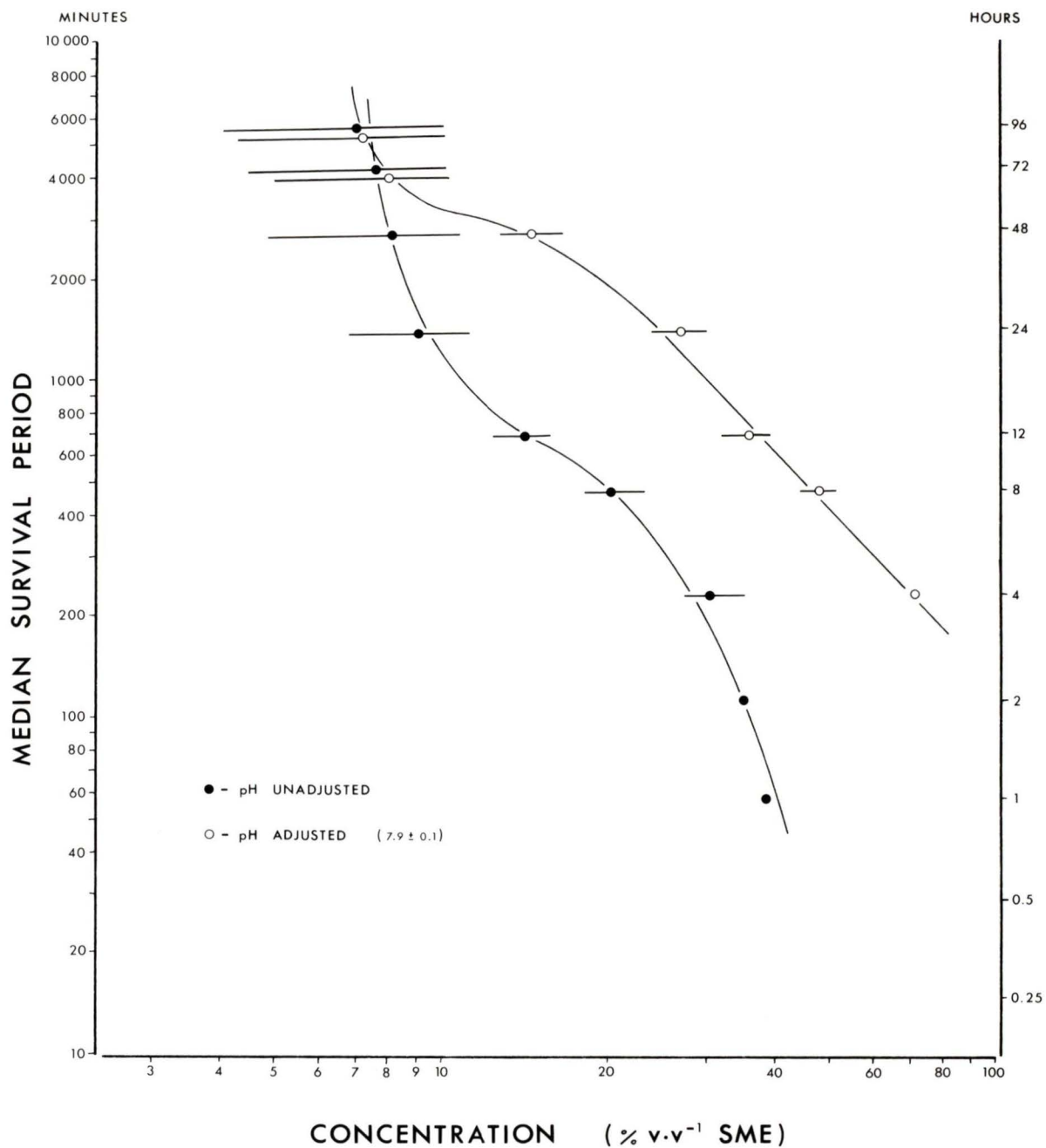
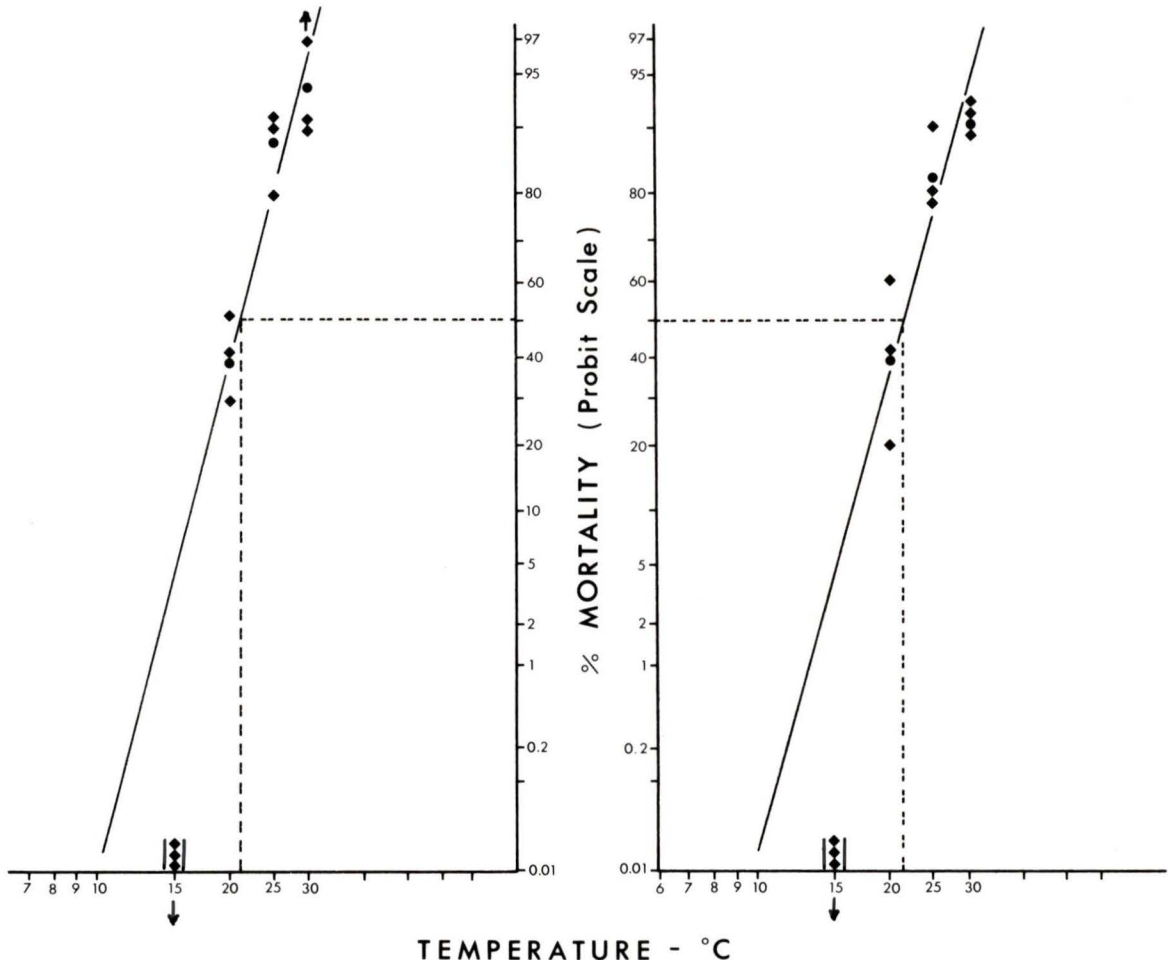
Oligochinus lighti

Figure 18 - Log/Probit regression figures for 96h acute lethal study of temperature tolerance in Allorchestes angusta and Oligochinus lighti. Descriptive statistics from the computer analysis, including the 96h-LC50, are provided for each species. Arrows refer to values corresponding to 0 or 100% response, i.e., probits of negative and plus infinity respectively. Points within parallel vertical bars are of equal value.

Allorchestes angusta

Oligochinus lighti



TEMPERATURE - °C

● MEAN MORTALITY
◆ REPLICATE MORTALITY

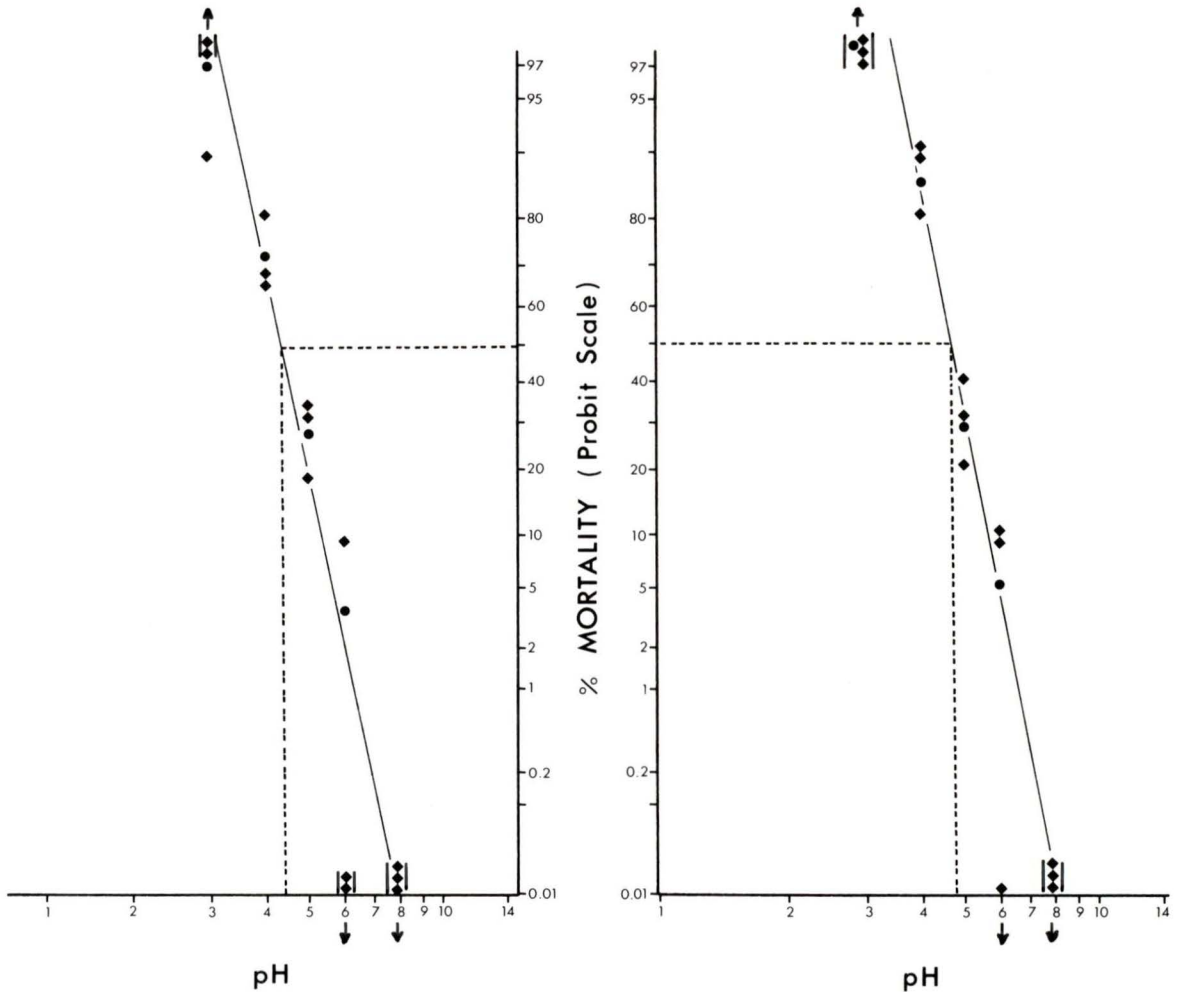
SLOPE (B)	12.8831
INTERCEPT (A)	-12.1130
VARIANCE OF SLOPE	10.9886
CHI-SQUARE	1.0661
DEGREES OF FREEDOM	3
LOG. LC50	1.3283
VARIANCE OF LOG. LC50	0.4499E-03
96h LC50	21.2973
VARIANCE OF LC50	1.0010

SLOPE (B)	11.6292
INTERCEPT (A)	-10.5156
VARIANCE OF SLOPE	8.8502
CHI-SQUARE	1.2572
DEGREES OF FREEDOM	3
LOG. LC50	1.3342
VARIANCE OF LOG. LC50	0.4978E-03
96h LC50	21.5872
VARIANCE OF LC50	1.0011

Figure 19 - Log/Probit regression figures for 96h acute lethal study of pH tolerance in Allorchestes angusta and Oligochinus lighti. Descriptive statistics from the computer analysis, including the 96h-LC50, are provided for each species. Arrows refer to values of 0 or 100% response, i.e., probits of negative and plus infinity respectively. Points within parallel vertical bars are of equal value.

Allorchestes angusta

Oligochinus lighti



● MEAN MORTALITY
◆ REPLICATE MORTALITY

SLOPE (B)	-12.4222
INTERCEPT (A)	130.132
VARIANCE OF SLOPE	9.7140
CHI-SQUARE	0.2406
DEGREES OF FREEDOM	3
LOG. LC50	0.6451
VARIANCE OF LOG. LC50	0.4528E-03
96h LC50	4.4165
VARIANCE OF LC50	1.0010

SLOPE (B)	-15.4195
INTERCEPT (A)	153.517
VARIANCE OF SLOPE	17.4905
CHI-SQUARE	0.1402
DEGREES OF FREEDOM	3
LOG. LC50	0.6713
VARIANCE OF LOG. LC50	0.3394E-03
96h LC50	4.6917
VARIANCE OF LC50	1.0009

asymmetrical sigmoid curve with the upper portion becoming asymptotic with the y-axis, suggesting that acute mortality was over, i.e., that a mortality threshold or incipient lethal LC50 had been reached. For each species toxicity curves plotted for the pH adjusted and the pH unadjusted bioassays converged to the same mortality threshold. A. angusta revealed an insipient lethal LC50 of approximately 13.5% volume/volume (v/v) SME while that for O. lighti was estimated at 7.3% v/v SME. No overlap of the 95% confidence intervals between the asymptotic portion of these curves is apparent, suggesting a significant difference (P 0.05) in the incipient lethal responses of these two species.

Figure 18 summarizes the tolerances of A. angusta and O. lighti to increases in temperature, as estimated by their acute lethal responses. The 96h-LC50 for A. angusta was estimated at 21.3°C (SD=1.0) while that for O. lighti was approximated at 21.6°C (SD=1.0). Comparison of these values suggests that there is no significant difference between the upper temperature tolerance limits of these two amphipod species.

Figure 19 establishes the lower pH tolerance limit for each of the test amphipods. For A. angusta the 96h-LC50 was 4.4 (SD=1.0) and for O. lighti it was 4.7 (SD=1.0). Again, no significant difference between these values is apparent.

5. DISCUSSION

The Canada Fisheries Act stipulates that the discharge of "deleterious materials" into waters "frequented by fish" is prohibited, with proof of such an effect not a requirement for prosecution by appropriate government agencies. Consequently, the direct and indirect effects of discharged pulping wastes represents one of the major Canadian environmental concerns due primarily to the tremendous volumes discharged (Walden and Howard, 1977).

The chemistry of the sulfite digestion of cellulosic materials is extremely complicated, and the disposal of spent sulfite liquor (SSL) is a potentially serious pollution problem since "more than 50% of the raw materials entering the process appear in the waste liquor as dissolved organic solids" (Shreve and Brink, 1977).

The high oxygen demand of these wastes makes it difficult to measure the toxic effects of the constituent components. Leach and Thackore (1973), however, revealed that the major compounds toxic to fish are resin acids, i.e., abietic, dehydroabietic, isopimaric, levopimaric, palustric, pimaric, sandaracopimaric, and neoabietic acids. Brownlee and Strachan (1977) showed that dehydroabietic acid, present in mill effluent in excess of 1 mg/l, was a persistent organic compound in the receiving waters of a kraft pulp mill and a good indicator of the areal influence of the effluent. Secondary toxic compounds, accounting for approximately 20-50% of the toxicity of a pulping sample, were derived from lignin degradation products, i.e., eugenol, isoeugenol, and 3,3'-dimethoxy-4,4'-dihydroxystilbene (Walden and Howard, 1977).

The environmental rehabilitation assessment of the receiving waters influenced by the Port Alice mill effluent was initiated to determine the effects of removal of spent sulfite liquor (SSL) from the waste stream. In addition to eliminating the toxic organic constituents associated with the SSL from the effluent discharged, a high BOD fraction of the waste was also removed. In response to the decreased oxygen demand on the receiving waters a significant increase in the available dissolved oxygen was observed within the water column. Davis (1975), in a review of the minimal dissolved oxygen requirements of aquatic life, pointed out the extreme interspecific variability in DO requirements and sensitivities. He also illustrated how toxic agents in conjunction with low oxygen, usually found in areas polluted with industrial wastes, can have an additive effect on resident populations.

Thus the direct abiotic benefits of SSL removal from Neroutsos Inlet may be species specific, i.e., a reduced toxicity, an increase in DO to a level above that of the lower tolerance limit of the species, or the additive effect of both of these factors.

Other abiotic factors will most certainly have an additional influence on the process of biological rehabilitation within the inlet. Reduced color in the surface waters for example, will directly affect light penetration and thus the primary productivity. A reduction in the loadings of suspended solids will enhance this effect, and also alleviate respiratory stress on migratory or resident populations of fish (Werner, 1978; Hughes, 1975).

The study undertaken here does not attempt to determine

definitively the cause-effect relationships in the biological rehabilitation process, but instead uses a number of biomonitoring techniques to define the extent of environmental damage, and subsequently to estimate the biological changes that occur in response to a reduced pollutional stress.

5.1 Rocky Shore Survey Method for Biomonitoring

Analysis of the qualitative rocky shore survey data has provided valuable information on both the delimitation of species with distance from the mill discharge source and thus the geographical zone of influence the waste sulfite has on the respective shorelines within Neroutsos Inlet. The results illustrate the tendency of the sulfite effluent to affect the east side of the inlet for approximately 10 km and to have a lesser effect on the west side of the inlet. The technique, although qualitative and of a 'reconnaissance' nature, provided baseline information as to the extent of environmental damage within the inlet.

5.2 Marine Oligochaeta as Biomonitoring

Marine oligochaete distributions have been used as an indicator of gross levels of SME pollution in this study. A region of shore within 1-2 km of the mill discharge is characterized by an assemblage of oligochaete species including Lumbricillus lineatus, Monopylephorus cuticulatus, and M. rubronivius.

Annelids, in general, have proven to be a very important benthic invertebrate group in regards to numerical dominance, biomass, etc., and to play an equally important role in assessing various degrees of water quality. Their use in the monitoring or the

assessment of water pollution, however, has been restricted primarily to studies involving the analyses of such variables as species composition, relative abundances, or of indicator species or of indicator communities. Examples of such approaches, utilizing enchytraeid and tubifid oligochaetes, include works by Brinkhurst and Kennedy (1965), Milbrink (1973), and Lang (1978). These papers, and others reporting similar situations, describe the delimitation of species in relation to distance from a pollutant discharge source.

Limitations as to the use of specific organisms as indicators of pollution must be realized. In many situations a single species found in abundance at an extremely polluted site may also occur in limited numbers at cleaner sites. Thus, to consider the mere occurrence of an organism as a reliable indicator of a perturbed environment, one should also be familiar with the morphological adaptations, physiological requirements, and the biological associations of that organism (Gaufin, 1973).

Lumbricillus lineatus has been recently found in B.C. waters only adjacent to three pulp mills (Coates and Ellis, 1980). The two species of Monopylephorus, although reported to be present in relatively unpolluted brackish water environments (Baker, 1981), was present only within the grossly impacted mill area at Port Alice. Therefore, at least within the confines of this study, this oligochaete species assemblage can be used as a pollution indicator group.

In reference to the E.R.A. it was shown that L. lineatus has been replaced by other oligochaete species since a 1976 sampling

(Coates and Ellis, 1980). This exchange coincides with the implementation of the pollution controls in 1977, and supports the initial hypothesis of a progressive shift of a diverse oligochaete community closer to the mill with an improvement in discharge quality.

Lack of observable changes in oligochaete species composition at sites closer to the mill may be attributable to one, or a number of possible factors. For example, an increased time lag in observable successional processes is likely to be more evident in areas of high impact than in areas of moderate or slight impact. Thus, a continued sampling program may not reveal significant changes for perhaps up to 5 years. A second consideration is that the present pollution controls are adequate for a substantial portion of the affected inlet shoreline, but that the effluent presently discharged remains toxic, either directly or indirectly, to all but the very tolerant species which are capable of existing within the grossly impacted area.

Chapman et al. (1982a) showed that Monopylephorus cuticulatus was very tolerant to 4 environmental factors (pH, temperature, salinity, and anoxia) and to 5 different pollutants (Cd, Hg, pentachlorophenol, pulp mill effluent, and sewage sludge). Of the 14 species tested this species was consistently the most tolerant, emphasizing its usefulness as a possible indicator species.

Tolerance of oligochaetes to organic effluents has been linked to the hypothesis that they can survive extended periods in anaerobic conditions. Chapman et al. (1982a) found that M. cuticulatus displayed a mean survival of 42 days under completely anoxic conditions. This value was twice that observed in the other

oligochaete species tested.

Tolerances of selected oligochaetes to a combination of pollutant and environmental factors have recently been studied by Chapman et al. (1982b). M. cuticulatus was again shown to be the most tolerant, with a reduction in black liquor (kraft pulping effluent) tolerance at lower pH and temperature regimes. Cross (1980) determined 96h-LC50 values for M. cuticulatus in relation to SSL and SME and found that the tolerances were decreased with salinity and pH, but not when temperature was increased.

In this study marine oligochaete populations have been effective biological indicators of gross levels of SME pollution. Their use as biomonitors of shoreline rehabilitation processes, however, has been far less productive. This contrast is most probably due to the fact that oligochaetes, in general, show an extreme range of tolerance to both environmental factors (e.g. anoxia, salinity, and pH) and various types of pollutional stresses.

In addition to this inherent physiological tolerance, a degree of physiological adaptation within resident populations is also apparent. Cross (1980) revealed a significantly greater acute lethal response (96h-LC50) in specimens of Monopylephorus cuticulatus collected at Port Alice than in those obtained from an unpolluted site in the Victoria area. Ferguson and Bingham (1966) provide evidence that genetic selection in natural systems results from the impact of chemicals. Although the actual mechanism for selection as a result of exposure to a pollutant is unknown, rates of mutation in some unicellular organisms, for example, are known to increase as a

result of such an exposure (Ames, 1979).

Consequently, the choice of marine oligochaete populations as a biomonitoring technique alone may not be appropriate for the type of assessment involved in this study. The resistance and apparent adaptation of these organisms to the effluent discharged limits their usefulness, considering the extent of environmental change which would be necessary to elicit an observable oligochaete community response over a logistically feasible monitoring period.

5.3 Fucus Amphipoda as Biomonitorers

The third biomonitoring technique employed in the Port Alice E.R.A. involved Fucus-epifaunal amphipoda. Restriction of amphipod populations from within the grossly impacted region around the mill, i.e., a 1-2 km radius, and regular occurrence within suitable habitats extending into Quatsino Sound, provided a group of organisms which were sensitive to moderate and low levels of effluent. This technique has proven to be the most successful in documenting shoreline rehabilitation processes within Neroutsos Inlet since the installation of the recovery boiler system.

As was shown by Read et al. (1978) with intertidal macrobenthic communities along a gradient of increasing pollution, the diversity and species richness of the amphipod community within Neroutsos Inlet decreased while numerical dominance increased in appropriate Fucus habitats closer to the mill. The reduced species richness and corresponding dominance increase was represented by the single amphipod species, Allorchestes angusta.

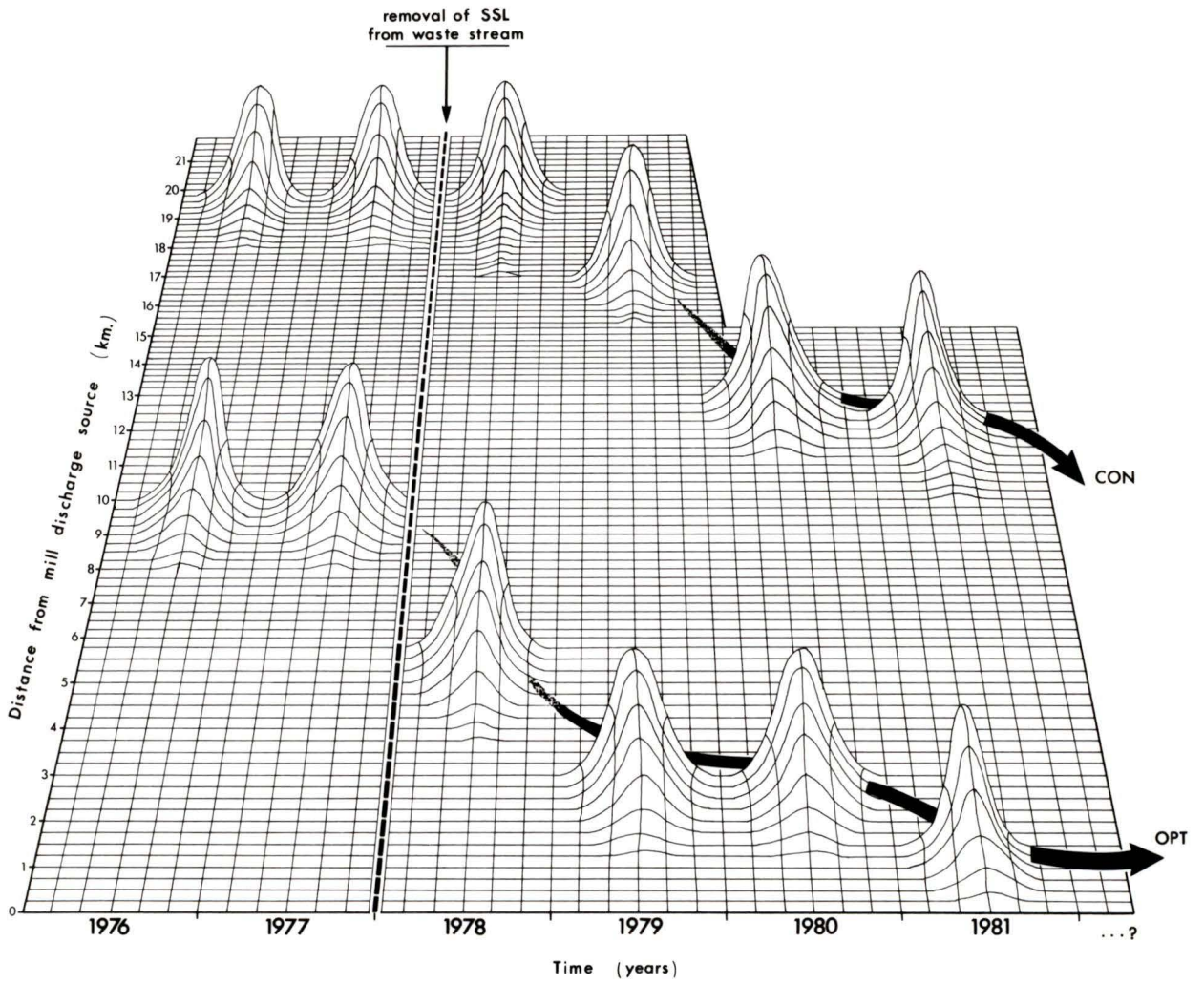
Pearson and Rosenberg (1978) suggested that the abundance of

an 'opportunistic' species at a moderate level of pollution results from the effects of organic enrichment. The maximum in abundance has been termed the 'peak of opportunists' (PO) and the change in the 'condition' of a polluted region may be assessed by recognizing changes in the PO-point with respect to time, i.e., if the PO-point moves away from the discharge source during successive sampling periods pollution is increasing, and if the PO-point approaches the discharge source, pollution is decreasing.

Opportunistic species are usually short-lived and show rapid seasonal variations with the PO-point becoming reduced in amplitude or even disappearing in response to environmental fluctuations (Pearson and Rosenberg, 1978). A single winter sampling revealed such a response in Allorchestes angusta. The abundance of this species remained low until June when numbers again increased to a maximum by August.

Working with this concept, a generalized trend for the PO-point with respect to time and seasonal fluctuations can be effectively portrayed graphically as in Figure 20. During the pre-recovery period (1976-1977) the PO-point remained in equilibrium at station 3 approximately 10 km from the mill. Since the implementation of pollution controls on the marine discharges from the Port Alice mill, the peak of opportunists has moved progressively closer to the mill. The 1980 survey revealed a stabilization of the PO-point at station 1A (2 km from the mill), a temporary position which was relocated closer to the mill in 1981 following the recolonization of appropriate rocky shores by suitable Fucus

Figure 20 - Generalized seasonal fluctuations (1976-1981) in abundance for the opportunistic (OPT) amphipod Allorchest-
es angusta and for the control (CON) amphipod assemblage
(characterized by Oligochinus lighti) with respect
to distance from the SME discharge source.



habitats. This supports the initial hypothesis and suggests a significant decrease in the effect of SME on the Fucus-epifauna within this region.

Figure 20 also reveals the movement of a second group of amphipods (CON) which is dominated by Oligochinus lighti, yet is representative of most of the 'control' species found within Neroutsos Inlet. A time lag of one year following the initial movement of the PO-point (for A. angusta) was observed with this group, i.e., no apparent movement in 1978. Stabilization of the group maximum at approximately 13 km from the mill was reached in 1980 and maintained through the 1981 sampling season.

There are few studies involving amphipod species as indicators of water quality conditions, particularly with respect to pulping effluents. Greer and Futer (1979) examined the macroinvertebrate recolonization of Fucus-filled mesh bags placed at varying distances from the Port Alice mill site. At a distance representative of moderate SME levels Allorchestes angusta dominated the Fucus community, supporting the findings of this study.

Harger and Nassichuk (1974) found large numbers of amphipods under rock cover close to the kraft mill at Port Mellon, while Waldichuk and Bousfield (1962) reported a profuse abundance of amphipods, primarily Allorchestes angusta, in the low oxygen (0.04-0.86 mg/l) surface waters adjacent to the Watson Island sulfite pulp mill near Prince Rupert, B.C.

Information available on amphipod distribution in relation to other pollutant sources is fragmentary. Barnard (1958) implicates

gammarid amphipods as an important group in marine fouling communities, and relates observed distributions to detrital-based turbidity resulting from domestic and industrial wastes. A recent paper by Bellan-Santini (1980), compiled 18 years of amphipod composition and distribution data collected from the French Mediterranean coast, and found species richness to be inversely related to the degree of pollution. Although no characteristic group of species was linked to areas affected by pollution, grazers were excluded by more severe perturbations and in some cases were replaced by suspension feeding types. Reish and Barnard (1979) provide a review of the use of amphipods in pollution ecology, illustrating the lack of information presently available and exemplifying the need to attain additional knowledge on this topic in view of the apparent sensitivity of this group to environmental changes.

Lethal and sublethal toxicity estimates have formed the basis for studies incorporating amphipod species and pollutants. Such studies have tested the toxicity of chloramine (Arthur and Eaton, 1971), detergents (Arthur, 1970; Arthur et al., 1974), power plant cooling effluents (Ginn et al., 1974), polychlorinated biphenyls (Wildish, 1970, 1972; Wildish and Zitko, 1971), petroleum oils (Lee et al., 1977; Lindén, 1976a, 1976b; Percy, 1976, 1977), and heavy metals (Arthur and Leonard, 1970).

Toxicity studies of pulping effluents utilizing gammarid amphipods are virtually non-existent. Levings et al. (1976) estimated the sensitivity of two estuarine species to varied salinity and temperature regimes and to bleached kraft mill effluent. BKME and

salinity were found to elicit a synergistic response in the organisms tested.

Acute lethal responses of Allorchestes angusta and Oligochinus lighti to SME were determined in the Port Alice E.R.A., not as an attempt to explain definitively the distributional movements of these species within Neroutsos Inlet, but to provide the basis upon which future investigation could elucidate the causative factors involved. Results of the 96h-LC50 bioassays clearly demonstrate a significant difference between the incipient lethal responses of these two species. Although provisional at best, these results nevertheless support the inferences made from field distribution data.

Results obtained from the Port Alice E.R.A. have proven the Fucus-epifaunal amphipod species to be the most useful biomonitors of shoreline rehabilitation within the inlet. The use of this technique may be important in other marine receiving waters, particularly if considered as one of several procedures, together satisfying a number of criteria which should be met in establishing a suitable test system. Included in these criteria (NRC, 1980) are that: (i) the test system be capable of replication by other laboratories; (ii) the procedure have sufficiently abundant source material to allow appropriate statistical analyses; (iii) the procedure be as economical and uncomplicated as possible while retaining realistic characteristics; (iv) it provide reliable data (validated with field studies) for mathematical prediction models; (v) it be capable of predicting effects for a wide range of pollutants; (vi) it be capable of being standardized for interlaboratory comparisons; and (vii) it

should be as realistic as possible, incorporating data records of behavioral responses for both disturbed and undisturbed situations so that reliable predictions can be made as to environmental condition.

With reference to these criteria amphipods are recommended as appropriate biomonitors, at least as one component of an ecotoxicological test system, for several reasons. Amphipods have a wide geographical distribution, and are found both in freshwater and in the marine environment from the abyssal depths to the infra-littoral fringe. In particular they are representative of the plankton and benthos in coastal waters where pollution problems are most evident and of immediate concern.

Amphipods have a short life cycle, a high reproductive potential, and are thus amenable to laboratory culturing methodology (Lee, 1977). A short life cycle becomes advantageous for long term toxicity studies which have important implications in regards to estimating the effects of toxicants on the more susceptible stages of the life cycle for the organism. Lindén (1976), for example, showed that juvenile amphipods had an acute toxic response several hundred times lower than that for adult specimens of the same species. Complete life cycle bioassays also permit estimation of chronic toxicant effects, as well as detecting abnormalities in behavioral responses such as light reaction, precopulation frequency, predator avoidance, prey location and habitat selection. The high reproductive potential of amphipods provides an abundant source of test organisms and thus permits appropriate statistical analyses.

The importance of gammarid amphipods as effective biomonitors

has been illustrated with the distribution data collected in this study, and with the further discussion of plausible complementary toxicity studies. Indeed, the Fucus-epifaunal amphipod species have been used effectively in this case study, but a question arises as to the site-specificity of this population-based monitoring technique, i.e., would it be applicable at other marine outfall locations?

Organisms which are taxonomically similar will undoubtedly have comparable physiological requirements, and with few exceptions (e.g., Allorchestes angusta), respond to environmental change in a similar fashion. Thus, the restriction of the Fucus-epifauna technique to a single group such as the amphipods should be avoided. The most appropriate approach, in this case, would dictate the use of the entire macroinvertebrate community associated with Fucus, providing an array of species characteristic of several functional groups (trophic levels) revealing differential sensitivities to an imposed pollutional stress. Consequently, a preliminary examination of the Fucus-epifauna community, with discussion of its suitability as a marine biological monitoring technique, will be undertaken in the following section.

III. FUCUS-EPIFAUNA AS A BIOMONITORING TECHNIQUE

IN OTHER COASTAL REGIONS

1. INTRODUCTION

1.1 General

Aquatic algae have been used extensively to assess the extent of pollution within receiving systems. Techniques employ mostly periphyton communities (e.g., Patrick, 1973) in artificial substrate and effluent bioassay studies. Macroalgae are used less frequently and follow primarily standard biological survey methodologies (APHA, 1976).

Although single species associated with macroalgae have been used in biological tests of receiving water quality, few studies have attempted to incorporate the entire associated faunal community to assess pollution-related stresses. Stoll (1975) first used the Fucus-epifauna technique at the Port Alice pulp mill, revealing a decreased species diversity within communities located closer to the waste discharge source. Incorporating a technique developed by Levings (1976), Greer and Futer (1979) used Fucus-filled mesh bags suspended in the intertidal water column at varying distances from the Port Alice mill to observe colonization by resident invertebrate populations. The dominant organisms thus recorded were similar to those reported in section II of this study.

Within natural, undisturbed environments animal populations associated with Fucus have been studied, primarily by European investigators. Colman (1939) described the faunas of seven species of

intertidal macroalgae, including Fucus spiralis, F. vesiculosus, and F. serratus. Although the density of organisms found on these species of Fucus differed, littorines, amphipods, copepods, and acarines dominated the communities. Hagerman (1966) quantitatively studied the micro- and macrofauna of Fucus serratus. He showed that the structure of the fauna indicated that an actual community existed. Haage and Jansson (1970) quantitatively illustrated that Fucus samples denuded of all epifauna were recolonized with a natural species assemblage after a week's exposure. Working with subtidal enclosures he found the algal habitat dominated by the crustaceans Idotea and Gammarus and the molluscs Hydrobia, Theodoxus, and Cardium. Competition for space between the sessile epifaunal species of Fucus serratus was studied by Stebbing (1973), and later the composition and zonation of these species were examined by Boaden et al. (1975). In 1979 Dunstone et al. examined the size and abundance of furoid algae and correlated these, and environmental parameters, with the associated fauna. Under harsh, exposed conditions the algae supported an impoverished epifaunal community in which the amphipod Hyale nilsoni, small littorine gastropods and acarines predominated. Recently, Seed and O'Connor (1981) related the diversity and zonation of sessile, encrusting epifauna to the structural complexity of Fucus serratus plants.

On this coast Nassichuk (1975) studied the structural and interactive relationships of faunal assemblages associated with Fucus. Working at two sites located in Howe Sound, he demonstrated seasonal fluctuations of the fauna with lowest numbers of organisms

occurring in the winter months and maximum numbers in the summer months. Macroinvertebrates dominating the Fucus community included species of littorine gastropoda, the amphipod Hyale, the isopod Idotea, the hermit crab Pagurus, and the mussel Mytilus. Although Howe Sound is influenced by a number of industrial waste sources, no attempt was made to establish whether or not these effluents had any effect on the community structure of the invertebrate populations associated with Fucus.

1.2 Objectives

This portion of the study discusses the potential of the Fucus-epifauna community as an effective marine biomonitoring technique. The faunal composition of Fucus over a range of environmental conditions is examined in order to establish natural community variation as a baseline to differentiating effects related to imposed pollutional stresses.

2. METHODOLOGY

2.1 Fucus Sampling

Fucus was collected at each of 107 shoreline sites around Vancouver Island and along the British Columbia mainland coast (Figure 21) during the month of August, 1981. Shaded areas A-D of this figure represent regions of extensive sampling, the specific site locations of which are shown in Figures 22-25, respectively.

The Fucus collection procedure employed at each site was the same as that outlined in the Port Alice E.R.A. field methods (section II, 2.2). Macroinvertebrates retained on a 1 mm sieve were identified to the lowest possible taxonomic level and enumerated. The actual volume (liters) of Fucus sampled at each station was estimated by displacement.

Salinity and temperature of the surface water (taken at a depth of 1 m) adjacent to each sampling site were recorded using a YSI Model 33 S-C-T (Salinity-Conductivity-Temperature) meter.

2.2 Data Analysis

Collected abundance data were compiled in a species by site matrix and analyzed descriptively using indirect ordination techniques. A Q-analysis, i.e., a site by site comparison incorporating both species composition and numerical contribution, was employed in all cases. The Cornell Ecological computer Program package (CEP-25B) ORDIFLEX was used, primarily due to its flexible graphics, editing, transformation, and data description options.

Specifically, Principal Components Analysis (PCA) and Polar Ordination (PO) procedures were applied to the data matrix. A priori

Figure 21 - Location of Fucus sampling sites used in general epifaunal survey study. Sites located within shaded areas are enlarged in subsequent figures.

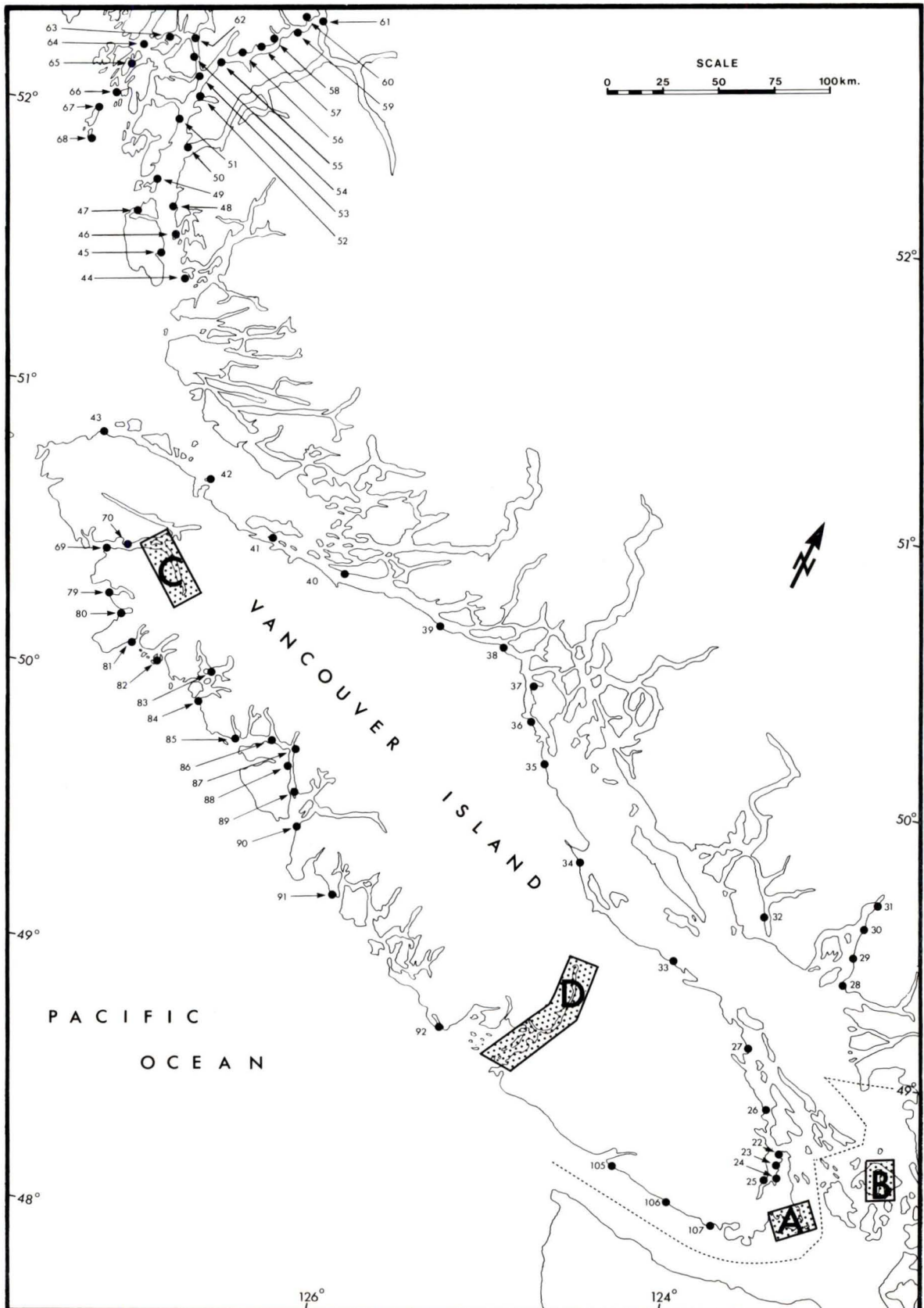


Figure 22 - Section A: Fucus sampling sites located in the Victoria area. Figure also shows source of major sewage outfalls within this region.

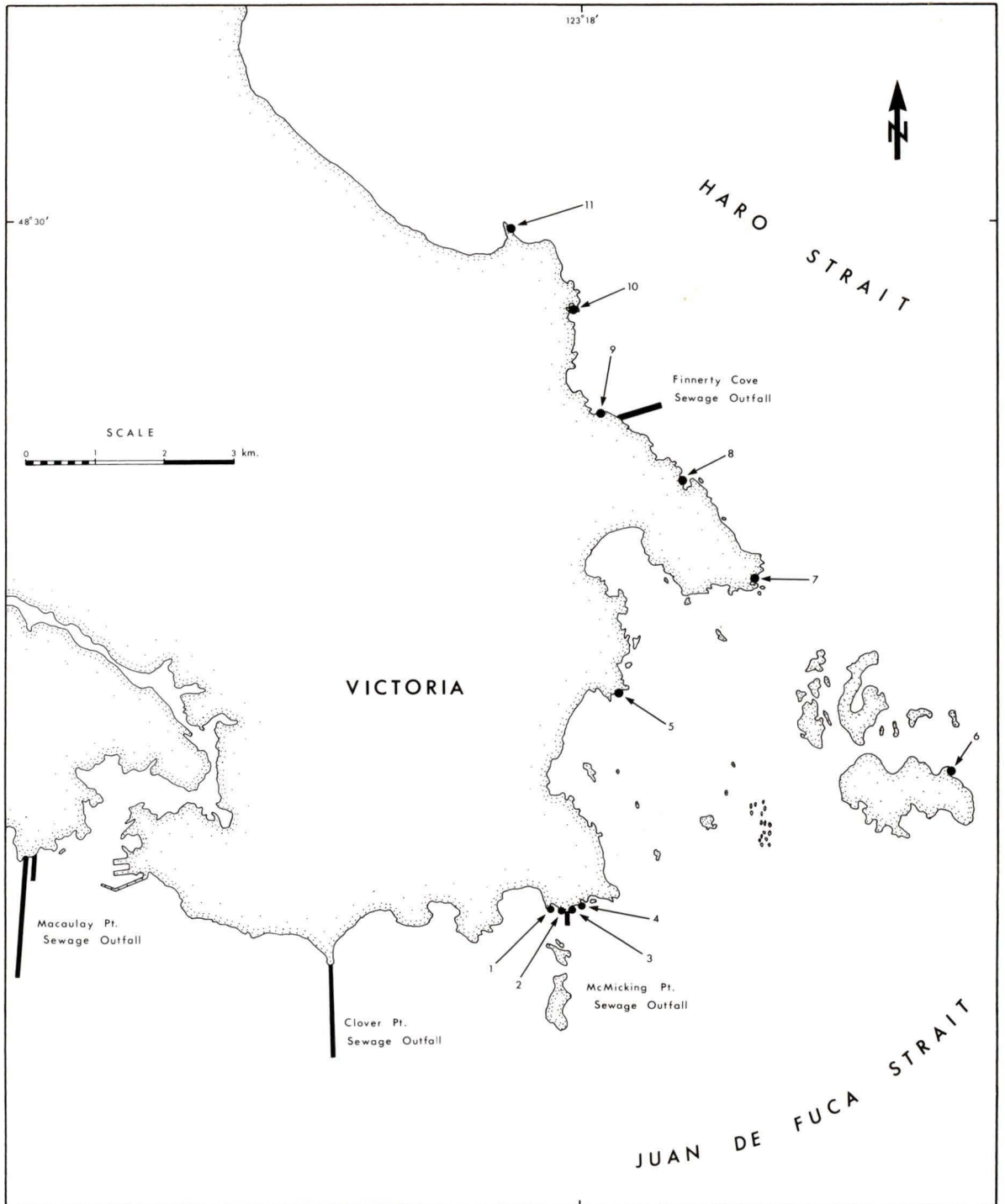


Figure 23 - Section B: Fucus sampling sites located within and in the vicinity of East Sound, Orcas Island, Washington.

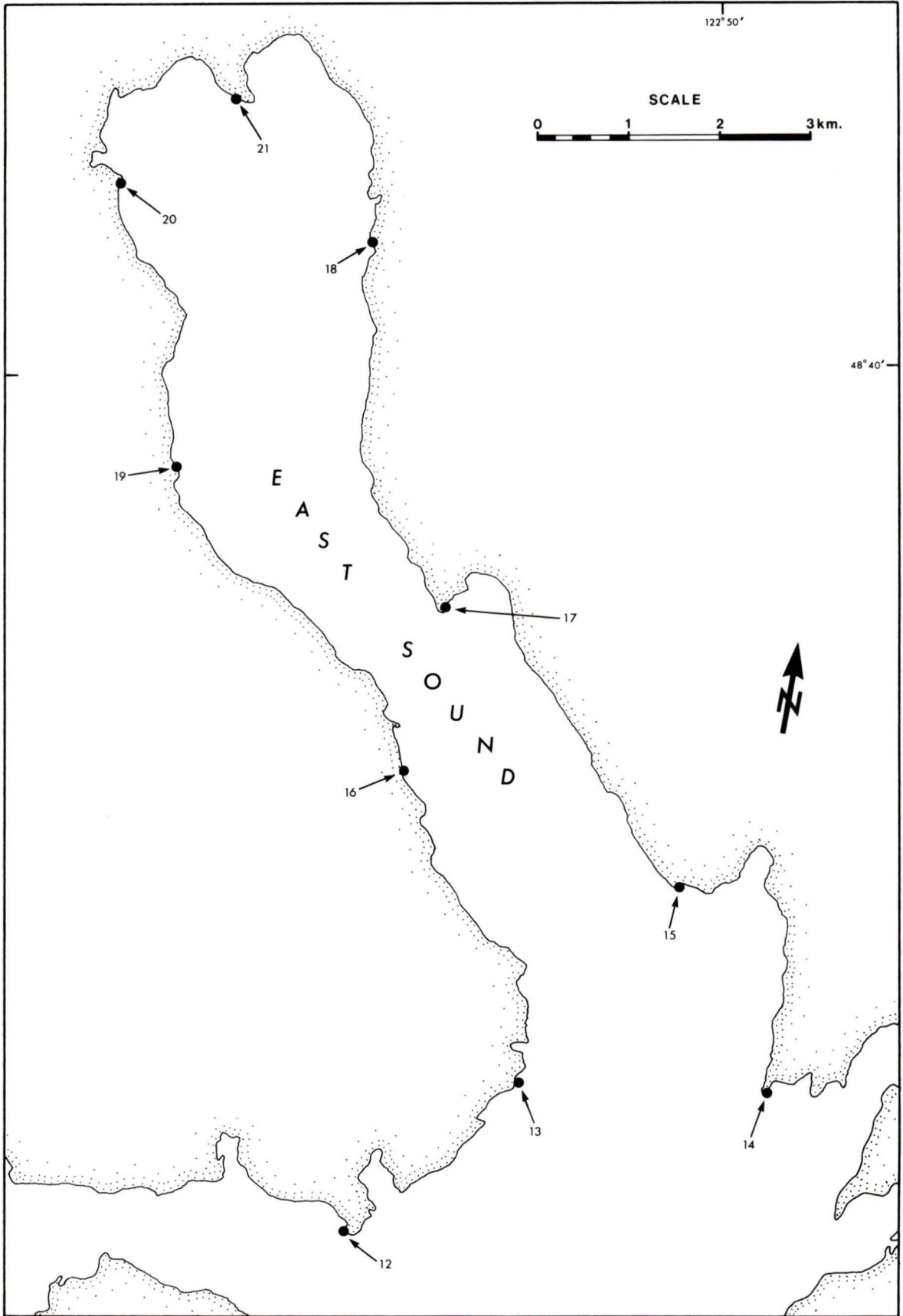


Figure 24 - Section C: Fucus sampling sites within Neroutsos Inlet and Quatsino Sound. Figure also shows location of Western Forest Products Ltd. sulfite process pulp mill.

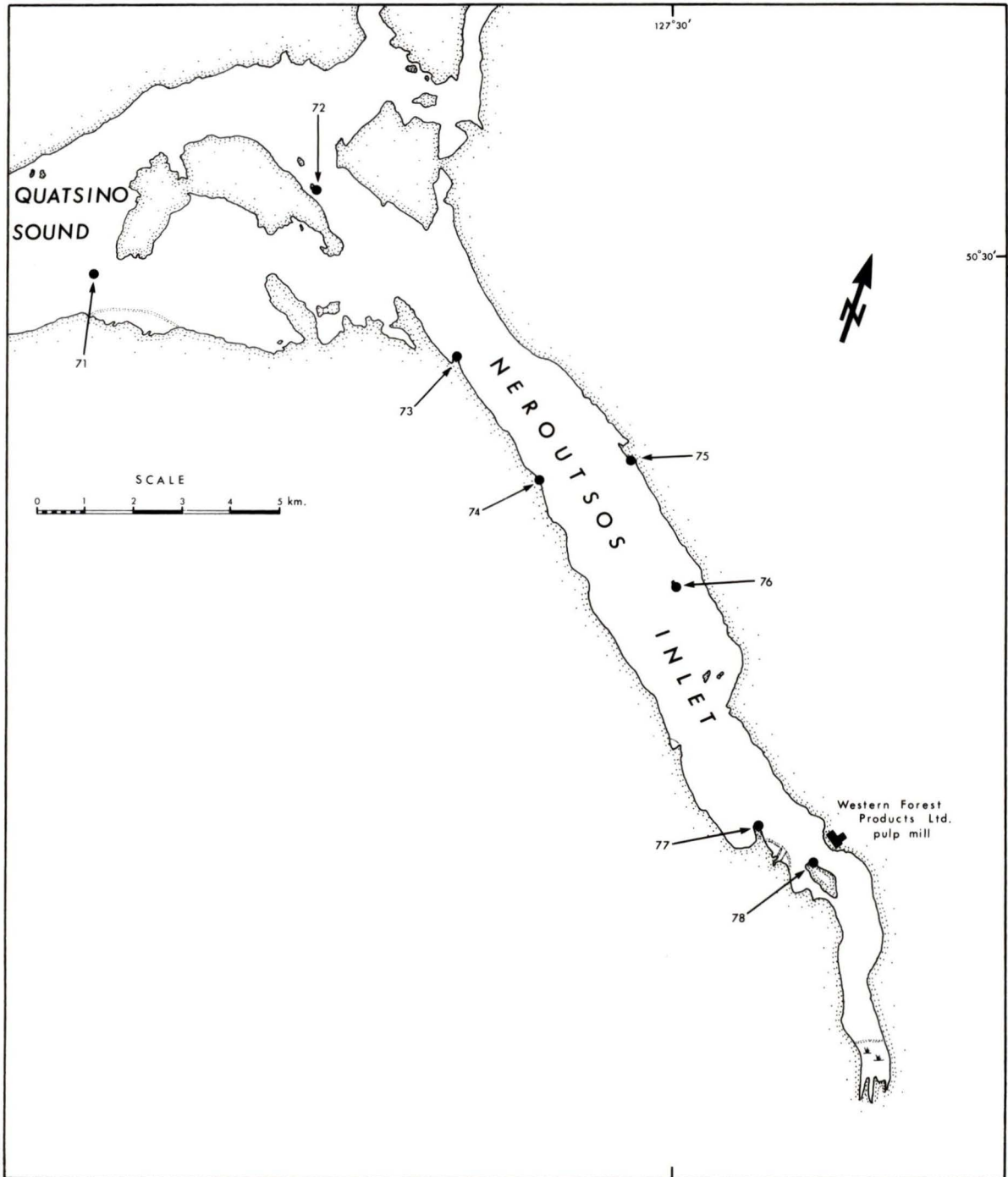
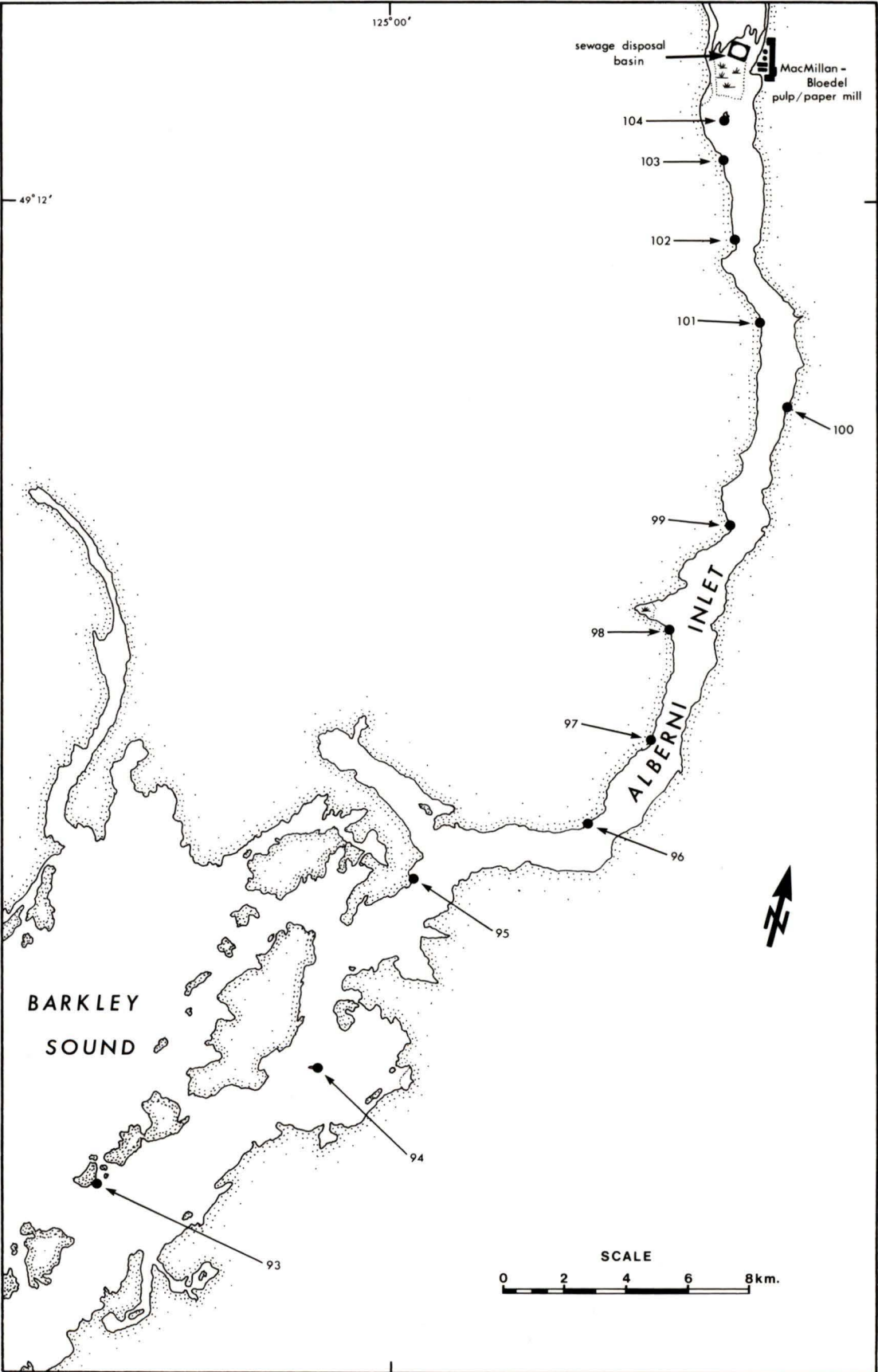


Figure 25 - Section D: Fucus sampling sites within Barkley Sound and Alberni Inlet. Figure also shows location of MacMillan-Bloedel kraft process pulp mill, and the city of Alberni's sewage disposal basin.



computer options chosen for the analysis included:

(i) deletion of species which occurred in fewer than 5 of the sites sampled;

(ii) data matrix values (abundance) transformed by $\log_{10}(x+1)$;

(iii) Wisconsin double standardization, i.e., species maxima standardized to 100 and sample totals relativized to 100;

(iv) non-centered adjustment for PCA with ordination scores scaled from 0-100;

(v) PO distance measure as percent distance (PD), calculated between two samples j and k using:

$$PD_{jk} = 100 - PS_{jk}, \text{ where } PS_{jk} = \frac{\min(D_{ij}, D_{ik})}{(D_{ij}, D_{ik})}$$

where the summations are over all species (N), and D_{ij} and D_{ik} are the abundances of species i in samples j and k, respectively;

(vi) automatic computation of PO endpoints using the pair of sites associated with the largest distance value for each axis; and

(vii) graphical displays for PCA (first 2 axes) and PO incorporating standardized scales.

Results were examined and discussed on the basis of information derived from a plot of the first two principal component axes. Samples considered representative of 'normal' or control sites were subjectively defined as a cluster on the basis of the proximity of

sample coordinates within the ordination space. Using a technique proposed by Bloom (1980), a 95% rejection envelope (R.E.) was calculated for this cluster of sample sites. Ordination scores for each of the two axes were tested for normality using a Chi-square goodness-of-fit test ($P < 0.05$). The mean (\bar{x}) and standard deviation (SD) of the control sample cluster on each axis were incorporated with the t statistic for individual observations to calculate the critical values above and below each mean. The following expression was used in this calculation:

$$x_c = \bar{x} \pm \frac{t \text{ (SD)}}{(N/(N+1))^{-0.5}} \quad df=N-1; \quad P=0.05 \text{ where}$$

N is the number of control sites and t the value obtained from appropriate statistical tables (Zar, 1974).

The pair of critical values obtained for the two axes represent the coordinates of the corners of the 95% rejection envelope.

A Polar Ordination analysis was employed to examine the variability of Fucus-epifaunal communities between sites falling within the 95% R.E. The distribution of these sites as a result of this analysis was related to collected environmental data.

Using computed ordination scores (axes 1 and 2) for points falling outside of the calculated 95% R.E., the respective site locations were established and considered with respect to distance from the control cluster and proximity to natural or anthropogenic sources of perturbation. Sites characteristic of particular coastal regions were joined by line segments on the graphic output and

discussed as a series of geographical transects moving away from a control cluster towards a perturbation source. Species composition and abundance data for these sites were compared on the basis of species richness (s) and two measures of community dominance. These measures included Simpson's index (D), defined as:

$$D = \frac{\sum p(p-1)}{N(N-1)}$$

where p is the abundance of species i and N is the total number of individuals of all species in the sample (Simpson, 1949); and

the inverse of Shannon-Weaver's evenness index (J'), that is:

$$1 - J'$$

where,

$$J' = H/H_{\max},$$

$$H = -\sum p_i \log p_i \quad \text{and}$$

$$H_{\max} = \log_{10} (s)$$

Each regional transect was considered separately, correlating species richness with each of the dominance measures for the sites (n) comprising these transects. Although the data did not appear to satisfy the conditions required for parametric statistical analysis, a simple linear correlation (r) in addition to the non-parametric Spearman rank correlation (r_s) were performed in each case. The significance ($H_0:p=0$) of each correlation coefficient was tested using a Student's t statistic for r and the appropriate critical values of the Spearman rank correlation coefficients (Zar, 1974).

A time series study using Port Alice Fucus-epifauna data

collected 3 years prior to and 4 years following the installation of the recovery boiler system was conducted using the aforementioned ordination techniques. Species composition and abundance data compiled from 7 sites over these years were subjected to a non-centered Principal Components Analysis (PCA). Stations 5 and 6 (Figure 1), considered representative of a 'normal' Fucus community, were used to define the 95% rejection envelope. Line segments were drawn between points characterizing each station over the 7 years sampled. As the proximity of one point to another is an indication of community similarity, those sites found to fall within the 95% R.E. after the implementation of the pollution abatement measures in 1977 were considered to have recovered from the pollutional stress.

2. RESULTS

Sorting and identification of the macroinvertebrates associated with Fucus samples taken over the 107 collection sites revealed a total of 26 species, a number of which were considered rare or transient. Table 4 lists the organisms found, ranking them according to frequency of occurrence.

The species by site abundance matrix resulting from this survey is presented as Table 1 of the Appendix. Table 2 of this appendix provides the geographical coordinates, the water temperature and salinity, and the displacement volume of Fucus sampled for each of the 107 sites. The volume of Fucus sampled was shown to vary only slightly among sites (mean = 2.82 L; standard deviation = 0.51 L).

Figure 26A summarizes the non-centered PCA ordination for the entire species-site abundance matrix. Each point on this graph represents one of the 107 sites sampled. The proximity of one point to another is an indication of how similar two sites are with respect to their Fucus-epifaunal communities, i.e., as measured by both species composition and the abundance of each species comprising the algal community. For example, the macroinvertebrate community characterizing site 93 is similar to that of site 76, and the community associated with site 103 is similar to that of site 104. However, the Fucus-epifaunal community at site 104 is extremely different from that found at site 93.

A 95% R.E. was calculated and encompasses a cluster of 66 sites considered to represent a 'normal' or control group (Figure

Table 4 - Macroinvertebrates associated with Fucus. % Frequency of occurrence within sites comprising the 95% rejection envelope.

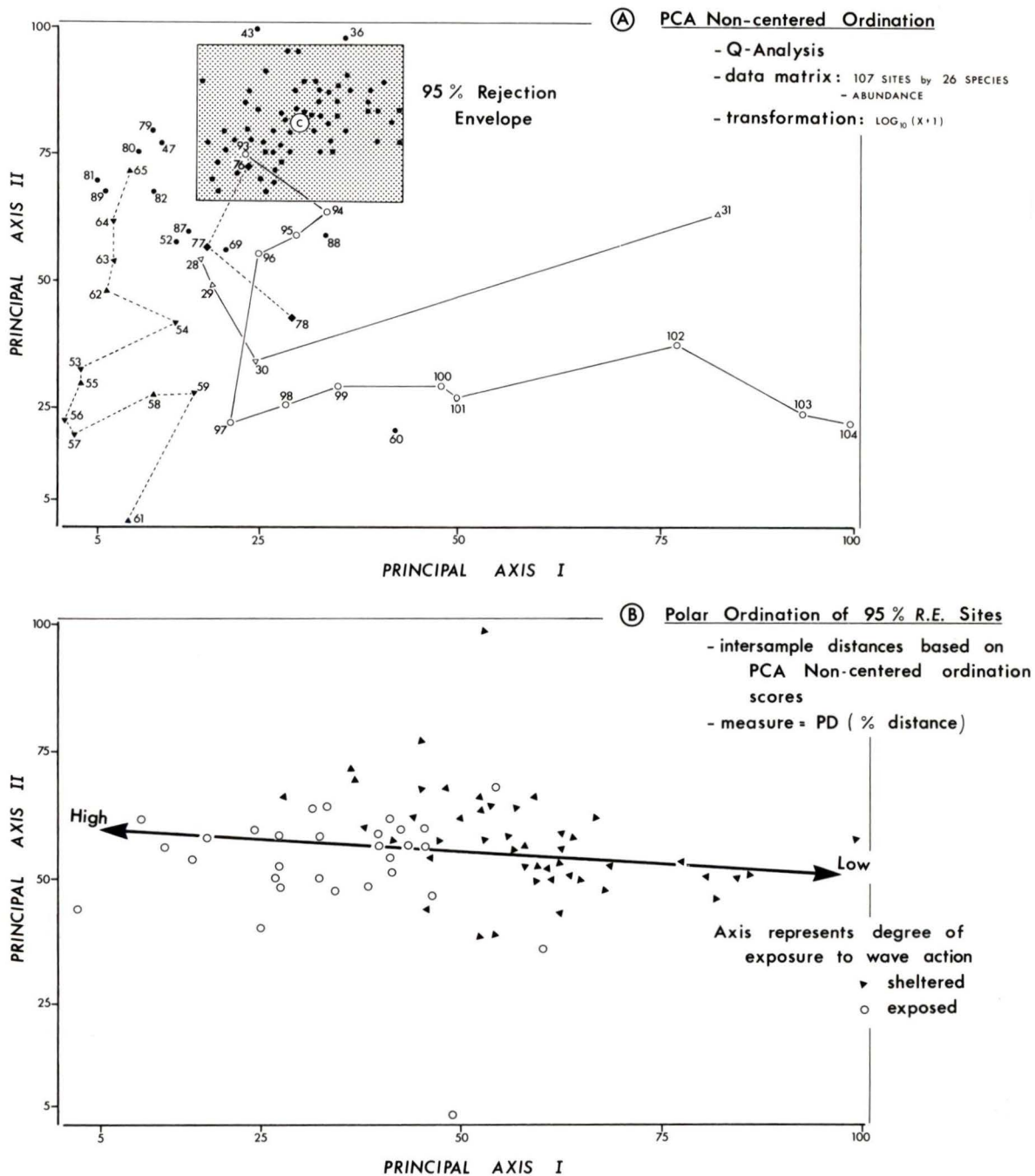
<u>Species</u>	<u>% Frequency</u>	<u>Code</u> *
<u>Littorina sitkana</u>	98.8	A
<u>Littorina scutulata</u>	97.6	B
<u>Mytilus edulis</u>	84.1	C
errant polychaete sp.	84.1	G
<u>Pagurus hirsutiusculus</u>	80.5	K
<u>Hyale californica</u>	79.3	I
<u>Oligochinus lighti</u>	78.0	O
chironomid larvae	73.2	E
<u>Idotea wosnesenskii</u>	71.9	D
<u>Ampithoe simulans</u>	70.1	L
<u>Gnorimosphaeroma oregonensis</u>	57.3	F
<u>Notoacmaea</u> sp.	50.0	U
<u>Hyale plumulosa</u>	48.8	H
<u>Hyale frequens</u>	37.8	J
<u>Collisella</u> sp.	37.8	T
<u>Allorchestes angusta</u>	32.9	Q
<u>Ampithoe valida</u>	25.6	M
<u>Onchidella borealis</u>	25.6	V
<u>Hemigrapsus nudus</u>	20.7	Z
<u>Corophium spinicorne</u>	19.5	P
platyhelminthes (polyclad)	15.9	W
<u>Paranemertes peregrina</u>	15.9	Y
<u>Ligia pallassii</u>	9.8	R
<u>Ampithoe plumulosa</u>	8.5	N
<u>Eogammarus confervicolus</u>	1.2	S
<u>Orchestia traskiana</u>	0.5	X

* abundance and site data for each of the above species is represented by the corresponding letter code in Table 1 of Appendix 3-12

Figure 26 - Fucus-epifauna community analysis.

- A. Results of PCA non-centered ordination incorporating data collected from all 107 sites; described using principal axes I and II. Sites common to particular coastal regions are joined by line segments.
- B. Results of Polar Ordination of 95% rejection envelope sites; described using principal axes I and II.

FUCUS-EPIFAUNA COMMUNITY ANALYSIS



26A). Ordination coordinates defining the site locations within the cluster were shown to be normally distributed (Chi-Square goodness-of-fit test for each axis; $P < 0.05$), and thus permitted use of parametric statistics in this calculation.

Sites falling outside of the 95% R.E. were assumed to be representative of disturbed communities or of communities not characteristic of 'normal' marine conditions. As illustrated in this figure, sites common to particular coastal regions were joined by line segments. Sites 28 to 31 represent a transect extending from the mouth to the head of Howe Sound (Figure 21), sites 76 to 78 moving towards the Port Alice pulp mill (Figure 24), and sites 93 to 104 a series of stations sampled in Alberni Inlet from Barkley Sound to the head of the inlet (Figure 25). Each of these areas is influenced by the imposition of pulping effluents at some point along the indicated transect. The fourth series of related sampling sites, represented by stations 65-62, 54, 53, 55-59, and 61, extends from Queen Charlotte Sound towards the head of Dean Channel (Figure 21) located along the British Columbia mainland coast. This area is uninhabited and free of any form of introduced pollutorial stress.

The distribution of points within the 95% R.E. suggested a distinct variation among the group of sites considered to be representative of a 'normal' Fucus-epifaunal community. Examination of this natural variation was achieved with a Polar Ordination of the 66 sites comprising the control cluster. Figure 26B summarizes the results of this analysis, revealing a distinct difference in community structure between sites found in exposed or wave-swept

areas and those located in sheltered regions. No correlation was found between community composition and water temperature or salinity.

Figure 27 illustrates the major macroinvertebrate species found within the control Fucus habitat. The species listed here occur in 70% of the 95% R.E. sites, and their proportions are presented as a percentage of their mean abundances. The two dominant organisms are represented by the gastropods Littorina scutulata and L. sitkana. The major fauna also include 3 species of amphipod crustacea, Oligochinus lighti, Hyale californica, and Ampithoe simulans, the hermit crab Pagurus hirsutiusculus, the mussel Mytilus edulis, the isopod Idotea wosnesenskii, chironomid larvae, and an unidentified errant polychaete species (contributing 0.8%).

Figure 28 compares the water temperature and salinity of sites falling outside the 95% R.E. with the confidence interval characteristic of the control sites. The shaded areas represent the 95% confidence intervals for the mean temperature and salinity of the 66 control sites. Each point in this figure corresponds to a site falling outside of the 95% R.E. Those points found within the shaded areas indicate no significant difference between the abiotic parameters measured at these sites and those taken at sites comprising the control group. Salinity of the surface water was shown to be significantly different at 24 of the 41 sites falling outside of the control cluster, with 20 of these stations incorporated in 3 of the established regional transects, i.e., Howe Sound, Port Alberni, and Dean Channel. Temperature revealed a much less apparent difference, with only 11 sites falling outside of the 95% confidence intervals

Figure 27 - Ten major macro-epifaunal species associated with a control Fucus habitat. Diagram summarizes their relative abundances as a percentage of mean abundance within the 66 sites comprising the 95% rejection envelope. Smallest segment is represented by an unidentified errant polychaete species contributing 0.8% of the invertebrate community.

MAJOR EPIFAUNA OF A 'CONTROL' FUCUS HABITAT

contribution as % of mean abundance

(BASED ON 95% R.E. SITES)

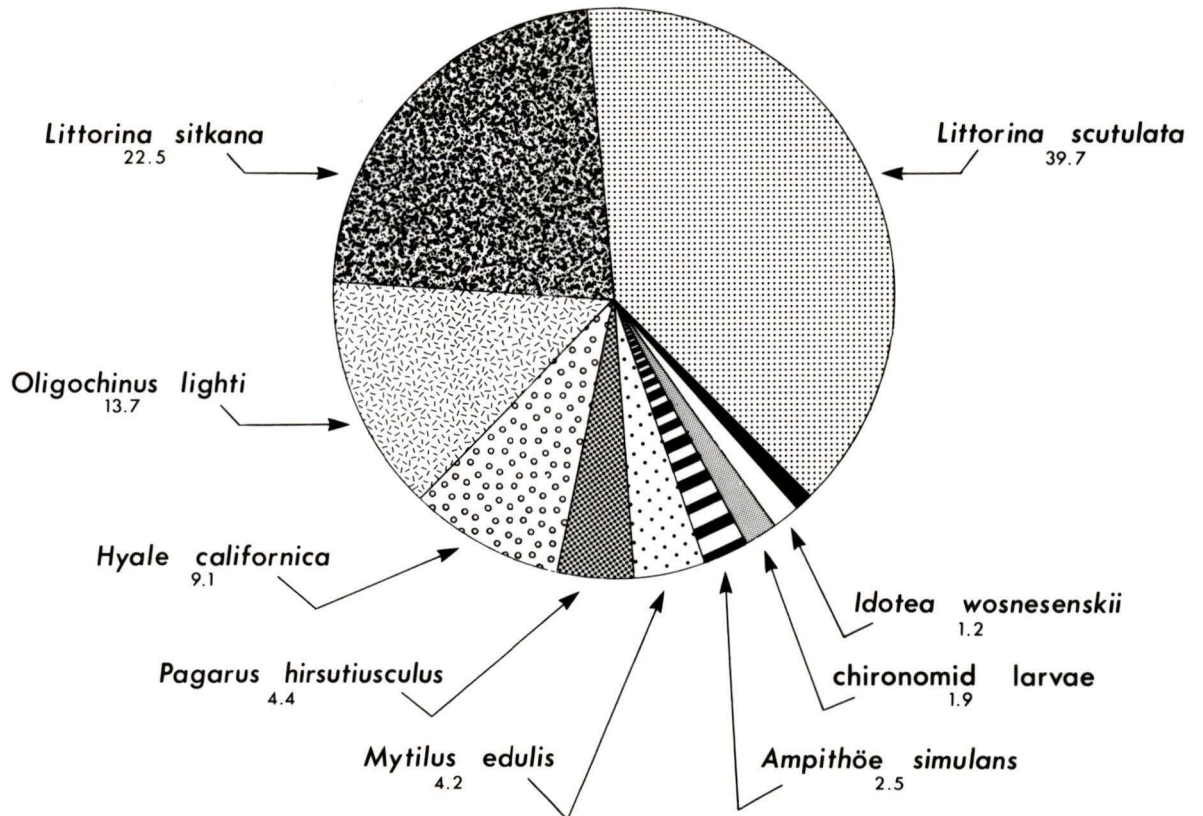
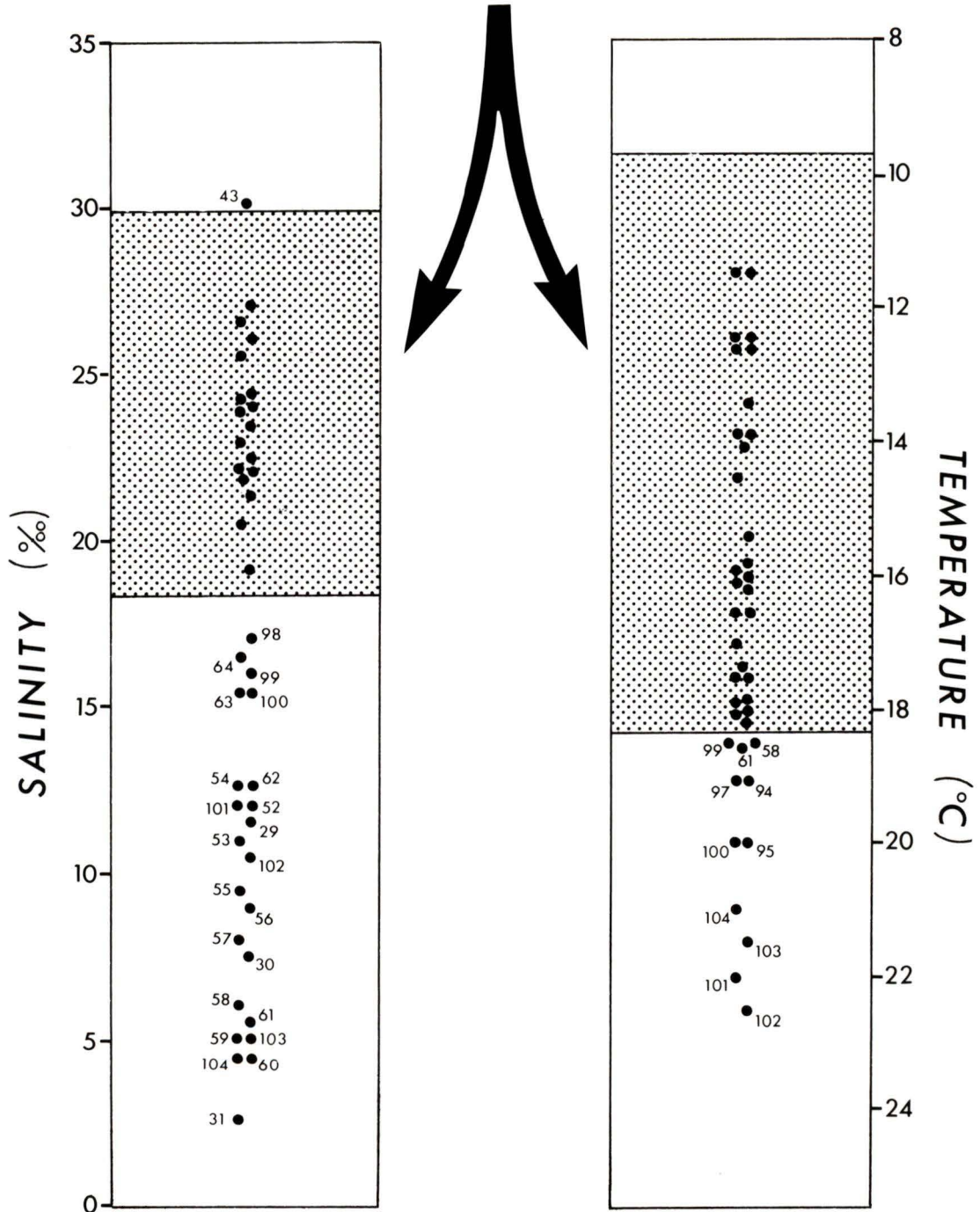


Figure 28 Summary of environmental data (salinity and temperature) collected at each Fucus sampling site. Shaded regions represent the 95% confidence intervals associated with the salinity and temperature values recorded at the 66 sites comprising the 95% R.E.. Points represent those sites falling outside of the 95% R.E.. Separation of shaded regions from numbered points (sites) suggests a significant difference between these sites and the 66 sites defining the control cluster.

95% Confidence Intervals

BASED ON MEASUREMENTS TAKEN AT
SITES FALLING WITHIN 95% R.E.



for the control cluster.

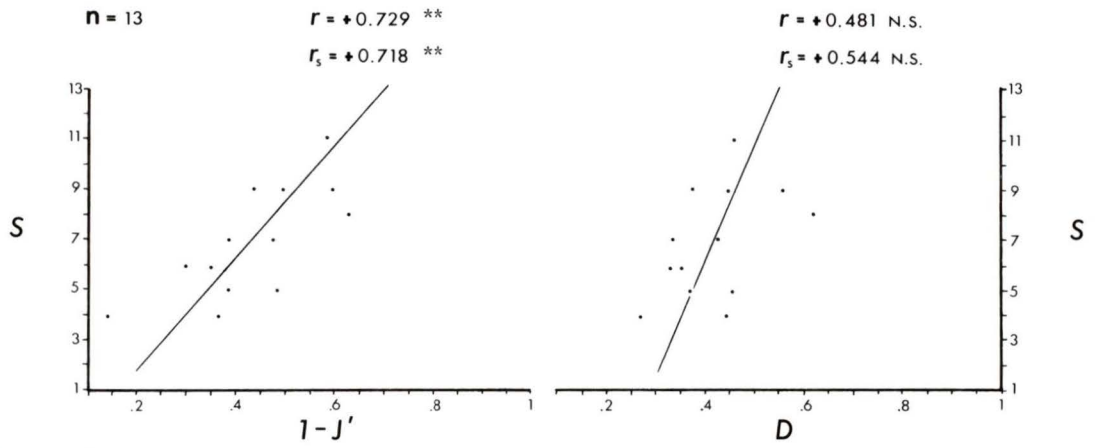
Figure 29 expresses species richness (s) as a function of two dominance measures for sites comprising three of the regional transects defined in Figure 26a. Dean Channel, characterized by a progressive reduction in surface salinity towards the head waters, shows a significant positive correlation between these variables. Port Alberni and Port Alice, in contrast, reveal a significant negative correlation indicating a distinct increase in dominance with a corresponding decrease in species richness. Both locations are influenced by a point source discharge of pulping effluent, and although Alberni Inlet is also subject to a decreasing surface salinity the species richness/dominance relationship remains the same as that at Port Alice where salinity is relatively constant.

The time series study of Port Alice shoreline rehabilitation using the entire macroinvertebrate component of Fucus was based on abundance data collected from 7 sites within Neroutsos Inlet. Species/abundance accumulated over 7 years (1975-1981) is presented in Table 5.

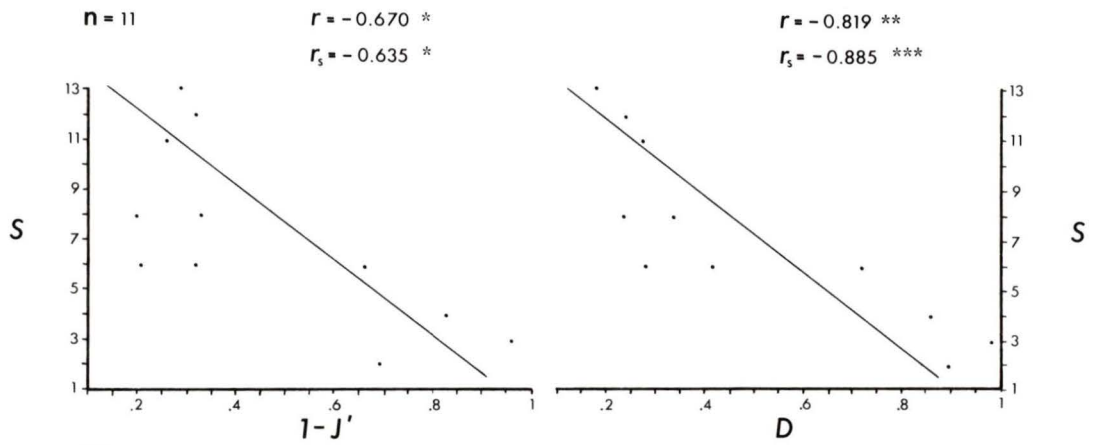
Figure 30 summarizes the results of the Fucus-epifauna recovery analysis, documenting the first two principal component axes of the PCA non-centered ordination. As evident in this figure Fucus recovery can be said to have occurred at stations 4, 3A, and 3. By 1981 each of these sites is shown to fall within the 95% rejection envelope, and thus show no significant difference from stations 5 and 6 which were considered as controls. Station 2, and to a lesser extent station 1A, also illustrate the recovery process within the

Figure 29 Linear correlations of species richness (s) and two dominance measures, i.e., the inverse of Shannon-Weaver's evenness ($1-J'$) and Simpson's Index (D), for each of 3 regional transects falling outside of the 95% R.E.. Values for both parametric and non-parametric correlations are given. Significance of each is indicated by one, two, or three asterisks, corresponding to probability levels of 0.05, 0.01, and 0.001, respectively.

DEAN CHANNEL



PORT ALBERNI



PORT ALICE

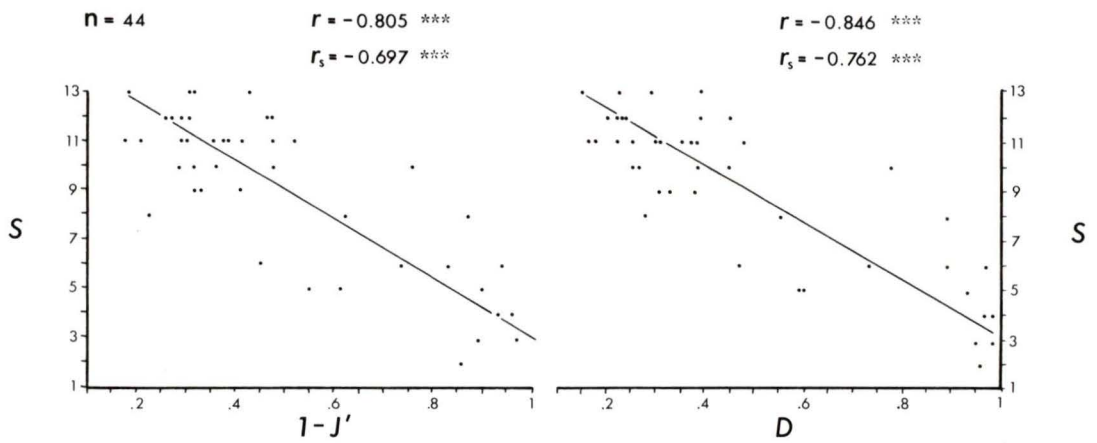


Table 5 - Fucus-epifauna species/abundance data accumulated over 7 years (1975-1981) from Neroutsos Inlet (Port Alice). Site codes correspond to those used in Figure 1 of this thesis.

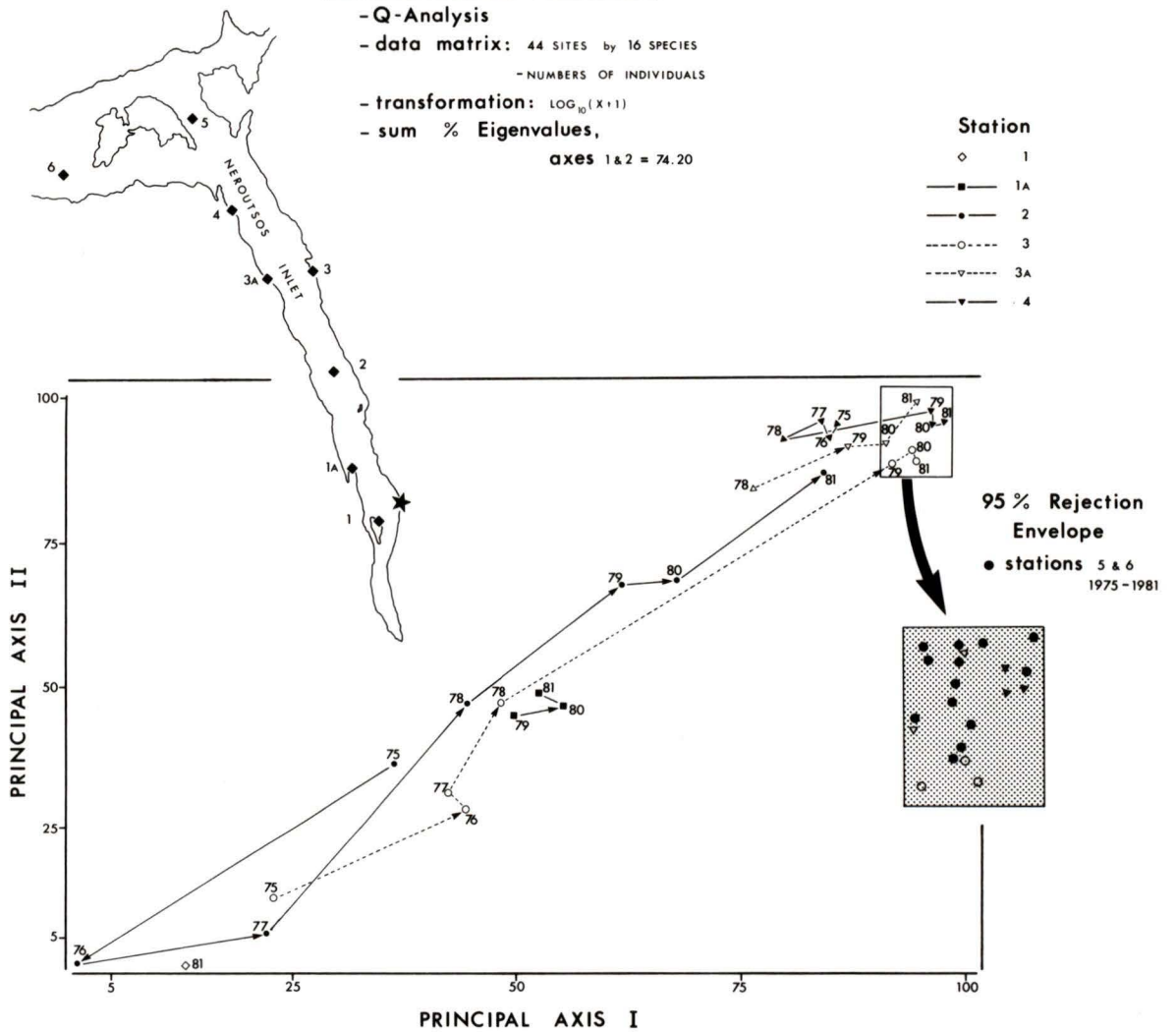
Species	Site/Year																	
	1975					1976					1977							
	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6			
<i>Ampithoe simulans</i>	0	0	2	36	62	0	0	2	5	7	0	0	4	19	17			
<i>Oligochinus lighti</i>	0	0	12	229	392	0	0	15	34	42	0	0	24	124	107			
<i>Hyale californica</i>	0	0	1	24	41	0	0	1	4	4	0	0	2	13	11			
<i>Hyale plumulosa</i>	0	0	1	12	21	0	0	1	2	3	0	0	1	7	6			
<i>Allorchestes angusta</i>	62	1994	1	0	0	378	3426	1	0	0	830	1637	1	0	0			
<i>Gnorimosphaeroma</i> sp.	0	4	1	0	0	0	116	1	0	0	0	3	0	2	0			
<i>Mytilus edulis</i>	0	0	6	10	1	0	1	2	1	0	0	0	1	3	0			
chironomid larvae	76	6	29	26	0	0	0	14	2	3	19	615	340	215	25			
<i>Littorina sitkana</i>	12	8	6	20	72	8	11	1	28	24	0	7	3	11	31			
<i>Littorina scutulata</i>	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0			
<i>Pagurus hirsutiussculus</i>	0	0	2	7	11	0	1	0	8	0	0	0	0	1	3			
<i>Siphonaria thersites</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
<i>Idotea wosnesenskii</i>	0	0	5	3	2	0	0	1	2	2	0	0	8	6	3			
<i>Notoacmaea</i> sp.	1	0	2	13	1	0	0	1	2	1	1	3	3	65	1			
<i>Onchidella borealis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
<i>polychaeta</i> sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0			
	1978																	
	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>				<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>	
<i>Ampithoe simulans</i>	0	0	0	1	80	31	3				0	0	2	8	14	6	9	
<i>Oligochinus lighti</i>	0	0	0	0	5	51	70				0	0	6	8	89	196	76	
<i>Hyale californica</i>	0	0	3	35	5	0	32				0	0	8	40	0	1	16	
<i>Hyale plumulosa</i>	0	0	0	0	0	0	20				0	0	0	0	0	0	0	
<i>Allorchestes angusta</i>	728	3541	1677	25	20	5	1				1341	232	0	0	0	0	0	
<i>Gnorimosphaeroma</i> sp.	6	0	6	6	2	0	0				20	0	0	1	1	0	0	
<i>Mytilus edulis</i>	2	3	3	71	39	69	17				18	4	34	19	85	6	11	
chironomid larvae	0	3	11	1	45	2	2				23	2	13	54	35	10	3	
<i>Littorina sitkana</i>	3	656	82	338	111	35	83				20	685	65	67	8	22	125	
<i>Littorina scutulata</i>	0	0	0	0	0	0	6				0	0	1	3	1	2	11	
<i>Pagurus hirsutiussculus</i>	0	0	0	2	1	2	4				1	1	1	0	1	7	12	
<i>Siphonaria thersites</i>	0	12	1	25	11	249	1				0	47	3	1	13	142	2	
<i>Idotea wosnesenskii</i>	0	0	1	2	10	3	1				0	1	2	1	2	3	2	
<i>Notoacmaea</i> sp.	0	0	0	0	0	1	4				0	0	0	0	0	0	2	
<i>Onchidella borealis</i>	0	0	0	0	0	0	0				0	0	25	0	26	0	0	
<i>polychaeta</i> sp.	0	1	0	1	1	2	0				0	1	3	3	1	1	0	
	1979																	
	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>				<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>	
<i>Ampithoe simulans</i>	0	0	10	12	18	17	5				0	0	41	29	10	22	21	8
<i>Oligochinus lighti</i>	0	0	5	13	143	25	36				0	0	2	54	145	119	20	53
<i>Hyale californica</i>	0	0	4	12	1	1	2				0	2	126	50	74	7	1	4
<i>Hyale plumulosa</i>	0	0	0	0	0	0	0				0	0	0	0	21	4	0	0
<i>Allorchestes angusta</i>	2780	186	0	0	0	0	0				2145	224	8	0	0	0	0	0
<i>Gnorimosphaeroma</i> sp.	20	0	1	1	0	0	0				0	20	2	29	0	2	3	0
<i>Mytilus edulis</i>	10	1	6	8	11	4	1				1	9	13	10	10	14	10	5
chironomid larvae	1	0	1	8	1	0	0				10	5	3	6	8	6	3	2
<i>Littorina sitkana</i>	14	818	95	107	16	3	75				0	99	304	53	140	27	36	15
<i>Littorina scutulata</i>	0	0	1	16	6	4	7				0	0	0	0	9	7	7	12
<i>Pagurus hirsutiussculus</i>	0	0	1	1	2	1	3				0	0	0	1	0	0	2	10
<i>Siphonaria thersites</i>	0	82	18	0	15	16	0				0	0	20	3	0	4	6	0
<i>Idotea wosnesenskii</i>	1	2	1	2	2	3	1				0	0	10	0	4	5	3	0
<i>Notoacmaea</i> sp.	0	0	0	0	0	1	5				0	0	0	0	0	0	1	0
<i>Onchidella borealis</i>	0	0	10	0	2	22	0				0	0	2	12	0	16	22	0
<i>polychaeta</i> sp.	2	5	3	2	1	0	0				0	0	1	3	1	1	0	0
	1980																	
	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>				<u>0</u>	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>
<i>Ampithoe simulans</i>	0	0	10	12	18	17	5				0	0	41	29	10	22	21	8
<i>Oligochinus lighti</i>	0	0	5	13	143	25	36				0	0	2	54	145	119	20	53
<i>Hyale californica</i>	0	0	4	12	1	1	2				0	2	126	50	74	7	1	4
<i>Hyale plumulosa</i>	0	0	0	0	0	0	0				0	0	0	0	21	4	0	0
<i>Allorchestes angusta</i>	2780	186	0	0	0	0	0				2145	224	8	0	0	0	0	0
<i>Gnorimosphaeroma</i> sp.	20	0	1	1	0	0	0				0	20	2	29	0	2	3	0
<i>Mytilus edulis</i>	10	1	6	8	11	4	1				1	9	13	10	10	14	10	5
chironomid larvae	1	0	1	8	1	0	0				10	5	3	6	8	6	3	2
<i>Littorina sitkana</i>	14	818	95	107	16	3	75				0	99	304	53	140	27	36	15
<i>Littorina scutulata</i>	0	0	1	16	6	4	7				0	0	0	0	9	7	7	12
<i>Pagurus hirsutiussculus</i>	0	0	1	1	2	1	3				0	0	0	1	0	0	2	10
<i>Siphonaria thersites</i>	0	82	18	0	15	16	0				0	0	20	3	0	4	6	0
<i>Idotea wosnesenskii</i>	1	2	1	2	2	3	1				0	0	10	0	4	5	3	0
<i>Notoacmaea</i> sp.	0	0	0	0	0	1	5				0	0	0	0	0	0	1	0
<i>Onchidella borealis</i>	0	0	10	0	2	22	0				0	0	2	12	0	16	22	0
<i>polychaeta</i> sp.	2	5	3	2	1	0	0				0	0	1	3	1	1	0	0
	1981																	
	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>				<u>0</u>	<u>1A</u>	<u>2</u>	<u>3</u>	<u>3A</u>	<u>4</u>	<u>5</u>	<u>6</u>
<i>Ampithoe simulans</i>	0	0	10	12	18	17	5				0	0	41	29	10	22	21	8
<i>Oligochinus lighti</i>	0	0	5	13	143	25	36				0	0	2	54	145	119	20	53
<i>Hyale californica</i>	0	0	4	12	1	1	2				0	2	126	50	74	7	1	4
<i>Hyale plumulosa</i>	0	0	0	0	0	0	0				0	0	0	0	21	4	0	0
<i>Allorchestes angusta</i>	2780	186	0	0	0	0	0				2145	224	8	0	0	0	0	0
<i>Gnorimosphaeroma</i> sp.	20	0	1	1	0	0	0				0	20	2	29	0	2	3	0
<i>Mytilus edulis</i>	10	1	6	8	11	4	1				1	9	13	10	10	14	10	5
chironomid larvae	1	0	1	8	1	0	0				10	5	3	6	8	6	3	2
<i>Littorina sitkana</i>	14	818	95	107	16	3	75				0	99	304	53	140	27	36	15
<i>Littorina scutulata</i>	0	0	1	16	6	4	7				0	0	0	0	9	7	7	12
<i>Pagurus hirsutiussculus</i>	0	0	1	1	2	1	3				0	0	0	1	0	0	2	10
<i>Siphonaria thersites</i>	0	82	18	0	15	16	0				0	0	20	3	0	4	6	0
<i>Idotea wosnesenskii</i>	1	2	1	2	2	3	1				0	0	10	0	4	5	3	0
<i>Notoacmaea</i> sp.	0	0	0	0	0	1	5				0	0	0	0	0	0	1	0
<i>Onchidella borealis</i>	0	0	10	0	2	22	0				0	0	2	12	0	16	22	0
<i>polychaeta</i> sp.	2	5	3	2	1	0	0				0	0	1	3	1	1	0	0

Figure 30 - Fucus-epifaunal recovery analysis incorporating data from 1975 through 1981. Results of the PCA (Principal Components Analysis) non-centered ordination are displayed on principal axes I and II. The 95% R.E. was estimated on the basis of data accumulated from stations 5 and 6 over this period.

FUCUS-EPIFAUNA RECOVERY ANALYSIS

PCA Non-centered Ordination

- Q-Analysis
- data matrix: 44 SITES by 16 SPECIES
- NUMBERS OF INDIVIDUALS
- transformation: $\text{LOG}_{10}(X+1)$
- sum % Eigenvalues,
- axes 1 & 2 = 74.20



inlet since 1977. The movement of these sites closer to the 95% R.E. suggests an increase in community similarity with the control sites, but indicates that by 1981 this recovery process remains incomplete, or that the present effluent discharge continues to have an effect on the Fucus community at least as far from the mill as station 2. Station 1, added to the sampling program in 1981, is the closest site to the mill discharge source and as shown in this figure remains the most dissimilar to the control sites.

4. DISCUSSION

4.1 Analytical approach

The examination of aquatic communities in biomonitoring programs has typically been approached in two ways, i.e., by use of a sample specific analysis or through application of a multiple sample analysis (Herricks and Cairns, 1982).

Sample specific analysis procedures generally include compilation of species presence and analysis of species abundance. The most common technique used in such an approach is the diversity calculation. Comparisons of index values either through space or time are normally related to corresponding water quality measurements taken at the same location. The major problem with the use of such indices is that large quantities of data are reduced to a single number or index. Thus, the information content within each sample is significantly reduced, particularly if this is the only analysis performed. Nevertheless, this form of analysis remains popular within the applied literature (e.g., Zand, 1976).

Multiple sample analysis extends the development of single sample parameters to incorporate comparisons between samples. Community comparisons can be made by using the relatively simple techniques of similarity or correlation coefficients, or by applying one of the more complex multivariate approaches. Green (1980) has reviewed multivariate techniques for analyzing the similarity or resemblance matrices produced by a number of samples. These procedures comprise two broad categories including cluster analysis and ordination. Both groups of community classification methods have

the distinct advantage of allowing visual interpretation of sample relationships.

Communities are not usually represented by distinct units, but instead change continually along an environmental gradient. Such gradients were apparent in Dean Channel and Alberni Inlet where significant reductions in salinity were observed. Gradients of pollutant concentrations within receiving waters also exist. Sulfite concentrations estimated at Port Alice illustrate this trend. Although not measured in this study Alberni Inlet undoubtedly supports a similar situation considering the presence of the kraft pulpmill at the inlet head.

As a consequence of the continuum concept, clustering, or hierarchal community classification methods, tend to impose sample groupings where groupings simply do not exist (Poole, 1974). Since ordination procedures are more noncommittal than clustering techniques they are considered more appropriate in such situations and thus were employed in this study.

Analysis of the Fucus-epifauna community was accomplished using two indirect ordination techniques, i.e., non-centered PCA and PO. These classification methods are considered 'indirect' since the resultant axes are mathematical constructs derived from a matrix of similarities calculated between the samples (as in this study) or species. The axes do not correspond directly to any quality of the environment. Once ordination scores have been plotted, the relationships between the samples (sites) can be related 'indirectly' to measured environmental parameters. Thus, in this study ordination

was used primarily as a mathematical tool, reducing the multidimensionality of the initial data matrix. The resultant scatter diagram expresses relative similarities of the ordinated samples (in this case), with trends in characteristics relating these samples readily observable. This approach to ordination characterizes a single-space ordination in the sense that it represents positions of the sites in a space directly defined by the variables, i.e., a species-dimensional space in which species scores are axes and samples are points.

In this study a non-centered PCA ordination was applied to the collected Fucus-epifauna data matrix. Much controversy exists with respect to the appropriate choice between centered and non-centered data for use in the ordination procedure. Noy-Meir (1973) provides a review of these approaches, stressing some of the advantages of non-centering.

Mathematically and geometrically, centering involves the specification of the origin, or the point of reference (Noy-Meir, 1973). It is commonly achieved by expressing the variables in terms of their mean and standard deviation. Thus, this point (mean) represents the point of zero information and anything at it is of little concern, while deviation from it can be considered as 'information'. This imposed scaling is logically necessary for variables such as pH or temperature where the zero-point is arbitrary. Centering by species, however, effectively transfers the reference point to an 'average' sample which is defined by the mean abundance of each species found over all sites sampled. Consequently,

a centered ordination will generally express inter-sample variation with respect to the mean or 'average' sample collected.

When non-centered data are used the reference point is the true zero, i.e., a site without species or a species which never occurs in a sample. Thus, a resulting ordination using non-centered data expresses community similarities in absolute values, which are directly dependent on initially recorded species abundances.

One point argued against the use of non-centered ordination is that it usually results in a unipolar first component. Noy-Meir (1973) illustrated that this commonly occurs only in an internally homogeneous or continuous data set. However, if a set contains one or more disjunctions a distinct bipolarity is introduced into the resultant ordinated data set, allowing an assessment of the number and sharpness of discontinuities in the samples. This property of non-centering was considered very important in applying ordination methods to analyze community variation in areas influenced by pollutional stresses. Since it is assumed that introduced pollutants will affect and thus alter the species composition within a community, the resulting differences would then appear as sample discontinuities in the data matrix. A non-centered ordination can therefore clearly differentiate these sites from those characteristic of normal environmental conditions.

Using a statistical technique proposed by Bloom (1980) a cluster of points within the two-dimensional ordination space was defined as a group of sites representative of a control Fucus epifauna community. Choice of this sample cluster, although objec-

tive, was considered unbiased in that the actual sample sites represented by the points within the ordination space were unknown prior to this decision. The validity of incorporating 95% confidence limits in further defining the bounds of this control cluster seems questionable. However, the rejection envelope created in this manner more closely approximates true cluster shape than any arbitrarily selected geometric shape. In addition, if this technique was considered practical for E.I.A. or E.R.A. programs, inclusion of these limits would add credence to the analysis in that interpretations and conclusions made in the subsequent decision-making process would be dependent on minimal subjectivity on the part of the monitoring agency.

4.2 Control species assemblage

A total of 10 macroinvertebrates were considered representative of a control Fucus habitat on the basis of both abundance and frequency of occurrence within the 66 sites comprising the 95% rejection envelope. Some of these genera, and in most cases species, are similar to those reported by Nassichuk (1975) in his Fucus-epifaunal study in Howe Sound, e.g., Littorina, Hyale, Mytilus, Pagurus, Idotea, and chironomid larvae. European investigators have also recorded species from a number of these genera, e.g., Littorina and Hyale (Dunstone *et. al.*, 1979; Colman, 1940); Ampithoe, Mytilus, Idotea, and chironomid larvae (Hagerman, 1966; Colman, 1940).

Hagerman (1966) showed that the structure of the fauna living on Fucus serratus indicated that an actual community exists. He revealed that although the majority of species inhabiting the Fucus

were herbivorous; species representing carnivores, detritivores, suspension-feeders, and scavengers were also residents of the algal habitat.

Of the 10 species considered representative of a Fucus-epifauna community in this study, the herbivores are represented by the two Littorina* species, Hyale californica, and the chironomid larvae*. Scavengers include Idotea* and Pagurus; Mytilus* is a suspension-feeder; Oligochinus lighti and the errant polychaete* are carnivores; and Ampithoe* simulans is considered a detritivore, although under unfavourable conditions may become carnivorous (Skutch, 1926). Asterisks indicate the organisms which are common to forms reported in the Fucus-epifaunal community described by Hagerman (1966). Amphipod species characteristic of Fucus habitats on this coast, although different from those recorded in Europe, have similar feeding strategies and are closely related taxonomically.

Polar ordination (PO) of the species abundance data associated with the 66 control sites indicated a community structural change between sites found in exposed or wave-swept areas with those located in sheltered regions. The type and number of organisms which are associated with Fucus in a wave-swept area may be determined by a number of factors. The so-called whiplash effect (Dayton, 1971) will influence settlement capability and likely prevent or dislodge organisms which might normally be present in less exposed areas. Dunestone et. al. (1979) supported this concept, and revealed that at sites of increased 'turbulence' Fucus-epifaunal communities were significantly less diverse. They suggested that in addition to the

physical removal of the more vulnerable species, wave action has an adverse effect on plant size which may, in turn, exclude some of the epifaunal species.

Indeed the structural complexity of the Fucus plants will determine the amount of available space within the habitat, and thus be an important factor in governing within-habitat diversity through the partitioning of this space (McArthur, 1965). Boaden et al. (1975) showed a reduction in Fucus size and degree of dichotomization under turbulent conditions. Jordan and Vadas (1972) correlated decreased vesiculation to similar conditions. In both cases structural complexity, which may act to separate populations of potentially competitive species, is reduced. As a result species diversity within the Fucus habitat is also decreased. In terms of the ordination plot this leads to the variation of sample points within the proposed 95% rejection envelope.

4.3 Irregular Fucus communities

As expected, results of the non-centered PCA clearly differentiated sample disjunctions inherent in the original data matrix. These discontinuities can be defined as the group of samples (41) which fall outside of the established 95% rejection envelope. Since it was assumed that the 66 samples comprising the 95% R.E. were representative of a 'normal' Fucus-epifauna community, then these outlying points can be considered characteristic of sites which have irregular, or at least different epifaunal communities.

The majority of sites falling outside of the control group were incorporated into 4 regional transects, each of which illustrat-

ed a significant decrease in community similarity as a function of at least one abiotic parameter. Port Alberni, Port Alice, and Howe Sound transects were each influenced by the discharge of pulping wastes. Port Alberni and Dean Channel transects revealed a significant reduction in salinity which appeared directly correlated to increasing community dissimilarity. The problem encountered at this stage was establishing a method for differentiating the effects of pollutants, e.g., pulping wastes, from natural community change due to an abiotic parameter such as salinity. This was considered of primary importance, particularly if this community analysis was to be used effectively in E.I.A. and E.R.A. programs.

The problem was solved by examining the actual Fucus community structure at each of the sites comprising the established regional transects. Community structure, in basic terms, can be defined as the number of species present (richness) and their relative abundances. For natural environments classical ecological generalizations have stated that there are typically fewer numerically dominant species in species-rich communities than in species-poor communities (Preston, 1960; McArthur, 1965; Hill, 1973). These conclusions, however, were based largely on results derived from terrestrial studies. Birch (1981) revealed that in marine communities the opposite was true and that there was a significant tendency for numerical dominance to be greater in species-rich than in species-poor conditions, under natural conditions. Comparable analysis of the Dean Channel transect data in this study supported his findings. In this case a reduction in species richness occurred at sites influenced by a corresponding

reduction in salinity.

Port Alice and Port Alberni transect data were also examined in this way. In contrast to the trend observed with the Dean Channel community data, both of these regions indicated a statistically significant negative correlation between species richness and dominance. That is, as the number of species within the community decreased, the dominance of one or two species within the assemblage increased. Both areas are influenced by the discharge of pulping effluent. This may contribute to organic enrichment of the water column, effectively supporting only the very tolerant, and thus community-dominant species (Pearson and Rosenberg, 1978; Read et. al., 1978). Although Alberni Inlet is also influenced by a reduced surface salinity, the effect of imposed pollutants becomes discernible when correlating changing species richness with an appropriate community dominance measure.

An important consequence of this analysis should also be considered at this time. If dominance in a natural marine community does tend to increase with a corresponding increase in species richness, then measures which are based on opposing assumptions should be used and interpreted cautiously (Birch, 1981). Single sample analyses, commonly employed in marine biomonitoring programs, include a number of these measures. Diversity (Shannon Weaver), for example, is based on such principles, with high diversity values dependent on a high degree of evenness ... not dominance.

4.4 Fucus-epifauna recovery analysis

A non-centered PCA was employed in the summarization of Port

Alice Fucus-epifauna data accumulated over seven years. Again, this multivariate technique was particularly useful, as explained above, due to the inherent sample disjunctions, i.e., sites associated with a depauperate epifaunal community due to imposed pollutional stress. The analytical objective here, however, differed slightly from the descriptive approach used in the general community survey discussed above. That is, epifauna samples taken at six sites over a period of seven years were analyzed concurrently to illustrate community similarities between sites, over time, and with respect to two control sites. The time frame used encompassed three years before and four years following the implementation of pollution abatement measures at the mill site (see section II). The technique effectively provides a multivariate model of shoreline recovery within Neroutsos Inlet using Fucus-epifaunal species/abundance relationships.

As illustrated in the resulting ordination plot, the Fucus communities at each of the sample sites indicates a distinct trend towards recovery. In fact, by 1981 total recovery had occurred at every site with the exception of stations 2, 1A, and 1. This corresponds with results of the biological surveys discussed in section II, and appears legitimate considering present sulfite concentrations and dissolved oxygen levels within 5 km of the mill. i.e., 200-300 ppm and 3-4 ppm, respectively.

Thus, non-centered PCA provides a multivariate analysis which appears useful in expressing environmental recovery in a meaningful way. Bloom (1980) carries this approach one step further by measuring the distance from each ordination point (sample) to the edge of the

95% R.E., obtaining a "distance to recovery". Once known, these data can be plotted against time. Theoretically, the general slope of the line obtained expresses the speed of environmental recovery. If the shape is not linear, but distinct plateaus or a 'staircase effect' results, a series of successional stages may be implied.

Whether this technique is used to its full extent, as described above, or whether it is used in a manner as outlined in this study, its usefulness in assessing environmental changes or in monitoring communities under chronic and acute pollutional stress, is quite evident.

IV. CONCLUSIONS

Use of biological monitoring techniques in environmental monitoring programs is an essential complement to physical and chemical methodologies. Although physical and chemical testing provides valuable information on water quality characteristics, biological data are necessary to establish the effects of deleterious materials on the receiving aquatic ecosystem. The most important advantage of biomonitoring is that a single series of samples can effectively reveal the status of existing biological communities which themselves represent the summation of prevailing conditions.

This study has been involved primarily with the effluent discharges associated with pulping wastes. The logistical limitations of various chemical and physical tests in these situations, for example, become particularly evident in view of the variability of types and amounts of chemicals used, types of wood processed, etc.. The complexities of the organic waste constituents prior to discharge are further compounded when dispersed and diluted in the marine receiving waters. The rates and pathways of chemical transformations and degradations compounded with environmental factors such as currents, tidal flushes, and freshwater inputs, make estimations of environmental pollutant concentrations next to impossible.

Biological monitoring techniques can be chosen and employed, singly or in combination, with reference to their pollutant sensitivities. The Port Alice E.R.A., for example, successfully documented the extent of environmental impact, in addition to shoreline recovery

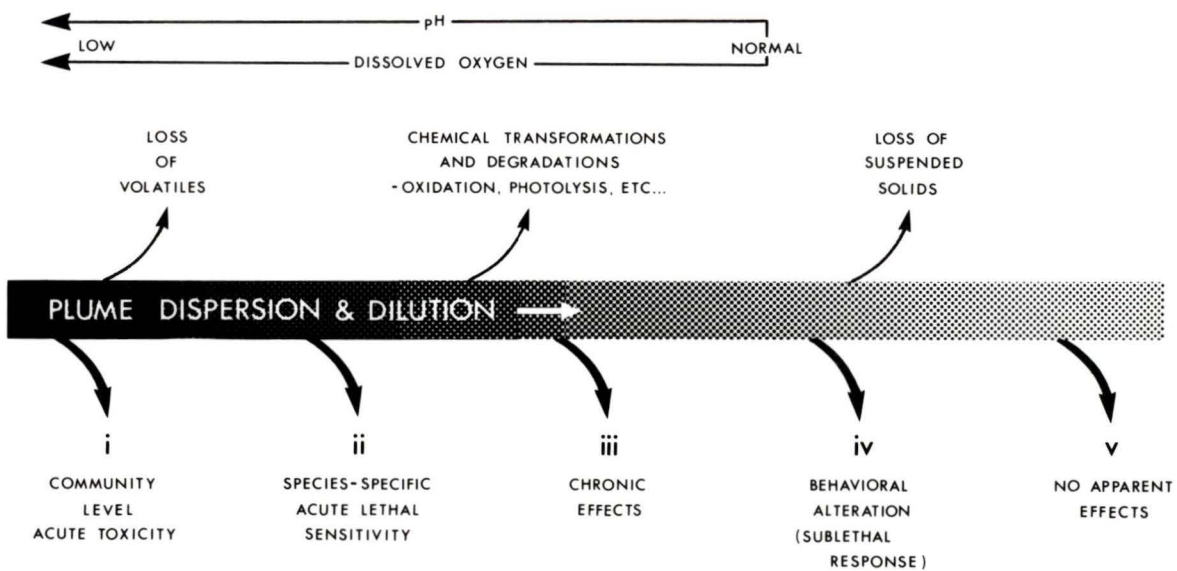
processes, using three biomonitoring techniques in combination. All three tests involved species distributional analyses, and thus could not lend themselves to an extrapolation of cause-effect relationships.

Cause-effect relationships are rarely attained in biomonitoring programs. Field studies could, theoretically, be used in conjunction with elaborate toxicity studies in an attempt to relate observed species distributional data to estimated sublethal toxicity thresholds. A refinement of the subjective categorization shown in the upper portion of Figure 31, for example, could be used to explain results obtained in the rocky shore intertidal survey at Port Alice if appropriate toxicity data were also provided. This approach, in addition to being very expensive and time-consuming, remains speculative in that it considers only a few direct and indirect abiotic effects of the pollutant. Sound cause-effect conclusions can only be drawn if all possible abiotic as well as biotic interactions are understood.

Biomonitoring techniques can be most effective if efforts are made to refine distribution-based methods. This type of approach can be used to define accurately the geographical extent of a pollutant on the basis of observed biological changes. Clear definition of an 'effective range' can then provide a baseline from which further changes in environmental quality can be monitored.

The Fucus-epifauna survey has been shown to be an effective distribution-based biomonitoring technique in this study. Initially restricted to the amphipod associates of Fucus, a refinement to incorporate the entire macroinvertebrate community was considered an

Figure 31 - Fate of effluent plume and possible resultant direct and indirect effects on the single species and community levels (Top). Functional composition and associated trophic level interactions are shown in a simple postulated 'web' diagram (Bottom).



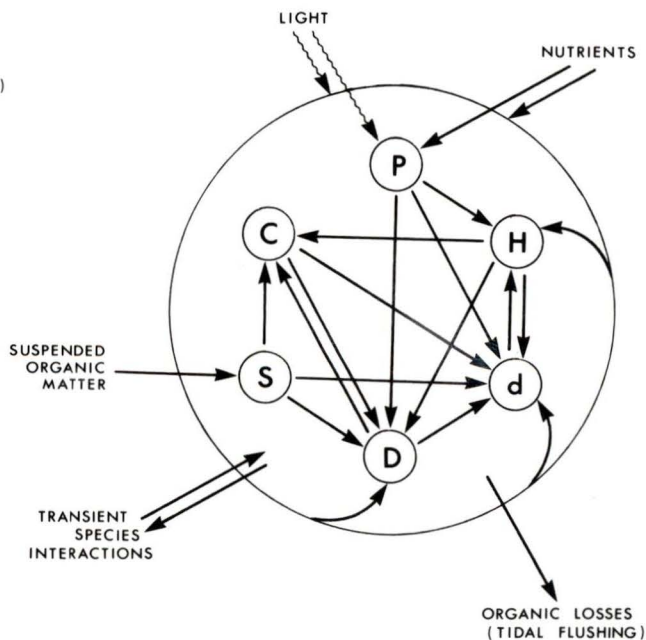
Fucus Community

- functional composition and interactions

(FUCUS REPRESENTED BY OUTER RING)

Key:

- P PERIPHYTON
- S SUSPENSION FEEDERS
- H HERBIVORES
- C CARNIVORES
- D DETRITIVORES (MACRO)
- d DETRITIVORES (MICRO)



important modification. Using all of the macroinvertebrates associated with Fucus provides an array of species characteristic of several taxonomic groups revealing differential sensitivities to an imposed polluttional stress.

The Fucus-epifauna technique considers changes in community structure as a function of a polluttional stress. Structural emphasis in a distribution-based method will reflect interactions between both abiotic and biotic factors. More important, documenting community structural differences provides information on possible functional changes due directly or indirectly to pollution effects.

The lower portion of Figure 31 presents a generalized Fucus community model, illustrating a number of functional groups and possible interactions. A number of indirect biotic effects due to a pollutant can be hypothesized. Restricting penetration of a particular wavelength of light from the water column, for example, could inhibit growth of periphyton on the Fucus. Although this type of response could be detected using single species distribution and toxicity tests of periphyton, the indirect response of other functional groups (e.g., herbivores) within the community would remain undetected. Conversely, toxicity tests utilizing herbivore species could provide a 'no effect' geographical range for this group around a discharge source. Such an approach would, however, exclude information on the delimitation of periphyton species, and thus on the indirect effects of the pollutant on the herbivore populations due to a missing food resource.

The design of an environmental impact or rehabilitation

assessment should include presentation of results in such a manner as to permit clear interpretation and communication of information. The multivariate approach used in this study reduces the multidimensionality of of the Fucus community structural data. Non-centered Principal Components Analysis (PCA) is particularly effective in pollution assessments in that community irregularities, evident as sample disjunctions within the data matrix, are clearly differentiated from communities representative of undisturbed environments. Additionally, graphical presentation of disturbed Fucus-epifaunal community samples with an appropriate group of samples characteristic of normal habitats can visually communicate information on the extent and/or rate of impact or recovery within a geographical region.

A holistic approach to biological monitoring of disturbed ecosystems is advocated. The Fucus-epifaunal community analysis supports this type of approach, and as a distribution-based method may be of particular use for monitoring displacements in shoreline ecosystem structure and function as a result of surface-imposed societal stresses.

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APPENDIX - Fucus-epifauna community data.

Site coordinates and measured environmental parameters.

Table 1 - Species by site abundance matrix resulting from general Fucus-epifauna survey. Species names corresponding to letter codes can be found within the text of the thesis (Table 4).

Species

<u>Site</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>	<u>F</u>	<u>G</u>	<u>H</u>	<u>I</u>	<u>J</u>	<u>K</u>	<u>L</u>	<u>M</u>	<u>N</u>	<u>O</u>	<u>P</u>	<u>Q</u>	<u>R</u>	<u>S</u>	<u>T</u>	<u>U</u>	<u>V</u>	<u>W</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
1	33	58	0	7	0	8	1	0	4	0	45	2	0	0	69	0	0	0	0	0	1	13	0	0	0	0
2	20	217	0	16	2	14	2	0	11	14	0	1	1	0	47	0	0	0	0	11	2	2	4	0	0	0
3	21	7	0	22	4	76	3	0	17	61	4	14	2	0	107	0	0	1	0	2	5	47	2	0	1	0
4	31	16	0	8	8	9	1	0	8	72	3	2	13	0	114	0	0	0	0	0	0	3	0	0	0	0
5	178	706	0	2	2	36	2	0	25	47	33	1	18	0	61	0	6	0	0	1	0	4	0	0	0	0
6	3	6	0	16	13	34	4	0	74	267	4	2	16	0	137	2	0	0	0	0	2	8	0	0	0	0
7	378	69	1	1	0	3	0	0	22	9	26	1	0	0	0	0	47	0	0	3	0	0	0	0	0	0
8	6	217	0	3	13	25	0	0	9	21	7	0	22	0	86	0	22	0	0	2	1	0	0	0	0	0
9	67	110	0	5	2	9	3	0	1	1	81	16	6	0	14	0	51	0	0	1	10	4	0	0	0	0
10	46	607	1	4	17	9	5	0	1	0	45	16	0	0	104	5	153	0	0	1	6	0	0	0	0	1
11	45	59	0	16	9	1	1	0	3	3	31	2	21	0	52	0	3	0	0	1	0	5	0	0	0	0
12	10	4	5	1	9	2	3	0	0	4	10	0	10	0	43	0	7	0	0	0	2	0	0	0	0	1
13	31	172	14	3	51	6	6	0	17	30	13	3	55	0	87	0	19	0	0	9	4	0	0	0	0	0
14	42	367	4	2	20	9	1	0	26	18	63	0	23	0	117	0	0	0	0	3	1	0	2	0	0	1
15	8	221	2	2	10	1	3	0	52	140	12	2	42	0	7	0	2	0	0	5	1	4	0	0	0	0
16	10	86	4	0	4	0	1	0	47	35	37	3	60	0	4	4	14	0	0	12	11	0	0	0	0	0
17	3	21	13	0	28	2	5	0	37	81	5	0	75	0	7	0	4	0	0	1	3	0	2	0	0	0
18	9	275	10	1	3	1	2	0	12	14	30	0	73	0	17	0	0	0	0	0	1	0	2	0	0	0
19	72	35	8	0	2	3	4	0	43	92	5	4	147	0	0	2	9	0	0	10	7	2	0	0	0	0
20	55	121	24	1	4	4	2	0	25	45	28	2	50	0	0	1	2	0	0	2	1	0	0	0	0	1
21	15	176	5	3	7	3	6	0	19	11	43	1	46	0	0	0	2	0	0	3	2	0	0	0	0	0
22	5	136	64	0	29	0	0	0	0	19	3	16	3	14	0	2	23	0	0	0	1	0	0	0	0	0
23	8	89	12	1	8	1	2	0	0	0	47	3	1	0	10	0	341	0	0	0	0	0	0	0	0	0
24	2	83	27	2	7	0	1	0	18	0	3	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0
25	2	19	9	0	8	0	3	0	12	13	8	0	0	0	3	0	31	0	0	1	3	0	1	0	0	0
26	15	598	6	17	0	0	3	47	86	0	0	1	0	0	31	0	0	2	0	0	0	0	1	0	0	7
27	18	296	9	10	0	0	3	21	22	0	119	0	0	37	2	0	6	0	0	0	0	0	1	0	0	1
28	35	97	4312	0	0	33	13	327	211	0	2	0	0	0	2	3	0	0	1	0	3	0	0	0	2	0
29	142	505	660	3	0	48	3	213	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	5	0
30	40	0	94	11	0	9	0	903	0	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0
31	0	0	0	0	4	0	0	0	0	0	0	0	0	0	10	0	0	0	5	0	0	0	0	0	0	0
32	102	202	269	11	0	0	3	0	8	0	0	0	0	0	0	0	0	0	0	6	4	0	0	0	1	0
33	3	136	12	5	2	0	2	67	99	0	40	0	0	22	169	0	20	0	0	0	0	0	0	0	0	1
34	48	18	0	0	2	0	14	0	0	0	11	0	0	13	0	12	5	0	0	2	0	0	0	0	0	1
35	10	213	3	1	2	0	1	0	0	0	18	9	0	14	84	3	75	0	0	1	3	0	0	0	0	3
36	6	97	0	2	0	0	0	0	1	4	7	8	0	8	93	0	0	0	0	11	2	0	0	0	0	1
37	1	2	3	1	2	0	0	0	0	15	0	19	0	8	141	0	0	0	0	14	3	1	0	0	0	1
38	8	162	0	0	1	0	0	0	0	11	3	1	0	0	2	0	0	0	0	9	3	0	0	0	0	1
39	27	4	1	4	2	0	0	0	4	30	1	7	0	26	54	0	0	0	0	2	0	0	0	0	0	0
40	103	22	1	18	3	5	2	0	0	46	1	22	0	0	187	0	0	0	0	20	21	33	0	0	2	0
41	395	5	0	1	0	0	0	0	6	0	18	1	0	0	3	0	2	0	0	0	0	0	0	0	0	0
42	282	33	12	0	0	1	3	0	6	0	12	3	0	0	35	0	0	0	0	1	4	1	0	0	0	0
43	29	149	0	0	0	2	0	0	96	0	6	0	0	0	29	0	0	0	0	0	1	2	0	0	0	0
44	184	7	7	3	4	1	2	35	30	0	24	26	0	0	27	0	0	0	0	2	0	0	0	0	0	0
45	38	66	2	15	2	0	4	2	9	13	48	17	0	0	20	1	0	0	0	0	2	0	0	0	0	1
46	63	3	2	5	0	3	1	8	0	0	16	50	0	0	80	0	0	0	0	1	22	1	0	1	0	0
47	166	239	2	0	0	0	3	20	15	0	10	1	0	0	7	0	0	1	0	0	0	0	0	0	0	0
48	25	159	9	6	2	18	3	3	0	0	5	33	0	0	76	0	0	0	0	0	0	13	0	0	0	0
49	137	53	53	0	2	1	10	2	0	0	2	3	0	0	34	0	0	0	0	0	0	0	0	0	0	0
50	0	0	16	1	5	0	9	0	0	0	1	9	0	0	224	0	0	0	0	0	87	39	3	0	2	0
51	238	30	45	0	0	0	3	730	0	0	30	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52	268	7	10	3	0	1	2	49	0	0	16	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0
53	69	2	328	0	2	0	2	174	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	30	5	615	0	24	0	8	362	0	0	2	4	0	0	0	0	0	0	0	0	1	2	5	0	0	0

Species

<u>Site</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>	<u>F</u>	<u>G</u>	<u>H</u>	<u>I</u>	<u>J</u>	<u>K</u>	<u>L</u>	<u>M</u>	<u>N</u>	<u>O</u>	<u>P</u>	<u>Q</u>	<u>R</u>	<u>S</u>	<u>T</u>	<u>U</u>	<u>V</u>	<u>W</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
55	90	2	49	0	3	1	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
56	20	1	50	0	2	0	1	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
57	12	0	73	0	0	0	2	34	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
58	17	0	49	0	1	0	1	348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
59	26	0	56	0	9	0	4	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	13	0	0
60	0	0	3	0	17	7	14	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
61	0	0	21	0	1	1	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	513	10	17	0	2	0	2	116	0	0	9	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
63	408	171	52	0	7	0	2	94	0	0	6	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0
64	460	110	7	0	1	0	1	45	0	0	12	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
65	125	713	8	0	0	0	1	226	31	0	24	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0
66	100	1100	4	3	2	3	2	16	20	0	5	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1
67	52	456	2	1	0	4	0	0	46	9	0	0	0	0	108	0	0	0	0	0	0	2	0	0	0	1
68	33	437	5	2	0	0	1	3	30	21	13	0	0	0	60	0	0	0	0	0	2	5	0	0	0	0
69	4	3	0	0	6	0	3	0	17	0	1	0	0	0	12	0	0	0	0	0	0	0	0	0	1	0
70	54	21	1	2	2	0	2	9	19	0	19	4	0	0	89	0	0	0	0	0	3	0	0	0	0	0
71	59	29	17	1	1	0	2	12	51	0	4	11	0	0	65	0	0	0	0	0	0	0	0	0	0	0
72	42	10	32	4	1	0	2	7	61	0	3	19	0	0	47	0	0	0	0	0	0	0	0	0	0	0
73	36	13	64	0	66	1	3	4	28	0	7	32	0	0	39	0	0	0	0	0	5	1	0	0	2	0
74	91	9	41	2	3	2	2	39	138	0	12	7	0	0	103	0	0	0	0	0	0	0	0	0	0	0
75	120	18	34	1	2	22	4	0	93	0	2	80	0	0	115	0	0	0	0	0	10	0	0	1	0	0
76	1245	0	36	18	0	0	6	0	193	0	0	78	0	0	3	0	11	0	0	0	10	0	0	0	0	0
77	111	0	46	0	5	0	65	0	6	0	0	0	0	0	0	1621	0	0	0	0	0	0	0	0	0	0
78	5	0	44	0	15	4	3	0	0	0	0	0	0	0	0	0	448	0	0	0	0	0	0	0	1	0
79	6	178	2	0	0	0	0	23	61	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	1
80	10	225	3	0	0	0	3	32	109	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
81	40	156	4	0	0	0	3	35	58	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	96	654	15	4	0	89	9	15	51	0	15	2	0	0	1	0	49	0	0	0	2	0	0	0	1	0
83	12	6	0	0	2	0	0	8	43	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
84	24	148	29	0	0	0	4	6	23	0	0	5	0	14	0	0	0	0	0	0	0	0	0	0	3	1
85	43	103	38	2	23	0	2	124	276	0	1	28	0	0	19	0	0	0	0	0	0	0	1	0	0	0
86	99	359	25	8	4	7	3	174	135	0	1	29	0	0	31	3	0	0	0	5	0	0	0	0	0	0
87	238	491	29	0	7	125	2	163	70	0	0	31	0	0	0	2	0	0	0	0	0	0	0	0	0	0
88	96	235	23	1	4	32	2	41	35	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
89	109	234	2	0	2	0	1	30	34	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
90	141	401	12	5	5	11	4	6	86	3	1	0	0	0	19	0	0	0	0	0	4	0	0	0	0	0
91	45	225	4	3	2	0	1	16	29	0	4	0	0	0	7	0	3	0	0	0	2	0	0	0	0	0
92	10	36	2	1	125	3	1	9	17	18	11	0	1	0	49	1	43	0	0	0	0	70	0	0	0	0
93	17	295	4	5	4	0	6	25	38	0	5	5	0	0	23	0	0	0	0	0	0	0	0	0	0	0
94	3	1	50	9	44	63	21	264	259	0	1	195	0	0	264	0	0	2	0	7	0	0	16	0	0	0
95	6	1	32	26	65	14	20	333	311	0	0	259	0	0	0	0	0	5	0	25	0	0	0	0	0	0
96	28	2	2	1	3	3	3	3	6	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0
97	43	0	8	4	2	24	0	388	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
98	3	0	4	0	14	13	14	34	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0
99	0	0	0	3	8	8	4	40	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
100	0	0	3	4	67	28	9	93	0	0	0	0	0	0	0	234	0	0	19	0	0	0	0	0	0	0
101	0	0	2	5	0	19	0	15	0	0	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0
102	0	0	0	6	0	189	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
103	0	0	0	0	0	2957	0	0	0	0	0	0	0	0	3	0	0	0	17	0	0	0	0	0	0	0
104	0	0	0	0	0	883	0	0	0	0	0	0	0	0	0	0	0	0	52	0	0	0	0	0	0	0
105	242	8	2	3	1	9	0	0	0	0	11	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
106	14	19	2	0	3	6	0	3	130	11	1	0	0	0	0	2	0	40	0	0	0	0	0	0	0	1
107	5	47	0	10	2	69	8	0	2	0	0	4	0	0	224	0	0	7	0	0	29	2	1	0	3	0

Table 2 - Geographical coordinates, water temperature, salinity, and displacement volume of Fucus sampled at each of the 107 sites.

Site	Coordinates		Temp. (°C)	Salinity (‰)	Fucus Vol. (l)	Site	Coordinates		Temp. (°C)	Salinity (‰)	Fucus Vol. (l)
1	123° 18.30' W;	48° 24.65' N	12.0	26.0	2.22	55	127° 45.70' W;	52° 14.70' N	17.7	9.5	2.38
2	123° 18.15' W;	48° 24.65' N	12.0	26.5	3.22	56	127° 38.40' W;	52° 17.20' N	17.0	9.0	2.88
3	123° 18.10' W;	48° 24.60' N	12.5	27.0	2.89	57	127° 33.85' W;	52° 10.15' N	18.0	8.0	2.78
4	123° 18.05' W;	48° 24.60' N	12.0	27.5	2.12	58	127° 28.70' W;	52° 21.65' N	18.5	6.0	2.68
5	123° 17.45' W;	48. 26.30' N	12.0	27.5	2.74	59	127° 22.05' W;	52° 24.25' N	18.0	5.0	1.95
6	123° 13.70' W;	48° 26.05' N	12.5	27.0	3.02	60	127° 19.00' W;	52° 27.00' N	18.0	4.5	3.20
7	123° 15.95' W;	48° 27.15' N	13.0	27.5	3.18	61	127° 14.10' W;	52° 27.25' N	18.5	5.5	3.06
8	123° 16.70' W;	48° 27.90' N	12.0	28.0	2.97	62	127° 56.65' W;	52° 18.55' N	17.5	12.5	2.90
9	123° 17.95' W;	48° 28.60' N	12.5	27.0	2.10	63	128° 05.75' W;	52° 18.00' N	16.5	15.5	2.64
10	123° 18.00' W;	48° 29.20' N	13.0	27.0	3.32	64	128° 11.50' W;	52° 15.50' N	16.0	16.5	2.95
11	123° 18.80' W;	48° 29.85' N	12.0	27.5	3.85	65	128° 16.30' W;	52° 10.90' N	15.5	22.0	3.00
12	122° 53.10' W;	48° 35.35' N	15.5	23.5	3.28	66	128° 19.25' W;	52° 04.25' N	11.5	30.0	2.92
13	122° 51.80' W;	48° 36.15' N	16.0	22.5	1.98	67	128° 25.45' W;	52° 00.00' N	12.0	29.0	3.12
14	122° 49.85' W;	48° 36.05' N	15.5	22.5	3.38	68	128° 28.30' W;	51° 54.45' N	12.0	26.5	2.94
15	122° 50.50' W;	48° 37.20' N	16.0	22.5	2.90	69	127° 55.90' W;	50° 27.95' N	11.5	25.0	3.88
16	122° 52.70' W;	48° 37.85' N	16.0	23.0	2.62	70	127° 50.40' W;	50° 29.35' N	11.5	25.0	2.70
17	122° 52.35' W;	48° 38.65' N	15.0	23.0	2.86	71	127° 39.60' W;	50° 29.50' N	13.0	26.0	2.18
18	122° 52.85' W;	48° 40.65' N	15.0	23.5	2.68	72	127° 37.00' W;	50° 30.85' N	13.5	25.5	2.92
19	122° 54.60' W;	48° 39.50' N	16.5	23.0	2.18	73	127° 34.00' W;	50° 28.80' N	13.5	24.5	3.10
20	122° 54.95' W;	48° 41.05' N	16.5	24.0	3.48	74	127° 32.30' W;	50° 27.05' N	14.0	24.5	2.75
21	122° 54.10' W;	48° 41.45' N	16.0	23.0	2.10	75	127° 30.95' W;	50° 27.30' N	15.5	24.0	1.98
22	123° 29.25' W;	48° 40.65' N	17.0	25.0	1.98	76	127° 30.00' W;	50° 25.80' N	16.0	24.0	2.56
23	123° 28.75' W;	48° 37.65' N	18.0	24.5	2.30	77	127° 28.50' W;	50° 23.35' N	15.5	24.0	3.62
24	123° 28.85' W;	48° 35.90' N	17.0	25.0	2.88	78	127° 27.45' W;	50° 22.80' N	16.0	24.0	2.68
25	123° 31.25' W;	48° 33.65' N	14.5	22.0	3.00	79	127° 51.35' W;	50° 18.05' N	12.5	27.0	2.60
26	123° 35.50' W;	48° 50.80' N	20.5	18.5	2.78	80	127° 45.35' W;	50° 14.30' N	13.5	26.0	2.82
27	123° 45.35' W;	49° 02.70' N	12.5	21.5	3.12	81	127° 40.70' W;	50° 08.05' N	14.0	23.0	2.98
28	123° 13.90' W;	49° 20.45' N	16.0	22.0	3.62	82	127° 32.30' W;	50° 04.95' N	12.5	22.5	3.42
29	123° 13.90' W;	49° 28.25' N	18.0	11.5	3.15	83	127° 12.05' W;	50° 04.95' N	15.0	25.0	1.95
30	123° 13.70' W;	49° 34.70' N	17.5	7.5	2.98	84	127° 14.75' W;	49° 58.25' N	13.5	28.0	2.15
31	123° 10.35' W;	49° 40.00' N	14.0	2.5	3.42	85	126° 59.00' W;	49° 51.90' N	14.5	22.5	3.48
32	123° 47.55' W;	49° 32.80' N	15.0	19.5	3.60	86	126° 47.80' W;	49° 53.05' N	14.5	20.0	3.16
33	124° 13.65' W;	49° 18.55' N	13.0	21.5	3.08	87	126° 38.95' W;	49° 51.00' N	14.5	24.0	2.78
34	124° 55.20' W;	49° 38.25' N	19.5	20.5	2.92	88	126° 39.25' W;	49° 46.95' N	14.0	25.5	2.45
35	125° 12.60' W;	49° 58.00' N	16.0	24.0	2.48	89	126° 35.70' W;	49° 41.75' N	16.5	24.0	3.02
36	125° 19.65' W;	50° 07.00' N	12.5	26.5	2.64	90	126° 33.70' W;	49° 34.65' N	14.5	27.5	2.20
37	125° 21.95' W;	50° 14.90' N	11.5	21.0	3.12	91	126° 16.90' W;	49° 21.45' N	14.0	24.0	3.62
38	125° 34.60' W;	50° 22.05' N	12.5	26.0	3.40	92	125° 31.50' W;	48° 55.50' N	13.0	28.0	2.12
39	125° 57.90' W;	50° 24.35' N	11.5	21.0	2.42	93	125° 09.40' W;	48° 54.30' N	17.0	23.5	1.88
40	126° 33.70' W;	50° 31.05' N	10.5	22.0	2.08	94	125° 03.35' W;	48° 52.35' N	19.0	22.0	3.52
41	127° 00.95' W;	50° 36.20' N	10.0	22.0	2.02	95	124° 59.95' W;	48° 58.05' N	20.0	19.0	2.75
42	127° 24.00' W;	50° 45.80' N	12.0	22.5	2.98	96	124° 54.95' W;	48° 59.05' N	16.5	21.5	2.40
43	128° 02.50' W;	50° 52.45' N	11.5	30.0	3.28	97	124° 52.20' W;	49° 00.90' N	19.0	20.5	3.10
44	128° 45.00' W;	51° 28.20' N	14.0	18.5	3.20	98	124° 51.65' W;	49° 03.35' N	18.0	17.0	3.35
45	127° 54.55' W;	51° 31.90' N	14.5	19.0	2.10	99	124° 49.80' W;	49° 05.30' N	18.5	16.0	3.42
46	127° 51.75' W;	51° 36.40' N	15.5	19.0	2.40	100	124° 47.95' W;	49° 07.80' N	20.0	15.5	2.98
47	128° 05.30' W;	51° 40.20' N	12.5	23.5	2.09	101	124° 48.35' W;	49° 09.35' N	22.0	12.0	2.10
48	127° 53.45' W;	51° 41.75' N	16.0	21.0	3.02	102	124° 49.25' W;	49° 11.40' N	22.5	10.5	3.30
49	128° 00.45' W;	51° 47.95' N	14.0	24.0	2.76	103	124° 49.60' W;	49° 12.95' N	21.5	5.0	2.92
50	127° 53.25' W;	51° 55.75' N	16.0	24.5	3.32	104	124° 49.40' W;	49° 13.70' N	21.0	4.5	2.50
51	127° 57.25' W;	52° 01.35' N	15.5	19.0	2.86	105	124° 26.30' W;	48° 31.50' N	11.0	28.0	2.46
52	127° 52.50' W;	52° 06.60' N	16.0	12.0	2.80	106	124° 06.20' W;	48° 25.80' N	13.0	27.0	2.86
53	127° 52.95' W;	52° 11.15' N	16.0	11.0	2.45	107	123° 49.30' W;	48° 21.20' N	12.0	28.0	3.46
54	127° 55.60' W;	52° 16.00' N	16.0	12.5	1.85						

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Stephen F. Cross

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