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Effects of Treated Sewage Effluent on Periphyton and Zoobenthos in the Cowichan River, British Columbia

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February 1988

Canadian Technical Report of
Fisheries and Aquatic Sciences
No. 1591

Fisheries and Oceans Pêches et Océans

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Canadian Technical Report of

Fisheries and Aquatic Sciences No. 1591

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EFFECTS OF TREATED SEWAGE EFFLUENT ON PERIPHYTON AND ZOOBENTHOS IN THE COWICHAN RIVER, BRITISH COLUMBIA

by

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Cat. No. Fs 97-6/ E ISSN 0706-6457

Correct citation for this publication:

Perrin, C.J., N.T. Johnston, and S.C. Samis. 1988. Effects of treated sewage effluent on periphyton and zoobenthos in the Cowichan River, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1591: 64 p.

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Perrin, C.J., N.T. Johnston and S.C. Samis. 1988. Effects of treated sewage effluent on periphyton and zoobenthos in the Cowichan River, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1591: 64 p.

Inorganic nutrient concentrations, periphyton accrual and zoobenthos biomass were measured at one upstream and three downstream sites in the Cowichan River to determine effects of effluent discharge from the upgraded Duncan-North Cowichan sewage treatment plant. Samples were taken during summer months in 1979 and before and after discharge began in August 1980.

Concentrations of total dissolved phosphorus (TDP) and ammonia (NH_3 + NH_4-N) and biomass of algal periphyton on artificial substrata increased by an order of magnitude after discharge began. NO_3 + NO_2-N levels did not change likely due to the reduced nature of the sewage effluent.

Taxonomic composition of the zoobenthos was greater between years than among sites within years. The relative abundances of taxa suggested that the three sites downstream of the discharge in 1980 differed from the spatial and temporal control sites. The abundances of cladocerans and oligochaetes generally increased at the sites subject to the sewage effluent discharge. Tardigrade abundance increased with increasing concentrations of algal biomass and macronutrients, and with decreasing intragravel dissolved oxygen concentrations. The abundances of Diptera, Ephemeroptera, Plecoptera, and Trichoptera were not influenced by the discharge.

The availability of fish food organisms is unlikely to have been greatly altered by the sewage effluent; neither oligochaetes nor benthic cladocerans are commonly important components of the diets of stream dwelling salmonids. The development of algal mats may have reduced availability of chironomids to juvenile salmon.

RÉSUMÉ

Perrin, C.J., N.T. Johnston and S.C. Samis. 1988. Effects of treated sewage effluent on periphyton and zoobenthos in the Cowichan River, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1591: 64p.

On a quantifié les concentrations de bioéléments inorganiques, l'accumulation de périphyton et la biomasse du zoobenthos à un site d'amont et à trois sites d'aval dans la rivière Cowichan afin de déterminer les incidences des effluents provenant de l'usine d'épuration rénovée de Duncan-North Cowichan. Des échantillons ont été prélevés pendant l'été 1979 et avant et après le déclenchement des déversements en août 1980.

Les concentrations de phosphore dissous total (TDP) et d'ammoniac (NH_3 + NH_4-N) ainsi que la biomass de périphyton algal sur des substrats artificiels ont augmenté d'un ordre de grandeur après le déclenchement des déversements. Les teneurs en NO_3 + NO_3-N n'ont pas varié, probablement à cause de l'état de réduction des effluents d'eau d'égout.

La diversité taxonomique du zoobenthos était plus importante d'une année à l'autre qu'entre les sites pendant la même année. L'abondance relative des taxons porte à croire que les trois sites en aval des déversements effectués en 1980 étaient différents des sites spatiaux et temporaux témoins. L'abondance des cladocères et des oligochètes a généralement augmenté aux sites soumis au déversement des effluents d'eau d'égout. L'abondance des tardigrades a augmenté en fonction d'une augmentation des concentrations de la biomasse algale et des macro-bioéléments et d'une baisse des teneurs en oxygène dissous dans les interstices du gravier. Par contre, l'abondance des Diptères, des Éphéméroptères, des Plécoptères et des Trichoptères n'a pas été touchée par les déversements.

Il est peu probable que la disponibilité de poissons proies ait été fortement modifée par les effluents d'eau d'égout; ni les oligochètes ni les cladocères benthiques ne constituent des éléments importants du régime alimentaires des salmonidés peuplant des cours d'eau. La prolifération de couches d'algues peut avoir diminué la disponibilité de chironomidés pour les saumons juvéniles.

INTRODUCTION

The effects of organic pollution on stream communities are well documented (Kolkwitz and Marsson 1909; Hynes 1971; Wiederholm 1984). Gross organic enrichment can result in decreased levels of dissolved oxygen (Hynes 1971), greatly increased abundance of algae (Fjerdingstad 1965; Whitton 1979), increased abundances of some groups of benthic invertebrates, such as oligochaetes and some chironomids, and decreased abundances of other groups (Hynes 1971; Gaufin 1973). These changes can adversely affect juvenile and adult fish through decreases in food availability and habitat quality (Mundie 1974). In contrast, mild organic enrichment or enrichment with inorganic nutrients can result in increased production of salmonid fishes in nutrient deficient streams (Warren *et al.* 1964; Slaney *et al.* 1986) by increasing standing stocks of the benthic invertebrates that are eaten by fish (Williams *et al.* 1977; Mundie *et al.* 1983; Perrin and Johnston 1985). Coastal rivers on eastern Vancouver Island have low background concentrations of macronutrients and respond to nutrient additions by greatly increasing periphyton production (Munro *et al.* 1985; Perrin *et al.* 1987). In some Vancouver Island streams, localized areas of thick algal mats have occurred where fish hatchery effluents have been discharged (Munro *et al.* 1985).

The interconnection of the City of Duncan and Corporation of North Cowichan sewage treatment facilities through a common discharge to the mainstem Cowichan River in August, 1980 raised the possibility of local degradation of an important salmonid spawning and rearing stream (Neave 1949). The treated sewage effluent had high concentrations of phosphorus and nitrogen and moderate biological oxygen demand (Derksen 1981) which could alter periphyton and zoobenthos communities in the vicinity of the discharge. This study was undertaken to assess the effects of effluent discharge on the periphyton and zoobenthos, and to infer the possible effects on salmonids from any observed changes.

MATERIALS AND METHODS

SITE DESCRIPTION

The Cowichan River is a third-order system that originates at Cowichan Lake, flows easterly for 47 km and discharges into Cowichan Bay, southern Vancouver Island (Fig. 1). At the point of study, the Cowichan River drains approximately 826 km^2 including the Cowichan Lake drainage. This area is 98% of the total Cowichan River Basin.

During summer months (mid-June through mid-September), river flow is normally maintained above 4.25 $m^3.s^{-1}$ by flow control at the B.C. Forest Products Ltd. (BCFP) dam, located at the Cowichan Lake outlet. Flow control by BCFP began in 1965 to provide a water supply for the pulpmill at Crofton, 10 km north of Duncan. Cowichan River flows are monitored by Water Survey of Canada at station number 08HA011, located 1 km upstream of the study area (Fig. 1).

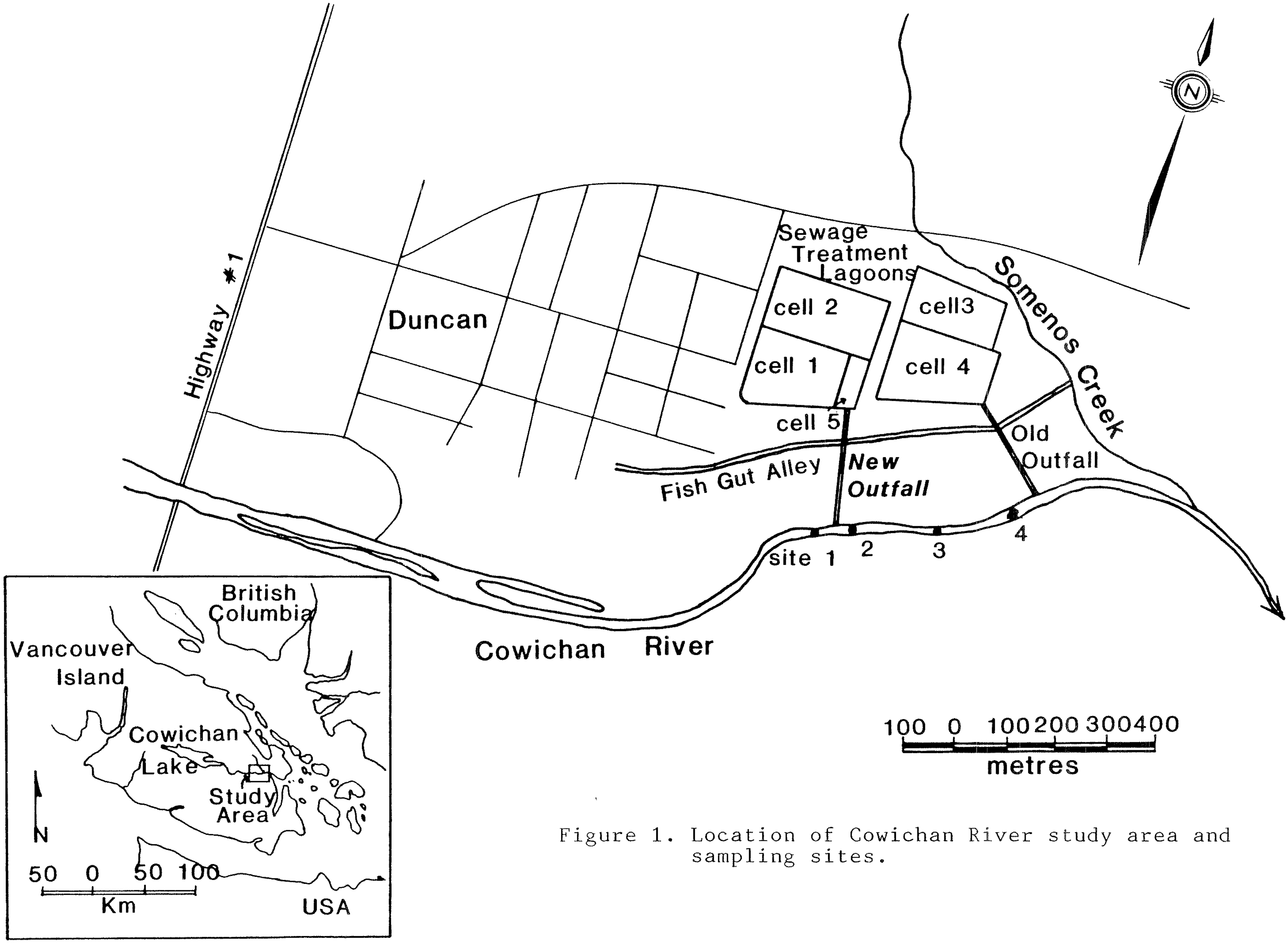

Figure 1. Location of Cowichan River study area and sampling sites.

In addition to effects from the Duncan - North Cowichan sewage lagoon effluent, chemistry of the Cowichan River is also affected by discharge from the Lake Cowichan sewage lagoon located near the townsite of Lake Cowichan (Fig. 1). This Lake Cowichan effluent is highly diluted at full mixing but has contributed to what are slightly higher cation concentrations than are typical of other streams on Vancouver Island (Perrin et al. 1987; Scrivener 1975). Anion concentrations are low and do not differ markedly from other sites. Specific conductance averages about 6.0 $mS.m^{-1}$ at 25°C, alkalinity is 30 $mg.L^{-1}$ $CaCO_3$ (Derksen, unpub. data) and pH ranges from 7.5 to 7.8. Nitrate plus nitrite nitrogen levels are generally <10 $ug.L^{-1}$ in mid-summer, but total ammonia concentrations are usually 6-10 $ug.L^{-1}$. Ammonia is usually undetectable (<5 $ug.L^{-1}$) in other Vancouver Island streams in summer. Total dissolved phosphorus levels are generally <5 $ug.L^{-1}$.

The Cowichan River has long been recognized as an important stream for salmonids (Neave 1949; Lill et al. 1974; Lister et al. 1971). Indigenous species include all 5 Pacific salmon in addition to cutthroat trout (Salmo clarkii), steelhead trout (Salmo gairdnerii), kokanee salmon (Oncorhynchus nerka), and Dolly Varden (Salvelinus malma). Attempts to introduce Atlantic salmon (Salmo salar), brown trout (Salmo trutta), and speckled char (Salvelinus fontinalis) before the Second World War were largely unsuccessful. The diverse nature of fish species in the Cowichan River is attributed to extensive low gradient channels that provide excellent spawning conditions and diverse rearing habitat. The hydrological buffering effect and nutritional characteristics of Cowichan Lake are also thought to optimize downstream rearing conditions (Lill et al. 1974). Average annual escapements to the Cowichan River include 7,000 chinook, 42,000 coho, and 55,000 chum salmon. These escapements, in addition to trout runs, support an important Indian food fishery, more than 12,000 angler days of sport fishing on the river, and a tidal sport fishery renowned for catches of large-sized chinook and coho. The Cowichan salmon stocks also provide a multimillion dollar contribution to the commercial salmon fisheries.

Sampling sites were located approximately 40 km downstream of Cowichan Lake on the Cowichan mainstem in the vicinity of the Duncan - North Cowichan sewage lagoon effluent diffuser (Fig. 1). Station 1 was located 50 m upstream of the diffuser and provided a spatial control site. Stations 2 through 4 (treatment sites) were located 50 m, 150 m, and 350 m respectively, downstream of the diffuser. The wetted width of the river during the study period averaged 15 m and water depth at periphyton sampling stations was 35-50 cm. Current velocities ranged between 40 and 60 $cm.s^{-1}$. Substrata consisted of mixed gravel and cobble and coarse sand.

PHYSICO-CHEMICAL VARIABLES

For the study periods of mid-June to mid-September in 1979 and 1980, Cowichan River flow data were obtained from Water Survey of Canada records for station 08HA011. This station has continuous water level recordings from which mean daily flows are determined using a calibrated flow-water height rating curve.

Water samples for chemical analysis of dissolved nutrients were collected at a mid-channel location at each site on four dates in 1979 and five dates in 1980 (Table 1) as described by Derksen (1981). Three replicate samples were collected for analysis of total dissolved phosphorus (TDP), nitrate plus nitrite nitrogen (NO_3 + NO_2 - N) and total ammonia nitrogen (NH_3 + NH_4 - N). With the exception of one date in 1980 when only one set of replicate samples were collected, replicate samples were collected three times over 24 hours to give a total of 9 replicates for each site on each sampling date.

Instantaneous temperature measurements were made using a hand-held thermometer at a mid-channel location on each sampling date.

At similar locations, duplicate samples of surface and subsurface (15 cm depth measured from uppermost substrate) water were collected in 600 mL stainless steel syringes for analysis of dissolved oxygen concentrations (Derksen 1981). Percent saturation of dissolved oxygen (DO) was determined from standard temperature-oxygen tables (Wetzel 1975).

PERIPHYTON ACCRUAL

Periphyton biomass was sampled from four replicate plexiglass substrata (20 x 30 cm plate mounted on a concrete anchor) incubated at each sampling site for 20 days (after Stockner and Shortreed 1976). Current velocities and water depths were similar between sites, ranging between 40 to 60 $cm.s^{-1}$ and 35-50 cm respectively. Replicates were located to suit these velocity and depth criteria at each site. In both 1979 and 1980, substrate incubations began in mid-June and were run consecutively until mid-September. This approach of repetitive incubations provided 4 separate experiments through time to examine differences in accrual between sites. Vandalism in 1979, however, resulted in only two completed experiments. Of these two, data from Sites 1 and 2 were incomplete. In 1980, two experiments were completed both before and after sewage discharge began on August 13.

After each 20-day incubation, one subsample for each of a chlorophyll-a and ash-free dry weight (AFDW) analysis was scraped from the plexiglass surface, placed in a light-tight plastic vial and shipped on ice to the Environment Canada, Water Quality Laboratory for analysis within 48 hours of sample collection. Laboratory procedures followed those outlined in Analytical Methods Manual (1979). Both chlorophyll-a and AFDW data provided estimates of periphyton biomass. After the plexiglass substrata were scraped, the sampling surface was cleaned and the plate assembly was reincubated for the subsequent experiment.

It is important to recognize that these biomass data do not represent periphyton growth (c.f. Bothwell 1985) or the net rate of periphyton accrual (c.f. Perrin et al. 1987). Sampling at a single point in time after a predetermined incubation period results in data subject to variation within and between sites due to differences in insect grazing, scouring, and passive settlement. In addition, a single data point through time does not allow a precise rate function to be calculated. Unless treatment effects are very large, variation imposed by this sampling approach may confound a real effect. Hence, our approach has been to use chlorophyll-a concentrations and AFDW from plexiglass substrata as relative biomass indices to detect large differences in periphyton accumulation due to sewage effluent that may impact the trophic structure of the Cowichan River.

Preliminary review of chlorophyll-a and AFDW data using log/log plots of the variances and means showed these two statistics not to be independent. Hence, before an ANOVA was run, data were transformed; x replaced by log (x+1) was found to be appropriate since the variance was generally greater than the mean. Each incubation series was treated as a separate experiment. Hence, a one-way ANOVA followed by the Newman-Keuls multiple range test was used for each experiment to examine treatment (location) effects. To display data, means were back-transformed and hence represent geometric means of the original data. Ninety-five percent confidence intervals were determined on log transformed data and back-transformed to match the geometric means.

PERIPHYTON TAXONOMY

Relative proportions of algal taxa were determined from three replicate samples collected from natural substrata in the vicinity of the plexiglass plates at each site. Samples were collected using a brush-piston assembly originally reported by Stockner and Armstrong (1971). The sampler collected biomass from a surface area of 20.4 cm^2. All samples were preserved in Lugol's solution and stored at room temperature prior to analysis. The relative abundance of each algal class or phyla and genera was determined after subsamples were allowed to settle in Utermohl chambers for at least 8 hours. Several transects were then examined at 500X magnification as described by Northcote et al. (1975). Extra-cellular products (e.g., gelatinous stalks) were included in the estimate. Reference works used for identification were Hustedt (1930), Cleve-Euler (1951-55), Prescott (1962), and Patrick and Reimer (1975).

ALGAL BIOASSAY

To examine the potential role of inorganic nitrogen and phosphorus in limiting algal growth in Cowichan River water, the Selenastrum capricornutum Printz algal assay bottle test (Miller et al. 1978) was conducted during September 16-30, 1981.

Since results of a batch culture bioassay have limited use when dealing with stream periphyton, the application of the technique in this study must be clearly defined. The basic approach of any batch culture bioassay is to examine the algal growth potential (AGP) of a test alga under suitable conditions of light and temperature in filtered and sterilized samples of water collected from the test site to measure the maximum biomass produced. The importance of chemical parameters (e.g., nutrients) in limiting AGP is examined by experimentally dosing cultures with several nutrient combinations. Relative differences in yield of the test alga provide insight into the order in which the various nutrients limit AGP. Hence, the bioassay only establishes the nature of the nutrient limiting maximum standing crop and does not examine nutrient-limited growth. This distinction between maximum biomass in batch culture and specific growth rate is most important when examining limitations to accumulation of algae in streams. In streams, the supply of nutrients is continuous and in third order or larger systems like the Cowichan River, periphyton biomass is low enough that the algae are ineffective in reducing the nutrient concentrations as does happen in an AGP bioassay. It is generally recognized that growth of algae in streams is limited by nutrient concentrations and the relationship between nutrient concentration and algal growth can only be examined in a continuous-flow apparatus where nutrient concentrations can be manipulated, such as that used by Bothwell (1983) or Stockner and Shortreed (1978). Specific nutrient concentrations are not maintained throughout the duration of a batch culture bioassay. Hence, differences in maximum biomass culture only elucidate the potential importance of N or P in limiting algal growth. A finding that P, for example, first limits AGP in the bioassay, does not mean that periphyton growth *in situ* is definitely P-limited. Actual P concentrations *in situ* or in culture may be adequate to saturate P-limited growth. In this study, then, the bioassay is used only to identify an element that potentially limits algal growth and would not otherwise have been identifiable from field sampling of sewage effluent that contains a wide range of chemicals that could affect algal growth. Results are then compared to *in situ* concentrations and supply ratios of potentially important elements which are useful in characterizing nutrient-deficiency in the *in situ* periphyton.

An important assumption in using the bioassay is that light and temperature do not limit algal growth. The bioassay is run at optimum light and temperature for growth of *S. capricornutum* in order to examine single factor effects: in this case, N and P were single factors. In testing Cowichan River water, this assumption seems reasonable. Both light and temperature are of secondary importance compared to phosphorus supply in limiting periphyton growth in other B.C. streams (Bothwell, unpub. data; Perrin and Johnston 1986)

Bioassay procedures followed those of Miller *et al.* (1978). The uni-algal culture of *S. capricornutum* was obtained from stock cultures of the U.S. Environmental Protection Agency Corvallis laboratory, and grown in autoclaved and filtered stream water collected from each Cowichan site to which N, P and a chelator were added in the following combinations:

1. control
2. site water + EDTA (1 $mg.L^{-1}$)
3. site water + EDTA + N(1 $mg.L^{-1}$ as $NaNO_3$ -N)
4. site water + EDTA + P (0.05 $mg.L^{-1}$ as K_2HPO_4 - P)
5. site water + EDTA + N (concentration as in 3) + P (concentration as in 4).

Treatments were run in triplicate using water from each of the four Cowichan sites. Cells of *S. capricornutum* were rinsed twice in sterile distilled water before inoculation in culture flasks to remove nutrients associated with the stock growth media. The initial cell concentration for each treatment was 1000 $cells.mL^{-1}$. Total culture volume was 50 mL. All flasks were incubated in a Labline Environ-Shaker 3597 at 24°C with continuous shaking of 100 rpm and continuous illumination using Vita-lite fluorescent lighting at 400 ft-c.

Algal growth was measured daily as *in vivo* fluorescence in a Turner fluorometer. When further increases in biomass were no longer detectable (usually day 14), cell number and median cell size were estimated with a Coulter Counter Model Z_{B1} using a 100 uL aperture tube. All cells greater than 20 um^3 were counted. An approximate conversion to standing crop (dry weight) was made as follows:

$$B = C_n \cdot V_m \cdot k$$

where: B = dry weight ($mg \cdot L^{-1}$)

C_n = final cell number

V_m = final median cell volume

k = volume to dry weight conversion factor (3.1×10^{-7} $ug.L^{-3}$)

N/P supply ratios (NO_3 + NH_3 - N:TDP) for each treatment were determined from duplicate NO_3 - N, NH_3 - N, and TDP analyses of autoclaved and filtered water from each of the 4 Cowichan sites before the bioassay was started.

BENTHIC INVERTEBRATES

Benthic invertebrates were sampled at the control site located about 50 m upstream of the effluent discharge and at the three treatment sites located 50, 150, and 350 m downstream (Fig. 1). Studies in other B.C. coastal streams had suggested that effluent (i.e., hatchery effluent) effects were largely confined to areas 60 to 700 m below discharge sites (Munro et al. 1985). Sampling sites were chosen for similar substrata, water depths, and water velocities (25-43 $cm.s^{-1}$). Six replicate samples were taken at each site using a modified Mundie sampler (Mundie 1971; Munro et al. 1985) with a 250 um mesh size and an area of 0.18 m^2. Samples were preserved in formalin. Samples were taken prior to (September 5, 1979) and after (September 9, 1980) the start of the discharge.

Samples were stained with rose bengal, large insects were removed from the whole sample, and the sample was split with a Folsom plankton splitter (McEwan et al. 1954) until a fraction (1/64 to 1/1024) containing 100-200 organisms was obtained. This sample size was found to give a reliable estimate of the total abundance (Munro et al. 1985). Four samples were enumerated from each date and site. Cladocerans were identified to species, chironomids to genera, other insects to families, and other invertebrates to orders. Count data were converted to numbers per m^2 for comparison with other data.

The experimental design for the zoobenthos sampling was a site-by-time factorial analysis of variance (ANOVA), which is the optimal design for an impact study (Green 1979). We assume that the spatial control differed from the impact area only in the absence of the effluent discharge. With both spatial and temporal controls, evidence for an impact is a statistically significant site-by-times interaction in the ANOVA (Green 1979). Abundance data for the major invertebrate groups were analysed as fixed effect, two-way factorial ANOVA's using the GENLIN general linear model (Greig and Bjerring 1980) at the University of British Columbia Computing centre. Comparisons of treatment means used Tukey's test at α =0.5; Zar (1984) gives reasons for favouring Tukey's test. All other statistical analyses used the MIDAS statistical package (Fox and Guire 1976).

The variances of the abundance data increased with the means. Consequently, abundance data were transformed as $\log(x + 1)$ to improve normality and homoscedasticity. Equality of variances of the transformed data was tested by Bartlett's test. Means and 95% confidence limits were back-transformed to the original scale and represent geometric means.

Cluster analysis was used to assess changes in benthic community composition between years and among sites. The clustering algorithm was the unweighted pair-group method using arithmetic average linkage (Sneath and Sokal 1973); single linkage and centroid linkage also gave identical results. Changes in species composition were examined using the complement of the Jaccard coefficient of association (Sneath and Sokal 1973) as the distance function while changes in relative abundance were examined using the complement of the correlation coefficient as the distance function.

We examined the effects of environmental conditions on the abundance of the zoobenthos by a stepwise regression of mean abundance against principal components formed from environmental variables. We used total organic carbon, total dissolved phosphorus, nitrate-nitrite nitrogen, chlorophyll-a concentration, ammonia nitrogen and intragravel oxygen concentrations as environmental variables. Low dissolved oxygen levels or high ammonia levels could be toxic to some benthic invertebrates while chlorophyll-a and macronutrients may reflect potential food quantity and quality. The chemical data measurements were made simultaneously with the invertebrate and periphyton sampling. Principal components were used to eliminate inter-correlations among the environmental variables. Taxa that accounted for more than 1% of the numbers of zoobenthos at any site were examined.

RESULTS

PHYSICO-CHEMICAL VARIABLES

Cowichan River flows were generally higher in 1979 than in 1980 (Fig. 2). In both years, flows were 10-12 $m^3.s^{-1}$ in early June but with flow control measures and lower runoff, flows declined to the minimum requirement of about 4.25 $m^3.s^{-1}$ in late June. Through July, 1979, flows increased to $\sim$10 $m^3.s^{-1}$ but declined to a minimum baseflow averaging 4.5 $m^3.s^{-1}$ from mid-August through mid-September. In 1980, flows remained at baseflow levels through July and August. In early September, flows increased rapidly to peak at 20.4 $m^3.s^{-1}$ due to release of storage water from Lake Cowichan during high runoff.

Surface water temperatures at Sites 1 through 4 were similar between years (Table 1). In mid-June, temperatures of 15-16°C were typical and by late July peak temperatures of 22.4°C and 20.8°C were recorded in 1979 and 1980 respectively. By the end of field sampling in mid-September of each year, temperatures were 16-17°C.

Table 1. Mean temperature and dissolved oxygen concentrations at Cowichan River sampling sites in 1979 and 1980.

A. TEMPERATURE (°C)

	June 16 - 20				July 7 - 11				July 28 - Aug. 1				August 20				Sept. 4 - 9			
Sites	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1979	16.0	16.0	16.2	16.3	17.6	17.6	17.6	17.6	22.1	22.1	22.4	22.4					17.5	17.5	17.5	17.5
1980	15.4	15.4	15.2	15.2	17.7	17.7	17.8	17.8	21.0	21.0	20.8	20.6	18.5	18.5	18.5	18.4	16.7	16.6	16.5	16.5

B. SURFACE DISSOLVED OXYGEN ($mg.l^{-1}$)

1979	9.6	9.7	9.8	9.8	9.4	9.4	9.4	9.5	8.9	8.9	9.0	9.0					9.6	9.6	9.9	9.6
Percent Saturation	99.8	101.1	103.0	103.1	101.5	101.6	101.9	102.0	104.4	104.6	106.7	106.4					103.6	103.8	106.8	103.4
1980	9.6	9.6	9.4	9.6	9.5	9.4	9.3	9.3	9.4	9.4	9.4	9.3	9.2	8.9	8.9	9.0	9.7	9.7	9.5	9.8
Percent Saturation	99.2	99.2	97.1	98.2	102.7	101.3	100.7	101.1	108.2	108.2	107.8	105.8	100.8	97.3	98.4	98.4	102.8	102.4	100.9	103.3

C. SUBSURFACE DISSOLVED OXYGEN ($mg.l^{-1}$)

1979	3.5	4.9	6.4	8.1	1.8	5.1	1.9	7.4	5.4	5.3	2.8	6.0					4.5	6.3	4.4	7.1
1980	7.1	7.7	7.3	8.0	7.2	7.6	7.2	7.1	6.9	7.4	7.2	7.4	6.2	4.0	3.6	5.7	6.8	5.0	4.1	5.2

Dissolved oxygen concentrations remained near saturation in surface waters but were consistently lower in subsurface gravels (Table 1). At the surface, DO levels always exceeded 8.9 $mg.L^{-1}$ and at all times were near 100% saturation with respect to change in oxygen solubility with increasing temperature. Thus, biological processes including organic matter accumulations at any site did not affect surface DO levels. In contrast, DO concentrations at a 15 cm depth in the substrata averaged 50% and 75% of surface levels in 1979 and 1980 respectively. In mid-July, 1979, DO levels in gravels at Sites 1 and 3 reached concentrations as low as 1.8 $mg.L^{-1}$. Assuming subsurface and surface temperatures to be similar, oxygen solubility was 20 to 70% of saturation in 1979 and 40 to 80% of saturation in 1980. DO levels less than 100% saturation indicate that biological processes (decomposition of organic matter for example) are regulating DO concentrations. Although oxygen demand in gravels was typical before discharge from the sewage treatment plant began, DO concentrations in gravel at sites downstream of effluent discharge were significantly lower (by 30%) than levels at the upstream control after sewage discharge began ($p<0.05$) when date and site data are examined for 1980. These data suggest oxygen demand was 30% greater at downstream sites compared to the control. It is important to note, however, that subsurface DO levels in 1979 were often lower than corresponding 1980 values. Hence, the wide range of DO concentrations reaching minimum levels near 2 $mg.L^{-1}$ and 30% saturation are typical of the Cowichan River regardless of sewage treatment plant discharge.

TDP concentrations increased markedly downstream of effluent discharge after plant start-up. Through summer 1979 and in June and July 1980, TDP levels ranged from 3 to 5 $ug.L^{-1}$ with no difference between sites ($p<0.05$) (Fig. 3). TDP concentrations at the control site in 1980 were similar to the pretreatment levels. After effluent discharge started, TDP concentrations increased at Sites 2 and 3 to 20 times greater than control levels. At Site 4, levels were about half those at Site 2 (Fig. 3).

Ammonia concentrations followed a similar temporal trend (Fig. 4). Before effluent discharge started, NH_3-N levels were 2-10 $ug.L^{-1}$. Immediately downstream of the diffuser in 1980, NH_3-N levels increased to 200-400 $ug.L^{-1}$, an increase of two orders of magnitude over control levels. Concentrations at Site 4 were half of those at Sites 2 and 3.

Nitrate concentrations downstream of the effluent discharge changed relatively little after plant start-up (Fig. 5). In mid-June 1979, NO_3 + NO_2-N concentrations were 7-10 $ug.L^{-1}$ and steadily declined to less than the detection limit of 2 $ug.L^{-1}$ by late July, with no difference between sites ($p<0.05$). In June through mid-August, 1980, concentrations were also similar between sites ($p<0.05$) and ranged from less than 2 $ug.L^{-1}$ to 5 $ug.L^{-1}$. In early September NO_3 + NO_2-N concentrations at Sites 1 and 2 were 2.2 and 2.6 $ug.L^{-1}$ but levels at Sites 3 and 4 were significantly greater (about two times, $p<0.05$).

NH_3-N concentrations increased downstream of the effluent diffuser. Effluent is dominated by organic nitrogen and reduced species of inorganic nitrogen as is clear from the very high concentrations of ammonia in Figure 4. On mixing with Cowichan River water, which is saturated with dissolved oxygen, nitrification of ammonia would be expected to proceed rapidly and thus result in some increase in nitrate concentrations downstream. However, there is never

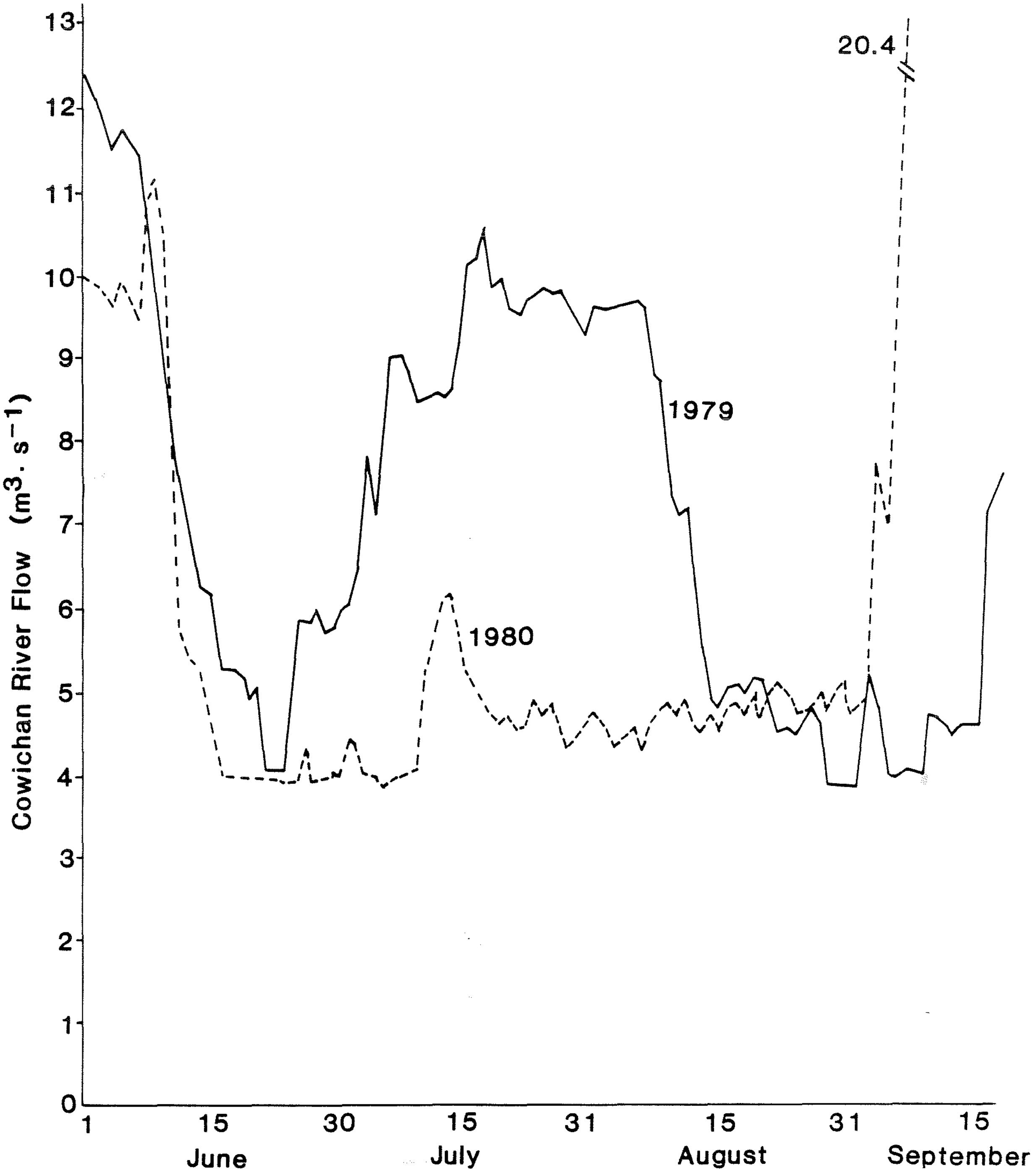

Figure 2. Mean daily flow of Cowichan River at Duncan; June through September 15, 1979 and 1980.

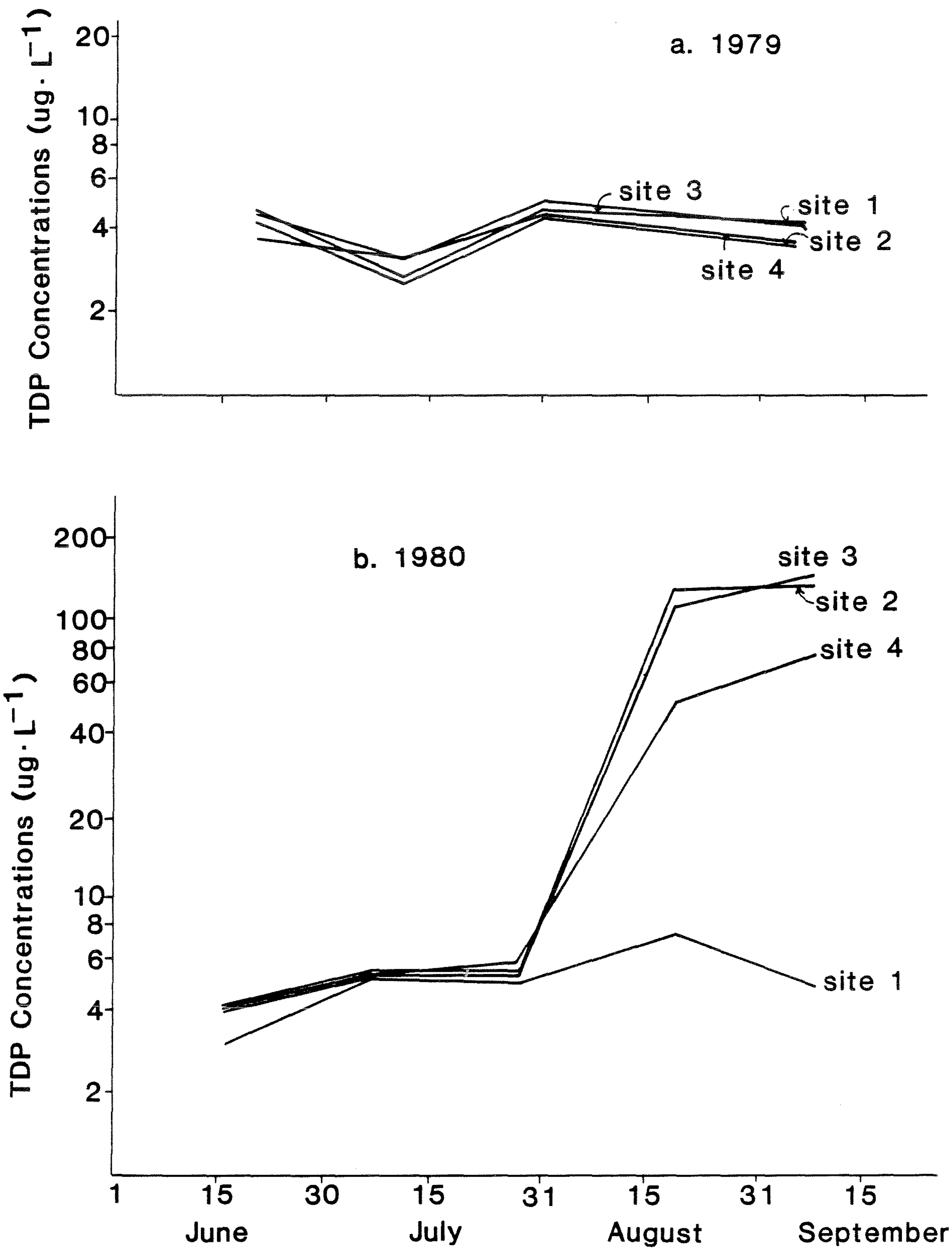

Figure 3. Mean total dissolved phosphorus concentrations at Cowichan River sites in 1979 and 1980.

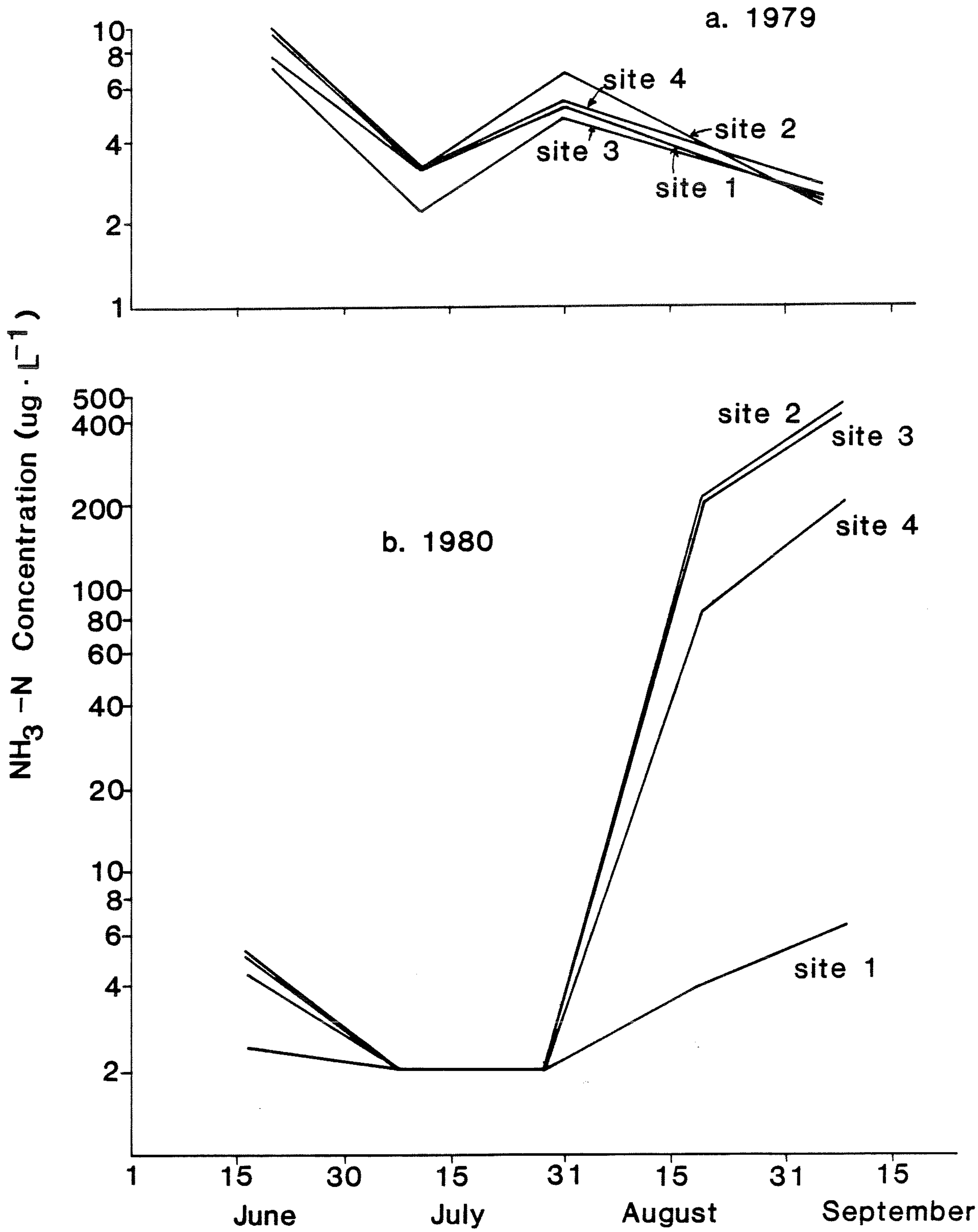

Figure 4. Mean ammonia concentrations at Cowichan River sites in 1979 and 1980.

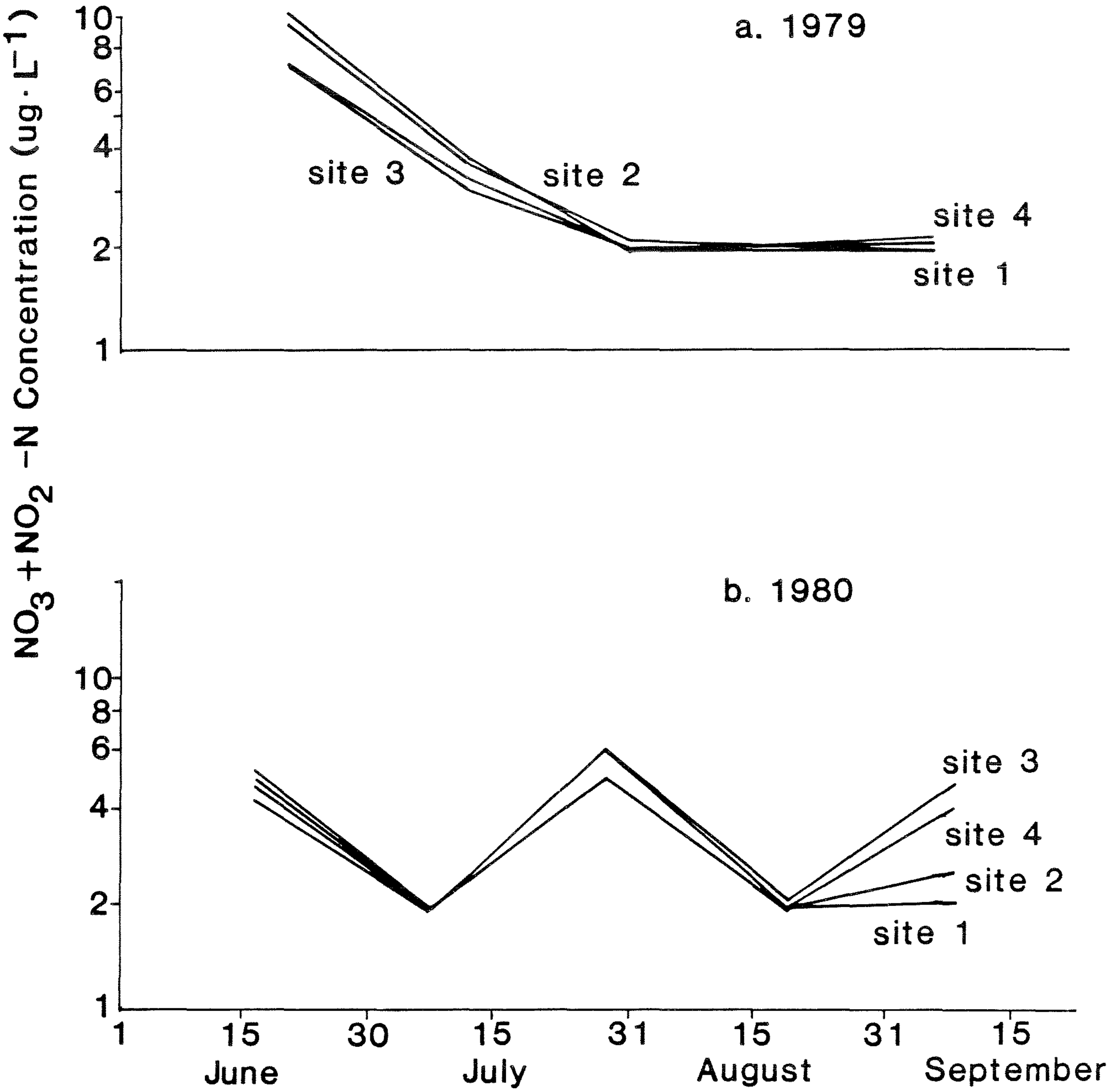

Figure 5. Mean nitrate plus nitrite concentrations at Cowichan River sites in 1979 and 1980.

a complete transformation of equal parts of ammonia to nitrate. Immobilization of ammonia will occur from biological uptake, some of the unionized form of ammonia will be volatilized and a large amount will be fixed from adsorption of NH_4^+ to particulates and colloids.

PERIPHYTON ACCRUAL

Large changes in periphyton chlorophyll-a concentrations and AFDW after 20-day substrata incubations followed changes in dissolved nutrient concentrations. Of the data that were successfully collected in 1979, chlorophyll-a concentrations approximated 2 $mg \cdot m^{-2}$ after an early summer incubation while in late summer, concentrations were between 3 and 6 $mg \cdot m^{-2}$ (Fig. 6). After an early summer incubation in 1980, chlorophyll-a concentrations were similar at all sites ($p<0.05$) and ranged between 0.5 and 0.9 $mg \cdot m^{-2}$ (Fig. 7). In July, concentrations were 0.3 to 0.6 $mg \cdot m^{-2}$ at Sites 2, 3 and 4 but were significantly greater by two times at Site 1 ($p<0.05$). From a biological perspective, these relatively small differences in biomass on plexiglass substrata between years and sites do not have great importance. One hundred percent differences in chlorophyll-a concentrations or AFDW are expected due to variation in orientation of the plates in the current (in part caused by vandalism in 1979), insect grazing, settlement, etc. After sewage discharge began, however, chlorophyll-a and AFDW increased significantly ($p<0.05$) by more than an order of magnitude at Sites 2, 3 and 4 compared to the upstream control after incubations in August and September (Figs. 7, 8). The large net increase in periphyton accrual correlates well with the timing of increased TDP and ammonia concentrations shown in Figs. 3 and 4.

PERIPHYTON TAXONOMY

In early summer of both 1979 and 1980, the periphyton community on natural substrata was dominated by diatoms (Fig. 9). Common genera included *Synedra*, *Achnanthes*, *Gomphonema*, and *Cocconeis* which are typical of other streams on Vancouver Island (Perrin *et al.* 1987; Stockner and Shortreed 1978). Chlorophytes were about 5% of sample volumes in early summer and were represented mainly by *Oedogonium*, *Ulothrix*, and *Stigeoclonium*. By late July, these chlorophytes increased to as much as 82% of sample volumes and diatoms declined to 10-20% of samples. This trend is consistent with seasonal taxonomic shifts of algae in coastal streams (Perrin *et al.* 1987; Perrin and Johnston 1986). Chlorophytes were again dominated by *Oedogonium*, *Stigeoclonium*, and *Mougeotia* and diatoms were represented by *Synedra*, *Cymbella*, *Gomphoneis*, and *Achnanthes*. With the exception of Site 2, the blue-green alga, *Dichothrix* was also found in up to 5% of sample volumes. An unusual occurrence of the Rhodophyte, *Andorimella* was found at Station 3.

After start-up of the sewage treatment plant, marked differences in periphyton community structure were found between control and treatment sites. At the control site, diatoms comprised up to 70% of sample volumes with the remaining 30% being chlorophytes. Downstream of effluent discharge, chlorophytes including *Stigeoclonium*, *Spirogyra*, and *Oedogonium* increased to 70% of sample volumes. Dominance by these chlorophytes seems typical in mid-summer under conditions of high irradiance levels, low stream flow, and nutrient enrichment (Perrin *et al.* 1987). Diatoms generally comprised less than 30% of

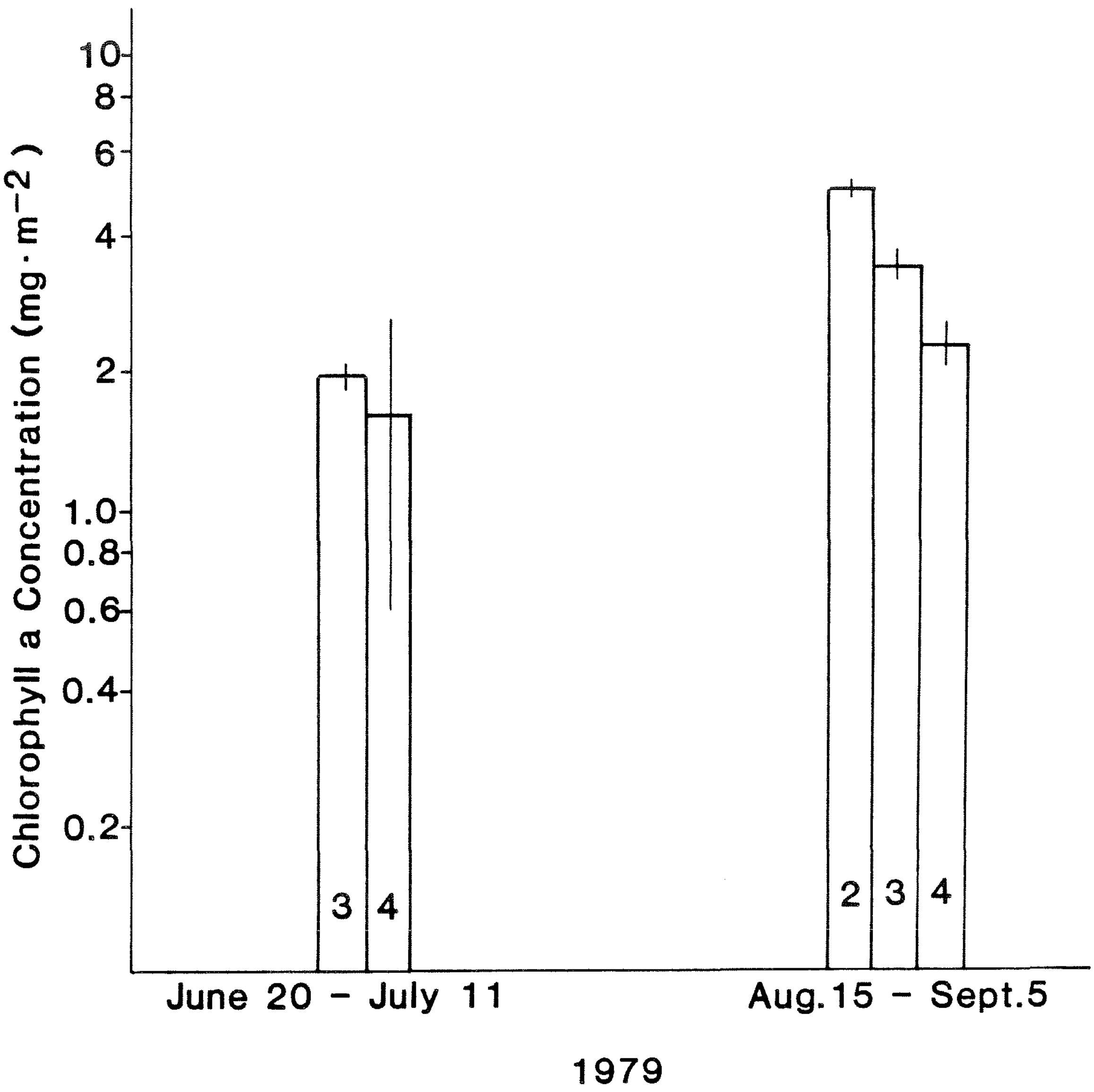

Figure 6. Geometric means (± 95% confidence limits) of chlorophyll *a* concentrations of periphyton on artificial substrata after 20-day incubations in summer, 1979. Numbers inside bars refer to sample stations shown in Figure 1.

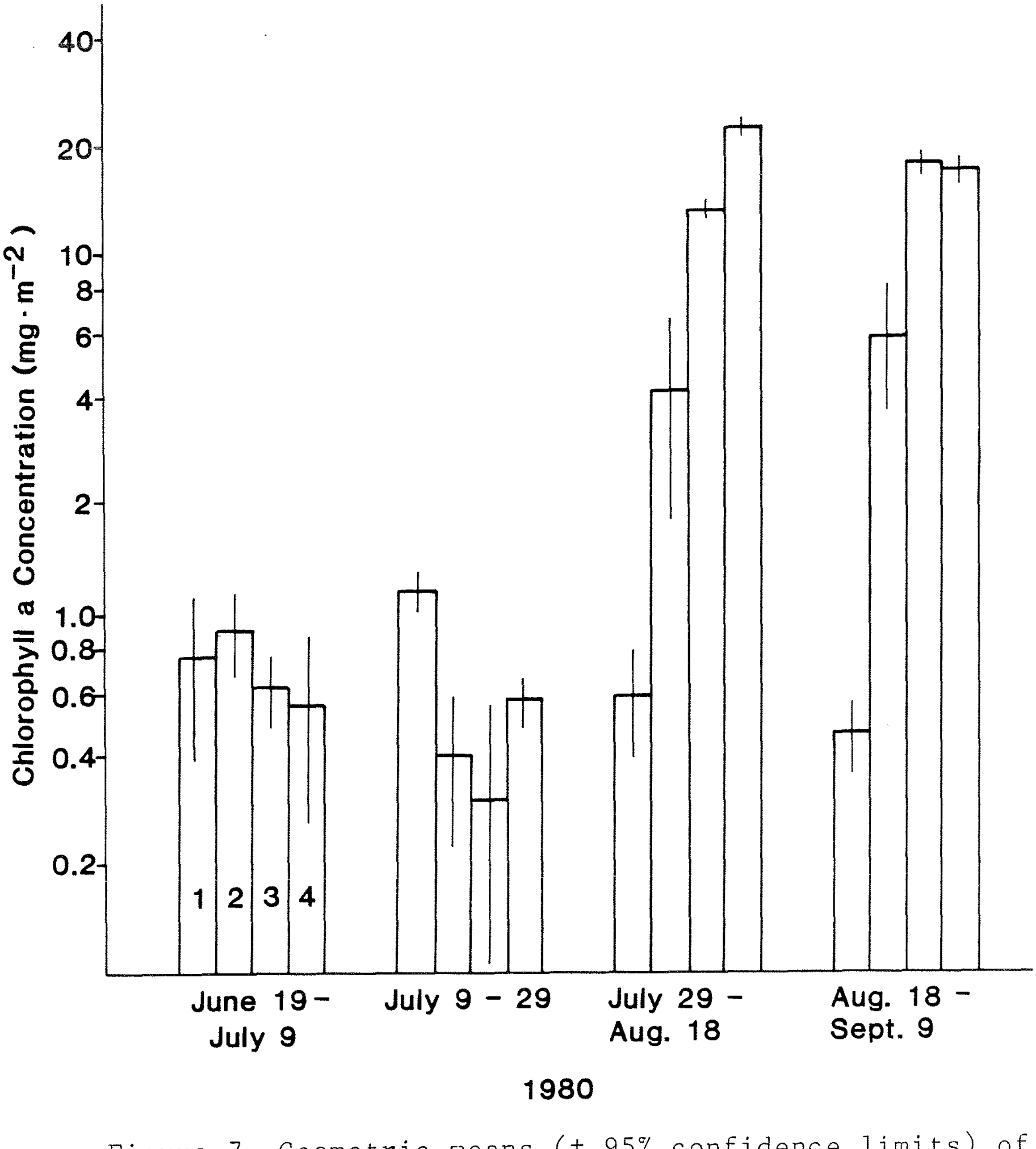

Figure 7. Geometric means (± 95% confidence limits) of chlorophyll a concentrations of periphyton on artificial substrata after 20-day incubations in summer, 1980. Numbers inside bars refer to sample stations shown in Figure 1.

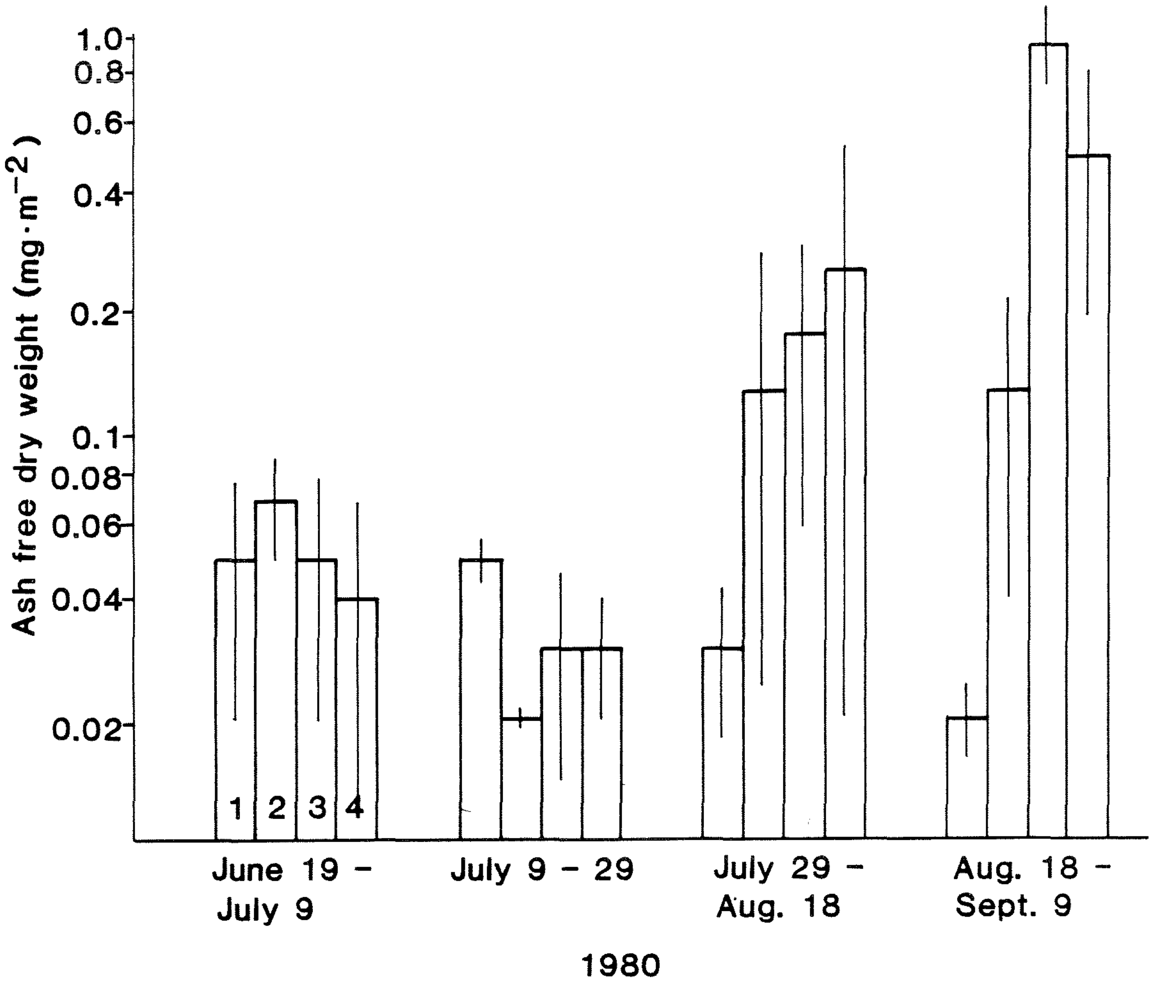

Figure 8. Geometric means (± 95% confidence limits) of ash-free-dry-weight of periphyton on artificial substrata after 20-day incubations in summer 1980. Numbers inside bars refer to sample stations shown in Figure 1.

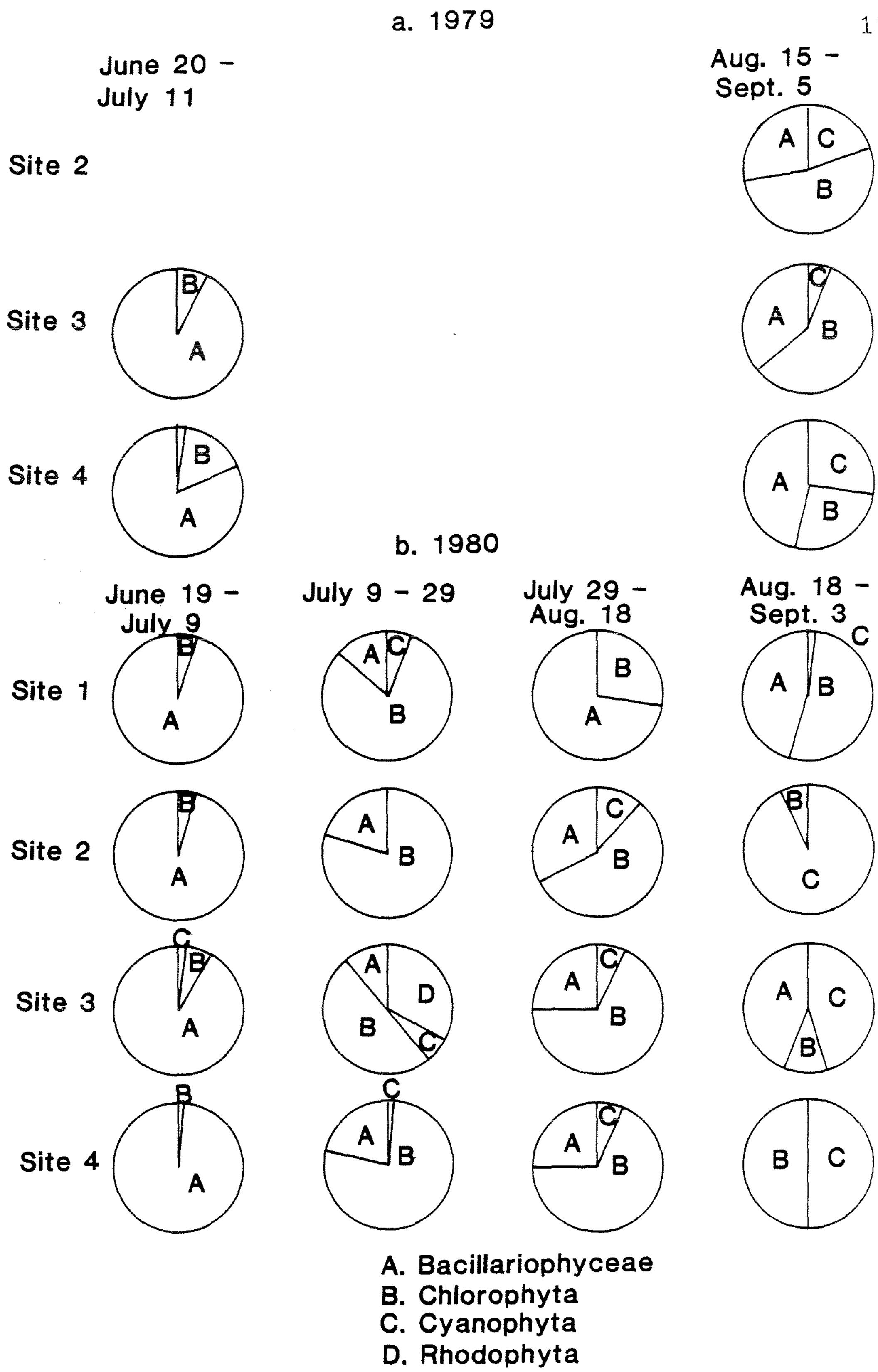

Figure 9. Relative percent composition by volume of periphyton algae on natural substrata at Cowichan River sites in 1979 and 1980.

sample volumes and genera were similar to those of the early summer community. Blue-greens also occupied up to 15% of sample volumes from sites downstream of effluent discharge yet they were not found at the control site. Blue-greens including Phormidium, Lyngbya, and Dichothrix gained increasing importance in treatment areas by early September 1980 by occupying from 45 to 90% of sample volumes. By comparison, blue greens were only found in trace amounts at the control site at the same time and in September of the previous year, blue greens did not exceed 30% of sample volumes at any location. Diatoms and chlorophytes accompanied the blue greens in mixed proportions, but at Sites 2 and 4, diatoms were absent. Chlorophytes were again dominated by Oedogonium, Spirogyra, and Stigeoclonium and common diatoms were Epithemia and Synedra.

ALGAL BIOASSAY

Experimental additions of nitrogen and phosphorus to Cowichan River water from Sites 1 through 4 provided media in several combinations of N and P concentrations with which to examine the AGP of S. capricornutum (Table 2). In control site media, NO_3-N, NH_3-N and TDP concentrations were low (12, 7 and 4 $ug.L^{-1}$ respectively) and yielded an untreated N:P atomic supply ratio of 10.5. Combinations of N and P additions changed this ratio to a low of 0.8 and a high of 564. Water from Sites 2, 3 and 4 was already enriched with NH_3 and P due to contribution from sewage effluent, thus giving background N:P supply ratios ranging from 3.6 to 3.9. Since the P concentrations in these water samples (Table 2) was far in excess of levels known to saturate P-limited growth of periphytic or planktonic algae (Bothwell 1985; Fuhs et al. 1972; Rhee 1973), AGP due to doses of nitrate were of greatest interest in water from these sites. Many algae will selectively use nitrate over ammonia as a primary nitrogen source, despite the necessary nitrate reduction being energetically more costly. S. capricornutum is one species that does preferentially use nitrate and its N-limited growth rate has been found to saturate at nitrate levels near 1 $ug.L^{-1}$ in batch culture (Steeman Nielson 1978). Background nitrate-N in samples from Sites 2, 3 and 4 ranged from 12 to 32 $ug.L^{-1}$, yet ammonia levels were an order of magnitude greater.

The finding of no significant change in AGP from N or P additions but a 60x increase when both N and P are added to Site 1 water (Fig. 10) suggests there was a close coupling between N- and P-limitation of AGP in Cowichan water not contaminated with sewage effluent. A similar effect is often observed in mixed species cultures in which the particular N:P supply ratio at which the transition from N- to P-limitation occurs or vice versa is species dependent (Rhee and Gotham 1980; Healey 1985). In our uni-algal culture, however, the coupling effect was only related to the addition of P immediately driving the AGP into N-limitation and vice versa. The addition of N and P together was likely sufficient to eliminate both N- and P-limitation of AGP. Since there was no significant difference in AGP between the control, N added, and P added treatments in Site 1 water ($p<0.05$), the element that was primarily limiting AGP cannot be determined. Clearly, however, there was a very close coupling between N- and P-deficiency.

In water affected by sewage effluent, N and P were present in concentrations (Table 2) that were sufficient to significantly increase AGP over control amounts ($p<0.05$). In water from Site 2, AGP was about one order of magnitude greater than that at the control. AGP declined to significantly lower levels

Table 2. Mean nitrogen and phosphorus concentrations and supply ratios in autoclaved and filtered water used in the Cowichan algal bioassay. Ratios are from analyses before the bioassay started and are given with and without experimental dosing with N and/or P.

	Treatment															
Site	1				2				3				4			
Dose	C[5]	N[3]	P[4]	N+P	C	N	P	N+P	C	N	P	N+P	C	N	P	N+P
NO_3-N[1]	12	1012	12	1012	32	1032	32	1032	23	1023	23	1023	12	1012	12	1012
NH_3-N	7	7	7	7	293	293	293	293	190	190	190	190	105	105	105	105
TDP	4	4	54	54	185	185	235	235	122	122	172	172	71	71	121	121
N/P[2]	10.5	564	0.8	42	3.9	16	3	12.5	3.9	22	2.7	15.6	3.6	34.8	2.1	20.4

1 units in $ug.L^{-1}$
2 expressed on an atomic basis
3 added at 1000 $ug.L^{-1}$ as N
4 added at 50 $ug.L^{-1}$ as P
5 control

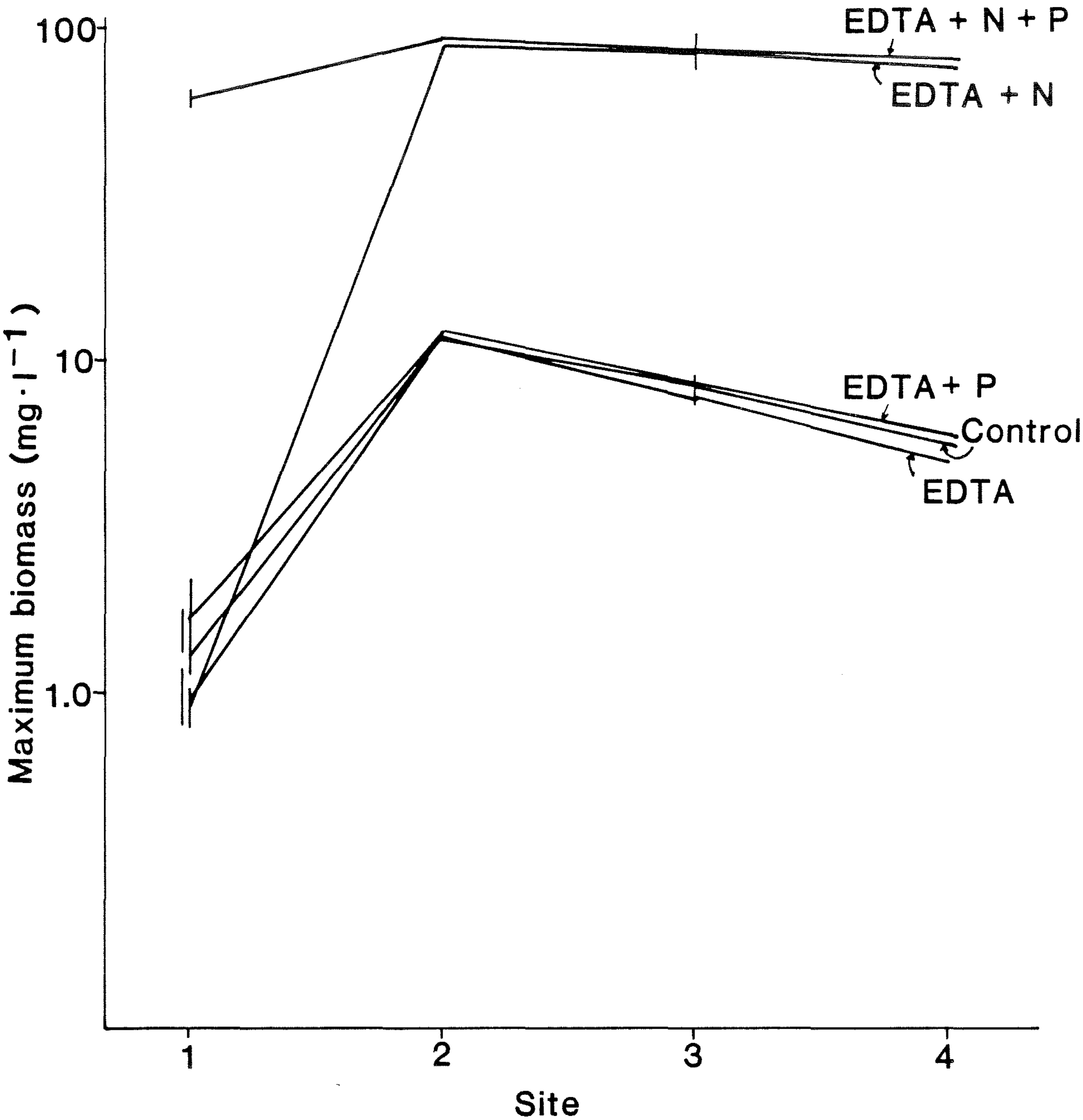

Figure 10. Interaction of treatment and site (± 95% confidence limits) in the S. capricornutum bioassay of Cowichan water, 1981.

in water from Sites 3 and 4 but remained at least six times greater than in upstream control water. The downstream gradient of declining N and P concentrations (Table 2) appears to set limits on AGP in water from each site affected by effluent. It is interesting that only nutrient additions including NO_3-N caused further increases in AGP in water from sites affected by effluent (Fig. 10). P supply in water from Sites 2-4 was not primarily limiting AGP. This demand for the nitrate form of inorganic N is undoubtedly related to the relatively low complement of NO_3 in effluent and the selective preference for nitrate by S. capricornutum for its nitrogen requirements.

BENTHIC INVERTEBRATES

A total of 50 invertebrate taxa occurred in the benthic samples (Appendix 1). However, the zoobenthos was dominated by a few groups (Table 3). In 1979, prior to the start of the effluent discharge, the benthic invertebrate communities at all sites were dominated by oligochaetes, benthic cladocerans, water mites, and Tanytarsini and Orthocladiinae chironomid larvae. Oligochaetes comprised between 24 and 36% of the total density of organisms at any site. Acroperus harpae, Alona affinis, and Chydorus sp. were the most abundant cladocerans and accounted for 11-31% of the total zoobenthos (Appendix 2). Hydracarina were 9-20% of the zoobenthos. Tanytarsini, especially Cladotanytarsus and to a lesser extent Microspectra, were the most abundant chironomids. Tanytarsini comprised 14-31% of the zoobenthos while orthoclads (predominantly Cricotopus) accounted for an additional 4-6%. Tardigrades, ostracods, harpacticoid copepods and Ephemeroptera, Plecoptera, and Trichoptera (EPT) considered as a group, were minor components of the benthos at all sites. The number of taxa found at any site ranged from 26 to 27 forms but only 16 were common to all sites. In total, 38 taxa were found in the 1979 samples, of which 14 occurred only in 1979.

The benthic samples taken in 1980, about six weeks after the start of the effluent discharge, showed a slightly different community composition. Tardigrades, which were a minor component of the benthic fauna in 1979, greatly increased their relative abundance at the sites (Site 2-4) that were subject to the effluent discharge and comprised 33-42% of the zoobenthos at those sites. The importance of Tanytarsini chironomids was greatly reduced at the impacted sites, from 14-31% in 1979 to 2-4% in 1980. Oligochaetes and water mites were also somewhat reduced in importance at the impacted sites, to about 14-22% and 4-8% respectively. Cladocerans, on the other hand, slightly increased their relative abundance to 18-42% of the fauna.

In contrast, the relative abundances of the dominant groups at the control site were more similar to those sampled in 1979 (Table 3). The proportions of cladocerans and chironomids (31% and 17% respectively) were unchanged. Oligochaetes decreased slightly from 30 to 18% of the benthos while water mites and tardigrades increased from 11 and 3% in 1979 to 17 and 10% in 1980.

The number of taxa remained high at the control site (25 taxa) and at the uppermost impacted site (24 taxa) but was reduced at the other sites (19-21 taxa). A total of 36 taxa were found in the 1980 samples of which 14 taxa occurred at all sites. Only fifteen taxa were found in 1980.

Table 3. Percentage composition of the commoner invertebrate groups in benthic samples from the Cowichan River, 1979 and 1980.

Taxonomic Group	1979 Sites				1980 Sites			
	1	2	3	4	1	2	3	4
Ephemeroptera, Plecoptera and Trichoptera	2.7	0.4	1.2	1.0	1.0	0.4	0.2	
Tanytarsini	13.8	31.4	19.6	16.0	10.5	4.4	3.3	1.7
Orthocladiinae	3.6	6.4	6.2	6.2	5.9	7.0	7.9	3.6
Oligochaetes	30.0	31.0	24.0	36.4	18.2	16.6	22.5	14.0
Hydracarina	11.2	9.3	15.6	20.4	16.6	8.1	4.3	3.6
Tardigrada	2.9	1.9	6.2	2.7	9.7	40.9	42.2	33.3
Ostracoda	0.3	1.7	0.9	1.2	0.5			
Cladocera	31.0	12.5	20.7	10.8	30.7	19.3	18.8	42.5
Number of Taxa	27	26	26	26	25	24	19	21
Mean Density* ($10^5.m^{-2}$)	2.02	1.88	2.01	1.85	1.05	3.68	4.77	11.8

* arithmetic mean

The large numbers of taxa that occurred in only one year suggest considerable between-year change in the taxonomic composition of the zoobenthos. Cluster analysis using a distance measure (Jaccard coefficient) which considered only presence-absence data separated the site-time pairs into two groups, with all the 1979 sites in one group and all the 1980 sites in another (Fig. 11a). Thus, when only the taxonomic composition of the benthic invertebrates was considered, differences between years were greater than differences among sites within a year. When the distance measure was considered with relative abundance, a slightly different pattern emerged (Fig. 11b). The three sites which were downstream of the effluent discharge in 1980 formed one group while the 1980 control site and all four sites in 1979 formed a second group. Thus, when both taxonomic composition and abundance were considered, the data suggest differences between the impacted sites and the spatial and temporal control sites.

Differences between the impacted sites and the spatial and temporal control sites were more clearly evident when the abundances of the dominant taxonomic groups were compared (Fig. 12-17). Abundance data for all taxa are given in Appendix 3. Cladocera (Fig. 12) showed a significant site-by-time interaction, which indicated an impact that was attributable to the sewage effluent discharge in 1980. Cladocera abundance appeared to decrease at the control site in 1980 compared to 1979 while increasing at all other sites. Multiple range tests (Table 4) confirmed that transformed abundances were generally higher at the impacted sites in 1980 than at either the control site in 1980 or the corresponding sites in 1979, prior to the discharge.

The oligochaete data also had a significant site-by-time interaction (Fig. 13) indicating an impact from the discharge. The multiple range tests showed a reduction in oligochaete abundance at the control site in 1980 when compared to the temporal control, increased abundance at Site 4 in 1980, and no between-year change in abundance at the other impacted sites (Table 4).

Tardigrades showed a quite different response (Fig. 14). The site-by-time interaction was not statistically significant, thus implying that no effect resulted from the discharge. There were, however, both among-site and between-years differences (Table 4). Densities in 1980 were considerably greater (about 36x on average) than in 1979, at all sites. Tardigrade abundances were identical at the three downstream sites but were always considerably greater than the densities at the control site (Table 4).

Diptera (almost wholly chironomids) also lacked a statistically significant site-by-time interaction, and thus showed no response to the discharge (Fig. 15). There were among-site differences (Table 4), with densities at the control site being lower than those at Sites 3 and 4.

Hydracarina did not vary in abundance, neither among sites nor between years (Fig. 16, Table 4). The sewage effluent also had no discernible impact on the abundance of Ephemeroptera, Plecoptera, and Trichoptera, which are considered as a group because of their general sensitivity to pollution; the interaction term in the site-by-time ANOVA was not significant. EPT densities were similar at all sites in either year, but were reduced in 1980 compared to 1979 (Fig. 17).

Table 4. Comparisons of mean densities of organisms among years and sites. Underlined means do not differ significantly at p=0.05 (Tukey's test). Data are coded as year (1979, 1980) and site (1,2,3,4). Note that comparisons refer to log(x + 1) transformed data. ns = ANOVA non-significant at p=0.05

Group	Comparisons
Cladocera	79-4 79-2 80-1 79-3 79-1 80-2 80-3 80-4
Oligochaeta	80-1 79-3 79-1 79-2 80-2 79-4 80-3 80-4
Tardigrada	1 < 2 4 3
	1979 < 1980
Diptera	1 4 3 2
E,P,T	1980 < 1979
Hydracarina	ns

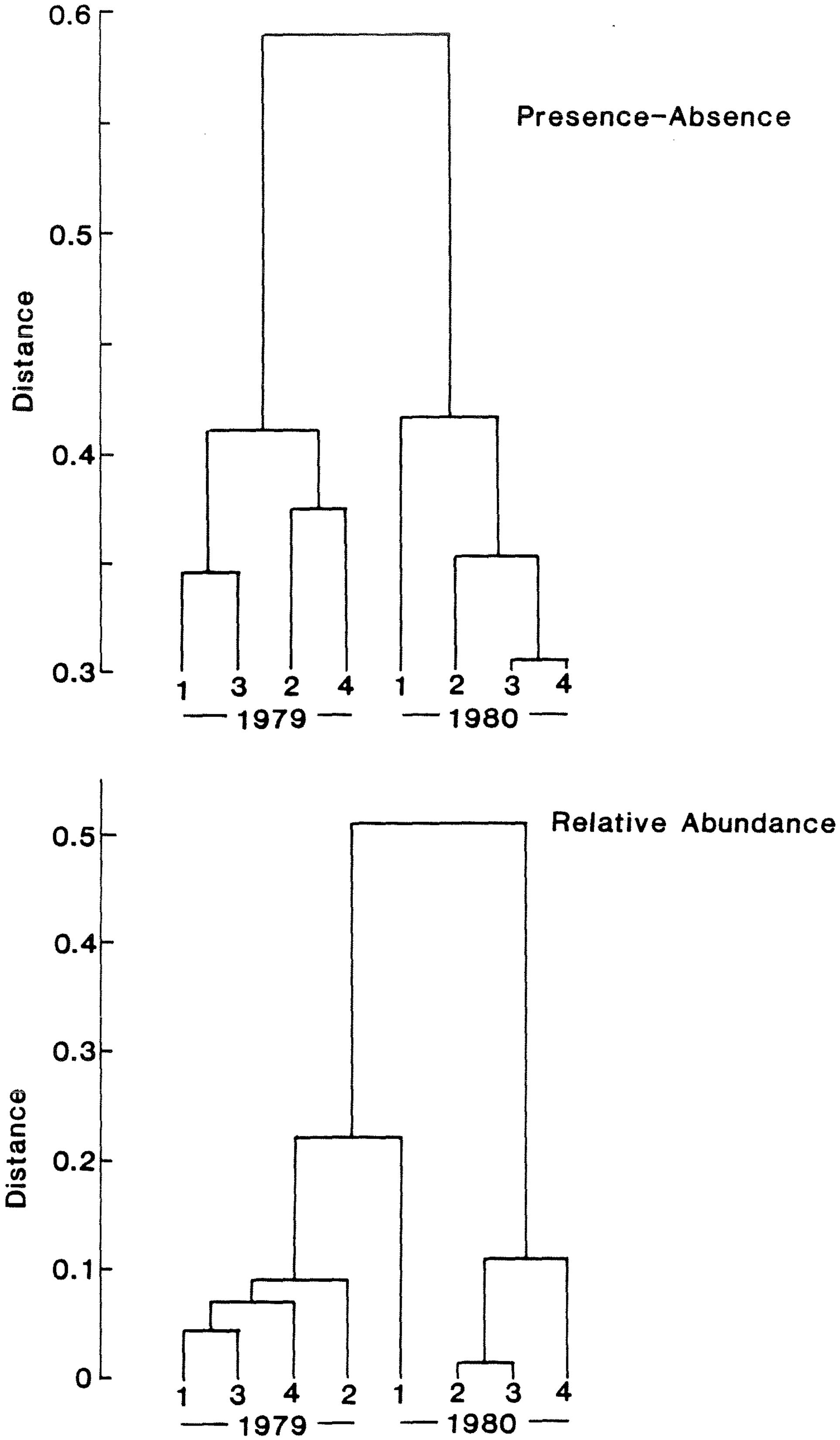

Figure 11. Groupings of benthic samples obtained by UPGMA on (a) presence - absence data and (b) relative abundance of taxa.

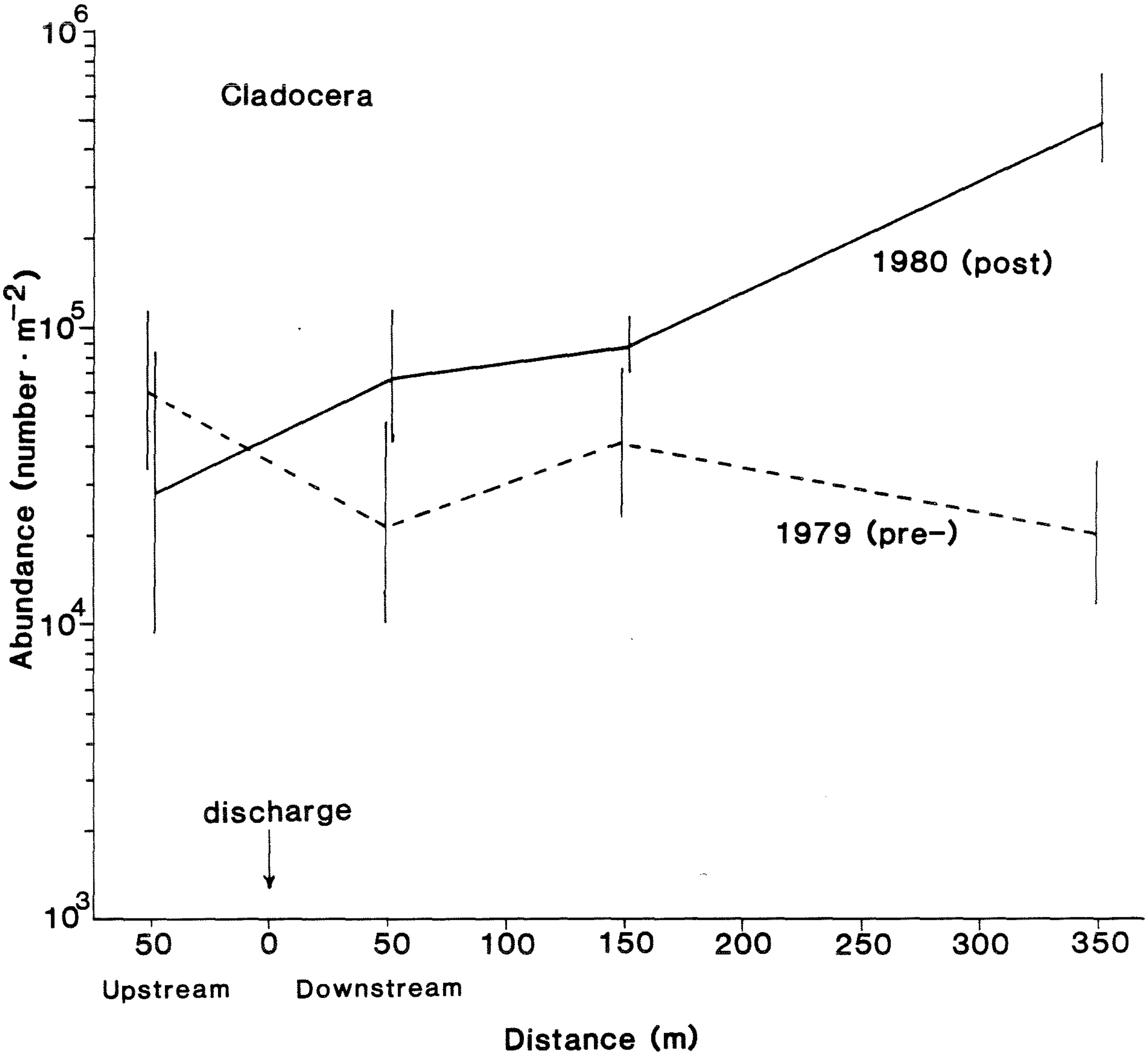

Figure 12. Abundance (± 95% confidence limits) of Cladocera at control and treatment sites, 1979 and 1980.

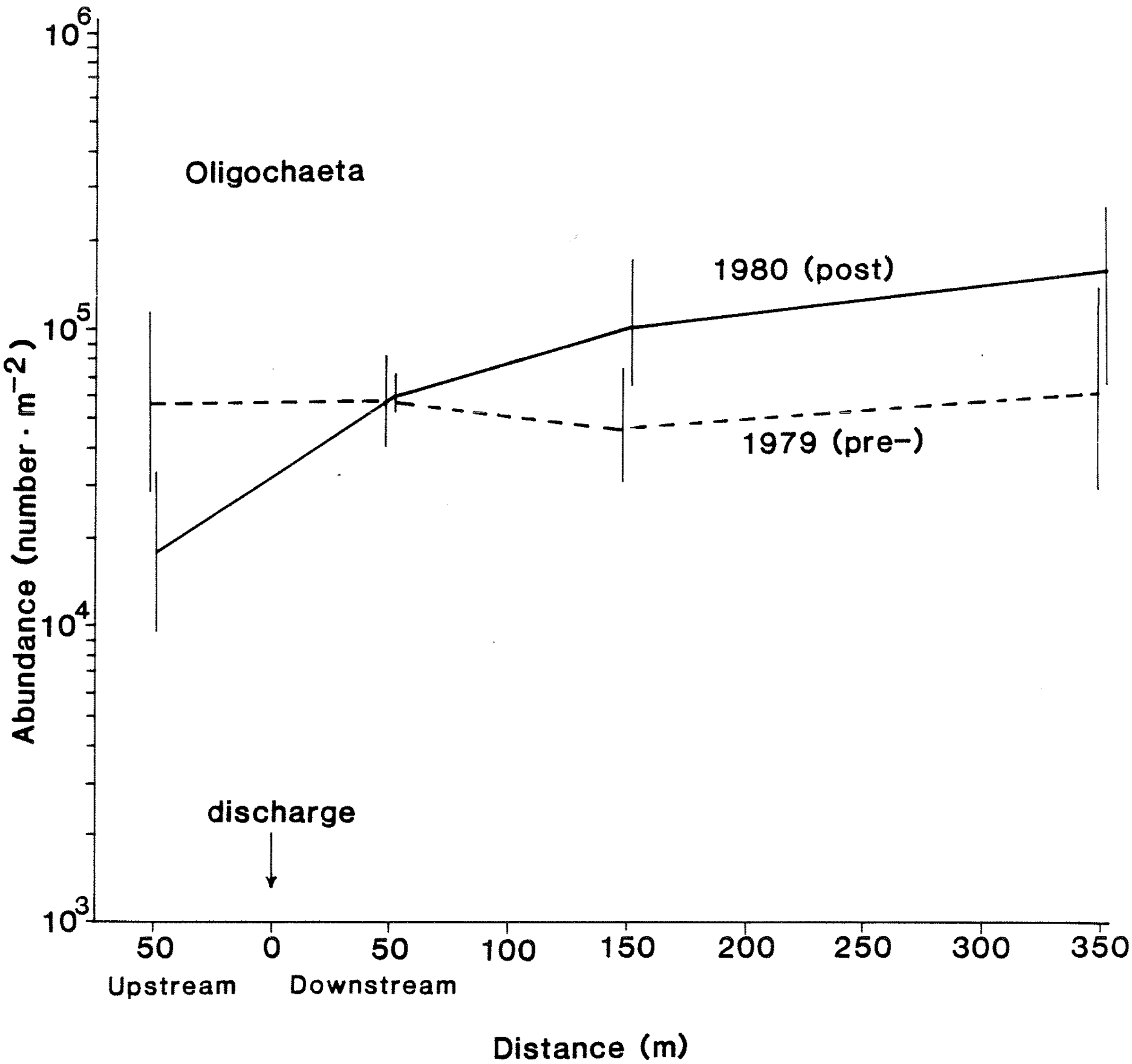

Figure 13. Abundance (± 95% confidence limits) of Oligochaeta at control and treatment sites, 1979 and 1980.

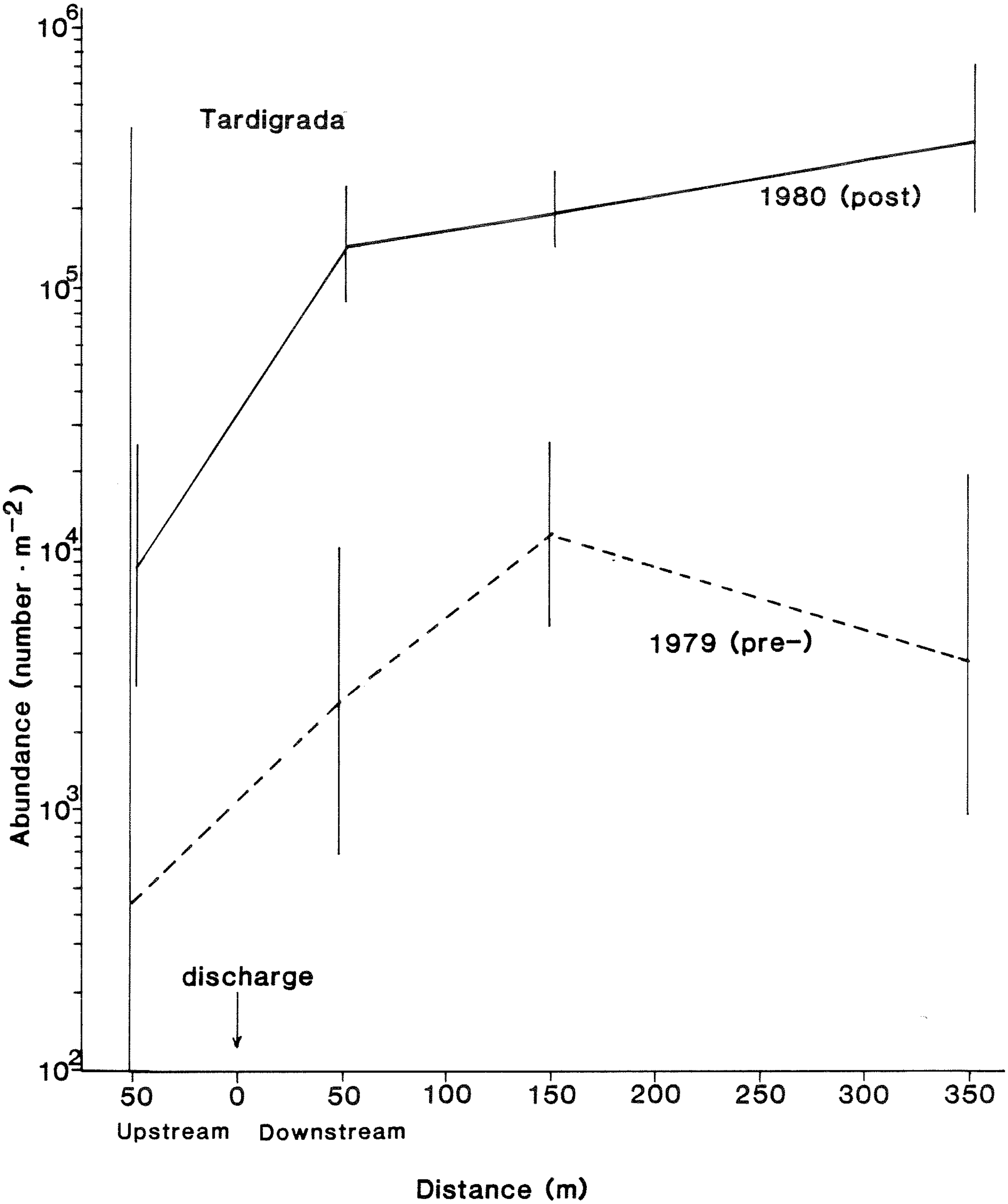

Figure 14. Abundance (± 95% confidence limits) of Tardigrada at control and treatment sites, 1979 and 1980.

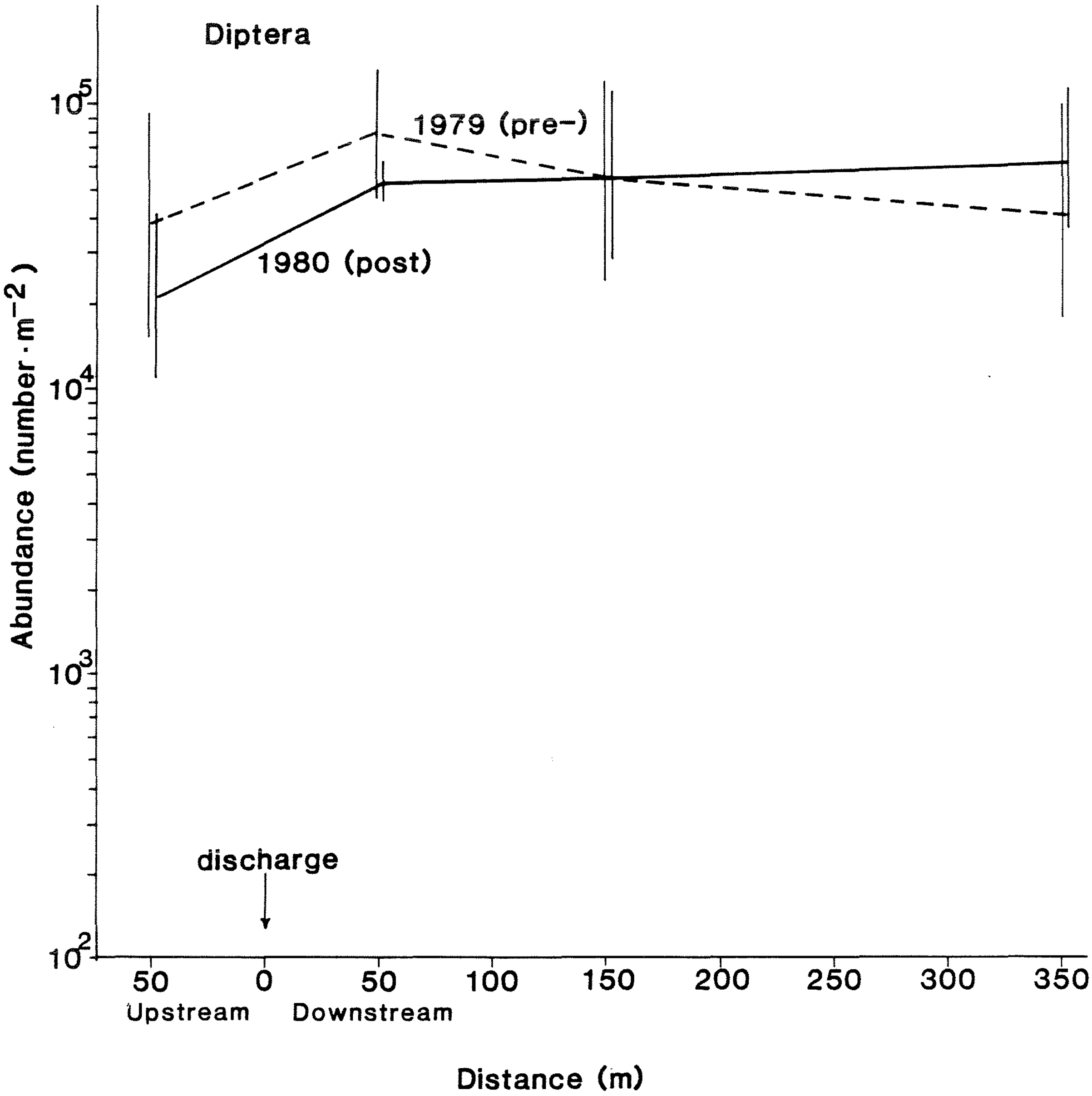

Figure 15. Abundance (± 95% confidence limits) of Diptera at control and treatment sites, 1979 and 1980.

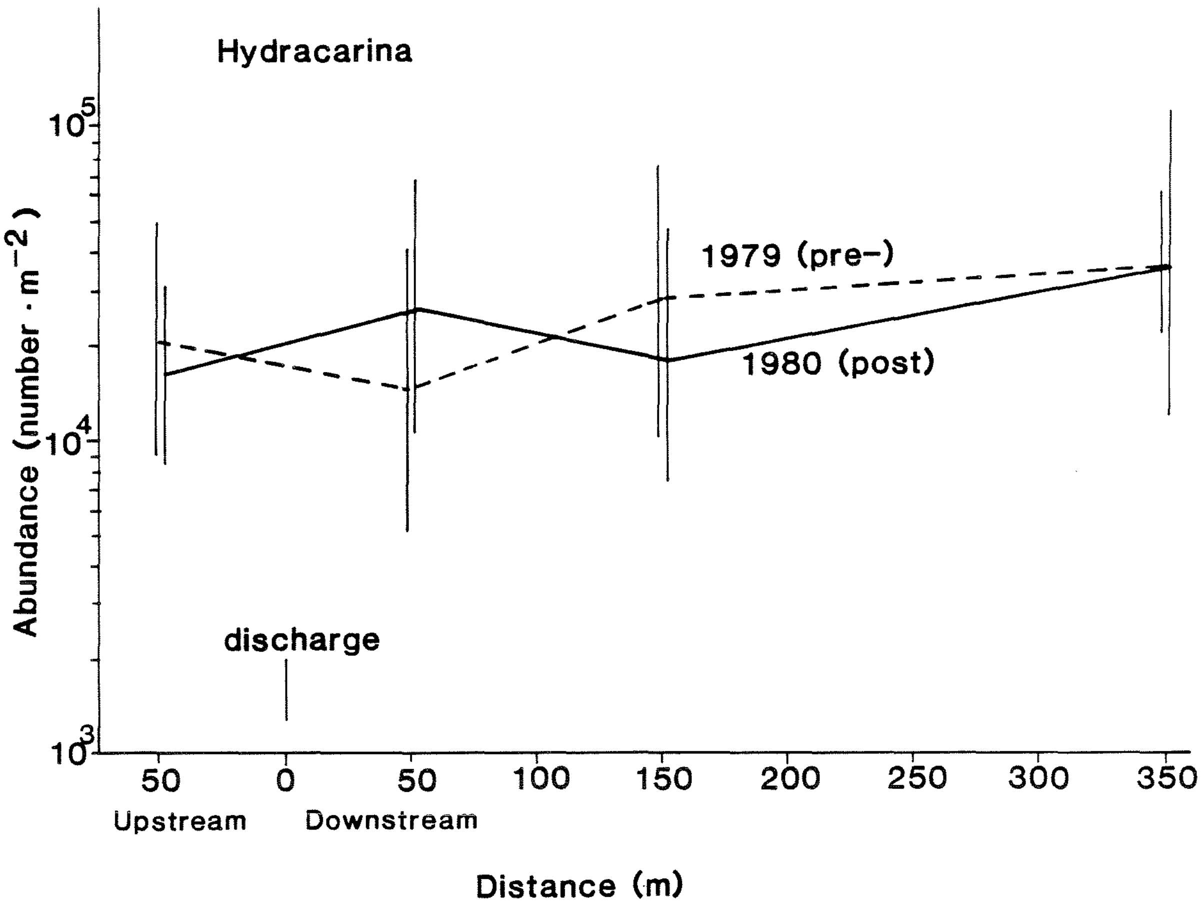

Figure 16. Abundance (± 95% confidence limits) of Hydracarina at control and treatment sites, 1979 and 1980.

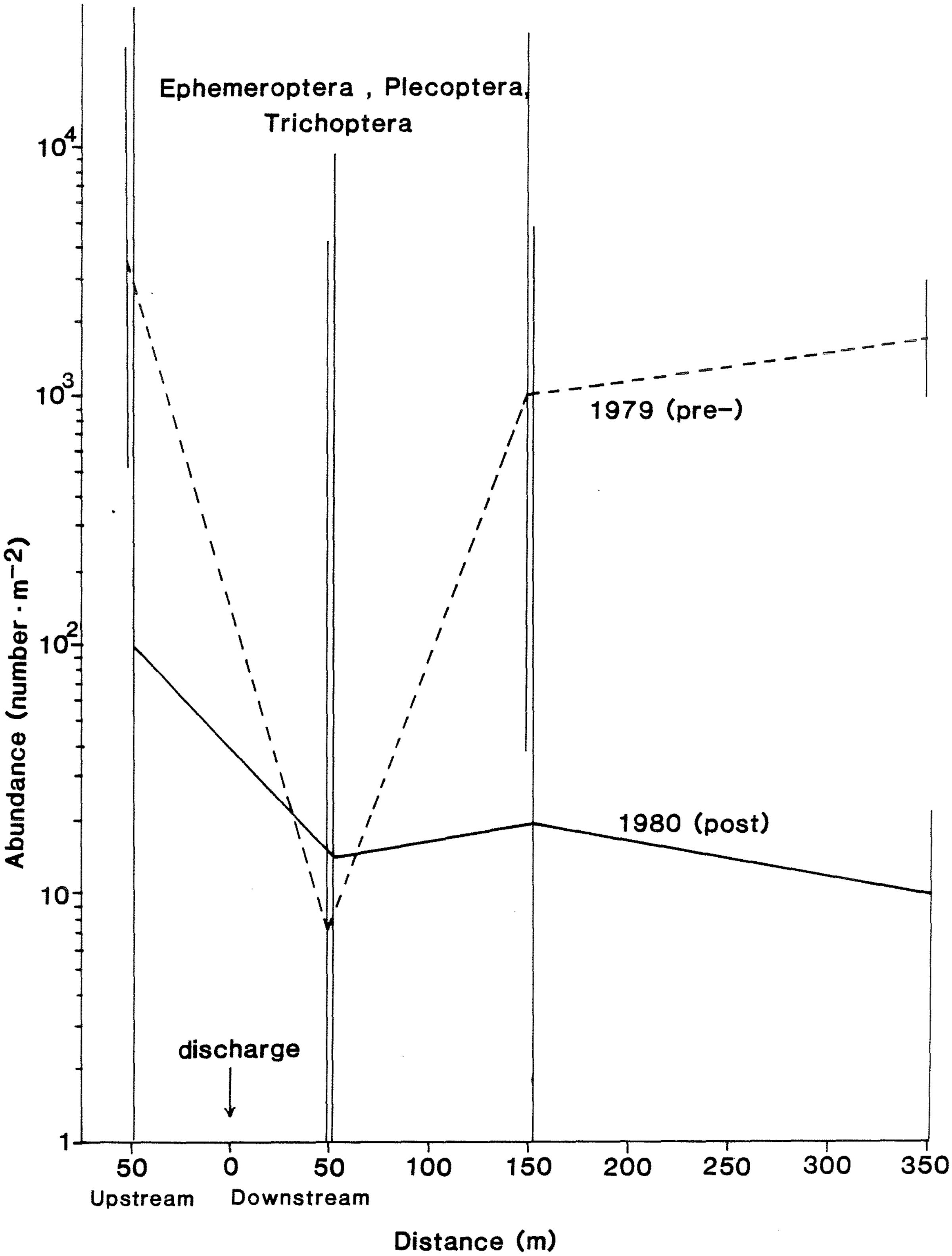

Figure 17. Abundance (mean ± 95% confidence limits) of Ephemeroptera, Plecoptera, and Trichoptera at control and treatment sites, 1979 and 1980.

For most taxa, a significant relationship between the mean abundances of the taxa and principal components formed from environmental variables was not found. Thus, the effects of the considerable variation in environmental characteristics between the pre-discharge and post-discharge conditions were not directly discernible in the abundance data. However, tardigrades, whose abundance changed between years independently of the effluent discharge, showed a significant positive relationship ($r^2 = 0.69$) with a principal component which had approximately equal positive loadings on TOC, TDP, NO_3-N, NH_3-N, and chlorophyll-a and a negative loading about half as great on intragravel DO. Thus, tardigrade abundance seemed to increase with macronutrient and algal levels and with lower intragravel DO.

DISCUSSION

Evidence from this study suggests that effluent from the Duncan - North Cowichan sewage treatment plant has changed some characteristics of the benthic community in the Cowichan River and that nitrogen and phosphorus in the sewage effluent were potentially important in causing those changes.

With due regard to limitations in using the batch culture bioassay in stream studies, the S. capricornutum test showed that additions of N and P to Cowichan River water that was unaffected by effluent can potentially increase algal growth. This finding is consistent with studies from Carnation Creek (Stockner and Shortreed 1978) and the Keogh River (Perrin et al. 1987) on Vancouver Island and at the Thompson River (Bothwell 1985) in the interior of British Columbia. It also supports the general view that phosphorus or nitrogen usually limits lotic productivity (Wilhm 1975). Since the sewage effluent from the Duncan-North Cowichan treatment plant contributed to relatively high concentrations of phosphorus and nitrogen in forms available for biological uptake (Fig. 3-5), there is little doubt that the order of magnitude increase in periphyton accrual downstream of effluent discharge (Fig. 7) was related to nitrogen and phosphorus associated with the sewage effluent. Characteristics of nutrient enrichment by effluent from sewage treatment plants are well known and this study adds another positive relationship between sewage, nutrients and algae to the list of other studies (e.g., Bahls 1973; Persoone and Depauw 1979) and general comment in engineering texts (e.g., Sawyer and McCarty 1978; Clark et al. 1971).

An important characteristic of effluent addition to the Cowichan River was an abrupt shift in nutrient deficiency and AGP in bioassay media (Fig. 10). Upstream of effluent discharge, N- and P-limitation of AGP appeared tightly coupled but immediately downstream of the discharge, high concentrations of available phosphorus and ammonia potentially caused NO_3-N to limit AGP.

These data, however, are difficult to relate to in situ conditions. Fig. 10 only suggests that S. capricornutum growth could potentially increase by about an order of magnitude between site 1 and 2 due to contribution from N and P in effluent but a further increase is limited by NO_3-N supply. The specificity of NO_3-N by S. capricornutum is an artifact of the bioassay and, hence, is not a good indicator of responses by the in situ periphyton mat.

Generally, there is a demand for ammonia in addition to nitrate as the nitrogen source in periphyton communities.

Results of in situ dissolved nutrient concentrations also suggest the bioassay may have been misleading. In streams in which the flow rates are high enough and the periphyton biomass low enough, such that the algae are ineffective in reducing the nutrient concentrations, supply of N and P can be closely approximated by the concentrations of DIN and DIP respectively. Since DIP cannot be measured, we assume that TDP measured in the Cowichan River reflects changes in DIP, acknowledging that TDP is an overestimate. TDP concentrations at sites not affected by effluent generally ranged between 3 and 7 ug.L^{-1}. Assuming the biologically available component of TDP to be half these concentrations, they remain near or above levels known to saturate P-limited growth in algae (Fuhs et al. 1972, Rhee 1973) including periphyton (Bothwell 1985). DIN levels upstream of effluent discharge were generally less than 20-40 ug.L^{-1} which has been found to be the level below which periphyton growth is N-limited (Bothwell, unpub. data). At sites affected by effluent discharge, however, DIN levels were an order of magnitude greater than those upstream and undoubtedly were not limiting the growth of the periphyton community based on the 20-40 ug.L^{-1} criteria. Where N and P do not limit periphyton accrual, temperature (Bothwell 1987) and passive settlement (Perrin et al. 1987) become important factors in regulating the net rate of periphyton accrual.

Although growth rates of algae on in situ substrata may be expected to be similar at sites downstream of the effluent discharge when nutritional criteria are considered, biomass accrual varied greatly yet consistently between sites (Figs. 7, 8). Generally, biomass increased in the downstream direction from Site 2. Usual explanations for variation on substrata include effects of insect grazing (Lamberti and Resh 1983) and sloughing. Passive settlement of drifting algae has also been found to contribute to biomass accrual on artificial substrata at consecutive sites downstream of a nutrient enrichment (Perrin et al. 1987) and may be important in the Cowichan River.

The design of the benthic invertebrate sampling program allowed an unambiguous assessment of the impacts of the sewage effluent discharge on the abundance of the zoobenthos. Although considerable change in taxonomic composition and abundance occurred between 1979 and 1980, changes in abundance which could be attributed to the impacts of the effluent occurred only for oligochaetes and cladocerans. Both oligochaetes and cladocerans were generally more abundant at the sites downstream of the discharge than at the spatial and temporal control sites; the exact patterns of differences were complex.

An increase in oligochaete abundance is a common response to organic pollution (Hynes 1971); their tolerance of low oxygen conditions and their bacterial food source (Learner et al. 1978) allow oligochaetes to tolerate saprobic conditions. The increased accumulations of periphyton (and presumably detritus) and lower intragravel oxygen levels in 1980 at the sites downstream of the effluent discharge may have provided conditions favourable for oligochaete worms. In other Vancouver Island rivers, oligochaetes increased in abundance in response to fish hatchery effluents (Munro et al. 1985) but not after inorganic fertilization (Perrin and Johnston 1985). However, the densities of oligochaetes in the Cowichan River, even in the pre-discharge sampling, were considerably greater than those reported in the above studies for the same time period. The arithmetic mean density of oligochaetes was $58,600 \cdot m^{-2}$ in the pre-discharge samples; in 1980, oligochaete densities were $19,000 \cdot m^{-2}$ at the control site and ranged from 61,200 to $165,000 \cdot m^{-2}$ for the impacted sites. In contrast, the maximum density found at sites enriched by hatchery effluents in the Quinsam, Puntledge, and Big Qualicum rivers was $34,300 \cdot m^{-2}$, while unimpacted sites averaged about $2,000 \cdot m^{-2}$ (Munro et al. 1985). The oligochaete data for the Cowichan River may indicate that the habitats sampled in this study were subject to mild enrichment possibly from the community of Lake Cowichan prior to the sewage effluent discharge, which produced further enrichment.

Cladocerans are, typically, minor components of the benthic invertebrate fauna of Vancouver Island rivers. The very large populations of cladocerans that occurred at sites downstream of the effluent discharge are attributable to the impact of the effluent and probably developed in response to the formation of algal mats, which may provide habitat and food. *Acroperus harpae* and *Alona affinis* are considered indicators of oligosaprobic to slightly mesosaprobic conditions (Sládeček 1973). The increased abundance of these benthic cladocerans therefore indicates mild enrichment rather than degradation of water quality.

No other major invertebrate group showed changes in abundance that could be attributed to the effluent discharge. The apparent lack of response by other benthic invertebrates may be an artifact of sampling only six weeks after the start of the discharge. In fertilization experiments on the Keogh River, differences between the benthos of enriched and control sites did not become apparent until four months after the start of the enrichment (Perrin and Johnston 1985). If the sewage effluent is not acutely toxic, changes in the abundance of the longer-lived components of the benthic invertebrate fauna may not be evident initially. Intragravel dissolved oxygen concentrations at the sites below the discharge ($4.1-6.8\ mg \cdot L^{-1}$) were low enough to adversely affect salmonids (Davis 1975), and so may also harm sensitive aquatic invertebrates, whose oxygen requirements are poorly defined (Davis 1975). A longer term response by the zoobenthos is quite possible and should be examined. The ammonia concentrations at the impact sites ($200-450\ ug \cdot L^{-1}$) should not be chronically toxic to invertebrates. Ammonia toxicity depends primarily on the concentrations of unionized ammonia rather than on total ammonia. Acute and chronic toxicity to ammonia is usually reported at total ammonia concentrations in the $mg \cdot L^{-1}$ range, which is much greater than those found in the Cowichan River.

The composition of the zoobenthic community in our Cowichan River samples differed from those seen in other Vancouver Island rivers (Munro et al. 1985) and in the upper reaches of the Cowichan River (Idyll 1943). In addition to higher abundances of oligochaetes and cladocerans, the abundances of tardigrades and water mites were much greater and the abundances of mayflies, stoneflies and caddisflies were much lower than elsewhere. The differences between the Cowichan and other Vancouver Island rivers were not methodological, since samples were taken with the same gear at the same time. Differing faunal compositions between the Cowichan and other Vancouver Island streams could reflect different background concentrations of macronutrients, temperatures, streamflows, or substrates.

The high densities of tardigrades (up to $392,000.m^{-2}$) were quite surprising since other studies rarely mention their presence. Tardigrade densities in lakes are typically around $10^4.m^{-2}$ but can be as high as $10^5.m^{-2}$ (Strayer 1985). The presence of high densities of tardigrades in "stone-in-current biotopes" in streams may indicate moderate organic enrichment since some biotic indices of water quality score tardigrades as pollution indicators (Chutter 1972). Although the large increase in tardigrade densities in 1980 apparently did not result from the effluent discharge, the unusually high densities found may indicate general enrichment of the river prior to the discharge. Tardigrades are usually herbivores (Marcus 1959) so that the high densities found in the lower Cowichan River may be related to algal standing crops.

The reduced abundance of the mayfly-stonefly-caddisfly group in the lower Cowichan River was also suggestive of enriched conditions. This group was the dominant constituent of the benthos of the upper Cowichan River in the past (Idyll 1943). Densities in other rivers (typically 5,000 - $15,000.m^{-2}$; Munro et al. 1985) were much greater than in our samples, although high variability makes comparisons difficult. Since the abundance of the EPT group was unaffected by the effluent discharge, their relatively low abundance may indicate previous nutrient enrichment. As a group, these animals are considered intolerant of pollution because of their high dissolved oxygen requirements (Hawkes 1979). Intragravel oxygen levels prior to the discharge were as low as $1.9\ ug.L^{-1}$ in midsummer, although levels in the river water remained high. Sensitive aquatic insects, such as EPT, would be adversely affected if exposed to such oxygen concentrations (Davis 1975). Aquatic insects are found in the hyporheic zone of the substrate (Coleman and Hynes 1970) so that they may experience the measured intragravel oxygen levels. The sampling sites were above the zone of tidal influence (Bell and Kallman 1976) so that physiological stress from salt water intrusions was unlikely.

In contrast to the EPT, the abundance and taxonomic composition of the Diptera (mainly the Chironomidae) were similar to those reported by Mundie (1971) and Munro et al. (1985). The chironomid fauna was dominated by the Orthocladiinae and Chironominae (especially the Tanytarsini). Most of the genera found (Appendix 2) are described as "collector-gatherers" (Cummins and Coffman 1978), which might be expected to increase as a result of the effluent discharge. Increased accumulations of periphyton and detritus should provide food and favourable habitat for collector-gatherers. However, an increase in the abundance of the chironomids may not be noticeable six weeks after the start of the discharge, as discussed above.

Changes in the zoobenthos that were attributable to the effluent discharge were unlikely to affect fish. The taxa that increased in abundance, oligochaetes and benthic cladocerans, are not major food items for juvenile salmonids. Chinook fry eat Alona, but the cladoceran comprised only 5% of the diet by volume (Northcote et al. 1979). Oligochaetes are rarely eaten (Aarefjord et al. 1973), but their true occurrence in the diet of fishes is likely to be underestimated (Milbrink 1973). Chironomids are a major food item for juvenile salmon (Mundie 1969; Northcote et al. 1979), but their abundance was not altered by the discharge. The development of algal mats may reduce the availability of chironomids and other food items to juvenile salmon which commonly feed from the drift.

The intragravel oxygen concentrations were low enough to reduce the survival of salmon eggs and alevins (Davis 1975). Since low subgravel oxygen levels were noted prior to the start of the discharge, they may be normal for this area. However, the development of algal mats will exacerbate the situation by impeding intragravel flow. The survival of salmon eggs varies directly with gravel permeability (Wickett 1958).

The responses of the periphyton and zoobenthic communities which were observed six weeks after the commencement of the discharge of treated sewage effluent, do not indicate gross degradation of water quality. However, the magnitude of the responses by the short-lived components of the biotic community suggest that considerable further change is likely. Monitoring should be done in the future to better establish the response of the long-lived organisms.

ACKNOWLEDGEMENTS

This study was completed with the assistance of several individuals. K. Munro, K. Masuda, B. Piercey and G. Carlson completed most of the field sampling. Some field assistance was provided by other staff of Dept. Fisheries and Oceans. Special thanks goes to G. Derksen for his contribution of water chemistry data. We also appreciate discussion with M. Nassichuk, I. Birtwell, H. Mundie and J. Stockner in early stages of the study. We thank G. Ennis and T. Tuominen for assistance with the algal bioassay. We also appreciate reviews of the manuscript by E. MacIsaac and M. Nassichuk.

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APPENDIX 1

The occurrence of taxa in benthic samples from the Cowichan River, 1979 and 1980

Appendix 1. The occurrence of taxa in benthic samples from the Cowichan River, 1979 and 1980. X=present in 1979, Y=present in 1980.

Taxanomic Group	Site 1 '79	Site 1 '80	Site 2 '79	Site 2 '80	Site 3 '79	Site 3 '80	Site 4 '79	Site 4 '80
Ephemeroptera								
Heptageniidae		Y		Y				
Leptophlebidae					X			
Plecoptera								
Perlidae		Y						
Trichoptera								
Hydropsychidae								
Hydropsyche						Y		
Hydroptilidae								
Hydroptila	X		X				X	
Oxyethura	X	Y	X	Y	X		X	
Rhyacophilidae								
Rhyacophila	X				X			
Unident. pupa		Y		Y	X	Y		Y
Diptera								
Unident.			X					
Dolichopodidae	X		X	Y	X		X	
Chironomidae								
Tanypodinae								
Ablabesmyia	X		X					
Potthasia		Y		Y		Y		Y
Orthocladiinae								
Brillia				Y				
Corynoneura		Y		Y		Y		Y
Cricotopus	X	Y	X	Y	X	Y	X	Y
Eukiefferiella	X			Y			X	
Heterotrissocladius		Y		Y		Y		Y
Psectrocladius	X		X		X			
Rheocricotopus			X				X	
Synorthocladius			X	Y	X	Y		Y
Thienemanniella	X		X		X		X	
Chirononinae								
Cladotanytarsus	X	Y	X	Y	X	Y	X	Y
Micropsectra	X	Y	X	Y	X		X	Y
Microtendipes		Y						
Polypedilum			X	Y			X	
Rheotanytarsus			X					
Unident. larvae	X	Y	X	Y	X	Y	X	Y
Unident. pupae	X		X	Y	X	Y	X	
Simuliidae		Y						
Tanyderidae							X	
Tipulidae	X				X			
Coleoptera					X			
Oligochaeta	X	Y	X	Y	X	Y	X	Y
Ostracoda	X	Y	X		X		X	

Appendix 1. (continued) The occurrence of taxa in benthic samples from the Cowichan River, 1979 and 1980. X=present in 1979, Y=present in 1980.

Taxanomic Group	Site 1		Site 2		Site 3		Site 4	
	'79	'80	'79	'80	'79	'80	'79	'80
Cladocera								
Unident.	X				X		X	Y
Acroperus harpae	X	Y	X	Y	X	Y	X	Y
Alona affinis	X	Y	X	Y	X	Y	X	Y
A. quadrangularis	X	Y		Y		Y	X	Y
A. rectangula	X	Y	X	Y	X	Y		Y
Chydorus	X		X		X		X	Y
Eurycercus			X				X	
Copepoda								
Cyclopoida								Y
Harpacticoida	X	Y			X		X	Y
Amphipoda								
Crangonyx		Y						
Hyalella		Y			X		X	
Tardigrada	X	Y	X	Y	X	Y	X	Y
Nematoda	X	Y	X	Y		Y	X	Y
Hydra	X							
Hydracarina	X	Y	X	Y	X	Y	X	Y
Turbellaria						Y		
Total number of taxa	27	25	26	24	26	19	26	21

APPENDIX 2

Zoobenthos abundance data for all stations

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	1	1	1	1	
Replicate	1	2	3	4	
Subsample	0.01563	0.00391	0.00391	0.00195	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	356	5689	2844	2844	2933
Oxyethura	356	4267	5689	0	2578
Rhyacophilidae					
Rhyacophila	0	0	0	6	1
Unid. pupae	0	0	0	0	0
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	711	0	5689	2844	2311
Cladotanytarsus	8178	24178	32711	36978	25511
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	356	0	0	0	89
Brillia	0	0	0	0	0
Corynoneura	0	0	0	0	0
Thienmaniella	0	1422	0	0	356
Cricotopus	4267	7111	7111	0	4622
Rheocricotopus	0	0	0	0	0
Psectrocladius	0	1422	4267	2844	2133
Synorthocladius	0	0	0	0	0
Heterotrissocla	0	0	0	0	0
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	0	0	0
Tanypodinae					
Ablabesmyia	0	0	1422	0	356
Potthasia	0	0	0	0	0
Unid. larvae	1422	1422	4267	5689	3200
Unid. pupae	711	2844	0	0	889
Tipulidae	0	0	1422	0	356
Dolichopodidae	711	1422	0	2844	1244
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	30578	56889	72533	82489	60622

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	1	1	1	1	
Replicate	1	2	3	4	
Subsample	0.01563	0.00391	0.00391	0.00195	Mean
Nematoda	356	0	0	0	89
Hydra	356	0	0	0	89
Hydracarina	9600	21333	31289	28444	22667
Ostracoda	711	1422	0	0	533
Amphipoda					
Hyalella	0	0	0	0	0
Crangonyx	0	0	0	0	0
Tardigrada	711	2844	19911	0	5867
Cladocera	1422	0	4267	2844	2133
Acroperus harpae	22044	29867	49778	36978	34667
Alona affinis	10667	11378	1422	17067	10133
A. quadrangularis	0	0	1422	0	356
A. rectangula	1067	2844	18489	0	5600
Chydorus	4267	7111	18489	17067	11733
Eurycercus	0	0	0	0	0
Harpacticoida	0	1422	1422	0	711
Cyclopoida	0	0	0	0	0
Total Organisms	98844	184889	284444	238939	201779

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	2	2	2	2	
Replicate	1	2	3	4	
Subsample	0.00195	0.00391	0.00391	0.00195	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	0	0	1422	0	356
Oxyethura	0	0	1422	0	356
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	0	0	0	0	0
Diptera	0	1422	0	0	356
Chironomidae					
Tanytarsini					
Micropsectra	17067	5689	5689	8533	9244
Cladotanytarsus	51200	34133	35556	73956	48711
Rheotanytarsus	2844	1422	0	0	1067
Orthocladiinae					
Eukiefferiella	0	0	0	0	0
Brillia	0	0	0	0	0
Corynoneura	0	0	0	0	0
Thienmaniella	2844	0	4267	0	1778
Cricotopus	5689	4267	11378	14222	8889
Rheocricotopus	0	1422	0	0	356
Psectrocladius	0	0	0	2844	711
Synorthocladius	0	0	1422	0	356
Heterotrissocla	0	0	0	0	0
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	2844	0	0	0	711
Tanypodinae					
Ablabesmyia	0	1422	0	0	356
Potthasia	0	0	0	0	0
Unid. larvae	0	5689	4267	14222	6044
Unid. pupae	2844	0	0	0	711
Tipulidae	0	0	0	0	0
Dolichopodidae	2844	1422	0	0	1067
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	65422	44089	52622	71111	58311

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	2	2	2	2	
Replicate	1	2	3	4	
Subsample	0.00195	0.00391	0.00391	0.00195	Mean
Nematoda	0	1422	2844	0	1067
Hydra	0	0	0	0	0
Hydracarina	36978	9956	8533	14222	17422
Ostracoda	8533	0	1422	2844	3200
Amphipoda					
Hyalella	0	0	0	0	0
Crangonyx	0	0	0	0	0
Tardigrada	8533	1422	1422	2844	3556
Cladocera					
Acroperus harpae	8533	15644	11378	25600	15289
Alona affinis	0	1422	2844	2844	1778
A. quadrangularis	0	0	0	0	0
A. rectangula	0	0	1422	0	356
Chydorus	0	7111	5689	8533	5333
Eurycercus	2844	0	0	0	711
Harpacticoida	0	0	0	0	0
Cyclopoida	0	0	0	0	0
Total Organisms	219022	137956	153600	241778	188089

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	3	3	3	3	
Replicate	1	2	3	4	
Subsample	0.00391	0.00195	0.00391	0.00195	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	17	4
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	0	0	0	0	0
Oxyethura	4267	2844	1422	0	2133
Rhyacophilidae					
Rhyacophila	0	0	0	11	3
Unid. pupae	1422	0	0	22	361
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	8533	11378	2844	14222	9244
Cladotanytarsus	21333	48356	17067	34133	30222
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	0	0	0	0	0
Brillia	0	0	0	0	0
Corynoneura	0	0	0	0	0
Thienmaniella	1422	2844	0	0	1067
Cricotopus	8533	19911	5689	5689	9956
Rheocricotopus	0	0	0	0	0
Psectrocladius	1422	0	0	0	356
Synorthocladius	1422	0	0	2844	1067
Heterotrissocla	0	0	0	0	0
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	0	0	0
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	0	0	0	0	0
Unid. larvae	1422	5689	1422	5689	3556
Unid. pupae	0	2844	0	5689	2133
Tipulidae	0	0	1422	0	356
Dolichopodidae	1422	0	0	0	356
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	5689	0	0	1422
Oligochaeta	35556	65422	52622	39822	48356

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	3	3	3	3	
Replicate	1	2	3	4	
Subsample	0.00391	0.00195	0.00391	0.00195	Mean
Nematoda	0	0	0	0	0
Hydra	0	0	0	0	0
Hydracarina	21333	45511	12800	45511	31289
Ostracoda	0	0	4267	2844	1778
Amphipoda					
Hyalella	0	0	0	2844	711
Crangonyx	0	0	0	0	0
Tardigrada	5689	11378	18489	14222	12444
Cladocera	1422	0	1422	0	711
Acroperus harpae	17067	28444	19911	36978	25600
Alona affinis	4267	5689	4267	17067	7822
A. quadrangularis	0	0	0	0	0
A. rectangula	2844	0	2844	0	1422
Chydorus	2844	8533	4267	11378	6756
Eurycercus	0	0	0	0	0
Harpacticoida	0	5689	0	2844	2133
Cyclopoida	0	0	0	0	0
Total Organisms	142222	270222	150756	241828	201257

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	4	4	4	4	
Replicate	1	2	3	4	
Subsample	0.00391	0.00195	0.00391	0.00391	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	1422	0	1422	1422	1067
Oxyethura	0	2844	0	0	711
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	0	0	0	0	0
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	1422	2844	1422	2844	2133
Cladotanytarsus	11378	42667	22756	32711	27378
Rheotanytarsus	0	0	0	0	
Orthocladiinae					
Eukiefferiella	0	2844	0	0	711
Brillia	0	0	0	0	0
Corynoneura	0	0	0	0	0
Thienmaniella	0	5689	0	0	1422
Cricotopus	1422	2844	4267	15644	6044
Rheocricotopus	1422	5689	2844	2844	3200
Psectrocladius	0	0	0	0	0
Synorthocladius	0	0	0	0	0
Heterotrissocla	0	0	0	0	0
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	1422	0	356
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	0	0	0	0	0
Unid. larvae	2844	2844	0	0	1422
Unid. pupae	1422	0	0	1422	711
Tipulidae	0	0	0	0	0
Dolichopodidae	1422	5689	0	1422	2133
Simuliidae	0	0	0	0	0
Tanyderidae	6	0	0	0	1
Coleoptera	0	0	0	0	0
Oligochaeta	51200	88178	32711	96711	67200

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-79	Sep-79	Sep-79	Sep-79	
Site	4	4	4	4	
Replicate	1	2	3	4	
Subsample	0.00391	0.00195	0.00391	0.00391	Mean
Nematoda	0	0	0	2844	711
Hydra	0	0	0	0	0
Hydracarina	55467	25600	36978	32711	37689
Ostracoda	1422	2844	1422	2844	2133
Amphipoda					
Hyalella	0	0	356	0	89
Crangonyx	0	0	0	0	0
Tardigrada	1422	2844	4267	11378	4978
Cladocera	0	2844	0	0	711
Acroperus harpae	5689	11378	11378	18489	11733
Alona affinis	2844	0	4267	1422	2133
A. quadrangularis	0	0	0	1422	356
A. rectangula	0	0	0	0	0
Chydorus	4267	5689	4267	7111	5333
Eurycercus	0	0	0	1422	356
Harpacticoida	1422	8533	0	5689	3911
Cyclopoida	0	0	0	0	0
Total Organisms	146494	221867	129778	240356	184624

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	1	1	1	1	
Replicate	1	2	3	4	
Subsample	0.00781	0.00391	0.00781	0.00391	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	1422	356
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	711	0	0	0	178
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	0	0	0	0	0
Oxyethura	711	0	0	1422	533
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	33	0	22	6	15
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	1422	0	0	0	356
Cladotanytarsus	10667	18489	3556	9956	10667
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	0	0	0	0	0
Brillia	0	0	0	0	0
Corynoneura	2133	1422	1422	4267	2311
Thienmaniella	0	0	0	0	0
Cricotopus	3556	2844	3556	4267	3556
Rheocricotopus	0	0	0	0	0
Psectrocladius	0	0	0	0	0
Synorthocladius	0	0	0	0	0
Heterotrissocla	0	1422	0	0	356
Chironomini					
Microtendipes	711	0	0	0	178
Polypedilum	0	0	0	0	0
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	2133	0	0	0	533
Unid. larvae	2133	8533	1422	2844	3733
Unid. pupae	0	0	0	0	0
Tipulidae	0	0	0	0	0
Dolichopodidae	0	0	0	0	0
Simuliidae	711	0	2133	0	711
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	11378	22756	14933	27022	19022

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	1	1	1	1	
Replicate	1	2	3	4	
Subsample	0.00781	0.00391	0.00781	0.00391	Mean
Nematoda	711	0	0	0	178
Hydra	0	0	0	0	0
Hydracarina	13511	9956	22044	24178	17422
Ostracoda	711	0	1422	0	533
Amphipoda					
Hyalella	711	0	0	0	178
Crangonyx	0	1422	0	0	356
Tardigrada	14933	5689	4267	15644	10133
Cladocera					
Acroperus harpae	25600	49778	10667	32711	29689
Alona affinis	2844	1422	0	0	1067
A. quadrangularis	0	0	0	2844	711
A. rectangula	1422	1422	0	0	711
Chydorus	0	0	0	0	0
Eurycercus	0	0	0	0	0
Harpacticoida	0	4267	711	0	1244
Cyclopoida	0	0	0	0	0
Total Organisms	96744	129422	66156	126583	104726

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	2	2	2	2	
Replicate	1	2	3	4	
Subsample	0.00195	0.00195	0.00195	0.00195	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	2844	711
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	0	0	0	0	0
Oxyethura	0	0	0	2844	711
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	6	0	0	0	1
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	0	0	5689	0	1422
Cladotanytarsus	8533	17067	8533	25600	14933
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	0	0	5689	0	1422
Brillia	0	0	2844	0	711
Corynoneura	22756	11378	11378	5689	12800
Thienmaniella	0	0	0	0	0
Cricotopus	8533	2844	5689	2844	4978
Rheocricotopus	0	0	0	0	0
Psectrocladius	0	0	0	0	0
Synorthocladius	5689	2844	5689	2844	4267
Heterotrissocla	0	0	2844	2844	1422
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	0	2844	711
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	0	2844	2844	0	1422
Unid. larvae	8533	5689	2844	2844	4978
Unid. pupae	2844	5689	2844	0	2844
Tipulidae	0	0	0	0	0
Dolichopodidae	0	2844	0	0	711
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	62578	54044	68267	59733	61156

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	2	2	2	2	
Replicate	1	2	3	4	
Subsample	0.00195	0.00195	0.00195	0.00195	Mean
Nematoda	0	2844	2844	0	1422
Hydra	0	0	0	0	0
Hydracarina	11378	31289	34133	42667	29867
Ostracoda	0	0	0	0	0
Amphipoda					
Hyalella	0	0	0	0	0
Crangonyx	0	0	0	0	0
Tardigrada	190578	93867	139378	179200	150756
Cladocera					
Acroperus harpae	34133	62578	45511	85333	56889
Alona affinis	8533	8533	11378	14222	10667
A. quadrangularis	0	2844	0	0	711
A. rectangula	2844	0	5689	2844	2844
Chydorus	0	0	0	0	0
Eurycercus	0	0	0	0	0
Harpacticoida	0	0	0	0	0
Cyclopoida	0	0	0	0	0
Total Organisms	366939	307200	364089	435200	368357

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	3	3	3	3	
Replicate	1	2	3	4	
Subsample	0.00195	0.00195	0.00195	0.00098	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	2844	0	711
Hydroptilidae					
Hydoptila	0	0	0	0	0
Oxyethura	0	0	0	0	0
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	0	6	0	6	3
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	0	0	0	0	0
Cladotanytarsus	17067	2844	14222	28444	15644
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	0	0	0	0	0
Brillia	0	0	0	0	0
Corynoneura	11378	28444	25600	28444	23467
Thienmaniella	0	0	0	0	0
Cricotopus	5689	0	5689	22756	8533
Rheocricotopus	0	0	0	0	0
Psectrocladius	0	0	0	0	0
Synorthocladius	2844	0	5689	11378	4978
Heterotrissocla	0	2844	0	0	711
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	0	0	0
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	2844	0	0	0	711
Unid. larvae	5689	0	5689	5689	4267
Unid. pupae	2844	0	0	0	711
Tipulidae	0	0	0	0	0
Dolichopodidae	0	0	0	0	0
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	71111	105244	99556	153600	107378

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	3	3	3	3	
Replicate	1	2	3	4	
Subsample	0.00195	0.00195	0.00195	0.00098	Mean
Nematoda	5689	0	0	0	1422
Hydra	0	0	0	0	0
Hydracarina	17067	22756	8533	34133	20622
Ostracoda	0	0	0	0	0
Amphipoda					
Hyalella	0	0	0	0	0
Crangonyx	0	0	0	0	0
Tardigrada	164978	190578	182044	267378	201244
Cladocera					
Acroperus harpae	36978	45511	62578	73956	54756
Alona affinis	39822	36978	11378	17067	26311
A. quadrangularis	5689	0	0	5689	2844
A. rectangula	0	5689	0	5689	2844
Chydorus	0	0	0	0	0
Eurycercus	0	0	0	0	0
Harpacticoida	0	0	0	0	0
Cyclopoida	0	0	0	0	0
Turbellaria	2844	0	0	0	711
Total Organisms	392533	440894	423822	654228	477869

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	4	4	4	4	
Replicate	1	2	3	4	
Subsample	0.00098	0.00098	0.00049	0.00098	Mean
Ephemeroptera					
Leptophlebidae	0	0	0	0	0
Heptageniidae	0	0	0	0	0
Plecoptera					
Chloroperlidae	0	0	0	0	0
Perlidae	0	0	0	0	0
Trichoptera					
Hydropsychidae					
Hydropsyche	0	0	0	0	0
Hydroptilidae					
Hydoptila	0	0	0	0	0
Oxyethura	0	0	0	0	0
Rhyacophilidae					
Rhyacophila	0	0	0	0	0
Unid. pupae	6	6	17	11	10
Diptera					
Chironomidae					
Tanytarsini					
Micropsectra	5689	5689	0	0	2844
Cladotanytarsus	5689	11378	22756	28444	17067
Rheotanytarsus	0	0	0	0	0
Orthocladiinae					
Eukiefferiella	0	0	0	0	0
Brillia	0	0	0	0	0
Corynoneura	11378	28444	68267	11378	29867
Thienmaniella	0	0	0	0	0
Cricotopus	11378	5689	0	0	4267
Rheocricotopus	0	0	0	0	0
Psectrocladius	0	0	0	0	0
Synorthocladius	11378	5689	0	5689	5689
Heterotrissocla	0	0	11378	0	2844
Chironomini					
Microtendipes	0	0	0	0	0
Polypedilum	0	0	0	0	0
Tanypodinae					
Ablabesmyia	0	0	0	0	0
Potthasia	0	5689	0	0	1422
Unid. larvae	0	0	0	5689	1422
Unid. pupae	0	0	0	0	0
Tipulidae	0	0	0	0	0
Dolichopodidae	0	0	0	0	0
Simuliidae	0	0	0	0	0
Tanyderidae	0	0	0	0	0
Coleoptera	0	0	0	0	0
Oligochaeta	216178	147911	182044	113778	164978

COWICHAN RIVER SEWAGE STUDY
(number per m2)

Date	Sep-80	Sep-80	Sep-80	Sep-80	
Site	4	4	4	4	
Replicate	1	2	3	4	
Subsample	0.00098	0.00098	0.00049	0.00098	Mean
Nematoda	5689	11378	0	0	4267
Hydra	0	0	0	0	0
Hydracarina	22756	17067	68267	62578	42667
Ostracoda	0	0	0	0	0
Amphipoda					
Hyalella	0	0	0	0	0
Crangonyx	0	0	0	0	0
Tardigrada	369778	238933	637156	324267	392533
Cladocera	11378	0	0	0	2844
Acroperus harpae	136533	204800	273067	216178	207644
Alona affinis	250311	164978	307200	290133	253156
A. quadrangularis	0	11378	22756	0	8533
A. rectangula	0	0	34133	0	8533
Chydorus	17067	45511	0	34133	24178
Eurycercus	0	0	0	0	0
Harpacticoida	11378	0	0	0	2844
Cyclopoida	0	0	11378	0	2844
Total Organisms	1086583	904539	1638417	1092278	1180454