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AN ON-SITE ASSESSMENT OF CHLORINATION IMPACTS ON BENTHIC MACROINVERTEBRATES

A Thesis Presented

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ABSTRACT

AN ON-SITE ASSESSMENT OF CHLORINATION IMPACTS ON BENTHIC MACROINVERTEBRATES

(May 1989)

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Biological and chemical samples were collected from 4 sampling stations over a 6-month period to assess the effects of a waste water treatment plant's chlorinated sewage discharge on the Lampson Brook's benthos. During the study, total residual chlorine (TRC) concentrations measured in individual sampling stations were inconstant, which relate to the variations in the quality of discharged effluents, the quantity of chlorine (sodium hypochlorite) WWTP applied, and stream dilution. Due to the decay of residual chlorine in the stream, the effluent TRC (2.4±0.65 mg/l) gradually lessened in concentration to one fiftieth of its original level at S-5 (0.048±0.010 mg/l), some 2.9 km downstream from the discharge.

Intrasite comparisons on community abundance and diversity were conducted to examine the community change from the period of non-chlorination to short-term and long-term continuous chlorination. The level of impact the

communities received is reflected in the loss of sensitive species, the dominance of tolerant species, and the irregular variation in both population abundance and species diversity. The identification of the collected macroinvertebrates in this study was restricted to the generic level. Individual limestone fragments in the basket-type substrates were regarded as individual sampling units. Each mean population density was estimated with a 95% confidence interval. Two-way ANOVA on mean density of community and major taxa were employed to afford intrasite comparisons. Pielou's formula (H) for estimating the total diversity of a collection was calculated for individual substrates. TRC was found to reduce the diversity but not the density of a community, located around 40 m downstream from the discharge. The suspect substances that impacted the community in both density and diversity were tentatively identified as chloroorganics in S-3, some 0.8 km downstream from the discharge. Community recovery during the continuous chlorination was noticed at S-5, presumably due to the seasonal succession and the tolerance of those successive species to low level of chlorine residuals.

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CHAPTER I

INTRODUCTION

1.1 The Status of Chlorination in the U.S.A.

Since 1893, when chlorine was first used to disinfect wastewater in the United States at Brewster, New York, the practice of chlorination has grown steadily. With the passage in 1977 of the National Pollution Discharge Elimination Systems (NPDES) Act and its stipulation that municipal wastewater treatment plant meet effluent bacterial standards, chlorination has become a virtually universal treatment (Lee, 1985). No other water-treatment process enjoys such popularity and almost universal adoption, particularly in the United States (Laubusch, 1971).

The water quality standards for disinfection of freshwater in Massachusetts approved by the Environmental Protection Agency (EPA) states "All wastes shall receive appropriate waste treatment which is defined as secondary treatment with disinfection .... to meet the objectives of the water quality standards, all as determined by the Division of Water Pollution Control. The amount of disinfection required shall be equivalent to a free and combined chlorine residual of at least 1.0 mg/l after 15 minutes contact time during peak hourly flow or maximum rate of pumpage. Disinfection from October 1 to May 1

however may be discontinued at the discretion of the Division of Water Pollution Control.... "(USEPA, 1973).

It is not surprising that this practice is so popular, for chlorine is a very effective bactericide at low concentrations. In addition, its rate of disinfection is satisfactory for water treatment where sewage is stored for hours or days before release. Another advantage of chlorine is that its residuals can be maintained in treated water to provide extra safety by inhibiting regrowth of bacteria (Schroeder, 1977).

More recently however, with the passage of federal legislation militating biological evaluation of discharges, a number of disadvantages have come to light (Foster, 1984). The discharge of toxic pollutants in toxic amounts was prohibited and the interim goal providing for the protection of aquatic life and wildlife by 1983 was adopted (Pratt and Coler, 1976). Unfortunately, in this instance, public health and ecological priorities are not congruent, for the residual chlorine or chlorine-combined wastewater constituents were revealed to be toxic to fish (Schroeder, 1977) and many other aquatic organisms (Mattice, 1976; Hall et al., 1982,1981). Hall et al. (1981) suggested that environmental perturbations caused by the discharge of chlorinated sewage are reflected in riverine community composition and diversity directly through toxicity manifestations and indirectly through alterations in the

food chain. Further, many of the chlorinated hydrocarbons, produced when organics-containing water is treated for domestic use, are known or suspected mutagens (Maruoka and Yamanaka, 1980).

1.2 Objectives

Consequently, the purpose of this study is to:

1. assess the acute and chronic toxic effects exerted by chlorinated sewage effluents on the benthic macroinvertebrate community colonizing artificial substrates.
2. develop techniques of data gathering and analysis as part of a practical methodology for the routine biological monitoring of chlorinated effluents.

The impacts of chlorination can be evaluated through the changes of structure and composition of benthic macroinvertebrate communities colonizing basket-type substrates. The density of individual species with 95% confidence intervals within a basket and the diversity of individual baskets will be calculated. The potential indicator species of chlorination will also be identified.

CHAPTER II

LITERATURE REVIEW

Research in chlorine toxicity for the most part has been devoted to defining in the laboratory lethal and sublethal toxicant levels (Hall et al., 1981,1982). Few studies have been actually conducted on-site to assess the acute and chronic effects of chlorinated sewage on resident macroinvertebrates (Mattice & Zittel, 1976). Further, of those studies published, the bulk were descriptive, cataloging dominant organisms or, as in the case of power plant discharges, confined to the response of fish populations (Hall et al., 1981,1982; Mattice, 1976).

After the following general review of publications pertinent to the biological impacts of chlorine, the derived methodology, adopted in this study, will be reviewed.

2.1 Chlorine and the Aquatic Biota

2.1.1 Macroinvertebrates Clarke (1977) reported the 96-hr LC50 values ranging from 0.65 to 0.83 mg/l TRC (total residual chlorine) for Amphipod, Hyallella azteca, and 1.08 mg/l TRC for the crayfish, Orconectes australis. He also found, however, higher LC50 values for organisms previously acclimated to low levels of TRC. Gooch et al. (Hall et al., 1981) noted that exposure to only 0.5 mg/l TRC for 100 hr

resulted in 100% mortality of the comparatively sensitive larval clam, Corbicula manilensis.

Ginn et al. (Hall et al., 1981) showed that elevated temperature increased the toxicity of chlorine for the amphipod, Gammarus tigrinus. They suggested that chlorination exposure may interact with other entrainment conditions (ie. temperature, velocity etc.) in causing greater mortality of this species.

Maturity may also be a factor in resistance to chlorine. The small juvenile leeches (Piscicola salmositica) were reported to be fairly sensitive to chlorine toxicity by Bower et al. (1985). They observed that all small juvenile leeches exposed to 0.044 mg/l of TRC for 24 hr died, and over half of such leeches exposed to 0.044 mg/l for 8 hr and 0.021 mg/l for 24 hr died. Predictably the large subadult and adult leeches were more resistant to chlorine than the juveniles. In a similar light, Roberts et al. (1975) noted that molluscan larvae were still more sensitive to chlorine, with 48 hr TL50 values less than 0.005 mg/l. McLean (1973) found, after an exposure to 2.5 mg/l total chlorine for 5 min, an 80% average mortality for barnacle larvae (Balanus improvisus). He observed little mortality however for the grass shrimp (Palaemonetes pugio) and the amphipod (Melita mitida). The mortality of P. pugio increased however to 70% 48 hr after exposure and to almost 100% after 96 hr. He suggested that

barnacle larvae might be regarded as indicators, demonstrating the effect of chlorination on susceptible organisms.

Mattice et al. (1981) reported that the 30- and 60-min LC 50 values were 0.97 and 0.063 mg/l, respectively, for Daphinia magna and 1.59 and 0.84 mg/l, respectively, for Gambusia affinis.

Murray et al. (1984) measured no effect of intermittent low-level chlorination (0.21-2.39 mg/l free chlorine) on hatching success of mayfly eggs. However, gill growth was significantly greater (by 51%) in nymphs previously exposed to chlorine as embryos than in the controls. He concluded that previous and subsequent chlorine exposures resulted in longer gills, a phenomenon apparently triggered during embryonic development.

The appearance and persistence of crustaceans, nematodes, flatworms, water mites, and chironomids found in potable water supplies and distribution systems, is regarded as a health hazard. They could adsorb associated bacteria and thus afford protection from chlorination (Levy et al., 1983). Interestingly, tubificid worms (Limnodrilus hoffmeisteri and Tubifex tubifex) were reported to accumulate many chlorinated chemicals (Oliver, 1984).

An on-site study on the effects of chlorinated municipal sewage discharges to the structure and composition of aquatic macroinvertebrate communities was

reported by Osborne (1984). The reduction in the numerical importance of mayflies, chironomids, caddisflies, and stoneflies was apparent. He suggested that certain Oligochaete taxa are much less susceptible to the toxic effects of TRC than are most aquatic taxa. In Osborne and Davies's studies (1986), stream stations receiving chlorinated municipal sewage effluent had more individuals and fewer numbers of taxa. Their data also indicate that sampling stations exposed to chlorinated effluent (left bank of the stream) have significantly lower diversity values than do stations not impacted by effluent (right bank of the stream).

Pagel and Landon (1981) compared the communities upstream and downstream from chlorinated sewage outfalls for several selected rivers. They suggested that the rapid flow of the river and high dilution ratio may help to reduce the impact of chlorinated waste water effluent on the benthic community. They noted that the Oligochaeta and Nematoda taxa are opportunistic and mostly pollution tolerant and that midges can tolerate limited chlorinated effluents, while mayflies, caddisflies, and stoneflies are generally considered sensitive organisms. They also suggested that the effluent discharges containing organic wastes would increase the midge population.

Heckman (1983) reported that the recovery of a temperate zone biotic community in a lotic freshwater

habitat was nearly completed in four months after the accidental release of a highly concentrated chlorine solution from the drinking water pipeline. Unlike Pagel & Landon (1981), he regarded Simulidae and Chironomidae as pioneer species because of their great abundance during the period immediately following the termination of chlorination. Case-buiding insect larvae resisted exposure to chlorine better than most other arthropods, and even the caddisflies had survived in moderate numbers. Those sensitive species, found abundantly upstream from the chlorine outlet but rarely in the chlorinated downstream waters, were Baetis rhodani, Pelmatohydra oligactis, Colurella uncinata, Pisidium casertanum, Gammarus pulex, Halipulus fluxiatilis, and Gasterosteus aculeatus.

The continuous chlorination effects, set at 0.00, 0.47, 0.94, and 1.41 mg chlorine-produced oxidant (CPO) l^{-1}, on numerical abundances of eight dominant species [Ampharete sp. Pista sp., Nereis succinea (polychaete), Molgula manhattensis (tunicate), Macoma tenta (bivalve), Corophium acherusicum, Melita nitida, and Haustorius sp. (amphipod)] were assessed in Sheridan and Badger's studies (1980). As compared to controls, chlorination significantly depressed the numbers of Ampharete, Molgula and Macoma in three concentrations (0.47, 0.94, and 1.41); and Pista, Corephium and Melita in two concentrations (0.94 and 1.41). Chlorination, however, induced significantly higher numbers

of Haustorius and Nereis in the chlorinated communities. They also described a significant decline in total numbers of individuals, since chlorinated communities contained 38 to 44% fewer organisms than controls did.

2.1.2 Fish Results collected from extensive laboratory investigations on freshwater fish suggest a greater tolerance for chlorine than invertebrates. Further, the data indicate that toxicity can be influenced by acclimation temperature, pH, chlorine species, exposure duration, and diel variation (Hall et al., 1981).

Brooks and Seegert (1977) categorized each of ten species as either sensitive or resistant based on their response to monochloramine. The sensitive species (emerald shiner, spotfin shiner, common shiner, channel catfish, white sucker, and sauger) yielded 72-hr LC50 values ranging from 0.35 to 0.71 mg/l while the resistant species, 72-hr LC50, were defined as falling between 1.15 to 1.50 mg/l (freshwater drum, white bass, bluegill, and carp). From these data, Hall et al. (1981) concluded that to protect the most sensitive species monochloramine exposures should not exceed 0.2 mg/l for a period of more than 160 min/day.

In some field studies, reviewed by Mattice and Zittel (1976), kills of cold and warm-water fish species have been attributed to chlorine. No fish were found in waters where chlorine residual was >0.02 mg/l. Paller et al.

(1983) concluded that residual chlorine in wastewater effluents was the main toxicant suppressing the diversity, size, and quantity of fish in two Illinois streams. Data collected from Grieve et al. (1978) demonstrated the influence of chlorinated discharges in affecting fish movements. Not surprisingly, in a chlorinated municipal waste discharge, a 100% mortality of caged black bullheads in a 24-hr exposure to 5.0 mg/l TRC was reported by Clarke et al. (1977).

Some physiological parameters, other than death, were used as endpoints to quantify the effects of chlorine. Loss of equilibrium was reported in fathead minnows when exposed to 1.5 mg/l monochloramine for 1 hour. Anoxia due to the oxidation of hemoglobin to methemoglobin was also reported in this species. Zeitour (1977) noted that TRC concentrations ranging from 1.09 to 3.86 mg/l resulted in thicker and darker blood in rainbow trout. Bass and Heath (1977) found that free chlorine kills fish by internal hypoxia induced by damage to the gills. Basch and Truchan (1976) suggested that water temperature and gas saturation levels can interact to cause changes in the concentrations of chlorine lethal to a salmonids.

2.1.3 Plankton A study of the impact of chlorination on plankton revealed high tolerance levels. By using respiration rates and net primary productivity (NP) of biological systems, Osborne (1982) recorded the acute

metabolic responses of lotic epilithic communities (ie. bacteria, algae, fungi, and protozoans) to total residual chlorine. When TRC was varied from 0.1, 0.5, 1.0, 1.5 to 2.0 mg/l, NP decreased significantly but the respiration rate decreased significantly only when concentrations were in excess of 0.5 mg/l. When exposed to 0.1 mg/l TRC, the respiration rate was significantly increased, a phenomenon generally recognized as an indicator of metabolic stress to sublethal concentrations of toxicants (Osborne, 1982). The chronic ramifications of a prolonged respiratory increase would likely be a decrease in the functional capabilities of the community as more energy is diverted for simple system maintenance.

Based upon measurement of ATP levels, Gentile et al. (Hall, 1982) reported a "complete destruction" of entrained phytoplankton exposed to applied rates of 0.35 to 0.55 mg/l TRC. Similarly a 50% reduced rate of C-uptake by phytoplankton was reported by Eppley et al. (Hall et al., 1982), upon 2-4 hr exposure to either 0.1 mg/l chlorine without ammonia or 0.06 mg/l chlorine with ammonia. In some cases, the phytoplankton recovered from the inhibitory effects of chlorine.

Though phytoplankton populations have received a fair amount of attention (Mattice 1976), few studies have addressed the sublethal effects of chlorination upon biological functions of zooplankton. Roberts et al.

reported a 48-hr TL50 value of <0.05 mg/l for the copepod Acartiad tonsa (Hall et al, 1982). Davis and Jensen speculated however that zooplankton mortality was not significantly impacted by chlorination at 1.00 mg/l of chlorine-produced oxidant (CPO) (Hall et al, 1982). There are several authors who concluded as well that chlorine exerts little effect on zooplankton survival (Hall et al., 1982). They attributed mechanical and thermal stresses as well as chlorine to zooplankton mortality. Bosnak and Morgan (1981) estimated a 96-hr LC50 value and associated 95% confidence level for Caecidotea bicrenata, a blind hypogean cave isopod, of 0.110 (0.042 to 0.286) mg/l TRC and for Lirceus alabamae, an epigean isopod, of 0.155 (0.082 to 0.295) mg/l TRC.

In summary, Mattice and Zitel (1976) presented a comprehensive compilation of chlorine toxicity data in tabular form for freshwater and marine organisms (fish and invertebrates). The fresh water data are predominantly for fish. Chlorine toxicity thresholds are also presented for the potential toxicity of a given dose-time combination to aquatic organisms. The acute toxicity threshold for freshwater macroinvertebrates approximates the line connecting the dose-time points 0.0015 mg/l-7200 min and 1.0 mg/l-1.1 min. Their recommended chronic toxicity threshold is 0.0015 mg/l. If such a conservative estimate is valid, most streams receiving chlorinated effluents will

be impacted over a greater length of their bed than initially suspected.

2.2 Benthic Macroinvertebrates As Tools to Evaluate the Environment

The evaluation of the structure and composition of aquatic macroinvertebrate communities to assess water quality has been widely practiced (Miller, 1986; Pratt, 1976,1977,1981; Olive, 1973). The reasons for this are eminent: First, much emphasis on the analysis of chemical and physical tests does not provide the biological aspect of the chronic synergistic effects under stressed environment. Second, they are superior to microorganism as ecological indicator due to their habitat preferences, longer life cycles, and relationship to fish (Pratt, 1976).

Macroinvertebrates, however, play a dominant role in energy flow. They occupy virtually all levels of the food web (Cummins, 1973; Lamberti, 1984; Benke, 1984) and fill well-defined ecological niches, as described in the "River Continuum Concept" (Merritt et al., 1984). Their characteristic assemblages or communities provide a kind of "faunal memory" reflecting environmental conditions not only during the time of collection but also from preceding periods. Some of them can assimilate and concentrate substances, such as heavy metals and pesticides that may be present temporarily or at undetectable levels (Pratt 1977). These organisms are also widely and abundantly distributed, and

easier to collect and analyze than are fish and microorganisms.

It was suggested that the design of macroinvertebrate studies should be based upon study goals; and the ideal must frequently be tempered by the realities of available resources, time limitations imposed on the study, and the characteristics of the habitat to be studied (USEPA, 1973).

2.3 Artificial Substrates As Devices to Colonize Macroinvertebrates

The construction and use of artificial substrates for experiments is really the creation of experimental islands (Osman, 1982). One important advantage of this experimental island is that of a relatively constant and uniform surface area available to the organisms. Standardized sampling can be achieved due to the identical characteristics of the substrate. In addition, the number of replicates of the artificial substrates will not be as limited as the natural islands, and their application to sampling provide convenience, economy, and greater flexibility. It follows that artificial substrates can be utilized to investigate many ecological hypotheses (Osman, 1982). In this regard Mason (1970) reported that artificial substrate samplers deployed upstream and downstream from sources of pollution could be compared on a quasi-quantitive basis to determine changes in environmental quality.

Mason, Anderson, and Morrison (1967) used a cylindrical barbecue basket filled with 9 kg of limestone as the sampler. Species composition and numerical values can be determined and the mutual comparisons from one sampler to another be obtained. However, this type of sampler does not provide a quantitative estimate of the fauna (Jacobi, 1971). The Division of Pollution Surveillance of the EPA uses a modified barbeque basket filled with limestone and suspended from a float (Mason et al. 1967; Dickson et al. 1971). They indicate that locating the basket within the euphotic zone creates a shallow stream environment that attracts a larger number and variety of organisms. A minimum of two of these kinds of baskets was suggested to be placed at each station to ensure retrieval of at least one substrate collection (100-300+ organisms) (Pratt & Coler, 1976).

However, Morin (1985) suggested that density estimates obtained with artificial substrates were as variable as those from natural substrates, although some artificial substrates (baskets of rocks) yielded less variable density estimates than average. Further, Fredeen and Spurr (1978) reported that these substrates are selective and that dragonfly larvae, hemipterans, beetle larvae and burrowing species of all taxa seldom occurred on artificial substrates even when relatively abundant in the immediate environment.

2.4 Estimating the Abundance of Stone-Dwelling Organisms

Qualitative sampling of macroinvertebrates is difficult because the distribution of macroinvertebrates within the substrate require large numbers of samples to be able to accurately estimate population densities (Hellawell, 1986). Calow (1972) suggested to use individual stone (actual habitat of organisms) as the most promising sampling unit. A new sampling protocol incorporating this approach was reported to be superior to conventional quadrat techniques in estimating population size by Wrona et al. (1986). They used individual stones as sampling units. Both the heterogeneity of the habitat and the spatial dispersion of a population among stones can be explained through the calculations of the density and error estimates.

In Wrona's study (1986), a predetermined number of stones is chosen within a defined size class and the numbers of organisms found per stone recorded. The method to categorize a random sample of stones into size classes was based on easily measured parameters such as length, breadth, or plane area. Once stone size classes have been decided upon, size class specific weighting factors can be derived and used to calculate organism population density and error terms. Although there are other ways to define it, the weighting factor may be expressed as the mean surface area of the stones occurring within each stone size

class. Using the reciprocal of the mean surface area as the weighting factor results in the final density estimate expressed as numbers per square centimeter of stone surface.

2.5 Estimating the Surface Area of A Limestone

Measuring the surface areas of the irregularly shaped stone has always been the main difficulty in making quantitative density estimates of stone-dwelling fauna. Muller (Calow,1972) roughly estimated the surface area of irregular stone by using the product of two longest measurements of each stone. Minshall (1984) suggested that a satisfactory estimate of area can be made, on relatively uniform particles, using the mean diameter and a standard formula for the approriate geometric shape. Calow (1972) presented a hypothetical linear relationship between surface area (Y) and some other linear parameter (X) in a population of stones. Based on their theories, estimating the surface area of stones with unique shape would be easier as long as the linear parameter (X) and the relative coefficient can be found.

While benthic macroinvertebrate density and diversity are largely a function of surface area, they are as well correlated with substrate (particle size, surface heterogeneity, porosity, texture etc.), stream current, and detritus (Erman & Erman, 1984; Shelley, 1979; McElhone & Davies, 1983).

CHAPTER III

SITE DESCRIPTION

3.1 Lampson Brook and Belchertown, Massachusetts

Lampson Brook, the study site, is located in Belchertown, a town of some 55.4 square miles and 7,863 inhabitants located in Hampshire County (Mass. Municipal Profiles, 1987). Town water and sewage lines service only the village of Belchertown, however (Smith, 1975).

The general climate of Belchertown is characterized by warm, humid summers and moderately cold winters. The period most conducive for stream recharge occurs between October and April. During late spring and summer, evapotranspiration is high as a result of increased water demands by plants and a high evaporation rate, thus causing generally low water levels (Smith, 1975).

Lampson Brook, a second order stream, is one of the principal perennial streams in Belchertown. It is in the area of the State Reservation, which is in the central west portion of Belchertown. It's headwaters originate at the swamp connecting State Street (Rt 202) and Hill Road. The stream flows past the Belchertown State School and across poorly sorted sands and gravels into Granby. In the upstream areas, the watershed is a combination of agricultural land, areas of dense mixed hardwood and softwood trees (21-40 feet), and WWTP-utilized filter bed.

In the midstream zone, it consists of a shallow marsh, areas of dense mixed hardwood and softwood trees (21-40 feet), agricultural land, and clustered residential land. In addition to having similar land types as midstream, the downstream watershed also contains wild land, areas with dense mixed higher hardwood and softwood (41-60 feet), and a light density of the residential land (Macconnell, 1975). Beaver activity is in evidence along the stream as indicated by felled trees, and dams.

Five study sites were chosen along the Lampson Brook and the confluence of Lampson Brook with Weston Brook (Fig. A-1): Station 1, some 100 meters upstream of the Waste Water Treatment Plant outfall. Station 2 was 40 meters downstream from the outfall. Stations 3 and 4 are located below the swamp, 740 and 1100 meters downstream from the outfall, respectively. Below station 4, Lampson Brook mixes with Weston Brook, which is a second order brook (Fig.A-1). Below its confluence the brook extends 970 meters to a small pond adherent to Mill Road. Along Mill Road, the brook flows past several private swimming pools. After this, it crosses Boardman Street and Rural Street, where Station 5 is located, 2900 meters from the WWTP outfall and ends, 640 meters downstream from station 5, in Forge pond, Granby (Fig.A-1). The general width and depth of the stream at the pools are about 5-9 feet and 2-4 feet, respectively. During summer, the flow rate within 500 feet downstream

from sewage outfall was estimated to be 0.47 feet/sec by the Department of Environmental Engineering, University of Massatusetts in Amherst.

3.2 Belchertown Wastewater Treatment Plant

The treatment facility is situated on State Street, Belchertown, and is under the authority of Massachusetts Department of Mental Health. It services the central Belchertown and State School, a total population of some 2500.

It was equipped as a secondary treatment plant with a designed flow of 0.5 mgd. The average daily flow and total yearly flow, as of 1985, are 0.311 mgd and 113.438 mg, respectively. Chlorine is applied within the period between April and October. The minimum-maximum total chlorine residuals required by EPA are 0.5-1.5 mg/l after 15 minutes contact at peak hourly flow. The effluent limitations and monitoring requirements are listed in Table A-1.

3.3 Stream Quality

Water quality for much of the length of Lampson Brook reflects the influence of the discharge from the WWTP. Their spacing was predicated on obtaining a spectrum of biological responses reflecting exposure to varied concentration of chlorinated sewage. From the chemical and physical parameters measured at all stations (Table A-2), it appears that except for residual chlorine, the most

biologically significant effluent constituent was ammonia (Table A-2), which concentration was above EPA water quality criterion (0.02 ppm) (Table A-3). All other chemical parameters exhibited little impact on water quality. The dissolved oxygen (DO) (Table A-2) between stations 2 and 4 was found to decrease after initiation of chlorination. However, the DO at S-5 was consistent, which implied a recovery at that point. Based on coliform counts, it appears that chlorination effectively controls the incidence of coliform bacteria.

CHAPTER IV

MATERIALS AND METHODS

4.1 Field Procedures

While biological samples were collected after 5 weeks exposure periods, chemical-physical samples were collected weekly whenever substrates were deployed.

4.1.1 Biological Sampling. Two sets of biological samples were collected during the entire experimental period. Wire barbeque baskets, each filled with 30, 2" to 3" diameter limestone fragments (garden marble chips) served as artificial substrates (Pratt, 1977).

The first set of samples was deployed in March of 1987, five weeks before chlorination resumed. Six of the substrates were secured at every station by connecting each with a loop of wire cable to a solid steel rod (one meter long by one centimeters in diameter) pounded into the river bed. The baskets were then lowered beneath the water surface to 5 cm above the river bed. After a 5-week recruitment period, just before the chlorination was resumed, two of the baskets were retrieved from each station. The remaining baskets were carefully netted (insect screening of fine mesh) to distinquish between avoidance behavior and lethality. The two remaining sets of netted baskets were recovered from each station after 4 and

14 days of exposure to assess acute and chronic toxicity, respectively.

The second set of samples was deployed for five weeks in the summer (May-June). Two substrates were placed at each station. The intent at this juncture was to assess succession under the impact of continuous chlorination.

Each collected basket contained 30 colonized stones, each as a sampling unit with a known surface area. Each sampling unit was immediately stored in its individually marked plastic bag (whirl-pak) half-filled with 70% ethanol, and then transported to the laboratory for subsequent processing (sorting, identification, and enumeration).

4.1.2 Chemical-Physical Sampling Water temperature, dissolved oxygen (D.O.), alkalinity, acidity, ammonia, nitrate, phosphates, calcium, hardness, chloride, fecal coliform and total residual chlorine (after chlorination resumed) were determined every week throughout the exposure period. Water temperature, and D.O. were determined on-site by YSI model 54A oxygen meter and pH by Altex Expand-Mate pH meter. The other parameters were determined in laboratory.

Four sets of sample bottles were routinely prepared for each station and the WWTP effluent :
Set 1, 1000 ml pyrex flasks, was treated at 550 C for 1 hr before being used as chlorine bottles. When chlorine samples were collected, opaque plastic bags were wrapped

over the bottles to avoid direct sunlight. Chlorine samples were immediately processed in the laboratory of the WWTP, with a Bausch & Lomb spectrophotometer (Spectronic 21). Determinations were made by the DPD colorimetric method which is sensitive to 10 ppb.

Set 2, 1000 ml high density polyethylene (HDPE) bottles, was used to collect samples for ammonia. When 1000 ml ammonia samples were collected, 0.4 mg $HgCl_2$ and sodium thiosulfate were added for respectively controlling bacteria growth, and dechlorination. These samples were also used for nitrite and phosphorus tests.

Set 3, 250 ml HDPE bottles, was sterilized before use as fecal coliform sampling bottles. Appropriate ammount of sodium thiosulfate were used for dechlorination.

Set 4, 1000 ml HDPE bottles, was prepared for collecting alkalinity, acidity, calcium, and hardness samples.

Furthermore, a fifth set of samples was collected twice throughout the exposure period to determine dissolved metals (copper, nickel, zinc, cadmium, chromium, silver, lead, aluminum).

4.2 Laboratory Procedures

As soon as the field samples from each station were returned to the laboratory, they were treated according to their assigned purposes.

4.2.1 Biological Protocol. Those collected sampling units, each in a whirl-pak plastic bag, were treated individually.

First, the contents of the bag were washed and filtered through a sieve fabricated from insect screening (fine mesh). Then, the sieve contents were concentrated by a sugar-floatation method (Fast, 1970; Lackey, 1971; Pratt, 1977). Those organisms (>2.0 mm) floating on the surface were picked up and stored in a vial, designating the station, substrate etc, containing 75% ethanol. The collected macroinvertebrates were only identified to the generic level and a few to species level because most ephemerid nymphs were in the early stages and most keys to species of chironomids were poorly organized. The identifications were confirmed by DEQE (Department of Environmental Quality and Engineering, Division of Water Pollution Control) staff.

The mounting methods adopted for midge identifications were in accordance with those suggested by DEQE. Specimens identified in this study were mounted in CMC-10 mounting media (Masters Chemical Inc., Illinois). The larva is mounted directly from the alcohol into CMC-10. The head is severed from the body and mounted ventral side up, while the body is mounted on its side under the same cover slip. A light pressure on the cover slip can spread out the mouthparts in the mounting media. The edges of the cover slip were subsequently rimmed with extra media. The prepared slides can be identified after one week. Species

names and number of individuals were recorded for each independent sampling unit.

The fecal coliform tests were completed within 6 hrs after the samples were collected. The m-FC broth base and agar were used to prepare the incubative petri dishes. The operating procedure for filtration and incubation and bacterial counting is in according to the MILLIPORE application manual AM302.

4.2.2 Chemical Protocol. Residual chlorine determination were performed immediately after the samples were taken. Alkalinity and acidity were measured within 24 hrs. All other tests were completed within a week. The methods for all the parameters measured (Table A-4) were in accordance with Standard Methods for the Examination of Water and Wastewater (1985).

4.2.3 Estimating the Density of Stone-dwelling Organisms Population densities of stone-dwelling organisms were estimated by Wrona's method (1986):

$$d = \sum_{i=1}^{k} (1/\bar{a}i)\bar{X}i$$

where d = mean density of organisms (mean number per unit habitat), $\bar{a}_i$ = mean surface area of the stones within the ith stone size class (eg. a_s, a_m, and a_l representing mean areas of stones in the small, medium, and large stone size classes, respectively), $\bar{X}_i$ = mean number of organisms per stone in the ith stone size class, and k = total number of

stone size classes. The stone size classes can be manipulated to be the same size for an artificial substrate, however. The estimated population density can be simplified to :

$$d = (1/\bar{a})\bar{X}$$

where $\bar{a}$ = mean surface area of the limestone chips in a basket, and $\bar{X}$ = mean number of organisms per limestone chip. In addition, the term $\bar{a}$ is estimated by using the general formula :

$$\bar{a} = \left(\sum_{r=1}^{R} a_r\right)/R$$

where a_r = surface area of the rth replicate stone from a basket, and R = total number of stones measured for surface area in a basket.

The errors from estimating the stone surface area and the mean counts of organisms per basket of stones were calculated through the equations developed in Wrona's study (1986). The total variance of the final weighted mean density estimate, assuming that X (number of organisms per stone) and Y (surface area per stone) are random variables, is :

$$V(d) = V(YX)$$

where V(d) = total variance of the weighted mean density estimate, and V(YX) = joint variance of stone surface area and mean organism estimates of stones. The estimated V(YX) is as:

$$V(YX) = \{V(X)+[E(X)]^2\}\{V(Y)+[E(Y)]^2\}-[E(X)E(Y)]^2$$

whose terms refer to the mean numbers of organisms, E(X), mean surface areas of stones, E(Y), and associated variances of V(X) for the organisms and V(Y) for the surface areas.

A convenient way of estimating the surface area of individual limestone chips was developed in this study. The correlation between the surface area and volume of individual limestone is developed through the mathematical transformations described below.

Limestones (garden marble chips) seem to be characterized by similar shapes. However, from the view of geometry (Mandelbaum & Conte, 1957), if two solids are similar, the areas (S_1 , S_2) of corresponding surfaces are proportional to the square of corresponding linear elements (a_1 , a_2) and their volumes (V_1 , V_2) are proportional to the cube of corresponding linear elements, as shown

$$\frac{S_1}{S_2} = \frac{a_1^2}{a_2^2}$$

$$\frac{V_1}{V_2} = \frac{a_1^3}{a_2^3} .$$

Then, the surface areas of two solids can be expressed as

$$S_1 = k(a_1)^2$$

$$S_2 = k(a_2)^2 ,$$

and the volumes as

$$V_1 = K(a_1)^3$$

$$V_2 = K(a_2)^3$$

where k and K are constants for surface area and volume, individually. Now, we divide the square root of surface with the cubic root of volume as

$$\frac{S_1}{V_1} = \frac{ka_1}{Ka_1} = k/K = R \text{ (constant)},$$

after mathemetic and logarithmic transformations, it becomes :

$$S_1 = R^2 (V_1)^{2/3}$$

then $$\log S_1 = \log R^2 + 2/3 \log V_1 \qquad (1),$$

also for S_2 and V_2,

$$\frac{S_2}{V_2} = \frac{ka_2}{Ka_2} = k/K = R$$

then, $$S_2 = R^2 (V_2)^{2/3}$$

and $$\log S_2 = \log R^2 + 2/3 \log V_2 \qquad (2)$$

where R is constant. However, a logarithmic transformation of both variables (X,Y) is applicable in situations in which the true relationship can be described by the formula $Y = a X^b$, where a and b are constants. The regression equation is represented as log Y = log a + b log X (Sokal & Rohlf, 1981). So, two similar solids with different linear elements obtain the same results (formulae 1 and 2) as a linear correlation between surface and volume after the transformations.

For the stipulated regressions, sixty limestone fragments were randomly chosen and their individual volumes and surface areas determined. A 500 ml beaker (Fig. A-2)

filled with water was used to measure the volume. The volume of overflowed water displaced by the stone, which would be its volume, was measured in a 100 ml volumetric cylinder. Subsequently, the surface area of each limestone piece was measured by weighing aluminum foil used to cover its surface (Minshall, 1984). Through the appropriate conversion (9 cm^2 of aluminum foil weighed 0.0372 g), the areas of individual stones were determined. The variability of this aluminum foil weighing method was estimated by repeating this process 10 times for a particular limestone chip.

The linear correlations (Kleinbaum et al., 1988) were employed for logarithmic surface area (log s) versus logarithmic volume (log v), volume (v) versus surface area (s), and cube of surface area (s^3) versus square of volume (v^2). The rationale for selecting the variables will be developed in section 4.4. The standard formula, for estimating the surface area of individual sampling units through measuring the volumes of individual stones, was evolved from these three linear correlations.

4.2.4 Diversity Index Pratt (1976) contends Brillouin's formula (B) is the most appropriate diversity index for the determination of substrate diversity. The collected organisms can be viewed as population instead of a sample from the macrobenthic community (Pratt, 1976). However, Pielou (1967) suggested a formula, H = B/N (N = the total

number of organisms in a collection), to consider the diversity per individual, rather than B, the total diversity of a collection.

In this study, the diversity index of a basket was calculated by the Pielou's method in which:

$$H = (C/N)(\log_{10} N! - \sum_{i=1}^{s} \log_{10} Ni!)$$

where N = the total number of individuals in the collection, s = the number of "species" represented in the collection, N_i = the number of individuals in a particular species, S_i, and C = 3.3219. The tables of log10N and log10N! are provided in the Appendix (USEPA, 1973; Lloyd et al, 1968).

The pooled diversity method (Pratt, 1977) is applied in this study to ascertain the obtained diversity of each basket. A asymptotic value, found through the pooled diversity versus cumulative sampling units, may indicate if the sampling units in the basket are adequent to estimate a confident diversity. The calculation for pooled diversity index is similar to the formula described above, except it is based on the cumulative value of individual sampling units rather than on the whole basket population.

4.3 Data Analysis

All of the samples units (limestone fragments) from each basket were examined one at a time and all of the members of every sampling unit were identified and counted.

The data recorded consisted of: (1) the name of each species (most to genus level) belonging to various higher taxonomic designations (e.g. phylum, order, etc.), (2) the number of individuals of each species for each sampling unit, (3) the total number of individuals of each species for each basket, (4) the total number of organisms of each basket, and (5) the estimated surface area of individual sampling units for each basket.

The density of individual species with a 95% confidence interval was calculated, and the diversity of each basket was estimated. Two-way analysis of variance on mean density of organisms was employed for intrasite comparisons before chlorination and after chlorination, also for intersite comparisons during the same sampling period. The significant tests of two-way ANOVA on mean density of organisms were accomplished by way of the logarithmic transformation on organism density. Levene's test for equality of variances was applied to evaluate the homogeneity of density data (Table A-5). The asymptotic diversity of pooled samples was applied to testify whether the number of sampling units in each basket was adequate.

A group of potential indicator species was selected from the residual chlorine-disturbed ecosystem. Those species exhibited a statistically significant shift in their density through the time of exposure to chlorinated effluents-receiving waters.

4.4 Limits of Experimental Methods

The applicability of the strategies in this study will be addressed in the following sections.

4.4.1 Basket-Type Artificial Substrates The use of limestone fragments- filled baskets as artificial substrates could avoid some variations from different particle sizes and surface heterogeneity that other basket-type substrates might experience. Although basket-type artificial substrates were reported to be very attractive to a variety of organisms (Mason, 1970; Fredeen & Spurr, 1978), they are still selective. Some common species of dragonfly larvae, and hemipterans were not found in this study, nor in the aquatic surveys of Fredeen and Spurr (1978).

Macroinvertebrates were separated from a basket by immediately transferring their inhabited rocks to individual storing bags when each basket was retrieved in the field. The allochthonous debris accumulated among rock spaces were also inhabited with organisms. Those organisms within the spaces were randomly delivered together with their respective stone to the storage bags. Time expended to process a basket from the moment of retrival to deposition of the last rock into its bag was less than two minutes. This process was repeated for every basket in the field, so the operating errors inherent to the technique would be consistent for each basket. However, this process may affect the organisms differently. It is easily applied

to organisms which are not readily dislodged from dwelling stones (ie. gastropods, leeches, simulids, tube-dwelling chironomids). It may also be applied to some more loosely attached organisms (ie. mayflies, stoneflies, amphipods, oligochaeta, tipulids), although requiring more care when sampling.

4.4.2 Estimating the Abundance of Benthic Macroinvertebrates

Benthic macroinvertebrates may often be shifted out of representative samples of their habitats, killed, counted, and the total population calculated by mathmatical formulae. Through this manipulation, the impacted ecosystem could be assessed by the way the numbers of organisms changed through time of exposure. The abundance can be expressed as density (number of organisms per unit area). The size of natural populations is also crucial for an understanding of population dispersion, dynamics, and demography. However, it should be noted that population abundance is determined by interaction of habitat and food suitability and availability.

The application of Wrona et al.'s method (1986) to estimate the abundance of stone-dwelling organisms in this study is appropriate because it allows for the calculation of variance and standard error estimates from both the physical heterogeneity of the habitat and the spatial despersion of the organisms. Colinvaux (1986) believes however that the best techniques developed to estimate the

number of organisms always leave some marginal uncertainty of the true number. The original design of Wrona's method is not for artificial substrates which do not provide the heterogeneity of natural habitats that many organisms prefer. However, the most promising idea derived from their method is the use of individual stone (a descrete natural unit) as the sampling unit. For an artificial substrate made by a limestone-filled basket, the stone size classes can be manipulated to be the same size, and the relationship can be modified as described in the laboratory procedures of Materials and Methods.

Barbeque baskets, each containing 30 limestone rocks, were used as artificial substrates for collecting benthic macroinvertebrates in this study. Individual limestone chips (coarse) were used as individual sampling units providing 29 degrees of freedom for each basket when estimating the density of organisms.

4.4.3 Estimating the Surface Area of Limestone Fragments.

The results from the linear correlations for the: (1) logarithmic surface area (log s) versus logarithmic volume (log v), (2) volume (v) versus surface area (s), and (3) cube of surface area (s^3) versus square of volume (v^2) are significant. The respective squared correlation coefficients (coefficient of determination) are (1) 0.906 for log s versus log v, (2) 0.909 for s versus v, and (3) 0.921 for s^3 versus v^2. Although the highest coefficient of

determination was obtained from S^3 versus V^2 (r^2 =0.921), its inconvenience of calculation militated against its adoption. Consequently, the adopted standard equation as a predictor for the surface area was directly derived from the convenient equation of S versus V. From the hypothesized test for B_1 (slope of the line), the test of statistic T exhibited a P-value less than 0.001, which suggested a significantly correlated relationship between surface area and volume of individual limestones. The test for lack of fit of the straight-line model failed (p>0.05). Consequently, a straight-line relationship may be assumed.

Behmer & Hawkins (1986) used longest horizontal and vertical dimensions to estimate total surface area of a cobble with a 0.994 of coeffient of determination (developed from ten cabbles). For getting a significant result, r^2 are relatively required to be higher in a small sample size than a big sample size. From the correlation coefficient (r) table, to get a p-value less than 0.001 for 9 degree of freedom, the r^2 should be higher than 0.72; and to get the same significance for 59 degree of freedom, the r^2 should only be higher than 0.17. So, the straight-line model (Volume versus Surface Area) developed in this study, is appropriate in estimating the surface area.

The use of limestone chips-filled baskets as artificial substrates is suitable for use in routine

biological observations with comparatively experimental designs.

4.4.4 Diversity Index. The estimated community diversities for S-1 to S-5, each with two duplicates, throughout the study period are listed in Tables A-9, A-11, A-13, and A-15, individually. Although the estimated diversity in this study was derived at the generic level, most genera were restricted to one species, except for Chironomus and Polypedilum.

The application of Pielou's index is appropriate to this study because it is relatively independent of sample size and can be estimated accurately from a randomly selected portion of a collection (Pratt, 1977). Basically, each basket was equipped with thirty limestone rocks. Some baskets however were retrieved with fewer rocks, resulting in different sample sizes.

The use of Pratt's (1976) cumulative pooled diversity to secure the number of sampling units in a basket is promising. The plots of the pooled diversity index (H_k) versus cumulative sampling units, derived from one of the replicated baskets before chlorination, were presented for each station (Fig.A-3). The use of 30 sampling units per basket ensured that the asymptotic diversity values were achieved, thus instilling confidence in the diversity values derived in this study.

CHAPTER V

RESULTS AND DISCUSSION

Although most research concerning the lethal or sublethal toxicity of residual chlorine to aquatic organisms have being undertaken in many laboratories, there is a paucity of data from actual on-site experiments. A similar study presented by Pagel and Langdon (1981) simply used the values expressed from 1 to 10 to reflect observed population changes from the "balanced community". Their identified organisms were confined to major taxa. In Osborne's study (1985), a crude description of the correlation analyses for evenness, number of individuals, species richness, and H' diversity with physicochemical parameters including TRC was presented. Although the classified organisms were major taxa and the mean abundance of each major taxon was in percentage, the average number of taxa within each major taxon was counted in his study. However, the real effects of TRC were not clearly differentiated from the other factors, such as temperature, and non-chlorinated sewage effluents.

The impact of chlorinated sewage on the Lampson Brook biota will be addressed sequentially at each station following a preliminary discussion of general trends.

5.1 General Discussion

The study, divided into two seasons (spring and summer), was designed to describe the biological response to varying periods of chlorination. The first experiment was comprised of three exposure periods: before chlorination (BC), 4 day acute exposure (AC), and 10 day chronic exposure (CC). The second experiment was set in the summer following continuous chlorination (SC) to observe the responses of macroinvertebrates to sustained exposure to chlorine. The two-way ANOVA test was applied only to intrasite comparisons of the first experiment. The reason for this is that the two sets of experiments are not of the same experimental design.

The species identified in this study were markedly different among stations due in part to the natural variability of riverine habitats (except for stations 1 and 2), but, as well, to the amount of treated sewage individual stations were subjected to. Further, in the two-way ANOVA on the total density of organisms, there is a highly significant interaction ($P < 0.01$) (Table A-5) stemming from the two grouping variables of site (four stations) and chlorination (BC, AC, CC). The stations did not show identical responses to chlorination as exemplified by density (Fig.1) and diversity (Fig.2). These basic intersite differences make it difficult to differentiate the real impact of chlorinated effluents by comparing each

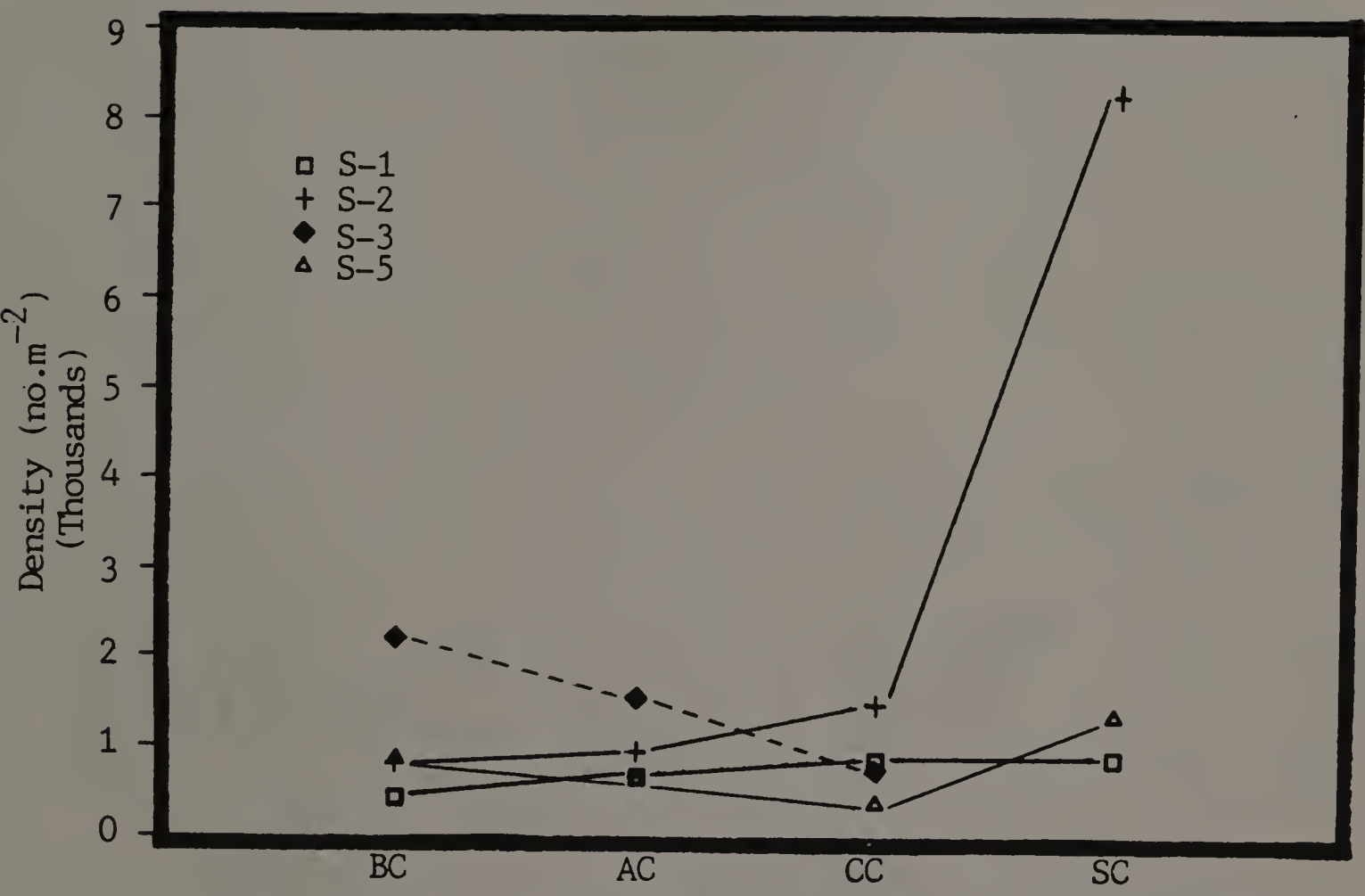


Figure 1. The intrasite variations on the abundance of organisms under four chlorination periods : before chlorination (BC), acute chlorination (AC), chronic chloriantion (CC), and summer chlorination (SC).

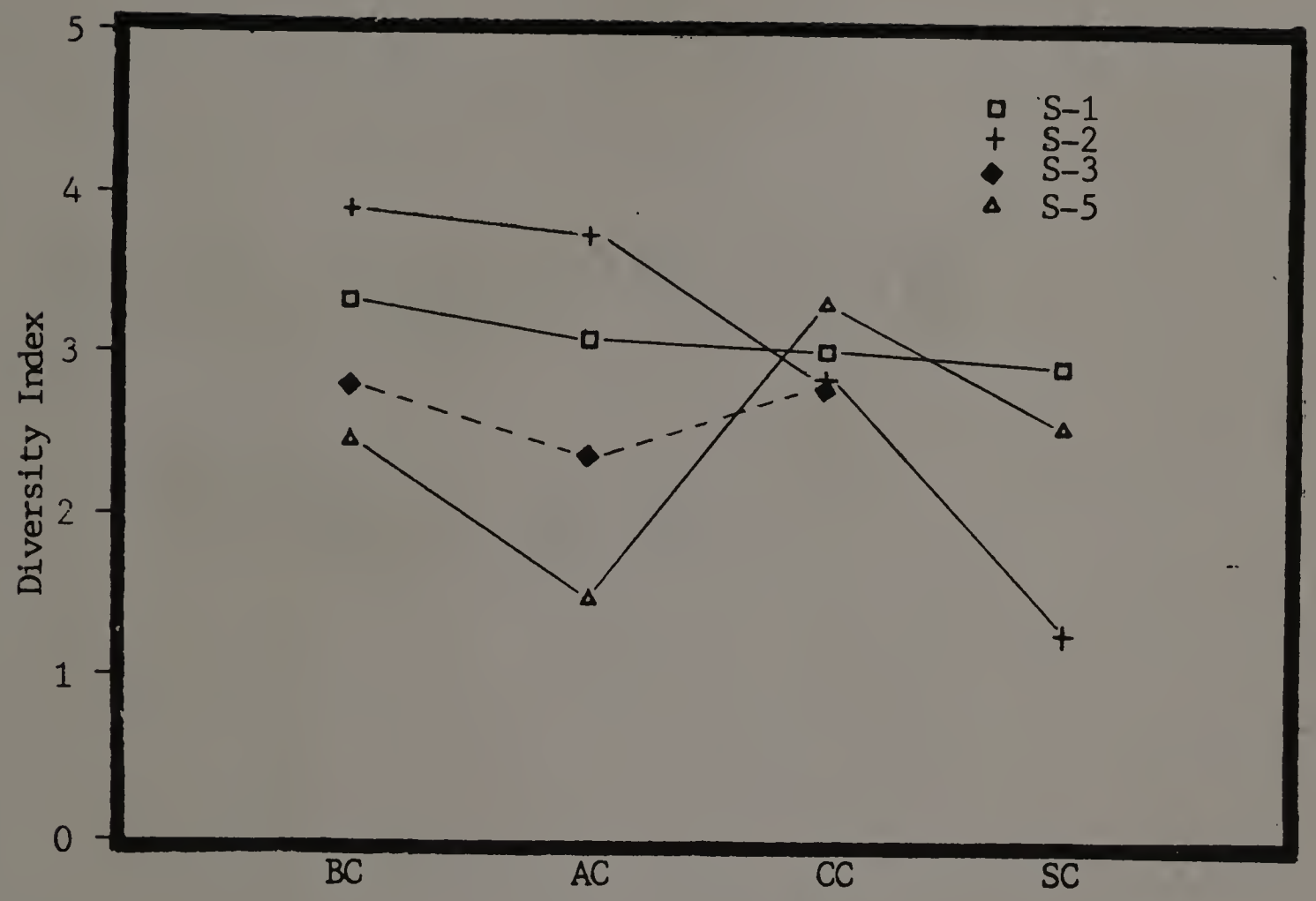


Figure 2. The intrasite variations of the diversity under four chlorination regimes during the spring, before chlorination (BC), acute exposure (AC), chronic exposure (CC) and summer, sustained exposure (SC).

impacted station with the reference station. However, intrasite comparisons could be made among the communities before and after chlorination.

The following discussions will concentrate on the effects of chlorination on benthic macroinvertebrates of each station. Through community succession, diversities and densities generated before and after chlorination, the aquatic biota were determined to be impacted. Rare species will not be addressed in the following discussion.

5.2 Reference Station (S-1)

The control station (S-1) was considered to be a unstressed habitat receiving no sewage discharge. During spring sampling, the density of organisms at S-1 increased significantly ($p<0.01$) (Table A-7). It increased from a mean 417 ± 75 m^{-2} (BC), to 681 ± 189 m^{-2} (AC), then to 856 ± 187 m^{-2} (CC), among which a significant difference was between BC and CC ($p<0.01$) (Table A-8). The communities collected in summer had a mean total density of 883 ± 354 m^{-2}. It implied that no density-independent catastrophic mechanisms affected the density of organisms because the populations resumed the tendency to exponential growth. The diversity of S-1, during the experimental period, exhibited a slight decrease from 3.3 (BC), to 3.1 (AC), then to 3.0 (CC); and became 2.9 in summer sampling. These collected communities with the gradually increased densities seemed to still be able to maintain their diversities, a phenomenom

characteristic of a healthy ecosystem. The almost constant biotic diversity is maintained partly by species replacement, as changing environmental seasonally associated conditions favored different assemblages of species. The general census data of collected macroinvertebrates are summarized in Table A-9, and the estimated densities of individual species are listed on Table A-10.

In the period from March to April, most organisms enumerated belonged to the order of Ephemeroptera, some to Coleoptera, Plecoptera, Trichoptera, and a few to Diptera. But in the period of early summer, almost all specimens belonged to Diptera and a few to Ephemeroptera. During spring sampling (Table 1), Ephemeroptera, Trichoptera, Coleoptera and Chironomidae increased significantly, but Plecoptera and some Diptera did not. The shifting trends of species composition and distribution above the sewage outfall during spring and summer chlorination are presented in Fig.3.

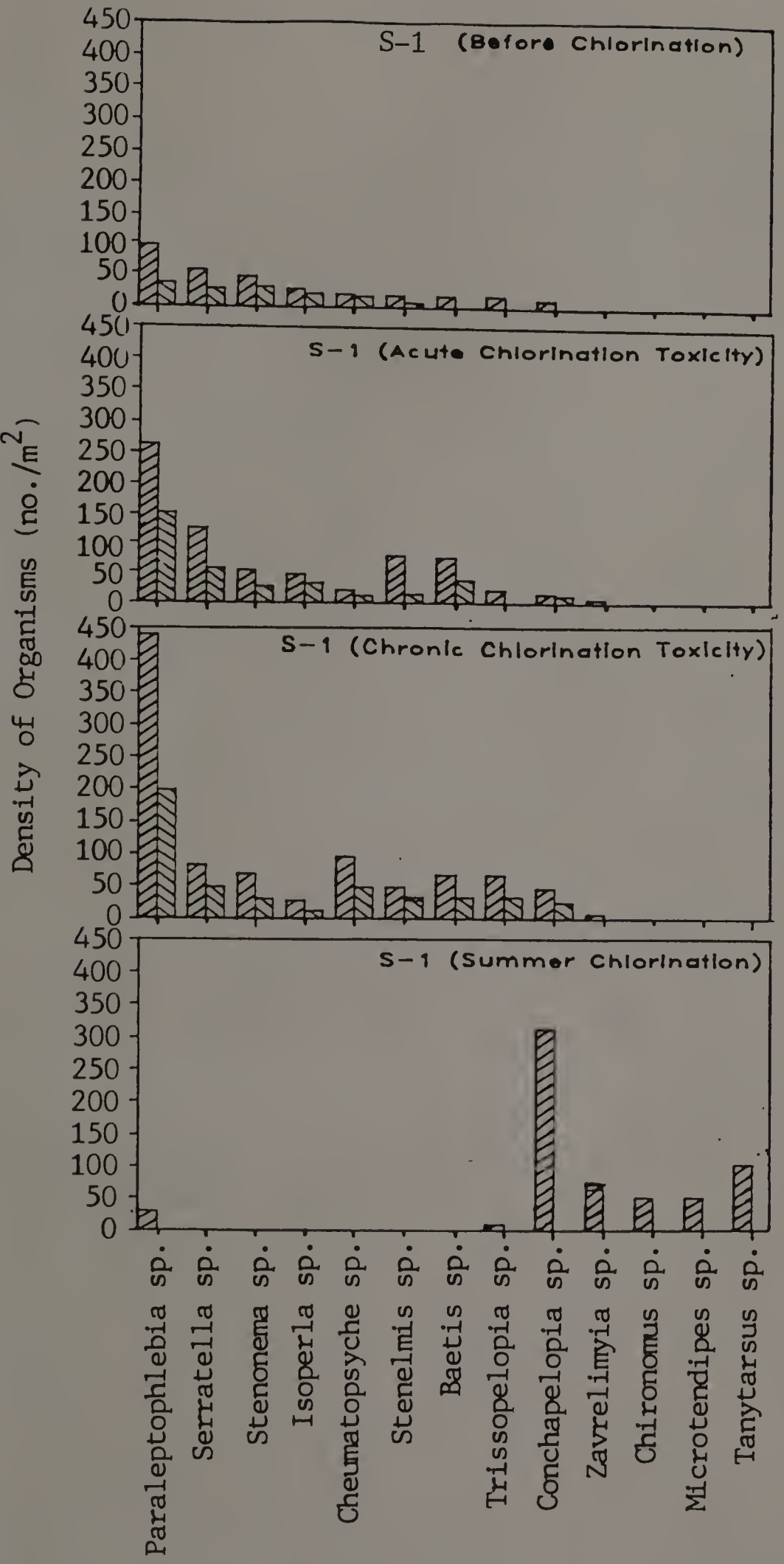
Most mayfly nymphs (Serratella sp., Stenonema sp., Baetis sp., Paraleptophlebia sp.) collected at S-1, are collectors or scrapers, feeding on a variety of detritus, algae, and some macrophyte and animal materials. Some newly hatched nymphs (<2mm), feeding largely on fine particle detritus, were also recovered. For the first set of sampling, mayfly nymphs significantly increased ($P<0.01$) in

Table 1. The mean densities of major taxa and their results of ANOVA on mean densities at S-1 under three periods of spring chlorination, Lampson Brook, 1987.

Time of Chlorination	BC	AC	CC	Significance[1]
No. of Sampled Rocks	43	46	47	
Organism Groups[2]				
Ephemeroptera	162	401	500	***
Plecoptera	41	58	42	
Trichoptera	21	17	74	***
Coleoptera	19	51	58	**
Chironomidae	80	71	131	**
Other Diptera[3]	23	48	23	

1. The significance marked as '***' represents $P<0.01$, as '**' represents $P<0.05$, as '*' represents $P<0.1$.
2. Except for the family Chironomidae, groups are listed by macroinvertebrate orders.
3. Other Diptera include the families Orthorrhapha, Tipulidae, and Ceretopogonidae.

Figure 3. The shifting trends of species composition and distribution in S-1 baskets from the period before chlorination, through spring chlorination, to summer chlorination, 1987.


S-1 (Before Chlorination)
S-1 (Acute Chlorination Toxicity)
S-1 (Chronic Chlorination Toxicity)
S-1 (Summer Chlorination)
Density of Organisms (no./m2)
0
50
100
150
200
250
300
350
400
450
Paraleptophlebia sp.
Serratella sp.
Stenonema sp.
Isoperla sp.
Cheumatopsyche sp.
Stenelmis sp.
Baetis sp.
Trissopelopia sp.
Conchapelopia sp.
Zavrelimyia sp.
Chironomus sp.
Microtendipes sp.
Tanytarsus sp.

abundance in S-1 baskets. Their mean densities were estimated as 162 m^{-2} at BC, 401 m^{-2} at AC, and 500 m^{-2} at CC. In summer, they appeared very rarely in S-1 collections.

Chironomid larvae occurred rarely in spring, but occupied most of the community in summer. Those collected chironomids (Table A-10) were about 5 mm and in the third or fouth instar. For spring sampling, densities of chironomids were found to increase significantly from 80 ± 38 m^{-2} (BC) and 71 ± 50 m^{-2} (AC), to 131 ± 65 m^{-2} (CC) (Table 1, p.44). In summer sampling, chironomids composed almost the whole collected community at S-1.

The Plecoptera (stonefly) collected at S-1 consisted of predators, shredders, and collectors. They were collected only in the spring sampling and only in small numbers. Their respective BC, AC, and CC mean densities of 41 ± 25 m^{-2}, 58 ± 31 m^{-2}, and 42 ± 24 m^{-2} were not significantly different.

Trichoptera (caddisfly), colonizing S-1 substrates were mostly of the genus Cheumatopsyche, which favor warm, erosional streams or rivers. Their densities were found to increase significantly from BC to CC (Table 1). Most Coleoptera found in S-1 were scrapers or gatherers, and dwelled in the same habitats as Cheumatopsyche sp. They had a tendency to increase in spring. The Megaloptera were

represented by the species Nigronia, which are predators with burrowing habits.

5.3 Station 2 (S-2)

Station 2 substrate populations were affected by chlorination with regard to diversity, but not with regard to total density of organisms. A significant difference ($p<0.01$) in organism density of S-2 was observed during spring chlorination (Table A-7). The mean organism densities of S-2 for each indicated period were 820 ± 230 m^{-2} (BC), 962 ± 270 m^{-2} (AC), and 1487 ± 293 m^{-2} (CC). Significant differences were found between BC and CC, and between AC and CC (Table A-8). The diversities of S-2 however decreased from 3.9 (BC), to 3.7(AC), then to 2.9 (CC). This situation implied that some chlorine-resistant species became dominant and some non-tolerant species left the substrates. A similar phenomenon was investigated in other studies (Osborne & Davies, 1986; Sheridan & Badger, 1980). Later during the summer chlorination, the substrate density at S-2 was estimated to be 8290 ± 1130 m^{-2}. The huge increase in density mostly resulted from the season-related emergence of some chironomids (midges). Relatively, the diversity at this point dropped to about 1.3, which implied a stressed ecosystem. The chlorinated effluents did stress the midge community of S-2 (decreased diversity), but it seemed to supply those chlorine-resistant organisms with rich organic material (increased density). The general

census data and estimated species density are summarized in Table A-11 and Table A-12, respectively.

Receiving discharged effluents 40 meters downstream from the outfall, S-2 supported a more diverse and abundant community than the reference station before the chlorination was resumed. The treated effluents from the Belchertown WWTP seemed to benefit the ecosystem instead of stressing it. A probable reason for this is that the discharged, treated, non-chlorinated effluents furnish the stream ecosystem with more available nutrients, which increase the primary production, and subsequently secondary production.

After chlorination was applied, however, the chlorine-treated discharge altered the basket populations tremendously. From spring sampling data (Table 2), chlorination of S-2 was found to significantly suppress the densities of Ephemeroptera, and Trichoptera, but not those of Plecoptera and Coleoptera. However, chlorination did not show a negative impact on the densities of Chironomidae and some other Diptera, which increased significantly during chlorination. The change in water quality was reflected in changes of species abundance, and an abrupt drop of diversity which decreased significantly through time of exposure from 3.9 (BC) to 1.3 (SC) (Fig.2, p.41). The shifting trends of species composition and distribution in

Table 2. The mean densities of major taxa and their results of ANOVA on mean densities at S-2 under three periods of spring chlorination, Lampson Brook, 1987.

Time of Chlorination	BC	AC	CC	Significance[1]
No. of Sampled Rocks	54	57	57	
Organism Groups[2]				
Ephemeroptera	313	125	6	***
Plecoptera	18	10	0	
Trichoptera	14	36	0	**
Coleoptera	65	82	34	
Chironomidae	283	465	1089	***
Other Diptera[3]	91	171	197	***

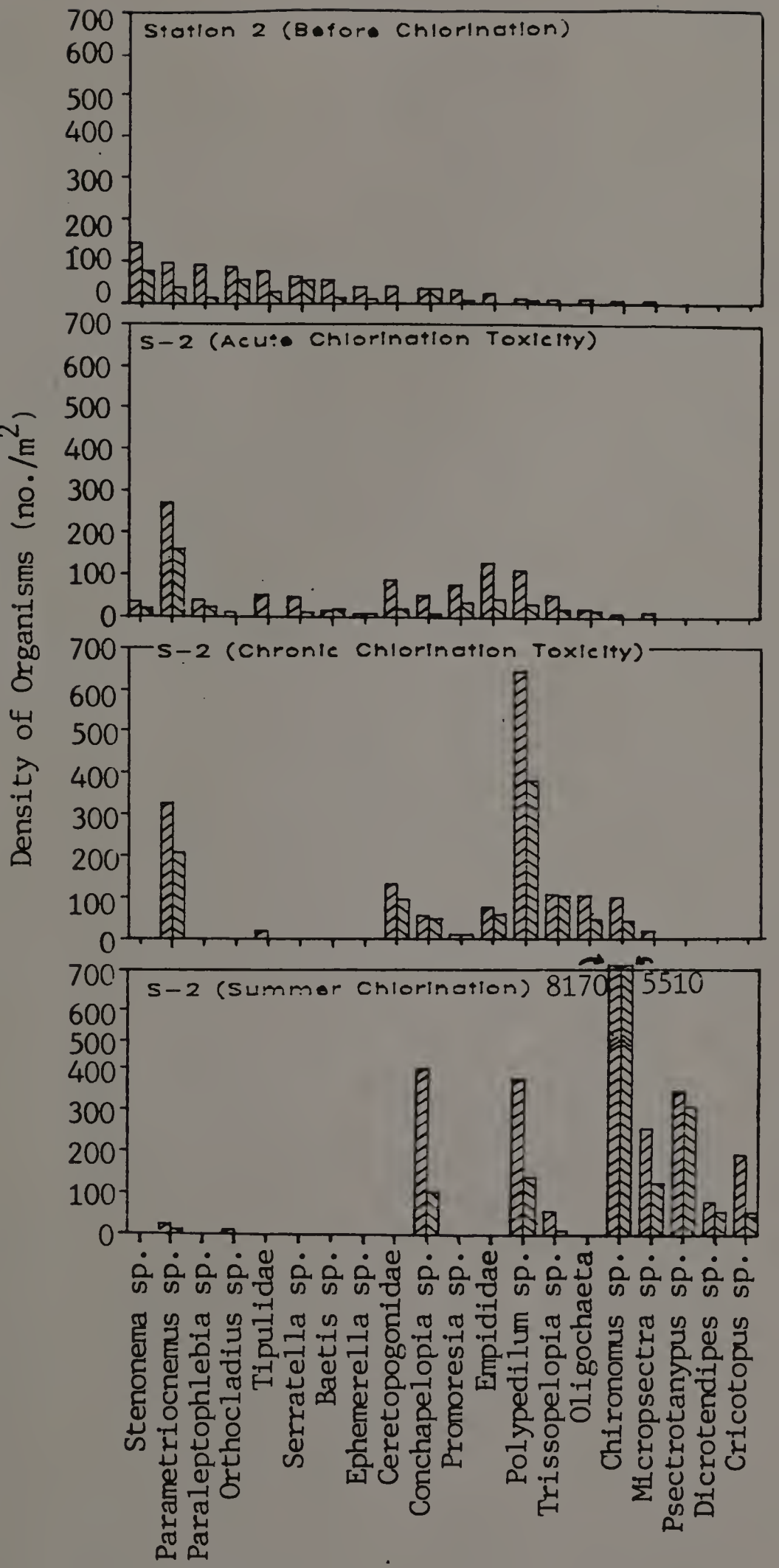
1. The significance marked as '***' represents $P < 0.01$, as '**' represents $P < 0.05$, as '*' represents $P < 0.1$.
2. Except for the family Chironomidae, groups are listed by macroinvertebrate orders.
3. Other Diptera include the families Orthorrhapha, Tipulidae, Ceretopogonidae, Tabanidae, and Empididae.

the period of chlorination from spring to summer are presented in Fig.4.

Most mayflies (Stenonema sp., Serratella sp., Paraleptophlebia sp., Baetis sp., and Ephemerella sp.) found abundantly before chlorination, totally disappeared upon 4 days exposure to an average TRC concentration of 0.677+0.232 (+SE). Stoneflies (Isoperla sp., Peltoperla sp.) although existing as a minor population before chlorination, could not be found in the collections after two weeks of chlorination, though they were isolated at the reference station. Although most caddisflies at this station were clingers (predominantly net spinners with a few case-builders), their retreats could not sustain them for two weeks of exposure though they were well represented at the reference station.

Coleopteran and Megalopteran larvae responded to chlorination in a similar manner when compared to reference site populations. Some species such as Empididae (Diptera) and Promoresia sp.(Coleoptera) sustained their numbers for 4 days of chlorination, but decreased after 2 weeks of exposure. Some Oligochaeta, Ceretopogonidae and Chironomidae (ie. Parametriochemus sp., Polypedilum sp., Trissopelopia sp.) tended to be opportunists during spring chlorination, but their populations became sparse in summer. A reason for their low numbers in summer could be competition or predation by the predatory genus Chironomus

Figure 4. The shifting trends of species composition and distribution in S-2 baskets from the period before chlorination, through spring chlorination, to summer chlorination, 1987.


Station 2 (Before Chlorination)
S-2 (Acute Chlorination Toxicity)
S-2 (Chronic Chlorination Toxicity)
S-2 (Summer Chlorination) 8170 5510
Density of Organisms (no./m2)
0
100
200
300
400
500
600
700
Stenonema sp.
Parametriocnemus sp.
Paraleptophlebia sp.
Orthocladius sp.
Tipulidae
Serratella sp.
Baetis sp.
Ephemerella sp.
Ceretopogonidae
Conchapelopia sp.
Promoresia sp.
Empididae
Polypedilum sp.
Trissopelopia sp.
Oligochaeta
Chironomus sp.
Micropsectra sp.
Psectrotanypus sp.
Dicrotendipes sp.
Cricotopus sp.

which comprised about 80% of the community. In addition, other subdominant midges identified in summer were Psectrotanypus sp., Conchapelopia sp., Polypedilum sp., and Micropsectra sp.. Conchapelopia sp. and Micropsectra sp. had increased gradually in numbers from the onset of spring chlorination.

The phenomenon of midges becoming dominant during exposure to chlorinated effluents has been observed by Carl and Richard (1981). They attributed the increase to the nutrient value of organic wastes and their tolerance to chlorine.

Comparatively, the population densities and patterns of community structures in S-2 varied more through time of exposure than the reference station (Fig.3, p.45; Fig.4, p.52).

5.4 Station 3 (S-3)

S-3 baskets yielded a significant decrease in mean total density of organisms in response to chlorination. The mean densities significantly decreased ($p<0.01$)(Table A-7, A-8) from 2226 ± 437 m^{-2} (BC) to 1550 ± 335 m^{-2} (AC), then to 809 ± 149 m^{-2} (CC). The diversities of S-3 decreased from 2.8 (BC) to 2.4 (AC), then returned back to 2.8 (CC) again. Those taxa that were significantly reduced (Table 3) by chlorination were Chironomidae, Simulidae and Amphipod,

Table 3. The mean densities of major taxa and their results of ANOVA on mean densities at S-3 under three periods of spring chlorination, Lampson Brook, 1987.

Time of Chlorination	BC	AC	CC	Significance[1]
No. of Sampled Rocks	55	60	59	
Organism Groups[2]				
Ephemeroptera	5	8	2	
Plecoptera	0	0	0	
Trichoptera	2	2	0	
Coleoptera	3	0	3	
Amphipoda	459	485	317	*
Simulidae	516	135	106	***
Chironomidae	983	736	243	***
Other Diptera[3]	19	17	32	

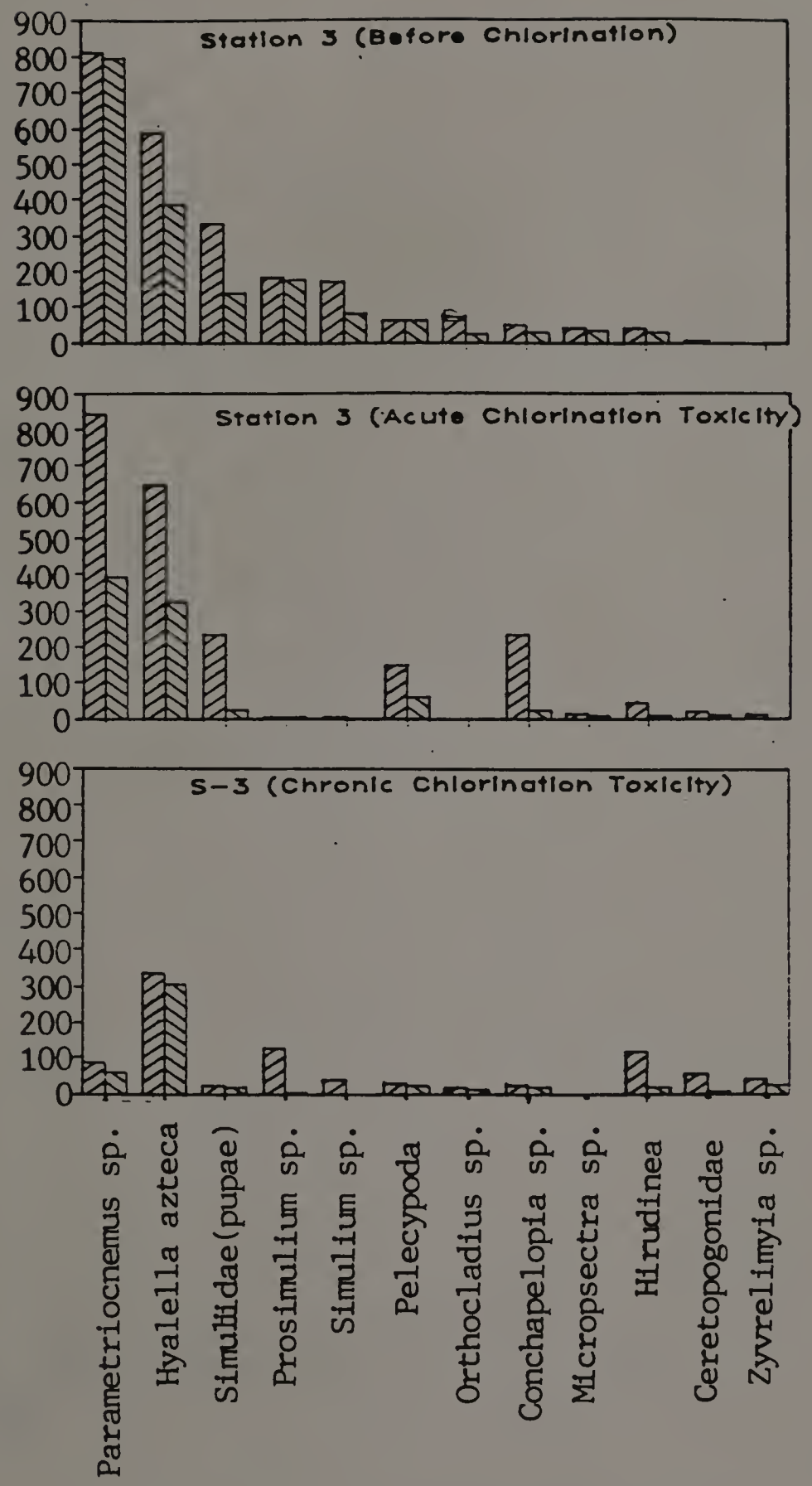
1. The significance marked as '***' represents $P < 0.01$, as '**' represents $P < 0.05$, as '*' represents $P < 0.1$.
2. Except for the families Simuliidae and Chironomidae, groups are listed by invertebrate orders.
3. Other Diptera include the families Ceretopogonidae and Tipulidae.

which comprised most of the basket population. The Chironomidae which increased significantly at other stations during spring chlorination, decreased significantly at S-3.

It is suspected that the chlorine-combined organic matter, detected between S-2 and S-3 by the Department of Environmental Engineering at UMASS, stressed the organisms of S-3 more harshly than the free and ammonia-combined chlorine. This concern finds support in many early studies (Kalmaz, 1981) indicating the high toxicity of chloroorganics. However, recent investigators suggested that a complex antagonistic interaction may result in the lack of effects of chloroorganics on the aquatic biota (Kalmaz, 1981). The criteria for those chemicals are vague. General census data and species density are summarized in Table A-13 and Table A-14, individually. Summer biological samples were totally destroyed by vandalism at S-3.

The concentrations of TRC measured at S-3 had exerted statistically significant effects on the density ($p<0.01$) but only slight effects on the community diversity. The originally balanced community (BC) responded with a decreased diversity (Fig.2, p.41) during the period of acute exposure. The decimation of Parametriocnemus sp., Simulium sp., and Prosimulium sp. also served to significantly decrease the total density of organisms (Fig.5). These species were perhaps stressed from the

Figure 5. The shifting trends of species composition and distribution in S-3 baskets from the period before chlorination, through spring chlorination, to summer chlorination, 1987.


Station 3 (Before Chlorination)
Station 3 (Acute Chlorination Toxicity)
S-3 (Chronic Chlorination Toxicity)
Density of Organisms (no./m2)
900
800
700
600
500
400
300
200
100
0
Parametriocnemus sp.
Hyalella azteca
Simulidae(pupae)
Prosimulium sp.
Simulium sp.
Pelecypoda
Orthocladius sp.
Conchapelopia sp.
Micropsectra sp.
Hirudinea
Ceretopogonidae
Zyvrelimyia sp.

toxicities of residual chlorine and chlorine-produced organics.

Those collections recovered after two weeks of chronic chlorination showed a more evenly distributed community composition and hence increased diversity (Fig.2, p.41) because of decreased dominance by Parametriocnemus sp., Hyallella azteca, and Simulium sp. (Fig.5). At the same time, the species composition and distribution became different from the original community. The total density of organisms decreased significantly ($P<0.01$) when compared to the communities collected before chlorination and after 4 days of chlorination.

Parametriocnemus sp. was dominant originally, but decreased by 25% in four days of chlorination and by 90% after two weeks of chlorination. This species exhibited the opposite response at station 2, where they gradually increased their numbers. It implies that Parametriocnemus sp. can tolerate the chlorinated environment better at S-2 than at S-3. Similarly, the amphipoda Hyallella azteca, a dominant species in spring sampling, decreased by 33% after chronic exposure. For Hyallella azteca, Clarke (1977) reported a lethal value ranging from 0.65 to 0.83 mg/l TRC (96-hr LC50). The TRC concentration of S-3 (0.044-0.094 mg/l) is distinctly lower than this value, but still suppressive to this species. This may be due to the chlorine-combined organics and chronic continuous

chlorination. In the family Simulidae, both larvae and pupae decreased significantly (Fig.5, p.57). The non-dominant Pelecypoda, Micropsectra sp., Orthocladius sp., and Conchapelopius sp. slightly reduced their numbers throughout the chronic study. Other non-dominant organisms (Hirudinea, Ceretopogonidae, and Zyvrelimyia sp.), on the other hand, maintained their number during spring chlorination.

5.5 Station 5 (S-5)

The total mean organism densities of S-5 dropped from 901 ± 172 m^{-2} (BC) to 650 ± 244 m^{-2} (AC), then to 397 ± 85 m^{-2} (CC). In all, spring chlorination significantly affected densities at S-5 substrats ($p<0.01$) (Table A-7). Significant difference existed between BC and CC ($p<0.01$), also between AC and CC ($0.01<p<0.05$) (Table A-8). The species diversities of S-5 dropped from 2.5 (BC) to about 1.5 (AC), then increased to 3.3 (CC). Summer chlorination samples (SC) yielded a mean total organism density estimated at 1033 ± 181 m^{-2} and a community diversity of 2.6. The situation at this time (SC) is very close to the one before chlorination (BC) although the community composition values were different due to the change in season. The collected data from S-5 in the period of AC were not complete because of the missing sampling in the field. The general census and estimated density of S-5 are summarized on Table A-15 and A-16, respectively.

The impact of chlorination at S-5 seemed to happen in the first few weeks of chlorination. From Table 4, the only group whose density is significantly impacted by chlorination was Simulidae. However, the Chironomidae, as in S-1 and S-2 baskets, significantly increased during chlorination. During continuous summer chlorination, the level of chlorine at this stretch of the stream did not exert a stress on the community.

The variability of community composition and distribution through time of exposure was shown on Fig.6. The diversity dropped severely during the first few days of chlorination, but increased significantly after two weeks of exposure. The relatively increased diversity in the period of CC (Fig.2, p.41) was due to the evenly distributed populations in the community, caused by (1) the loss of dominant species of Simulidae larvae, and (2) the gradually hatched populations (Zavrelimyia sp., Paramerina sp., and Tanytarsus sp.) (Fig.6).

The species of Parametriocnemus sp. seemed to be unaffected by the chlorination of S-5 during the period from spring to summer (Fig.6). Simulium sp. and Prosimulium sp. larvae were not found when chlorination was resumed. However, Simulidae pupae maintained their numbers during spring chlorination.

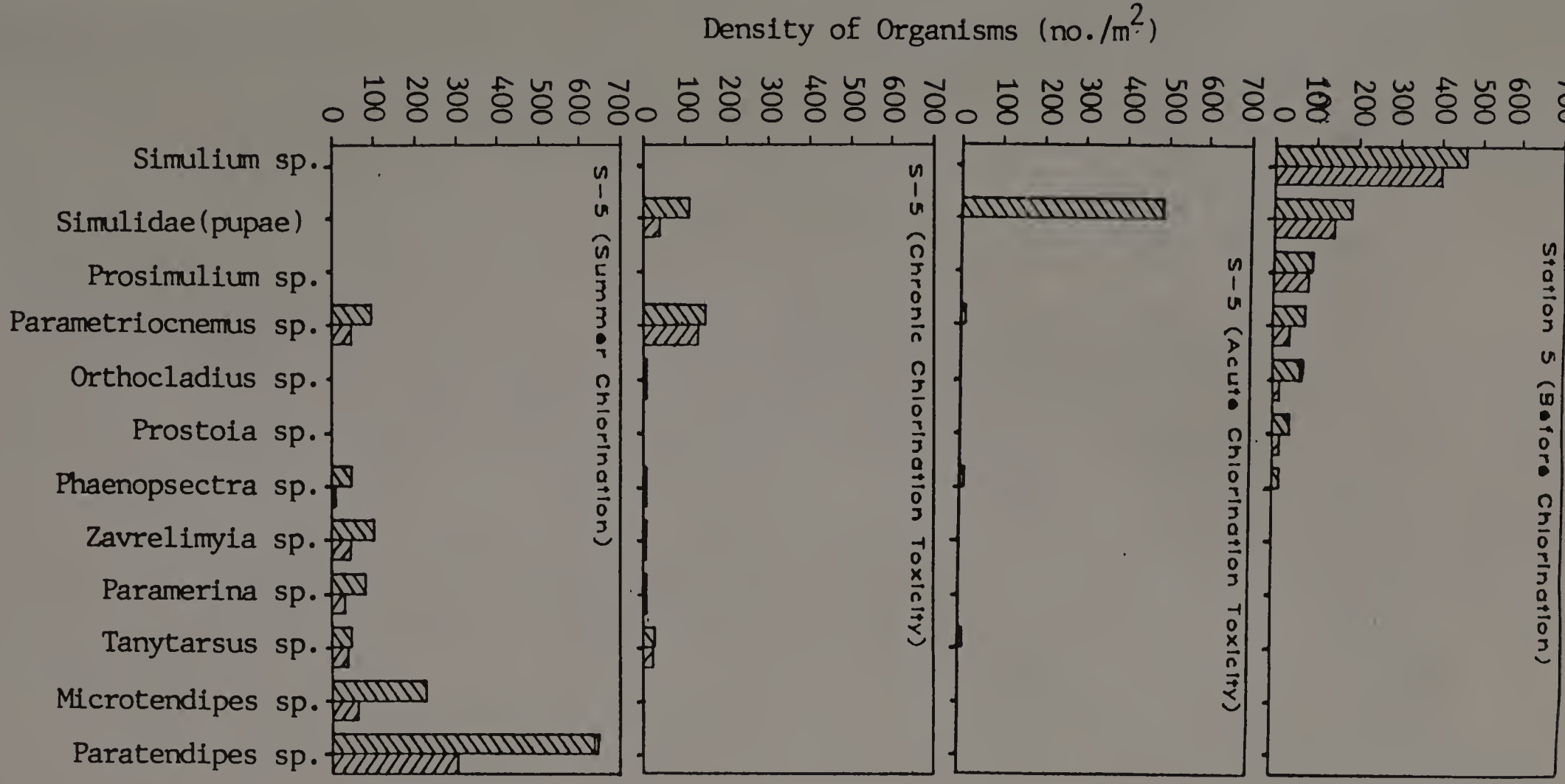
During summer chlorination, a new community was reestablished. Its diversity seemed to be restored to

Table 4. The mean densities of major taxa and their results of ANOVA on mean densities at S-5 under three periods of spring chlorination, Lampson Brook, 1987.

Time of Chlorination	BC	AC	CC	Significance[1]
No. of Sampled Rocks	54	18	60	
Organism Groups[2]				
Ephemeroptera	6	0	12	
Plecoptera	25	0	19	
Trichoptera	3	0	6	
Coleoptera	8	0	0	
Amphipoda	0	0	3	
Simulidae	680	500	76	***
Chironomidae	173	131	323	***
Other Diptera[3]	19	9	18	

1. The significance marked as '***' represents $P < 0.01$, as '**' represents $P < 0.05$, as '*' represents $P < 0.1$.
2. Except for the families Simuliidae and Chironomidae, groups are listed by invertebrate orders.
3. Other Diptera include the families Tipulidae, Empididae, and Ceretopogonidae.

Figure 6. The shifting trends of species composition and distribution in S-5 baskets from the period before chlorination, through spring chlorination, to summer chlorination, 1987.


Density of Organisms (no./m²)
0
100
200
300
400
500
600
700
Simulium sp.
Simulidae(pupae)
Prosimulium sp.
Parametriocnemus sp.
Orthocladius sp.
Prostoia sp.
Phaenopsectra sp.
Zavrelimyia sp.
Paramerina sp.
Tanytarsus sp.
Microtendipes sp.
Paratendipes sp.
S-5 (Summer Chlorination)
S-5 (Chronic Chlorination Toxicity)
S-5 (Acute Chlorination Toxicity)
Station 5 (Before Chlorination)

prechlorination levels (Fig.2, p.41) because Paratendipes sp. became dominant as Simulium sp. larvae did before chlorination. Any species of S-5 with a tendency to decrease its population would be considered as a species sensitive to subtle amounts of chlorine residuals.

5.6 Indicator Species

Biological indicators are used to evaluate the impairment of environmental quality by chlorination through shifting population densities and patterns of community structures. The potential indicator species with their respective tolerances to the estimated ranges of TRC concentrations, are listed on Table 5. Comparatively, these selected indicator species exhibited a more significant variation in densities during chlorination. Some sensitive species were chosen due to their decreased densities in a chlorinated environment, when compared to their increased populations in the reference station. Generally, tolerant species were distributed among Chironomidae, Ceretopogonidae, Empididae, Oligochaeta, and Hirudinea, while the sensitive species were among Ephemeroptera, Plecoptera, Amphipoda, Simulidae, Tipulidae, and one particular chironomid genus (Orthocladius sp.).

Mayflies, caddisflies, and stoneflies have been previously reported to be susceptible to the toxicity of TRC by Osborne (1985) and Carl & Richard (1981). Other studies, however have reported the oligochaetesthe to be

Table 5. Potential indicator macroinvertebrates existing in chlorination-impacted downstream stations along Lampson Brook, 1987.

Station	S2	S3	S5
TRC (mg/1)	0.677 ± 0.232	0.069 ± 0.025	0.048 ± 0.010
Ephemeroptera			
stenonema sp.	-	N	*
Paraleptophlebia sp.	-	*	N
Serratella sp.	-	N	*
Baetis sp.	-	N	*
Ephemerella sp.	-	*	N
Chironomidae			
Parametriocnemus sp.	+	-	+
Orthocladius sp.	-	-	-
Conchapelopia sp.	+	+	*
Polypedilum sp.	+	*	*
Trissopelopia sp.	+	*	*
Chironomus sp.	+	*	*
Micropsectra sp.	+	-	*
Psectrotanypus sp.	+	N	*
Dicrotendipes sp.	+	N	N
Cricotopus sp.	+	*	*
Zavrelimyia sp.			
Phaenopsectra sp.	+	*	+
Tanytarsus sp.	*	*	+
Microtendipes sp.	*	N	+
Simuliidae			
Prosimulium sp.	N	-	-
Simulium sp.	N	-	-
Plecoptera			
Prostoia sp.	N	N	-
Amphipod			
Hyalella azteca	N	-	N
Tipulidae	-	*	*
Ceretopogonidae	+	+	*
Empididae	+	N	N
Oligochaeta	+	*	*
Hirudinea	N	+	*

Estimate of relative tolerance is expressed as '+'= tolerant, and '-'= sensitive.
'N'= non-existing, and '*'= scarcely existing.

the most TRC-tolerant groups (Oliver, 1984; Richard et al., 1983; Osborn, 1985; Carl & Richard, 1981; Sheridan & Badger, 1981). In this study, however, midges, especially Chironomus sp., were found to be more tolerant than the oligochaetes. In Osborne's study (1985), chironomids were reported to be TRC-sensitive; but they were found to be TRC-tolerant in many other studies (Richard et al., 1983; Carl & Richard, 1981; Heckman, 1983).

Most chironomids found at S-2 proved TRC-tolerant, except for Orthocladius sp.. On the other hand, most of the TRC-sensitive indicator species belonged to Ephemeroptera (Serratella sp., Ephemerella sp., Stenonema sp., Paraleptophlebia sp., Baetis sp.). None of these species could sustain itself longer than a two week exposure to chlorination. Some Diptera (Ceretopogonidae, Empididae) and Oligochaeta were seen to be tolerant to chlorination because of their consistent appearance at S-2. The Tipulidae at S-2, and Orthocladius sp. at S-2, S-3 and S-5 tended to decrease in abundance during chlorination and were identified consequently as sensitive species.

From S-3, few tolerant species were found in the Chironomidae because S-3 was mostly dominated by Amphipod and Simulidae. The groups of Ceretopogonidae and Hirudinae were judged to be tolerant at S-3. The sensitive species mostly belonged to the group of Amphipod (Hyalella azteca). According to the literature (Pennak, 1978), the species of

Hyalella azteca is widely distributed and common in unpolluted clear waters. The decreased population (≈33%) of this species at S-3 during spring chlorination demonstrated their moderate sensitivity to TRC concentrations of S-3. Simulids seemed to be sensitive to residual chlorine at S-3. However, their coexitences of larvae and pupae in S-3 and S-5 baskets implied the emergence was happening during spring. Therefore, it is hard to determine their susceptibility to chlorine residuals. On the other hand, Heckmam (1983) considered Simulidae to be a pioneer taxa following the termination of chlorination.

The sensitive species belong to Chironomidae were Parametriocnemus sp., Orthocladius sp., and Micropsectra sp. at S-3. The genera Parametriocnemus and Micropsectra exhibited tolerance to chlorination at S-2, but seemed to be sensitive at S-3. This may suggest a sensitivity to the chlorine-combined organics, which were mentioned previously as being detected at S-3.

In S-5 baskets, most tolerant species were restricted to the group of Chironomidae (Parametriocnemus sp., Zyvrelimyia sp., Phaenopsectra sp., Paramerina sp., Tanytarsus sp., Microtendipes sp.). The sensitive genera, on the other hand, were Prostoia sp. and Orthocladius sp.

5.7 Conclusion

From a clear aspect of this study, all stations downstream from the sewage outfall were impacted, when

compared to the reference station during the chlorination regimes (Table 6). In all, Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, and Chironomidae were stressed by a 10 day exposure to a TRC concentration of 0.69 mg/l, but not by 0.048 mg/l. However, Ephemeroptera, Chironomidae, and Amphipoda were also shown to be impacted by 10 days of exposure to a TRC concentration of 0.068 mg/l. Although Simuliidae larvae decreased significantly upon 10 days wxposure to a TRC concentration of 0.048 mg/l, they might emerge during that period because many pupae existed simultaneously. Otherwise, they seem to be good indicators of chlorination.

Before initiation of chlorination (BC), S-3 basksts were found to support the most abundant populations (Fig.7), and S-2 the most diverse (Fig.8). When compared to the lower population densities in control station (S-1) baskets, upstream from the sewage discharge, the sewage effluents seems to stimulate periphyta growth, and thus in turn increase the population of the S-2 baskets two fold (Table 6, p.70). Aquatic insect production was catalyzed by increased primary production, which provided more habitats, shelters, and food (Wiederholm, 1986). Organic matter, however, through bacterial metabolism, reduces the amount of oxygen that many species of aquatic insects require (Wiederholm, 1986).

Table 6. Chronic toxicity of chlorine on the density and composition of higher taxa of benthic macroinvertebrates at Lampson Brook, 1987.

SITE	BEFORE CHLORINATION	*	**	AFTER CHLORINATION (10 DAYS)		TRC CONC. (mg/l)
1	Ephemeroptera(5):	162		Ephemeroptera(5):	500	0
	Plecoptera(3):	41		Plecoptera(3):	42	
	Trichoptera(1):	21		Trichoptera(1):	74	
	Coleoptera(2):	19		Coleoptera(3):	58	
	Chironomidae(6):	80		Chironomidae(6):	131	
	SUM(17):	323		SUM(18):	805	
	DIVERSITY:	3.34		DIVERSITY:	3.02	
2	Ephemeroptera(6):	313		Ephemeroptera(1):	6	0.68
	Plecoptera(1):	18		Plecoptera(0):	0	
	Trichoptera(3):	14		Trichoptera(0):	0	
	Coleoptera(5):	65		Coleoptera(3):	34	
	Chironomidae(18):	283		Chironomidae(13):	1089	
	SUM(33):	693		SUM(17):	1129	
	DIVERSITY:	3.90		DIVERSITY:	2.86	
3	Ephemeroptera(2):	5		Ephemeroptera(1):	2	0.069
	Chironomidae(13):	983		Chironomidae(10):	243	
	Amphepoda(1):	459		Amphepoda(1):	317	
	Simuliidae(2):	516		Simuliidae(2):	106	
	SUM(18):	1963		SUM(14):	668	
	DIVERSITY:	2.80		DIVERSITY:	2.80	
5	Ephemeroptera(2):	6		Ephemeroptera(2):	12	0.048
	Plecoptera(1):	25		Plecoptera(1):	19	
	Trichoptera(1):	3		Trichoptera(2):	6	
	Coleoptera(1):	8		Coleoptera(0):	0	
	Chironomidae(9):	173		Chironomidae(15):	323	
	Simuliidae(2):	680		Simuliidae(2):	76	
	SUM(16):	895		SUM(22):	436	
	DIVERSITY:	2.47		DIVERSITY:	3.34	

'*' refers to the number of genera found in that taxa.

'**' refers to the density (no.m^{-2}) of the taxa.

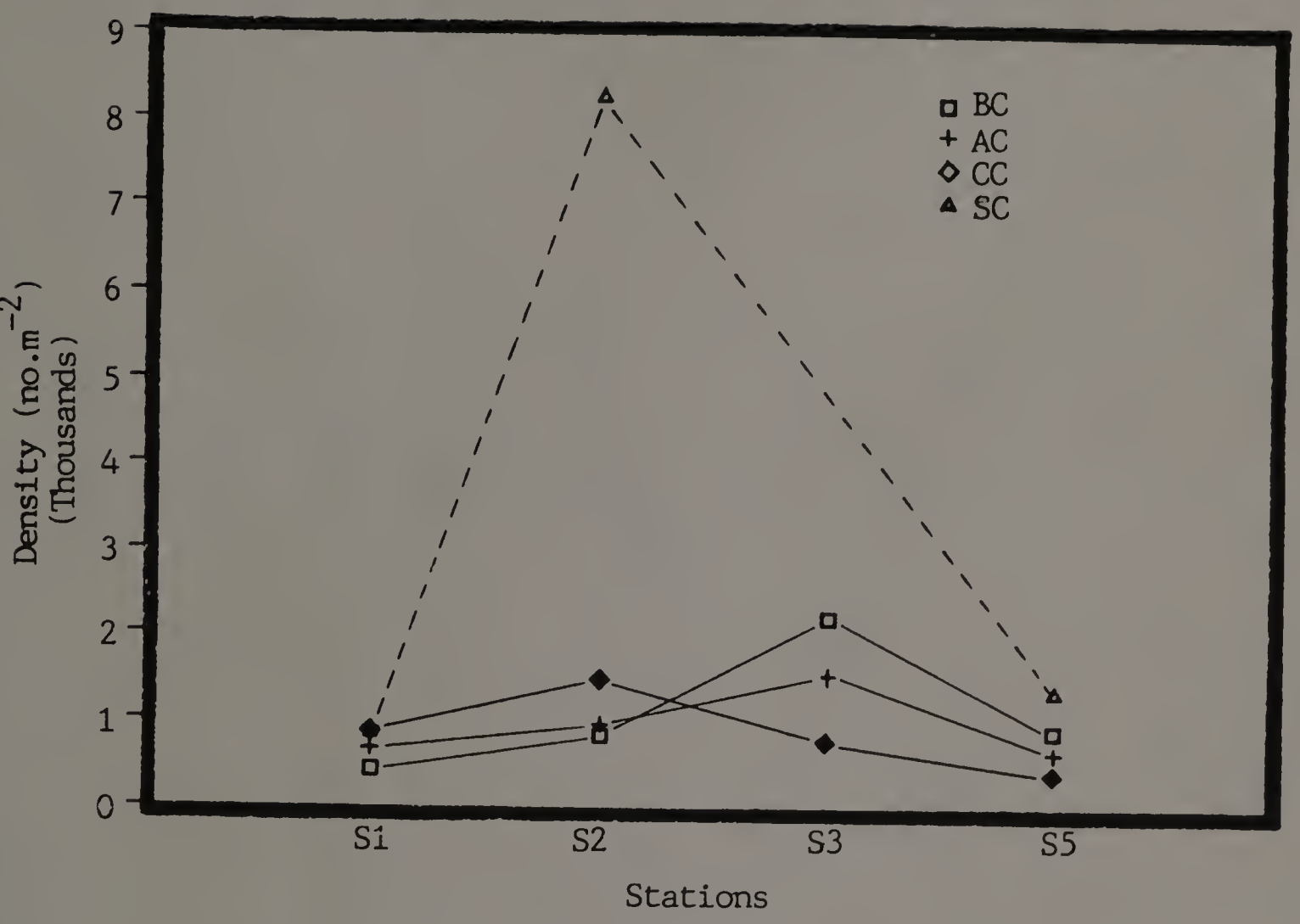


Figure 7. The intersite variations on the abundance of organisms before and after chlorination.

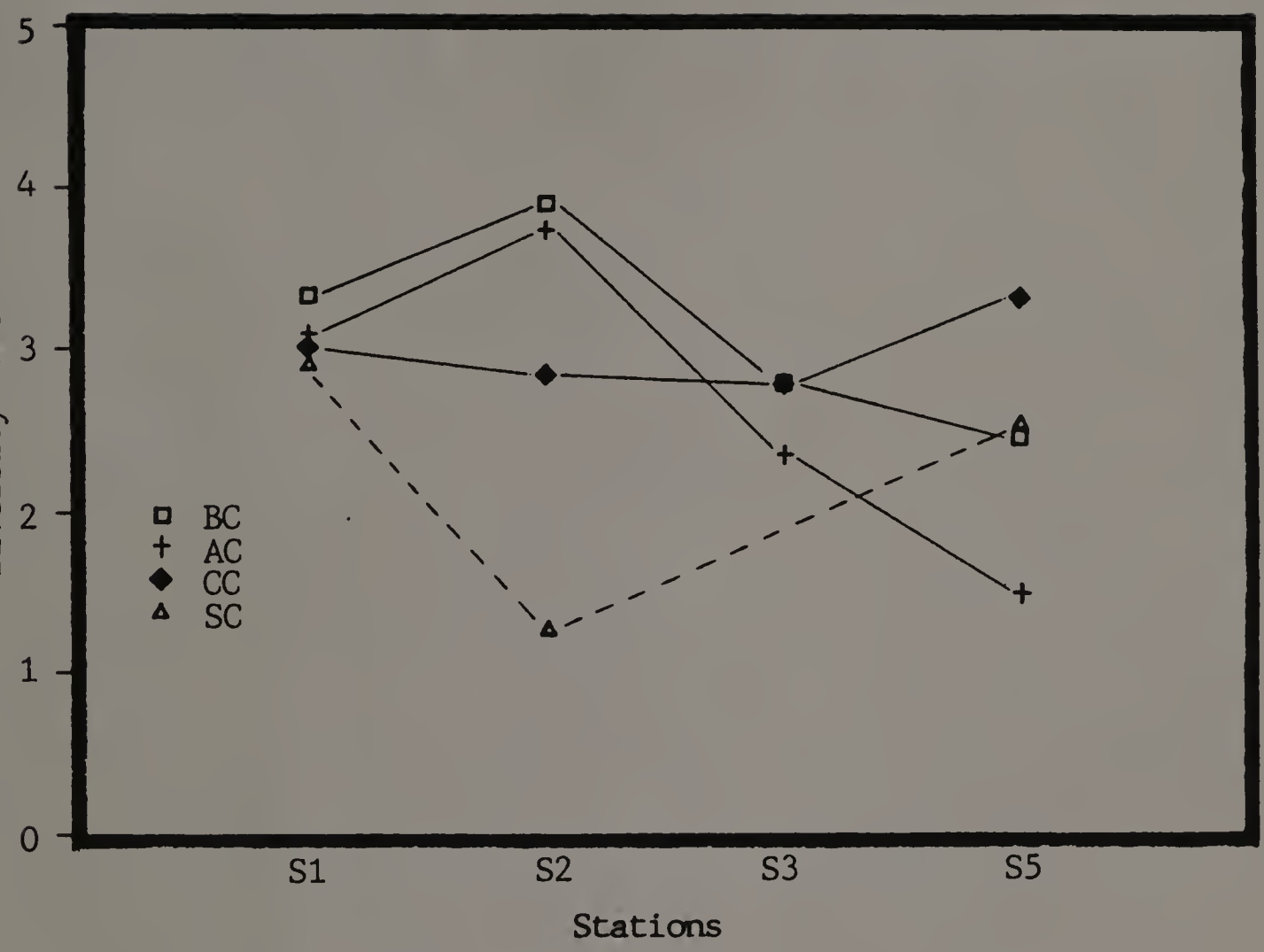


Figure 8. The intersite variations on the diversity of organisms before and after chlorination.

Four days after chlorination (AC), S-3 baskets still supported the most abundant community, although the density decreased from 2226±437 to 1550±335 m^{-2} while those in S-1 and S-2 baskets increased from 417±75 to 681±189 m^{-2} and from 820±230 to 962±270 m^{-2}, respectively (Fig.7, p.71). Surprisingly, S-2 baskets still supported the most diverse community among the stations (Fig.8, p.72).

After 10 days of chlorination (CC) (Fig.7, p.71), the S-2 baskets yielded the most abundant populations (1487±293 m^{-2}), but with reduced diversities (Table 6, p.70). The S-3 basket populations (809±149 m^{-2}) became as abundant as S-1 (856±187 m^{-2}), but suffered the greatest degree of population decimation (about 1/3) compared to the prechlorination period (Table 6, p.70). When compared to BC populations, the maximum increase in density was found in S-2 baskets (from 820±230 to 1487±293 m^{-2}), followed by S-1 baskets (from 417±75 to 856±187 m^{-2}). The maximum decrease in density was found in S-3 baskets (from 2226±437 to 809±149 m^{-2}, and then S-5 baskets (from 901±172 to 397±85 m^{-2}). The diversity index of S-3 and S-5 baskets were found to increase from about 2.4 and 1.5 (AC), to 2.8 and 3.3 (CC), respectively. The diversity index of S-2 baskets decrease significantly from 3.7 (AC) to 2.9 (CC) (Fig.8).

In spring sampling from BC to CC, with regard to the variation in density (Fig.7) and diversity (Fig.8) at each station due to chlorination, similar patterns

were revealed between S-1 and S-2, and between S-3 and S-5, which explain the highly significant interaction (Table A-5) between site and chlorination. However, control station (S-1) had the minimum variations in both density and diversity during chlorination. Maximum variation of density due to chlorination happened at S-3, then S-2 and S-5 following in order (Fig.7).

In the second set of samplings, the continuous chlorination and summer weather resulted in a midge-dominated stream system. The S-3 basket samples were not processed at this period. Except for a few mayflies found at S-1 and few stoneflies and caddisflies found at S-5, the baskets were colonized exclusively by chironomids. The numbers of organisms collected at all stations were more abundant than those collected from the spring samples. In the period of summer chlorination, S-2 had the maximum increased density and the maximum decreased diversity when compared to the period of spring chlorination. S-1 and S-5 basket diversities were similar to these before chlorination, which imply that S-5 was not influenced by chlorination in the long term.

In S-2, the genus Chironomus sp. was found to be most tolerant to TRC at a concentration of 0.67 ± 0.23 ppm. Excluding intraspecific competition, most midge populations found in S-2 were active under their chlorinated environment. However, mayflies, stoneflies and caddisflies

numbers in S-2 baskets could not be sustained in the same environment for two weeks. The suspected substances of stressing the aquatic biota most was chlorine-combined organics, which supressed Amphipoda, Simulidae, and even Chironomidae at S-3. In addition, the midge genus Orthocladius sp. were found to be sensitive to TRC at the concentration of 0.048+0.01 ppm. Depending on the TRC concentration (0.038--0.058 mg/l) of S-5, which is viewed as the least impacted station, it seems that to protect the benthic community continuous TRC exposures should not exceed 0.02 mg/l. However, for some sensitive species, which may not originally exist in S-5, this estimated value may still be too high.

APPENDIX A. Tables for chemical methods and criteria, data and computer printouts.

Table A-1. Effluent limitations and monitoring requirements of Belchertown sewage treatment plant.

Effluent Characteristic	Discharge Limitations			Monitoring Requirement	
	Average Monthly	Average Weekly	Maximum Daily	Measurement Frequency	Sample Type
Flow- m^3/day (MGD)			(0.50)	Continuous	see footnote 1
BOD	30 mg/1	45 mg/1	50 mg/1	Weekly	Composite- 8 hrs
TSS	30 mg/1	45 mg/1	50 mg/1	Weekly	Composite- 8 hrs
Settleable Solids		0.1 ml/1	0.3 ml/1	1/Day	Grab
pH	6.5 - 8.0 at any time			1/Day	Grab
Fecal Coliform[2] (per 100 ml)	200	400	400	Weekly	Grab
Chlorine Residual[2]	Minimum total chlorine residual 0.5 mg/1, maximum 1.5 mg/1 after 15 minutes contact at peak hourly flow.			1/Day	Grab

1) Report maximum and minimum daily rates and total flow for each operating day.
2) Applicable from April 1 to October 31.

Table A-2. Analyses of water quality of selected stations along Lampson Brook within the period from March 19 to June 19, 1987. Except for TRC, all parameters presented with two sets of data for the analyses before (upper case) and after (lower case) chlorination.

All data are presented as Average + S.D. in mg/l unless otherwise indicated.

Water Quality Parameter	Station 1	Station 2	Station 3	Station 4	Station 5
DO	14.2 ± 5.0	11.6 ± 1.9	10.2 ± 0.3	13.5 ± 2.0	11.4 ± 2.1
	9.6 ± 1.4	9.2 ± 1.4	6.6 ± 2.5	5.4 ± 1.5	9.7 ± 3.6
$N\text{-}NH_3$	0.21 ±0.24	0.84 ±0.44	0.52 ±0.41	0.22 ±0.01	0.13 ±0.09
	0.04 ±0.05	0.77 ±0.52	0.26 ±0.19	0.29 ±0.28	0.04 ±0.07
$N\text{-}NO_3$	0.38 ±0.13	0.79 ±0.13	0.41 ±0.03	0.46 ± ...	0.63 ±0.24
	0.12 ±0.05	0.45 ±0.15	0.62 ±0.22	0.63 ±0.17	0.11 ±0.04
Total P (ug/l)	225 ± 104	1281 ± 79	805 ± 48	523 ± 109	348 ± 28
	104 ± 117	1381 ± 521	826 ± 207	669 ± 250	575 ± 174
Alkalinity	23 ± 8	29 ± 12	26 ± 11	19 ± 6	16 ± 6
	37 ± 7	51 ± 8	44 ± 8	50 ± 7	37 ± 12
Chloride	22 ± 2	38 ± 1	33 ± 4	33 ± ...	32 ± ...
	62 ± 25	59 ± 15	51 ± 17	51 ± 14	30 ± 7
Hardness	32 ± 14	42 ± 16	37 ± 14	47 ± 11	38 ± 17
	89 ± 26	78 ± 14	80 ± 18	86 ± 18	67 ± 18
Temperature	18 ± 7	15 ± 8	17 ± 8	...	14 ± 6
	15 ± 4	15 ± 3	16 ± 4	...	14 ± 5
Fecal Coliforms	146 ± 114	179 ± 156	77 ± 57	45 ± 40	56 ± 63
	282 ± 238	36 ± 63	119 ± 143	86 ± 81	143 ± 180
pH	6.9 ± 0.3	7.4 ± 0.5	6.9 ± 0.4	...	7.0 ± 0.4
	6.9 ± 0.2	7.5 ± 0.1	6.8 ± 0.3	6.9 ± 0	6.9 ± 0.2
Total Residual Chlorine[1]	0	0.677±0.232	0.069±0.025	0.067±0.036	0.048±0.010

[1]Total residual chlorine was measured since chlorination was assumed.

Table A-3. The criteria of water quality for freshwater aquatic life and public health (EPA, 1976; Legal Compilation, 1973).

Water Quality Parameter	Criterion
Alkalinity	20 mg/l or more as $CaCO_3$ for freshwater aquatic life except where natural concentrations are less.
Ammonia	0.02 mg/l for fresh water aquatic life.
Chlorine	TRC 2.0 ug/l for salmonid fish; 10.0 ug/l for other freshwater organisms.
Facal Coliform	not to exceed a log mean of 200 per 100 ml, nor more than 10% of the total samples taken during any 30 day period exceed 400 per 100 ml for bathing waters.
Hardness	Concentration 0 - 75 mg/l $CaCO_3$ is rated as soft water; 75 - 150 as moderately hard; 150 - 300 as hard; and 300 up as very hard.
Nitrates; Nitrites	10 mg/l nitrate nitrogen (N) for domestic water supply (health).
Dissolved Oxygen	5.0 mg/l for freshwater biota.
pH	6.5 - 9.0 for freshwater aquatic life; and 5 - 9 for domestic water supplies (welfare).
Total Phosphate	not to exceed an average of 0.05 mg/l as P during any monthly sampling period for fish and wildlife (Class C in Massachusetts).
Temperature	A maximum of 90 F with a maximum permissible rise above the naturally existing temperatures of 5 F in stream and 3 F in lakes.

Table A-4. The applied methods for chemical protocol, most in accordance with Standard Methods for the Examination of Water and Wastewater (1985).

Parameter	Applied Methods
Chlorine Residuals	DPD colorimetric method
Acidity	Potentiometric titration (to pH 8.3)
Alkalinity	Potentiometric titration (to pH 4.3–4.7)
Ammonia	Preliminary distillation step and Nesslerization method
Nitrate	Low range nitrate test model NI-14 of HACH Inc. with Spectronic 21
Phosphorus	Persulfate digestion method and Stannous chloride method
Chloride	Mercuric nitrate method
Calcium	EDTA titrimetric method
Hardness	EDTA titrimetric method
Fecal Coliforms	MILLIPORE application manual AM302
D.O.	Oxygen meter YSI model 54A
Temperature	Temperature meter YSI model 54A
pH	pH meter ALTEX Expand-Mate

Table A-5. The results of two-way ANOVA on mean density of organisms, developed for all stations during spring chlorination, Lampson Brook, 1987.

ANALYSIS OF VARIANCE

Source	SS	DF	MS	F Value	P Value
Site	50.5	3	16.9	26.9	0.00*
Chlorination	1.1	2	0.6	0.9	0.41
Interaction	49.5	6	8.2	13.2	0.00*
Error	373.5	596	0.6		

LEVENE"S TEST FOR EQUALITY OF VARIANCES

Site	3, 596	2.0	0.11
Chloriantion	2, 596	0.2	0.80
Interaction	6, 596	1.6	0.15

Table A-6. Average weekly concentrations of chlorine residuals, detected in all stations along Lampson Brook, during the period of chlorination from April 10 to June 19, 1987.

All data in mg/l presented as average + S.E.

Station	S1	WWTP	S2	S3	S4	S5
Free Cl_2	0	0.102±0.054	0.023±0.011	0	0	0
NH_2Cl	0	2.161±0.718	0.551±0.260	0.002±0.002	0	0.020±0.007
$NHCl_2$	0	0.085±0.030	0.102±0.029	0.065±0.026	0.067±0.036	0.027±0.005
NCl_3	0	0.052±0.042	0	0.002±0.002	0	0
TRC	0	2.400±0.652	0.677±0.232	0.069±0.025	0.067±0.036	0.048±0.010

'WWTP' refers to the discharge of waste water treatment plant.

Table A-7. Two-way ANOVA on the mean density of organisms, developed for the effects of spring chlorination in individual stations along Lampson Brook, 1987.

EFFECT	STATISTIC	F	DF	P
Chlorination at S-1	SS= 6.206			
	MS= 3.103	5.17	2, 585	0.0060*
Chlorination at S-2	SS= 12.363			
	MS= 6.181	10.29	2, 585	0.0000*
Chlorination at S-3	SS= 21.384			
	MS= 10.692	17.80	2, 585	0.0000*
Chlorination at s-5	SS= 13.136			
	MS= 6.568	10.94	2, 585	0.0000*

Table A-8. The result of two-way ANOVA on mean density of organisms, developed for the intrasite comparisons of spring chlorination (BC, AC, CC) at individual stations along Lampson Brook, 1987.

EFFECT	STATISTIC	F	DF	P
S1 BC & AC	SS= 1.103 MS= 1.103	1.76	1, 596	0.1850
S1 BC & CC	SS= 5.792 MS= 5.792	9.24	1, 596	0.0025*
S1 CC & AC	SS= 1.889 MS= 1.889	3.01	1, 596	0.0830
S2 BC & AC	SS= 0.190 MS= 0.190	0.30	1, 596	0.5825
S2 BC & CC	SS= 9.899 MS= 9.899	15.80	1, 596	0.0001*
S2 CC & AC	SS= 9.258 MS= 9.258	14.78	1, 596	0.0001*
S3 BC & AC	SS= 2.481 MS= 2.481	3.96	1, 596	0.0470*
S3 BC & CC	SS= 20.480 MS= 20.480	32.68	1, 596	0.0000*
S3 CC & AC	SS= 9.258 MS= 9.258	14.78	1, 596	0.0001*
S5 BC & AC	SS= 0.711 MS= 0.711	1.13	1, 596	0.2874
S5 BC & CC	SS= 12.444 MS= 12.444	19.86	1, 596	0.0000*
S5 CC & AC	SS= 2.570 MS= 2.570	4.10	1, 596	0.0433*

Table A-9. General census data of S-1, collected from March to June, 1987.

Time[1] of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b	a	b	a[2]	b[3]
No. of Collected Rocks	25	18	23	23	23	24	16	-
Major Taxa (No./Basket)								
Insecta								
Ephemeroptera.....	35	17	43	68	93	58	3	-
Plecoptera.....	9	5	7	11	9	4	0	-
Trichoptera.....	3	3	2	3	14	8	0	-
Odonata.....	1	0	0	0	0	0	0	-
Megaloptera.....	1	0	1	0	5	5	0	-
Coleoptera.....	5	1	13	4	8	10	0	-
Diptera.....	17	8	19	22	23	24	81	-
Mollusca								
Gastropoda.....	1	0	0	0	0	0	0	-
Annelida								
Oligochaeta.....	0	0	1	0	0	0	0	-
Crustacea	1	0	0	0	0	0	0	-
Total No. of Collected Organisms	73	34	86	108	152	109	84	-
Estimated Mean Density (m^{-2})	450	370	520	842	1045	674	883	-
Species Diversity	3.47	3.20	3.12	3.07	2.92	3.13	2.92	-

[1]BC= Before Chlorination
AC= Acute Chlorination
CC= Chronic Chlorination
SC= Summer Chlorination

[2]Some rocks of this basket were missing.

[3]The whole basket was missing.

Table A-10. Estimated density of benthic macroinvertebrates collected at S-1 from March to June, 1987.

All data in no.m^{-2} presented as Average + 95% C.I.

Time of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b	a	b	a	b
Specimens (no.m^{-2})								
Ephemeroptera								
Serratella sp.	28± 6	57±9	60±11	124±20	81±10	48± 7	0	-
Eurylophella sp.	11± 4	0	7± 3	21± 5	7± 3	0	0	-
Stenonema sp.	45± 5	29±5	27± 6	55± 9	68±11	30± 6	0	-
Paraleptophlebia sp.	96±12	36±6	153±19	263±30	440±43	199±22	31±11	-
Baetis sp.	17± 5	0	40± 7	76± 9	34± 8	66± 9	0	-
Ephemerella sp.	0	0	0	7± 3	0	6± 2	0	-
Plecoptera								
Tallaperla sp.	17± 5	7±3	7± 3	14± 4	0	0	0	-
Peltoperla sp.	6± 2	7±3	0	7± 3	7± 3	0	0	-
Isoperla sp.	28± 5	21±5	33± 7	48± 8	27± 5	12± 3	0	-
Alloperla sp.	0	0	7± 3	7± 3	27± 7	12± 3	0	-
Trichoptera								
Cheumatopsyche sp.	17± 5	21±5	13±4	21± 6	95±12	48± 8	0	-
Odonata								
Cordulegaster sp.	6± 2	0	0	0	0	0	0	-
Megaloptera								
Nigronia sp.	6± 2	0	7± 3	0	34± 7	30± 8	0	-

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Table A-10. Cont.

Coleoptera								
Stenelmis sp.	17± 4	7±3	80±13	14± 4	34± 7	48± 6	0	-
Optioservus sp.	6± 2	0	0	7± 3	0	0	0	-
Promoresia sp.	0	0	7± 3	7± 3	7± 3	6± 2	0	-
Anchytarsus sp.	0	0	0	0	14± 4	6± 2	0	-
Heteroceridae	6± 2	0	0	0	0	0	0	-
Diptera								
Chironomidae								
Trissopelopia sp.	17± 4	0	20± 6	0	34± 9	66±11	10± 5	-
Conchapelopia sp.	17± 4	0	7± 3	14± 4	14± 4	12± 3	73±13	-
Polypedilum sp.	6± 2	7±3	27± 8	7± 3	20± 5	6± 2	21±10	-
Parametriocnemus sp.	11± 3	0	13± 4	14± 4	47± 8	24± 5	313±43	-
Orthocladius sp.	6± 2	21±6	0	0	0	0	0	-
Corynoneura sp.	6± 2	0	7± 3	14± 4	7± 3	6± 2	0	-
Heterotrissocladius sp.	0	7±3	7± 3	7± 3	0	0	42±12	-
Synorthocladius sp.	0	7±3	0	0	0	0	0	-
Cricotopus sp.	0	7±3	0	0	0	0	0	-
Zavrelimyia sp.	0	0	7± 3	0	7± 3	0	73±21	-
Rheocricotopus sp.	0	0	0	0	0	12± 3	0	-
Macropelopia sp.	0	0	0	0	0	0	21± 7	-
Phaenopsectra sp.	0	0	0	0	0	0	21± 7	-
Chironomus sp.	0	0	0	0	0	0	52±10	-
Microtendipes sp.	0	0	0	0	0	0	52±12	-
Endochironomus sp.	0	0	0	0	0	0	31± 8	-
Thienemanniella sp.	0	0	0	0	0	0	10± 5	-
Nanocladius sp.	0	0	0	0	0	0	10± 5	-
Tanytausus sp.	0	0	0	0	0	0	104±22	-
Micropsectra sp.	0	0	0	0	0	0	10± 5	-
Tipulidae	22± 5	7±3	7± 3	14± 4	20± 6	18± 5	0	-
Ceretopogonidae	11± 4	0	7± 3	14± 4	7± 3	0	0	-
Oligochaeta	0	0	7± 3	0	0	0	0	-
Orthorrhapha	6± 2	0	0	69±11	0	0	0	-
Crustecea	6± 2	0	0	0	0	0	0	-

Table A-11. General census data of S-2, collected from March to June, 1987.

Time[1] of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b	a	b	a	b
No. of Collected Rocks	29	25	30	27	29	28	28	27
Major Taxa (No./Basket)								
Insecta								
Ephemeroptera.....	70	36	20	22	0	2	0	0
Plecoptera.....	4	3	2	1	0	0	0	0
Trichoptera.....	1	4	5	8	0	0	0	0
Megaloptera.....	1	3	3	2	1	1	0	0
Coleoptera.....	12	7	18	9	6	0	0	0
Diptera.....	75	53	149	55	236	208	1598	1286
Mollusca								
Pelecypoda.....	0	0	2	0	0	0	0	0
Platyhelminthes								
Turbellaria.....	2	0	0	0	2	6	0	0
Annelida								
Oligochaeta.....	2	0	3	3	8	19	0	0
Nematoda	2	1	3	0	0	0	0	0
Total No. of Collected Organisms	169	107	205	100	253	236	1598	1286
Estimated Mean Density (m^{-2})	1008	602	1288	500	1532	1441	9717	6914
Species Diversity	4.04	3.75	3.90	3.57	2.78	2.93	0.91	1.66

[1]BC= Before Chlorination
AC= Acute Chlorination
CC= Chronic Chlorination
SC= Summer Chlorination

Table A-12. Estimated density of benthic macroinvertebrates collected at S-2 from March to June, 1987.

All data in no.m^{-2} presented as Average + 95% C.I.

Time of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b	a	b	a	b
Specimens (no.m^{-2})								
Ephemeroptera								
Serratella sp.	63± 7	54± 6	12± 3	48± 7	0	0	0	0
Eurylophella sp.	6± 2	22± 4	6± 2	18± 4	0	11± 3	0	0
Stenonema sp.	143±10	76± 8	35± 6	18± 4	0	0	0	0
Paraleptophlebia sp.	91±11	16± 4	41± 5	24± 4	0	0	0	0
Baetis sp.	57± 5	16± 4	17± 3	18± 4	0	0	0	0
Ephemerella sp.	40± 5	11± 3	6± 2	6± 2	0	0	0	0
Plecoptera								
Peltoperla sp.	0	0	12± 3	0	0	0	0	0
Isoperla sp.	23± 5	16± 4	0	6± 2	0	0	0	0
Trichoptera								
Cheumatopsyche sp.	6± 2	11± 3	12± 3	30± 4	0	0	0	0
Diplectrona sp.	0	5± 2	0	6± 2	0	0	0	0
Hydropsyche sp.	0	5± 2	6± 2	12± 3	0	0	0	0
Ceratopsyche sp.	0	0	6± 2	0	0	0	0	0
Lepidostoma sp.	0	0	6± 2	0	0	0	0	0
Megaloptera								
Nigronia sp.	0	11± 3	17± 3	12± 3	6± 2	6± 2	0	0
Sialis latreille	6± 2	5± 2	0	0	0	0	0	0
Coleoptera								
Stenelmis sp.	23± 5	0	12± 3	6± 2	18± 4	17± 3	0	0
Optioservus sp.	34± 5	11± 3	0	0	0	0	0	0
Promoresia sp.	29± 5	5± 2	76± 9	36± 6	12± 3	11± 3	0	0
Anchytarsus sp.	0	0	0	6± 2	0	6± 2	0	0
Cleptelmis sp.	11± 4	0	0	0	6± 2	0	0	0
Oulimmus sp.	6± 2	16± 5	17± 5	6± 2	0	0	0	0
Limnichidae	6± 2	0	0	0	0	0	0	0

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Table A-12. Cont.

Diptera								
Chironomidae								
Trissopelopia sp.	11± 3	0	52± 7	18± 4	107±11	105±10	12± 4	58± 9
Conchapelopia sp.	34± 5	33± 6	52± 6	6± 2	48± 7	55± 6	105±16	402± 29
Polypedilum sp.	11± 3	5± 2	111±14	30± 5	642±46	380±24	139±14	379± 35
Parametriocnemus sp.	97±11	38± 8	268±23	14± 4	208±19	325±22	23± 6	12± 4
Halocladius sp.	0	0	0	12± 4	0	11± 3	0	0
Orthocladius sp.	57± 7	136±14	23± 4	0	0	0	12± 4	0
Stempellinella sp.	6± 2	0	0	0	6± 2	6± 2	0	0
Corynoneura sp.	0	11± 4	6± 2	0	0	0	0	0
Heterotrissocladius sp.	11± 3	0	12± 3	0	0	11± 3	0	12± 4
Synorthocladius sp.	11± 3	0	0	0	0	0	0	0
Cricotopus sp.	0	0	0	0	0	0	58±10	198± 20
Acricotopus sp.	0	0	0	0	0	6± 2	0	0
Zavrelimyia sp.	0	0	0	0	6± 2	0	46±10	35± 7
Rheocricotopus sp.	6± 2	0	6± 2	0	6± 2	0	0	0
Psectrotanypus sp.	0	0	0	0	0	0	314±33	350± 31
Paraphaenocladius sp.	6± 2	5± 2	6± 2	6± 2	0	0	0	0
Phaenopsectra sp.	11± 4	0	17± 3	0	0	6± 2	163±15	93± 11
Chironomus sp.	6± 2	0	6± 2	0	101± 9	44± 7	8170±372	5510±197
Cryptochironomus sp.	0	0	0	0	6± 2	0	0	0
Parachironomus sp.	0	0	0	0	6± 2	0	0	0
Endochironomus sp.	0	0	0	0	0	0	0	12± 4
Stictochironomus sp.	0	0	0	0	0	0	0	6± 2
Microtendipes sp.	0	0	6± 2	6± 2	0	0	23± 6	0
Dicrotendipes sp.	0	0	0	0	0	0	58±11	82± 12
Thienemanniella sp.	6± 2	0	0	0	0	0	0	0
Nanocladius sp.	6± 2	0	0	0	0	0	12± 4	0
Tanytausus sp.	11± 4	0	70± 8	6± 2	18± 4	11± 3	0	41± 9
Rheotanytarsus sp.	0	0	0	6± 2	0	0	0	0
Paratanytarsus sp.	6± 2	0	0	0	6± 2	0	23± 6	47± 8
Cladotanytarsus sp.	11± 3	0	17± 3	0	0	0	0	0
Micropsectra sp.	0	0	0	12± 3	18± 4	0	128±16	262± 22
Tipulidae	74±13	27± 5	52± 6	0	18± 5	0	0	0
Ceretopogonidae	40± 9	0	87±11	18± 4	131±12	94±10	0	0
Tabanidae	6± 2	5± 2	0	6± 2	0	0	0	0
Empididae	0	22± 4	128±12	42± 7	77± 8	61± 8	0	0
Oligochaeta	11± 3	0	17± 5	18± 4	48± 6	105±11	0	0
Orthorrhapha	0	0	6± 2	0	0	0	0	0
Nematoda	11± 3	5± 2	17± 3	0	0	0	0	0
Turbellidae	11± 3	0	0	0	12± 3	33± 6	0	0
Pelecypoda	0	0	12± 4	0	0	0	0	0

Table A-13. General census data of S-3, collected from March to June, 1987.

Time[1] of Chlorination	BC		AC		CC		SC[2]	
Basket Replicates	a	b	a	b	a	b	a	b
No. of Collected Rocks	26	29	30	30	30	29	-	-
Major Taxa (No./Basket)								
Insecta								
Ephemeroptera.....	0	2	4	0	1	0	-	-
Trichoptera.....	0	0	0	1	0	0	-	-
Megaloptera.....	0	0	0	0	1	0	-	-
Coleoptera.....	0	1	0	0	1	0	-	-
Diptera.....	248	302	263	99	98	37	-	-
Hemiptera.....	0	0	0	1	0	0	-	-
Crustacea								
Amphipoda.....	100	66	133	60	55	57	-	-
Mollusca								
Pelecypoda.....	10	10	12	28	4	5	-	-
Annelida								
Oligochaeta.....	6	0	2	3	5	3	-	-
Hirudinea.....	7	5	2	8	3	20	-	-
Nematoda	0	0	0	4	0	0	-	-
Total No. of Collected Organisms	371	386	416	204	168	122	-	-
Estimated Mean density (m^{-2})	2258	2198	1994	1106	877	693	-	-
Species Diversity	2.73	2.87	2.21	2.51	3.15	2.45	-	-

[1]BC= Before Chlorination
AC= Acute Chlorination
CC= Chronic Chlorination
SC= Summer Chlorination

[2]Missing data due to human vandalism.

Table A-14. Estimated density of benthic macroinvertebrates collected at S-3 from March to June, 1987.

All data in no.m^{-2} presented as Average $\pm$ 95% C.I.

Time of Chlorination	BC		AC		CC	
Basket Replicates	a	b	a	b	a	b
Specimens (no.m^{-2})						
Amphipod						
Hyalella azteca	581±41	382±29	646±42	323±23	303±19	334±22
Ephemeroptera						
Neoephemera sp.	0	6± 2	5± 2	0	0	0
Ephemerella sp.	0	6± 2	0	0	0	0
Leptophlebia sp.	0	0	5± 2	0	0	0
Paraleptophlebia sp.	0	0	10± 3	0	6± 2	0
Trichoptera						
Limnephilidae	6± 2	0	0	5± 2	0	0
Coleoptera						
Agabus sp.	0	6± 2	0	0	6± 2	0
Megaloptera						
Chauliodes sp.	0	0	0	0	6± 2	0
Odonata						
Coenagrionidae	0	0	5± 2	0	0	0
Hemiptera						
Neoplea sp.	0	0	0	5± 2	0	0
Diptera						
Simuliidae						
Simulium sp.	81±13	168±14	5± 2	0	39± 6	0
Prosimulium sp.	180±25	174±14	5± 2	5± 2	127±14	6± 2
(pupae)	139±11	330±24	233±17	27± 6	22± 4	18± 4
Stratiomyidae						
Odontomyia sp.	17± 4	0	0	0	0	0
Chironomidae						
Trissopelopia sp.	17± 4	17± 4	24± 4	5± 2	17± 3	12± 3
Conchapelopia sp.	52± 7	29± 5	107± 9	32± 4	83± 7	41± 5
Zavrelimyia sp.	0	0	0	22± 4	33± 5	47± 8
Chironomus sp.	0	6± 2	0	5± 2	11± 3	0
Polypedilum sp.	6± 2	0	5± 2	5± 2	33± 5	6± 2
Thienemanniella sp.	17± 5	17± 4	0	0	0	0
Parametriocnemus sp.	796±78	810±66	841±53	393±32	88± 6	59± 6
Cricotopus sp.	0	23± 5	5± 2	5± 2	6± 2	0
Rheocricotopus sp.	6± 2	17± 4	0	0	6± 2	0
Hererotrissocladius sp.	0	0	0	5± 2	6± 2	0
Paraphaenocladius sp.	12± 3	0	0	0	0	0
Synorthocladius sp.	6± 2	0	0	0	0	0
Orthocladius sp.	23± 4	81± 8	5± 2	0	17± 3	12± 3
Diplocladius sp.	6± 2	12± 3	0	0	0	0
Nanocladius sp.	0	0	5± 2	5± 2	0	0
Tanytarsus sp.	17± 4	12± 3	0	0	0	0
Paratanytarsus sp.	6± 2	17± 4	5± 2	0	0	0
Cladotanytarsus sp.	0	0	0	0	0	6± 2
Micropsectra sp.	41± 5	35± 6	15± 3	11± 4	0	0
Phaenopsectra sp.	0	0	0	0	0	6± 2
Ceretopogonidae	6± 2	0	19± 4	11± 3	55± 6	6± 2
Tipulidae	0	0	5± 2	0	0	0
Hirudinea	41± 8	29± 6	10± 2	43± 6	17± 3	117±13
Oligochaeta	35± 8	0	10± 2	16± 3	28± 5	18± 4
Nematoda	0	0	0	22± 5	0	0
Pelecypoda	58±10	58± 9	58±10	151±16	22± 4	29± 4

Table A-15. General census data of S-5, collected from March to June, 1987.

Time[1] of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b[2]	a	b	a	b
No. of Collected Rocks	27	27	18	-	30	30	28	30
Major Taxa (No./Basket)								
Insecta								
Ephemeroptera.....	2	0	0	-	2	2	0	0
Plecoptera.....	2	6	0	-	5	2	5	9
Trichoptera.....	0	1	0	-	2	0	0	3
Megaloptera.....	0	1	0	-	0	0	0	0
Coleoptera.....	0	2	0	-	0	0	0	0
Diptera.....	118	142	65	-	69	78	98	249
Crustacea								
Amphipoda.....	0	1	0	-	0	1	0	0
Mollusca								
Pelecypoda.....	0	0	0	-	1	0	0	0
Gastropoda.....	1	1	0	-	0	0	0	0
Annelida								
Oligochaeta.....	1	0	1	-	6	5	0	1
Hirudinea.....	0	0	0	-	1	0	0	0
Nematoda	1	0	0	-	0	0	0	0
Total No. of Collected Organisms	125	154	66	-	86	88	103	262
Estimated Mean Density (m^{-2})	751	1061	649	-	477	311	562	1457
Species Diversity	2.32	2.62	1.22	-	3.46	3.21	2.24	2.88

[1]BC= Before Chlorination
AC= Acute Chlorination
CC= Chronic Chlorination
SC= Summer Chlorination

[2]Missing data due to the operating errors and human vandalism.

Table A-16. Estimated density of benthic macroinvertebrates collected at S-5 from March to June, 1987.

All data in no.m^{-2} presented as Average + 95% C.I.

Time of Chlorination	BC		AC		CC		SC	
Basket Replicates	a	b	a	b	a	b	a	b
Specimens (no.m^{-2})								
Ephemeroptera								
Stenonema sp.	7± 2	0	0	-	0	6± 2	0	0
Isonychia sp.	7± 2	0	0	-	0	0	0	0
Sattatella sp.	0	0	0	-	11± 3	0	0	0
Baetis sp.	0	0	0	-	0	6± 2	0	0
Plecoptera								
Prostoia sp.	13± 3	41± 8	0	-	0	0	0	0
Allocapnia sp.	0	0	0	-	0	0	6± 2	23± 4
Claassenia sp.	0	0	0	-	0	0	23± 5	28± 4
Perlodidae	0	0	0	-	28± 4	12± 3	0	0
Trichoptera								
Cheumatopsyche sp.	0	7± 3	0	-	6± 2	0	0	0
Chimarra sp.	0	0	0	-	6± 2	0	0	0
Pycnopsyche sp.	0	0	0	-	0	0	0	11± 3
Coleoptera								
Optioservus sp.	0	14± 4	0	-	0	0	0	0
Megaloptera								
Nigronia sp.	0	7± 3	0	-	0	0	0	0

Cont., next page

Table A-16. Cont.

Diptera								
Simuliidae								
Simulium sp.	397±22	459±33	0	-	0	0	0	0
Prosimulium sp.	91±12	82±12	0	-	0	0	0	0
(pupae)	143±15	185±21	487±45	-	39± 5	110±14	0	0
Chironomidae								
Trissopelopia sp.	0	0	0	-	17± 3	18± 4	0	28± 5
Conchapelopia sp.	0	7± 3	0	-	11± 3	12± 3	17± 4	11± 3
Zavrelimyia sp.	0	0	0	-	11± 3	12± 3	75± 8	188±13
Zavrelia sp.	0	0	0	-	17± 4	0	0	0
Polypedilum sp.	0	7± 3	0	-	17± 3	18± 4	12± 3	17± 3
Chironomus sp.	0	0	0	-	0	0	0	17± 3
Parametriocnemus sp.	39± 6	75± 8	10± 4	-	133±13	147±15	46± 6	0
Psectrotanypus sp.	0	0	0	-	0	0	0	17± 3
Cricotopus sp.	33± 5	0	0	-	17± 3	18± 4	0	0
Microtendipes sp.	0	0	0	-	0	0	63± 7	228±15
Paratendipes sp.	0	0	0	-	0	0	306±24	649±37
Orthocladius sp.	13± 5	69± 9	0	-	6± 2	6± 2	0	0
Diplocladius sp.	13± 5	0	0	-	0	0	0	0
Nanocladius sp.	0	0	29± 7	-	17± 3	18± 4	0	6± 2
Procladius sp.	0	0	0	-	0	0	0	11± 3
Heterotrissocladius sp.	0	0	0	-	0	0	0	23± 5
Micropsectra sp.	7± 2	0	10± 4	-	17± 3	18± 4	0	11± 3
Phaenopsectra sp.	0	14± 5	10± 4	-	6± 2	6± 2	6± 2	46± 6
Corynoneura sp.	7± 2	0	29± 7	-	11± 3	12± 3	0	0
Thienemanniella sp.	0	14± 4	0	-	0	0	0	0
Stempellinella sp.	0	7± 3	0	-	6± 2	6± 2	0	23± 5
Tanytarsus sp.	0	0	10± 4	-	22± 4	25± 4	40± 5	46± 6
Paratanytarsus sp.	0	7± 3	0	-	0	0	0	0
Rheotanytarsus sp.	13± 3	14± 4	29± 7	-	11± 3	12± 3	0	0
Cladotanytarsus sp.	7± 2	0	0	-	0	0	0	0
Empididae	7± 2	27± 5	10± 4	-	17± 3	12± 3	0	0
Tipulidae	0	7± 3	0	-	0	0	0	0
Ceratapogonidae	0	0	0	-	11± 3	6± 2	0	0
Oligochaeta	7± 2	0	10± 4	-	33± 5	31± 7	0	6± 2
Nematoda	7± 2	0	0	-	0	0	0	0
Gastropoda	7± 2	7± 3	0	-	0	0	0	0
Hirudinea	0	0	0	-	6± 2	0	0	0
Pelecypoda	0	0	0	-	6± 2	0	0	0

APPENDIX B. Figures for site description laboratory facility, and pooled diversity.

Figure A-1. Five stations selected along Lampson Brook, Belchertown, Massachusetts, 1987 (mapped from Geological Survey, 1979).


Belchertown State School
STATE RESERVATION
Sewage Disposal
Lampson Brook
Gravel Pit
Sportsmen Club
Cooks Corner
Forge Pond
Rural Cem
RURAL
MILL ROAD
GEORGE HANNUM STREET
Weston
Cem

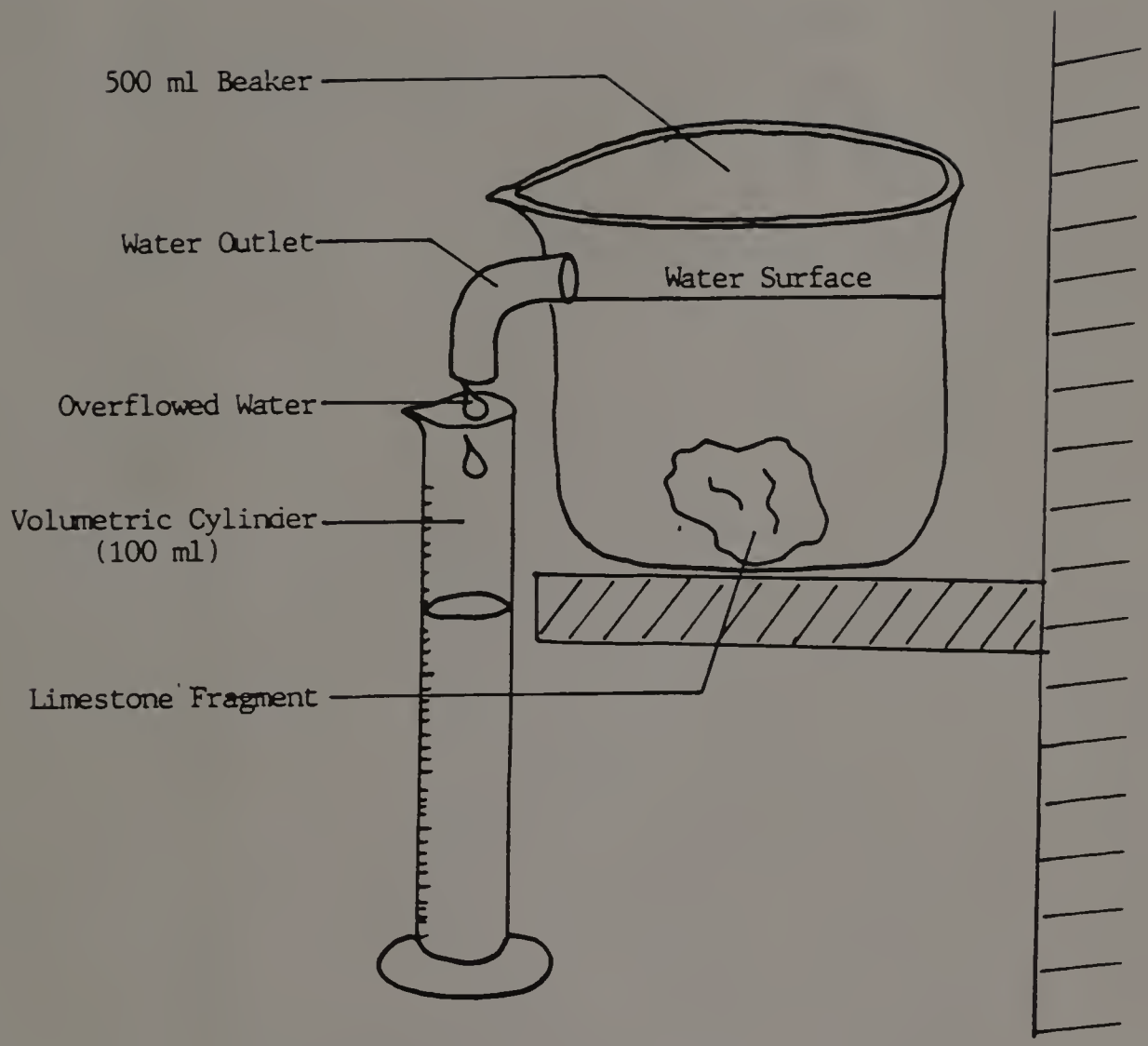
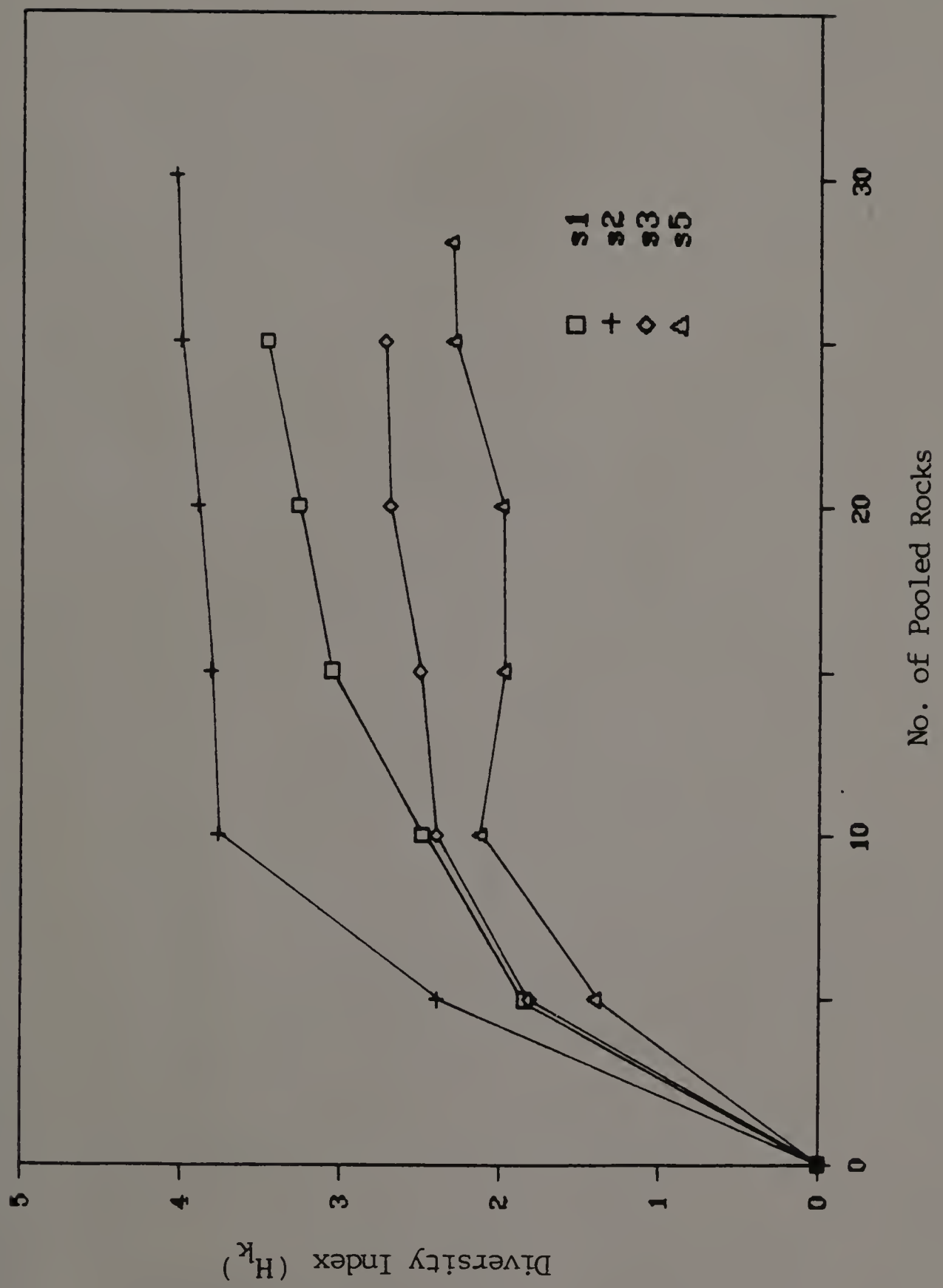


Figure A-2. Facilities for measuring the volumes of limetone fragments.

Figure A-3. Pratt's cumulative pooled diversity (H_k), derived from one of the replicated baskets for each station before chlorination (BC), 1987.


s1
s2
s3
s5
0
1
2
3
4
5
0
10
20
30
No. of Pooled Rocks
Diversity Index (H_k)

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