

CHANGES IN COLONIZATION DYNAMICS AND COMMUNITY STRUCTURE OF  
PROTOZOANS AND MACROINVERTEBRATES IN RESPONSE TO STRESS

by

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(ABSTRACT)

The response of macroinvertebrate and protozoan colonization dynamics to industrial pollution was determined in a thermal organic effluent and in a metals effluent. The relationship of the time course data to the MacArthur-Wilson model is considered, along with Pinkham and Pearson's community similarity index and traditional community structural parameters. The response of both communities is similar with respect to equilibrium number of taxa at both sites; however, the fitted rate constant,  $G$ , was accelerated in the organic effluent for the protozoans. The macroinvertebrate  $G$  in the same effluent showed a dose response. There were no differences in rate between stations in the metals effluent for either the protozoans or the macroinvertebrates, although the equilibrium number of taxa was decreased downstream.

Community similarity indices shows similar

macroinvertebrate and protozoan communities at all three stations in the organic system at equilibrium, although some differences occur in the early stages of colonization. The communities in the metals effluent are most often significantly different at the downstream station, which was the most impacted. Community diversity indices indicate the same results for the equilibrium community as do the other methods of evaluation. While community colonization studies have been suggested as a method of water quality assessment, colonization dynamics and particularly the fitted rate constant,  $G$ , may be misleading unless considered in conjunction with equilibrium information.

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## INTRODUCTION

The response of an aquatic community to changes in water quality, as a result of pollution, may be evaluated by structural or functional methods. Structural measures, such as species richness, diversity indices, total abundance, and community structure have traditionally been used in the assessment of community changes resulting from environmental stress (Mason, et al., 1970; Minshall et al., 1983). Community respiration and adenylate ratios have been used as functional measures for comparison of clean and impacted communities (Matthews et al., 1982; Toczydlowski and Buikema, in preparation). The entire community (i.e., all of the species at all levels of organization in a given ecosystem) is seldom analyzed due to time and money constraints. In addition, the information to be gained from analyzing all levels of the community is redundant (Kaesler and Cairns, 1972); that is, the same information may be gained by observing selected portions of the community as may be gained by collecting information about the entire community. It is easier to obtain structural information such as species richness, diversity, and total abundances from higher taxonomic levels (e.g., macroinvertebrates, fish, or vascular plants) than from microscopic communities in which the number of individuals per square centimeter may be  $10^5$  or greater.

Macroinvertebrate and/or fish community structure is the variable most often used to assess water quality. However, functional parameters are easier to measure in the compact microcommunity than in the groups of larger organisms mentioned above.

Many ecological models, including diversity indices, community respiration equations, immigration/emigration and colonization models, are designed for application to all or most of the taxonomic groups within a community, rather than to only one level. Indeed, if there is as much information to be gained by inspecting selected portions of a community as may be gained by evaluating the community in its entirety, the component parts must be assumed to have similar structural and functional characteristics. Because it is easier to compare functional parameters across communities than it is to compare community structure (Odum, 1971), a means of measuring function in assemblages of representative organisms, including macroinvertebrates in aquatic communities (Kaesler and Cairns, 1972), is desirable. The colonization dynamics of communities (as described by MacArthur and Wilson, 1963, 1967) or subcomponents of communities provides both a functional parameter, colonization rate, and structural indicator, equilibrium number of species (i.e. species richness).

The MacArthur-Wilson species equilibrium model (1963,1967) was developed to describe the pattern of species accumulation on oceanic islands colonized by a source pool located on a continent. Simberloff (1974) states that "any patch of habitat isolated from similar habitat by different, relatively inhospitable terrain traversed only with difficulty by organisms of the habitat patch may be considered an island." Thus, individual rocks, fallen leaves or twigs, or other introduced substrates may serve as an initially uninhabited island, for the purpose of studying species colonization.

Colonization dynamics of aquatic communities can be conveniently determined using artificial substrate samplers. Size, surface area, and location of the substrates are easily controlled, and the number of replicates is not limited to the amount of similar natural habitat available. These replicable islands have an additional advantage in that sampling is nondestructive. This advantage makes them particularly appealing for time-course sampling (Cairns and Henebry, 1982; Rosenberg and Resh, 1982; and Osman, 1982).

Protozoans and diatoms have been favored components of aquatic microbial communities for artificial substrate colonization studies (Cairns and Henebry, 1982; Kuhn, et al., 1980; Patrick, 1967). There is evidence that the

distribution of diatoms in the water column prevents them from colonizing in a manner consistent with the MacArthur-Wilson model (Stewart et al., 1985). Conversely, protozoan data generally support the model

$$S_t = S_{eq} (1 - e^{-Gt})$$

(where  $S_t$  is the number of species present at time  $t$ ,  $S_{eq}$  is the estimated number of species at equilibrium, and  $G$  is the fitted rate constant (colonization rate)) when using Polyurethane Foam (PF) unit artificial substrates. Both general adherence to the MacArthur-Wilson model and to the predicted conditions affecting the model, including island size, distance of islands from the source pool, and size of the source pool, have been well-demonstrated by protozoans (e.g., Cairns and Henebry, 1982).

A factor predicted to affect colonization is the relative harshness of the habitat found between source pools and islands. A terrain that is difficult to traverse will hinder or prevent individuals of some species from reaching an island. In a laboratory experiment, species from epicenter source pools were slower to accumulate on substrates in the presence of high and intermediate doses of copper sulfate (Cairns et al., 1979) than in control systems. In contrast, enrichment of the intervening terrain may facilitate colonization. Kuhn et al. (1980) and Henebry and Cairns (1984) demonstrated that protozoan

colonization was faster in Michigan lakes and bogs with higher nutrient (nitrogen and phosphorus) levels, even in acid bogs which had previously been considered dystrophic due to low pH, than in bodies of water with circumneutral pH containing less nutrients. Treated sewage effluents produced a higher number of taxa at equilibrium, which was accompanied by an increase in the colonization rate (Shen, unpublished data).

Information about macroinvertebrate community structure is frequently used in water quality assessment; there are a number of artificial substrate studies which present species richness, diversity, and biomass data (Minshall et al., 1983; Beak, et al., 1973; Khalaf and Tachet, 1977; Bournaud et al., 1978; Anderson and Mason, 1968; Benfield et al., 1974). The conformity of macroinvertebrate colonization to the MacArthur-Wilson model has been less well-documented. Dickson and Cairns (1974) found that species accumulation on blocks of 3M conservation webbing did not fit the model. However, Minshall et al. (1985) show that macroinvertebrate colonization does follow the MacArthur-Wilson model. In several published studies (Cover and Harrell, 1978; Ulfstrand et al., 1974; Nilsen and Larimore, 1979), time course sampling, i.e., samples taken at increasing lengths of time from the initial date of artificial substrate

placement, has been used to determine whether equilibrium has been reached as assessed by species richness, biomass, or total numbers of individuals. Each of these published studies presents time-course data; application of the MacArthur-Wilson model shows that macroinvertebrate colonization generally does follow the model (Table 1). When equilibrium data rather than colonization dynamics are sought, the artificial substrates serve as surrogates to direct sampling methods. Cairns (1972) cautions that equilibrium samples should be recovered before a density-dependent, interactive equilibrium is established. The effect of substrate size, structure, and complexity on the structure of the macroinvertebrate community have been described (Wise and Molles, 1979; Trush, 1979) and their effects on species richness and total abundance concur with the predictions of the MacArthur-Wilson model. Minshall et al. (1983), while not directly applying the species equilibrium model, found that macroinvertebrates recolonizing a defaunated stretch of the Teton River (ID) were affected by fluctuating water levels and local municipal and agricultural runoff, indicating that the condition of the intervening habitat affects macroinvertebrate colonization as well. There are virtually no studies which present time course data and/or the fit of the species equilibrium model in polluted streams or effluents,

nor has there been an analysis of the effect of single compounds on colonization of this portion of the aquatic community. Such information is needed to fill out the data base for colonization if this method is to be used for water quality assessment.

Based on the commonly encountered assertion that the levels of a given community are comparable to one another with respect to composition and function, I hypothesize that selected components of the aquatic community (i.e., protozoans and macroinvertebrates) should respond similarly to stress, and that colonization dynamics of both groups will be explained by the MacArthur-Wilson model. Because macroinvertebrates are considered highly representative of the entire aquatic community, changes in their colonization pattern may be a useful tool in water quality assessment. If, in turn, protozoan colonization response is similar to that of the macroinvertebrates, then that quicker process could be substituted for the longer macroinvertebrate colonization study.

The purposes of this research are to: 1) determine if macroinvertebrate and protozoan taxa accrual patterns are similarly affected by stress, 2) to assess the usefulness of the MacArthur-Wilson model, and particularly the parameters  $S_{eq}$  (number of species at equilibrium) and  $G$  (the fitted rate constant) in predicting the impact of

industrial effluents, and 3) to determine whether the colonization data can be compared to traditional community structure parameters.



Table 1. Nonlinear regression parameters for published time-course sampling of macroinvertebrates on artificial substrates.

	$S_{eq}$	G	$t_{90}$	pr>F
Cover & Harrel(1978)	50.6	0.09	25.5d	0.001
Ulfstrand et al.(1973)	55.0	0.32	7.23	0.001
Nilsen and Larimore (1968)	15.9	0.24	9.5	0.01

## MATERIALS AND METHODS

Time-course samples of organisms accumulating on artificial substrates were taken from two industrially impacted streams in Southwestern Virginia. The data were used to construct colonization curves and to generate community structural information. The two sites were chosen for their distinct differences in effluent composition and the availability of similar riffle areas at all stations.

### SITE CHARACTERIZATIONS

**Thermal-Organic effluent:** Study 1 was carried out on the New River in Montgomery County, VA (Fig. 1A) in late summer of 1984. The site is approximately 16km downstream of Claytor Lake, an impoundment from which water is released daily depending on hydroelectric demand. Water flows over a bed of sheetrock and gravel at depths ranging from 0.6 m to greater than 3 m at the sampling stations. The site receives a warm-water effluent containing secondary treated domestic waste, nitro-organic wastes, surface runoff, and some fly-ash residues.

Sampling stations were selected above, within, and downstream from the effluent, in riffle areas with substrate sizes ranging from a single layer of gravel to large cobbles and boulders at each station. The upstream

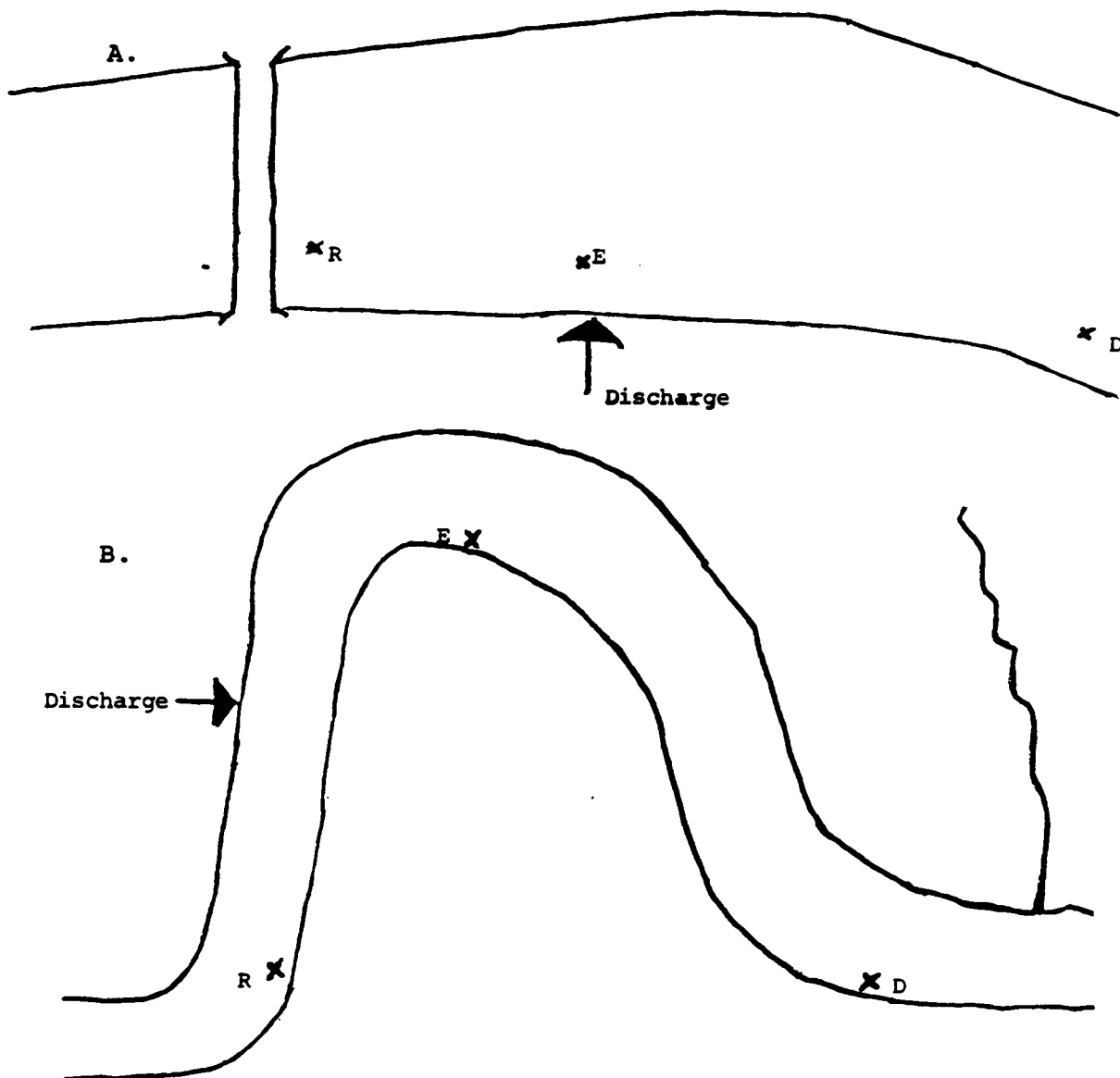


Figure 1. Location of sampling stations in the thermal-organic (A) and metals (B) effluents. R/reference E/effluent D/downstream stations.

(reference) station was about 70 m upstream of the effluent. The effluent station was located approximately 10 m below the discharge to minimize stranding of substrates on dry ground during low water. Visual inspection of the discharge plume verified that this station was impacted during low river-flow (as opposed to discharge flow), and probably during periods of high water as well. The downstream (or recovery) station was about 100 m below the effluent, near a bend in the river. There was no visible evidence that the effluent impacted this station.

Metals effluent: Study 2 was conducted in Peak Creek in Pulaski County, VA (Figure 1B) in late winter/ early spring, 1985. The site received a metal effluent containing iron (Fe) species and chromium; this effluent passed through a series of four settling ponds before discharge into the stream. At the point of discharge the effluent represents approximately one-half of the average stream flow. The creek flows over a bedrock and gravel substrate; the size of the loose substrate increased from gravel upstream to cobbles and boulders downstream, with a concurrent decrease in the number of loose rocks along the same gradient.

Sampling stations were chosen upstream, near, and downstream with respect to the discharge. The reference

station was located outside the plant property about 150 m upstream of the discharge. The effluent station, located about 50m downstream of the discharge, was within the plant boundary. The downstream station was located approximately 200m below the discharge, above the confluence of a small tributary.

#### ARTIFICIAL SUBSTRATES

Protozoans were collected on Polyurethane Foam (PF) unit artificial substrates, 5 x 6.5 x 7.5 cm (Cairns, 1971). The units were tied around the middle with cotton string, at 20 cm intervals. A string of 18 to 21 units was prepared for each station. Upon placement in the streams, the units were squeezed to saturate them with ambient water. The saturated substrates floated just below the surface of shallow or low-flow water. During high flow due to dam release in the New River they were at a depth of >2 m.

Macroinvertebrate artificial substrates consisted of cylindrical wire baskets, filled to a uniform weight of 7.3 kg with uniformly sized (minimum axis 1.5cm, maximum axis 7cm) local basalt or limestone rocks. Nylon rope was used to connect 15 to 18 baskets at each station; the rope was knotted so that individual substrates could be randomly

selected and removed without disturbing the other substrates.

#### SAMPLING REGIME

The PF units were placed in the thermal-organic effluent on August 20, 1984, and in the metals effluent on March 3, 1985. Three randomly selected PF units were collected from each station at 2 hours, 1, 2, 4, 7, and 15 days at both sites. Each PF unit was placed into a new ziploc bag and sealed with as much air as possible to prevent desiccation or loss of sample while allowing aerobic respiration to continue. The sealed bags were placed in a container of ambient (reference station) water to prevent the warm air temperature from affecting community composition during the trip back to the laboratory. The units were returned to the laboratory within 1 hr of collection.

The PF units were harvested by squeezing each over a clean beaker. The squeezings were allowed to settle for at least 30 min. at ambient temperature. Subsamples of 2 to 4 drops taken from the meniscus and bottom of the beaker were placed on clean glass slides and covered by 22 x 22 mm glass coverslips. The area under each coverslip was then thoroughly and systematically inspected for live protozoans. Two or 3 subsamples were usually counted for

each PF unit. Stewart et al. (1985), using the methods of DeCaprariis et al. (1978) noted that for PF unit samples two to three subsamples (slides) were usually sufficient to establish an asymptote in species number. Individuals were identified to genus or species when possible.

Unidentifiable individuals were sketched and described for later identification. Unusually abundant taxa were noted, as were unusually scarce taxa.

Macroinvertebrate substrates were placed in the thermal-organic effluent on August 16, 1984, and in the metals effluent on February 23, 1985. Three substrates from each station were randomly chosen and removed at 4, 8, 16, 35, and 44 days after placement in the organic effluent, and at 4, 8, 19, 32, and 56 days after placement in the metals effluent. At 4 days the substrates at the reference station in Peak Creek were moved to ensure that the substrates remained submerged when the water level dropped. Stream flow rate was measured on that date and on subsequent dates to verify that the flow was not different at the level of the substrates between the three stations in Peak Creek.

Baskets were collected by gently lifting them into a net positioned downstream to collect escaping invertebrates. On the stream bank the basket contents were emptied into a 0.5 in. mesh hardware cloth basket set

inside a plastic washtub. The net was inspected for clinging invertebrates, which were added to the tub. Alcohol (isopropyl or ethyl) or a 5% formalin solution was poured over the rocks in the tub, and the macroinvertebrates were removed with a gentle swishing in the preservative. The rocks were also individually inspected for clinging macroinvertebrates. The resulting sample was concentrated through a 235  $\mu$ m standard sieve and placed in a mason jar containing preservative.

Samples were hand-picked in the laboratory under a dissecting scope. Macroinvertebrates were identified to genus or species except for the Chironomidae (Diptera), Tricladida, and Annelida: chironomids were identified to tribe, and the worms to phylum or order as indicated.

#### STATISTICAL METHODS

Mean numbers of species were plotted against time for the protozoans and macroinvertebrates colonizing the artificial substrates. The data were compared to the MacArthur-Wilson equilibrium model by nonlinear least-squares regression using SAS (SAS Statistics manual, 1984). An  $r^2$  test was performed to determine if the model adequately described the variation in the data. An estimate of the time required to reach 90 percent of the equilibrium number of species, or  $t_{90}$ , was calculated using



the formula

$$t_{90} = \frac{2.303}{G}$$

Differences in the number of species at equilibrium and overall differences between species numbers at the different stations were analyzed by analysis of variance (ANOVA) tests.

Community similarity coefficients computed from presence-absence data (Pinkham and Pearson, 1978) were used to produce dendrograms to show differences in community composition between stations and times. The Pinkham and Pearson equation was designed for pollution studies; its strength lies in its ability to distinguish between taxa within an assemblage, while accounting for the differences in species richness and total abundance that traditional diversity indices use (Pinkham and Pearson, 1978). Community diversity, changes in the proportion of functional groups, and mean total numbers for the macroinvertebrate equilibrium data were compared by inspection to the colonization curves, regression coefficients, and community similarity information to determine which measures contribute the most to the assessment of impact or stress. Changes in the proportion of functional groups in the protozoan component of the community were also assessed using the groupings described by Pratt and Cairns (1985).

## RESULTS

### COLONIZATION DYNAMICS

Thermal-organic effluent: Colonization curves for the three stations in the thermal-organic effluent indicate that protozoan colonization rate ( $G$ ) was accelerated at the effluent and downstream stations as compared to the reference station (Figure 2 and Table 2). A Duncan's Multiple Range (DMR) test indicates no significant differences between 8 d and 15 d data except for the 8 d reference. In addition, the 4 d data for the reference and effluent are not significantly different from the 8 d and 90 15 d samples, supporting the  $t$  estimates of 2.3 to 4.6 d. The depression in  $G$  value at the effluent and an intermediate  $G$  value downstream for the macroinvertebrate colonization curves were more predictable (Table 2 and Figure 3). With the exception of the effluent samples on days 4 and 16, the number of taxa collected on all baskets on all dates were similar as indicated by the DMR test. This fact agrees with the  $t_{90}$  estimates of 4-6 d for the reference and downstream stations, and about 9 d for the effluent. There were no differences in the number of species at equilibrium ( $S_{eq}$ ) in either the protozoan or macroinvertebrate communities.

The initial stages of the protozoan colonization process differed between the reference and effluent

Table 2. Nonlinear regression parameters for artificial substrate colonization in a thermal-organic effluent.

	$S_{eq}$	G	$t_{90}$	pr>F
Protozoans				
REFERENCE	26.1	0.493	4.66 d	0.001
EFFLUENT	24.8	0.967	2.38	0.005
DOWNSTREAM	24.6	0.998	2.30	0.005
Macroinvertebrates				
REFERENCE	15.6	0.467	4.93 d	N.S.
EFFLUENT	16.2	0.253	9.10	0.005
DOWNSTREAM	16.4	0.395	5.80	0.010

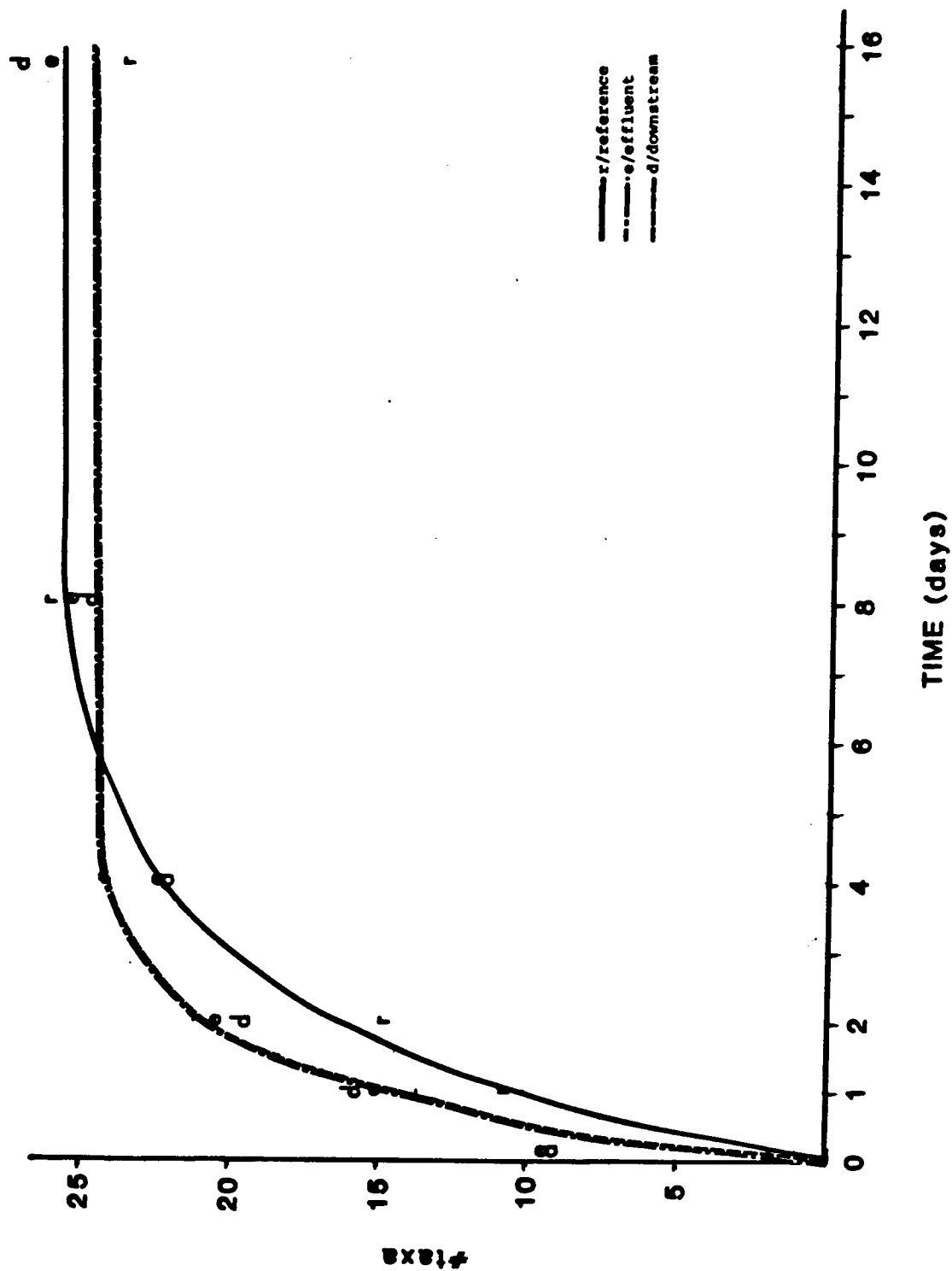


Figure 2. Nonlinear regression curves for protozoan colonization of artificial substrates in a thermal-organic effluent. Letters represent the mean of three sample values; curves are from regression of sample data using the equation

$$S_t = S_{eq} (1 - e^{-Gt})$$

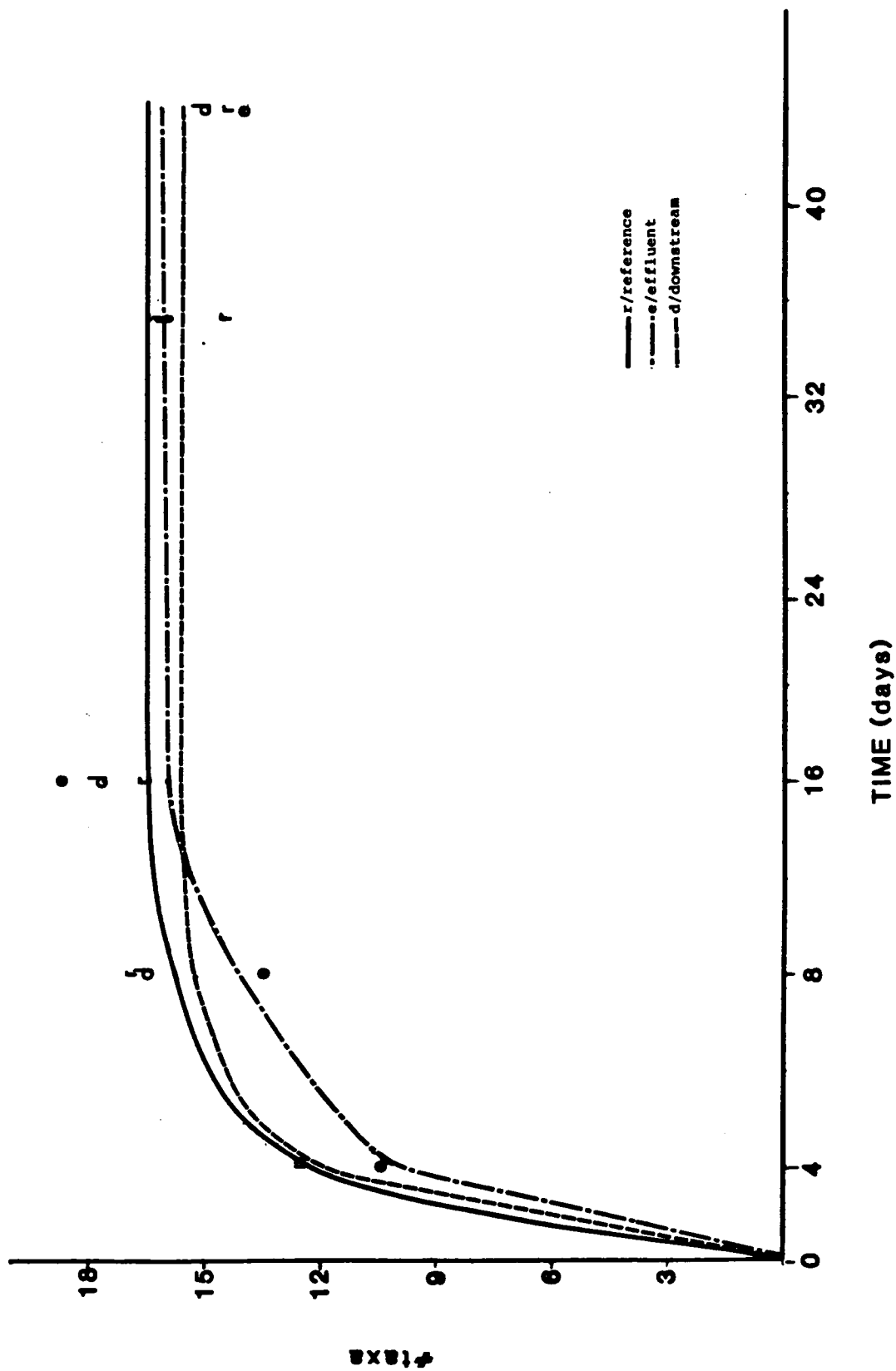


Figure 3. Nonlinear regression curves for macroinvertebrate colonization of artificial substrates in a thermal-organic effluent. Letters represent mean sample values; curves are from regression of sample data using the formula  $S_t = S_{eq}(1 - e^{-Gt})$ .

stations: the taxa commonly noted as "pioneers" (Yongue and Cairns, 1978) were present at all stations, but at the effluent impacted stations the community was augmented by larger flagellates and ciliates most common to late colonization stages. The large ciliates began to appear at the reference station at about 4 days.

Macroinvertebrate colonization in the thermal-organic effluent did not follow as distinct a pattern as protozoan colonization. Early colonizers at the reference station included Ephemeroptera nymphs (mostly Tricorythodes sp), gastropods, amphipods, and Brachycentridae. Bivalves (Corbicula fluminea) did not accumulate in baskets at the reference station in as great numbers as they did at the other two. Chironomids were scarce at that station throughout colonization, although they were common at the effluent-impacted stations; Orthocladiinae dominated early colonization; predaceous Tanypodiinae appeared as colonization progressed. The pulmonate snail Physella hendersoni appeared at the two lower stations late in colonization period.

Data from qualitative kick samples taken on days 16 (Figure 4) and 44 indicate that the basket samplers collect a few more species than does the surrounding natural habitat at the New River site, though there was little difference in species composition between the two. Total

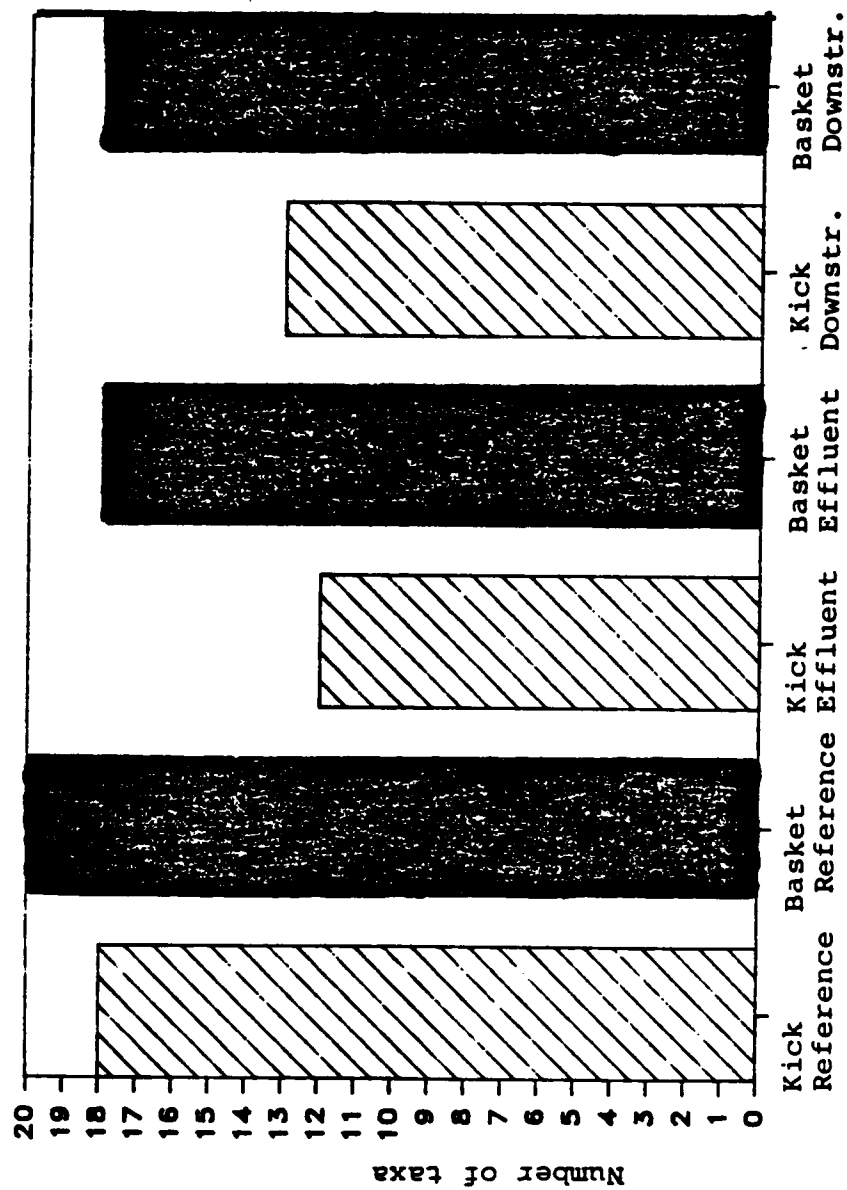


Figure 4. A comparison of the number of taxa collected on natural and artificial (Basket) substrates at stations in a thermal-organic effluent.

numbers and density may not be compared, because the surface area of both substrates is unknown.

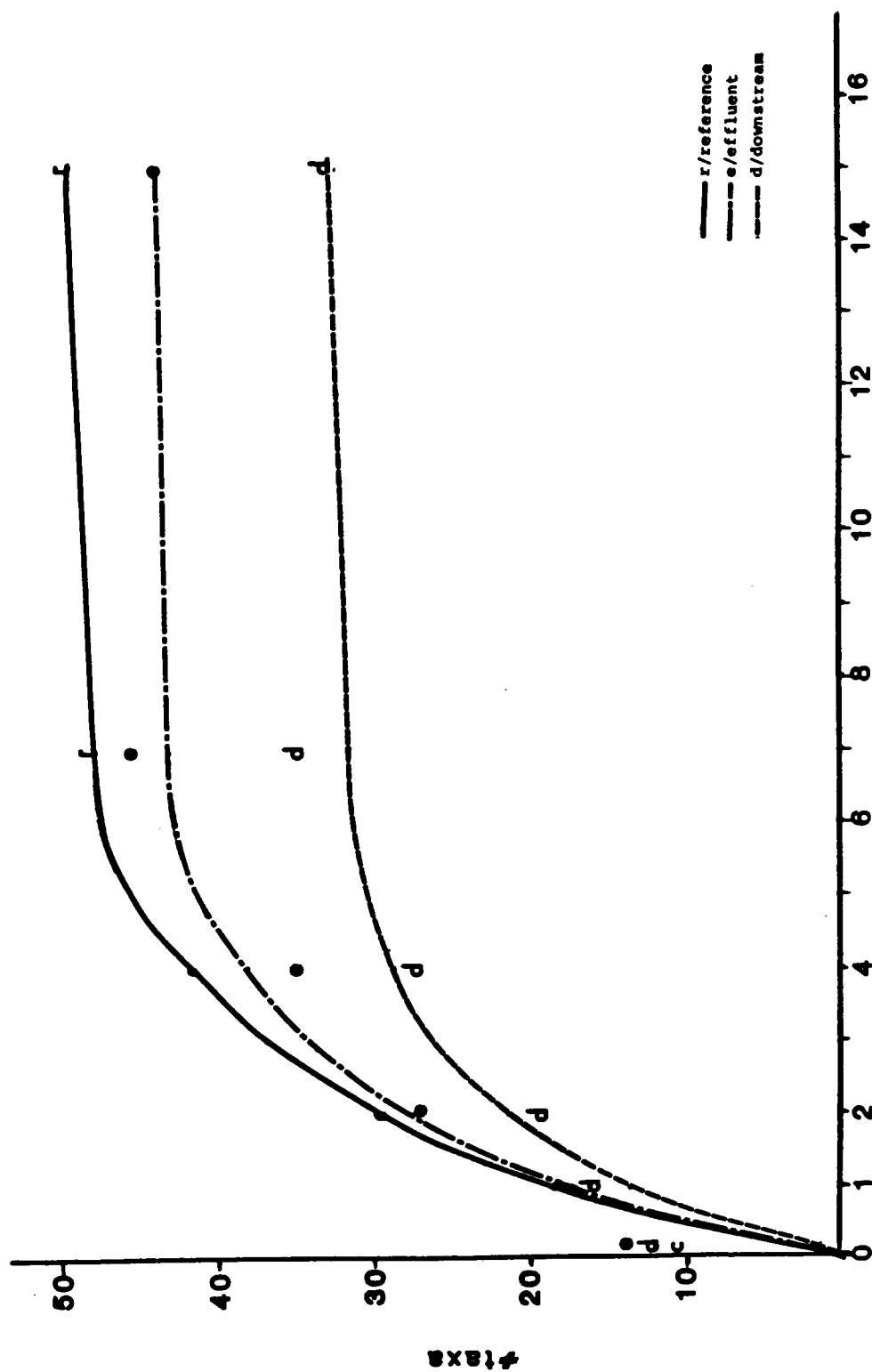
Metals effluent: Colonization patterns of protozoans and macroinvertebrates were more similar in the metals effluent. Protozoan colonization rates (G values) were not significantly different for the three stations (Figure 5 and Table 3), although the number of species at equilibrium was much lower at the downstream station. The number of species collected on the downstream PF units at 7 d and 15 d is comparable to the number collected at 2 d at the other two stations. The number of macroinvertebrate taxa was also depressed at the downstream station (Figure 6 and Table 3); in this case the number of taxa collected on the baskets at 56 d was significantly different at all 3 stations. No significant differences were observed in the G values.

Protozoan colonization patterns followed the frequently cited (Yongue and Cairns, 1978) sequence from small, biflagellated pioneers (such as Bodo sp., Cryptomonas sp., and Monas sp.) through moderately sized flagellates (Euglenoids) and small ciliates (like Holophrya sp. and Litonatus sp.), to include large ciliates (Paramecium sp., Oxytrichia sp., and dileptus sp.) at the later stages of colonization. Autotrophic taxa were again less common at the effluent-impacted stations at equilibrium. Diatoms were the most abundant autotrophs in



Table 3. Nonlinear regression parameters for artificial substrate colonization in a metals effluent.

	$S_{eq}$	G	$t_{90}$	pr>F
<b>Protozoans</b>				
REFERENCE	49.8	0.463	5.00 d	0.005
EFFLUENT	44.4	0.491	4.69	0.001
DOWNSTREAM	32.3	0.550	4.20	0.001
<b>Macroinvertebrates</b>				
REFERENCE	15.8	0.11	20.90 d	0.001
EFFLUENT	13.2	0.07	33.02	0.001
DOWNSTREAM	8.9	0.10	23.10	0.001



TIME (days)

Figure 5. Nonlinear regression curves for protozoan colonization of artificial substrates in a metals effluent. Letters represent the mean of three sample values; curves are from regression of sample data using the equation  $S = S_0 (1 - e^{-Gt})$

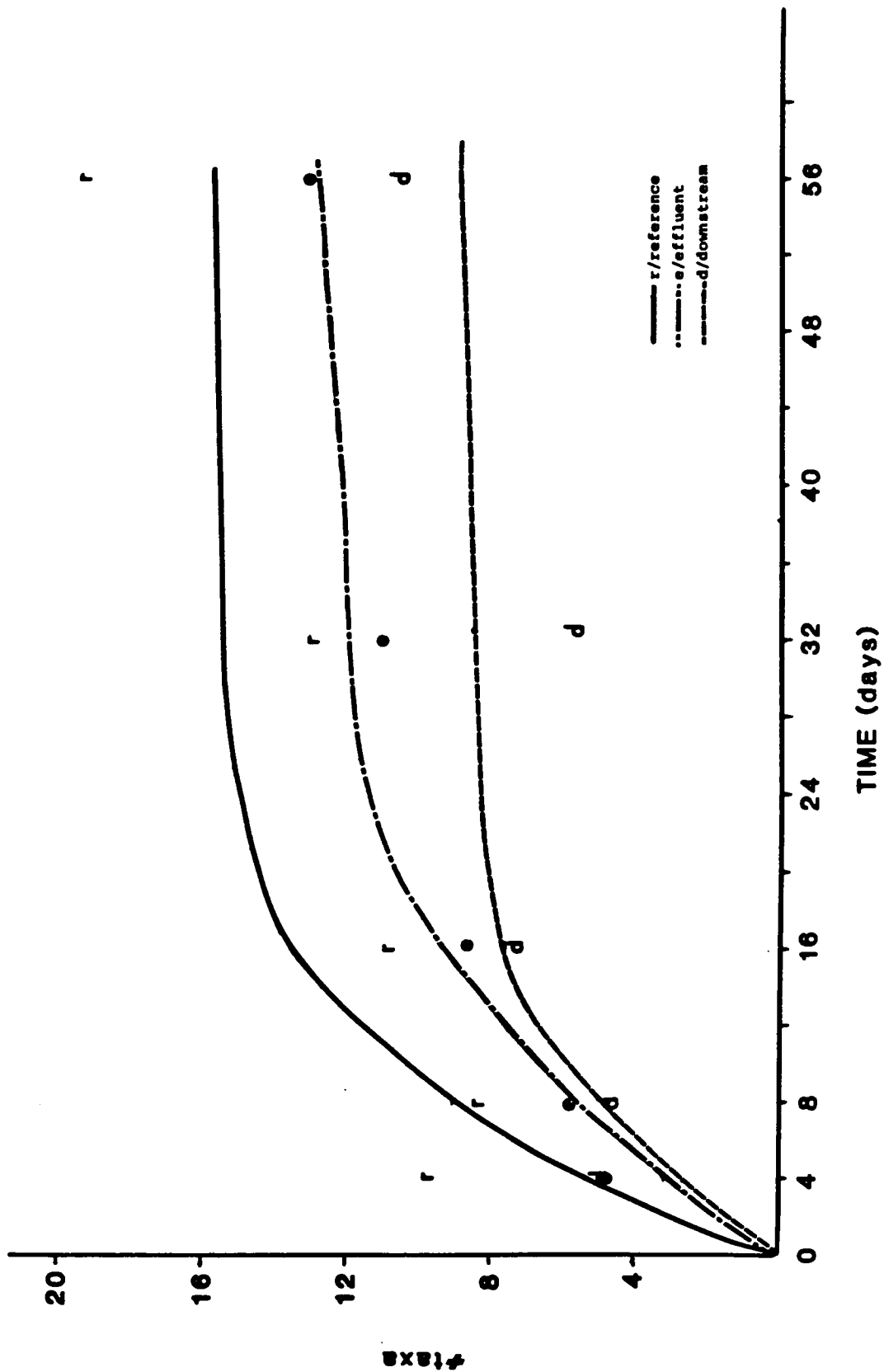


Figure 6. Nonlinear regression curves for macroinvertebrate colonization of artificial substrates in a metals effluent. Letters are the mean of three sample values; curves are from regression of sample data using the formula  $S_t = S_{\infty} (1 - e^{-Gt})$ .

the smaller stream.

The macroinvertebrate colonization pattern in the metals effluent may have been the result of seasonal succession or colonization. Prosimulium sp. dominated the early stages of colonization, especially at the two impacted stations. Later, Chironomidae were numerically dominant, even at the reference station. The taeniopterygid stoneflies present during February and early March sampling disappeared on later sampling dates, and several taxa of Trichoptera appeared later in the study.

Analysis of qualitative kick samples again indicated that the basket samplers collect about the same or a greater number of taxa than are present in the surrounding natural substrate (Figure 7), except at the reference station at 56 d, when there was an exceptionally large number of Trichoptera larvae. Some species of Hydropsychidae were more common in the kick samples, but the most striking difference was in the large numbers of Simuliidae collected on the baskets at the downstream and effluent stations early in colonization.

#### COMMUNITY SIMILARITY INDICES

Pinkham and Pearson "B" values were generated and the corresponding  $r^2$  values plotted for pooled data for each station and time. For the sake of clarity, truncated

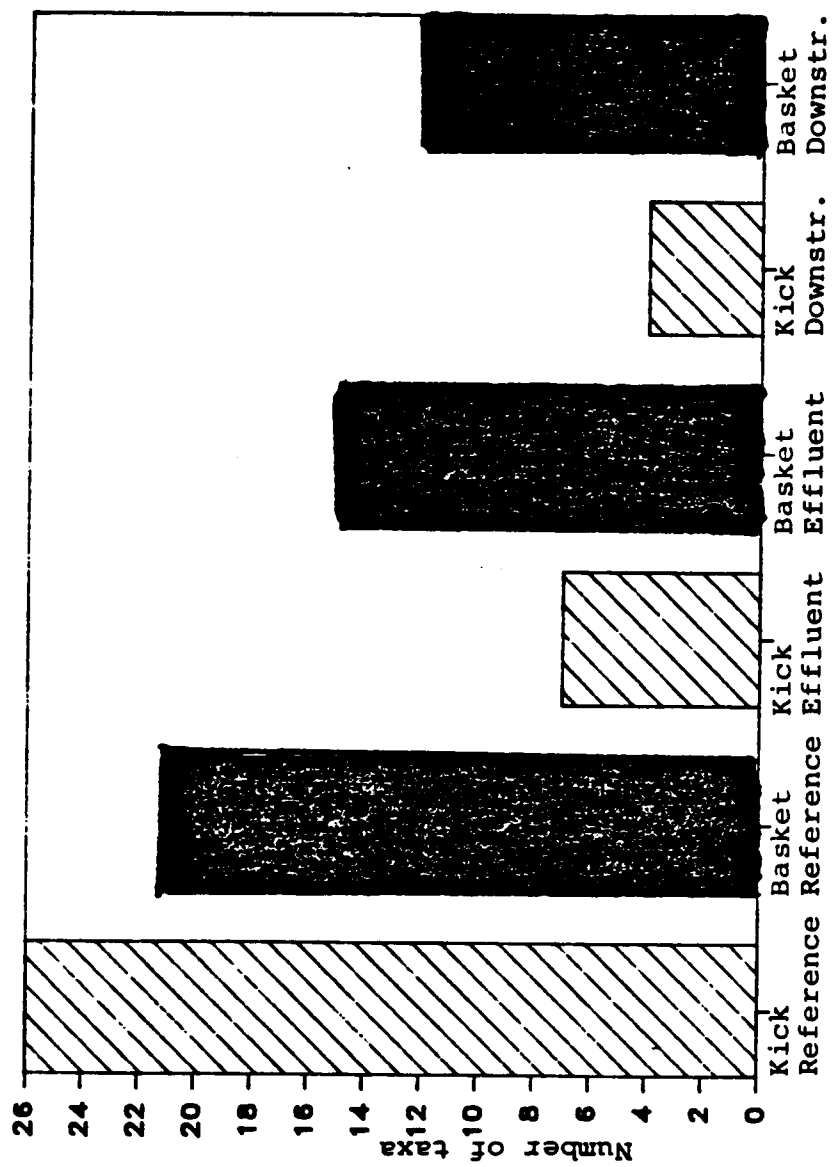


Figure 7. A comparison of the number of taxa collected on natural and artificial (basket) substrates at stations in a metals effluent.

dendrograms are presented in Figures 8-11; the plots are separated by date to determine trends in community similarity. The original dendrogram plots are presented in the Appendix.

The thermal-organic effluent protozoan dendrograms show no consistent trends (Figure 8): on three of the sample dates the reference community was distinctly different from the stations receiving the effluent; this was most notable after only 2 hr of colonization, when the reference PF units had only one or two taxa each. After 24 h and again at 15 d, the downstream samples are different. The difference between  $r^2$  values on all but the first sample time were negligible.

The thermal-organic effluent macroinvertebrate data (Figure 9) indicate that the three communities begin to converge later in the study, if a commonly accepted cutoff point of  $r^2 > 0.6$  (E.F. Benfield, pers. comm.) is used. The 44-day community (Appendix, Fig. A-2) was slightly less similar than the 35-day samples; this was probably a result of an upstream disturbance. The downstream station did not consistently cluster with either the reference or effluent community; effluent impact was evident on some sample days, and recovery evident on others.

The metals-effluent protozoan dendrograms (Figures 10 and A-3) indicate a distinctly different downstream

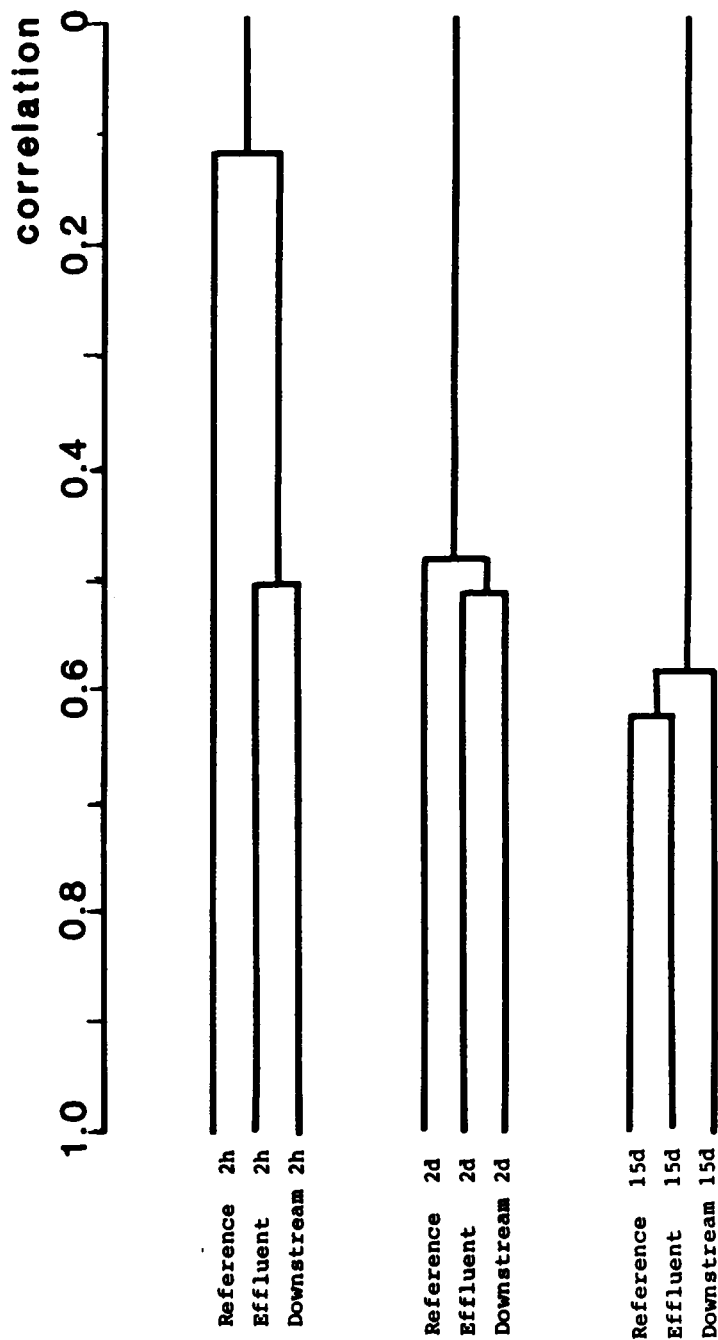


Figure 8. Community similarity dendrograms for protozoans in a thermal-organic effluent. The original dendrogram plot is shown in figure A-1.

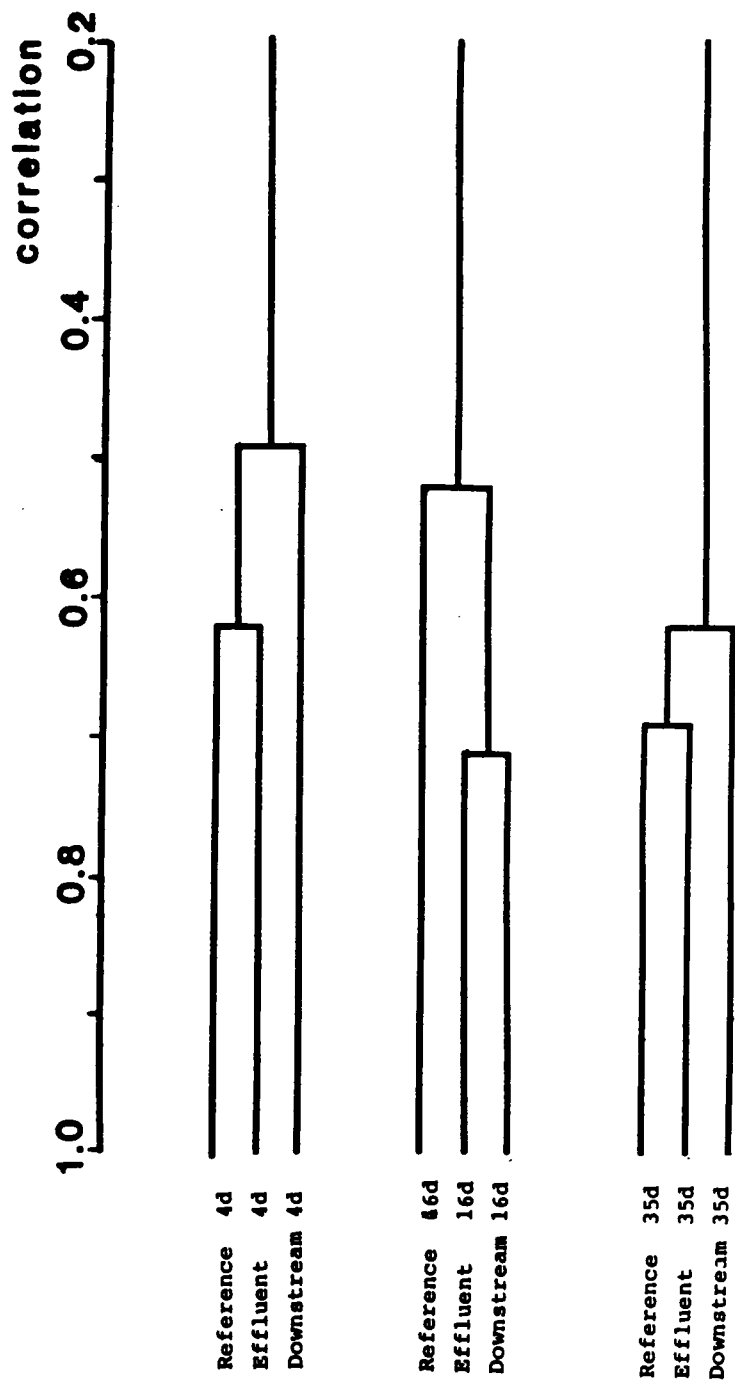


Figure 9. Community similarity dendrograms for macroinvertebrates in a thermal-organic effluent. The original dendrogram plot is shown in Figure A-2.



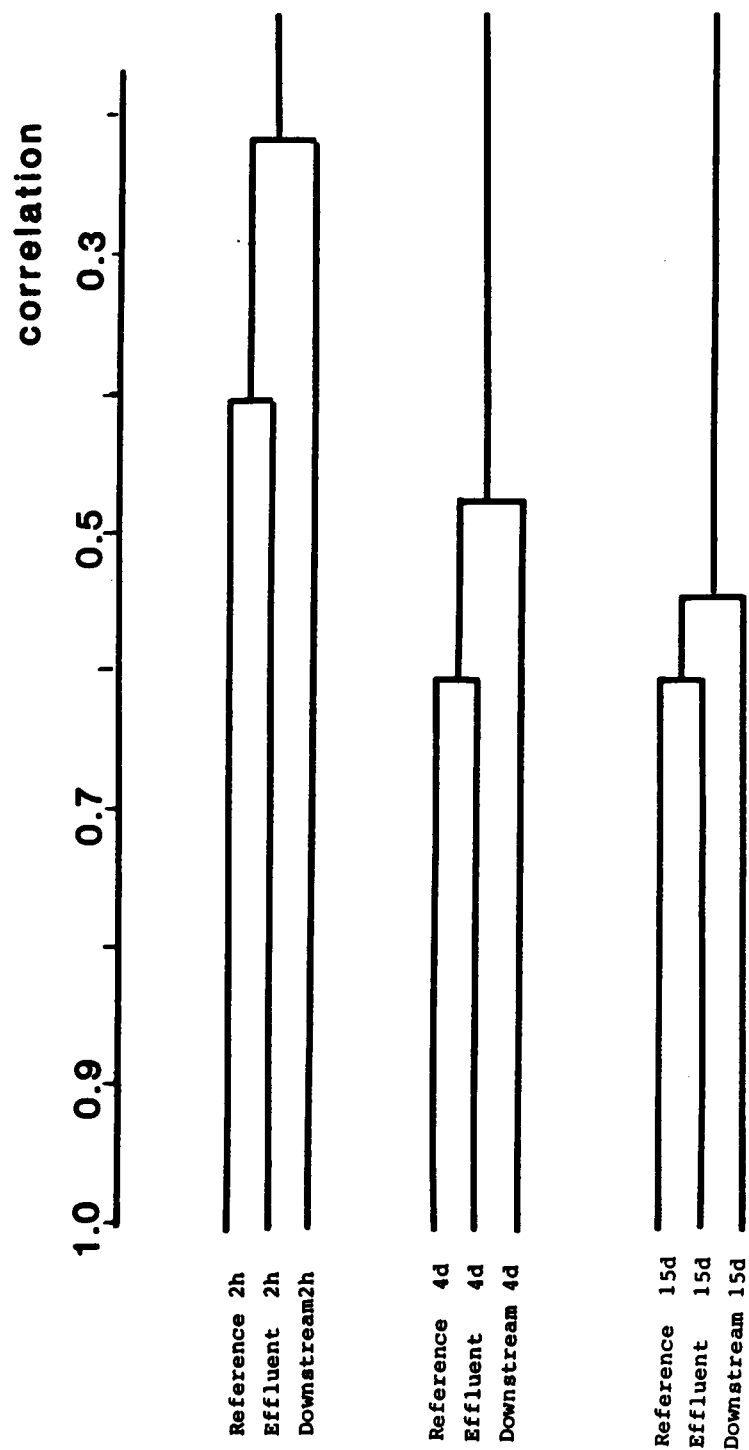


Figure 10. Community similarity dendrograms for protozoans in a metals effluent. The original dendrogram plot is shown in Figure A-3.

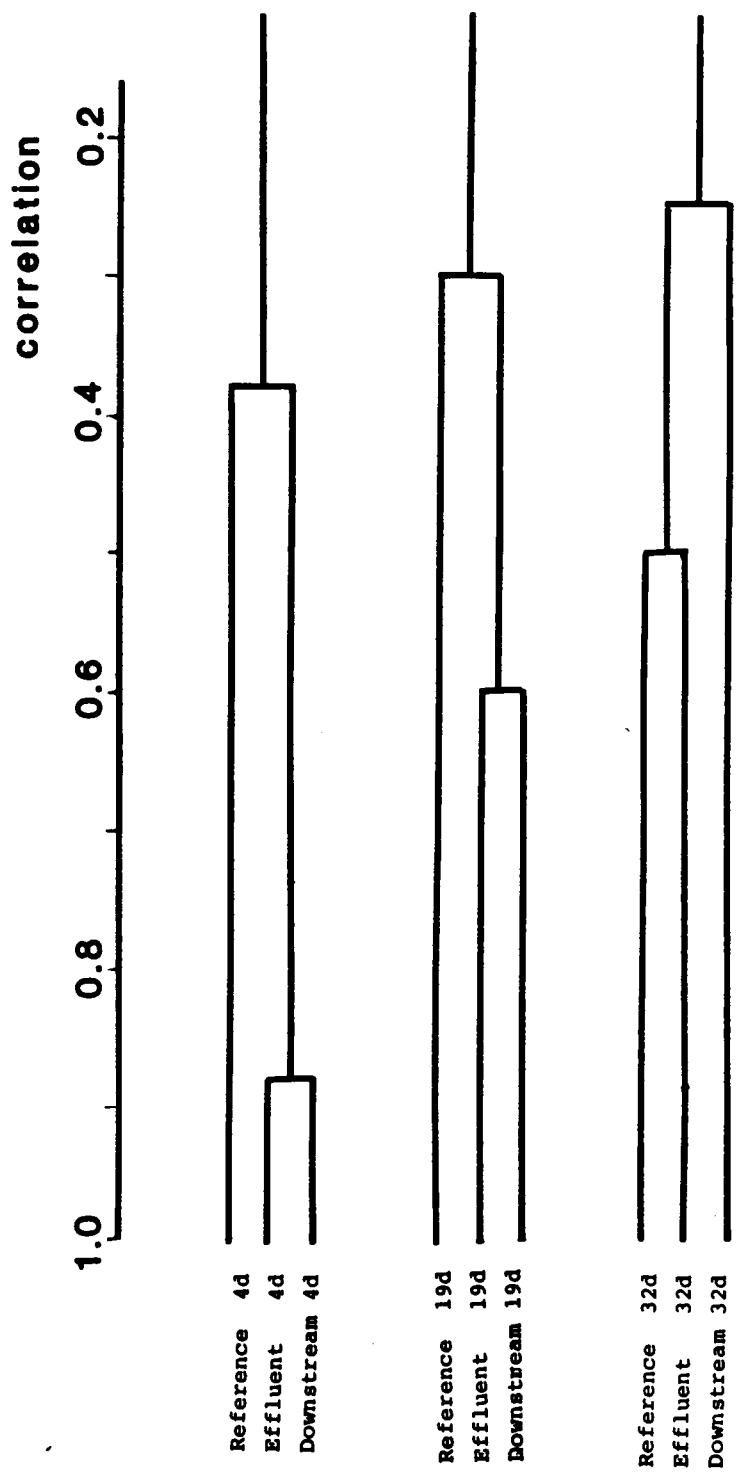


Figure 11. Community similarity dendrograms for macroinvertebrates in a metals effluent. The original dendrogram plot is shown in Figure A-4.

community on all sample dates except at 8 d (Fig. A-3), when the effluent community was significantly different. The macroinvertebrate dendrograms (Figure 11) for the same stream indicate that the reference station was different on the first three sampling dates; at 32 d (Fig. 11) and 56 d (Appendix, Figure A-4), the downstream station was different, due to the lower number of taxa colonizing the substrates. The differences in  $r^2$  values were considerable on all but the last sample date.

#### COMMUNITY STRUCTURAL CHARACTERISTICS

The macroinvertebrate community was also analyzed using a traditional structural parameter, the diversity index (Table 4). The diversity ( $d'$ ) values for the thermal-organic effluent equilibrium community were virtually equal, again indicating limited impact from this effluent. The total abundance of individuals per sampler was not different in mean numbers, but there was better replication (i.e., smaller standard deviations) on the effluent baskets. The control and effluent stations each collected three rare taxa (occurring only once or only at one station). There is no indication (EPA, 1973) that those taxa are particularly tolerant of organic pollution.

The reference station in Peak Creek had a much higher  $d'$  than the other two stations (Table 4); that station also

Table 4. Structural features of macroinvertebrate communities collected on artificial substrates. Figures describe the equilibrium community. Number per sampler is mean plus or minus one standard deviation.

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	#taxa	d'	# per sampler
<hr/>			
Organic Effluent			
REFERENCE	14	2.99	282+/-64
EFFLUENT	16	2.88	209+/-8
DOWNSTREAM	16	2.81	252+/-106
Metals Effluent			
REFERENCE	18	2.85	525+/-260
EFFLUENT	13	1.16	759+/-342
DOWNSTREAM	10	1.13	405+/-88

---

had a larger number of rare taxa. The large numbers of Simuliidae and Chironomidae at the effluent-impacted stations is largely responsible for their decreased diversity. The samplers at the downstream station collected a smaller mean number of individuals than baskets at the other two stations did, with a smaller standard deviation. However, the standard deviation of the means at the other two stations are quite large, and their ranges overlap that of the downstream station, so there is no significant difference between the means.

#### FUNCTIONAL GROUPS OF PROTOZOANS AND MACROINVERTEBRATES

Differences in the protozoan functional groups among the stations were determined using the classifications of Pratt and Cairns (1985). There were between 8 and 11 percent fewer autotrophic taxa at the effluent impacted stations at both the thermal-organic and the metals site (Figures 12 and 13). There was also a 14 percent increase in the number of bacterivores in the bacteria-rich organic effluent (Figure 13) as compared to the reference station. The proportion of non-selective species, algivores, saprotrophs, and predators varies from zero to 11 percent, but the differences in the number of species in each of these groups among the stations at each site is negligible.

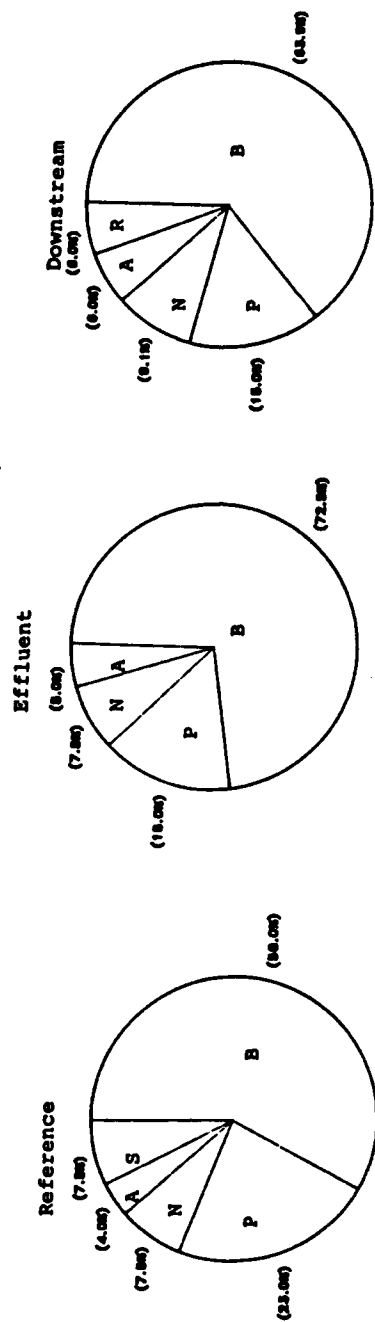


Figure 12. Changes in protozoan functional groups in a thermal-organic effluent. B = bacterivore, P=photosynthesizer, A = Algivore, N = nonspecific consumer, R = raptor, S = saprotroph.

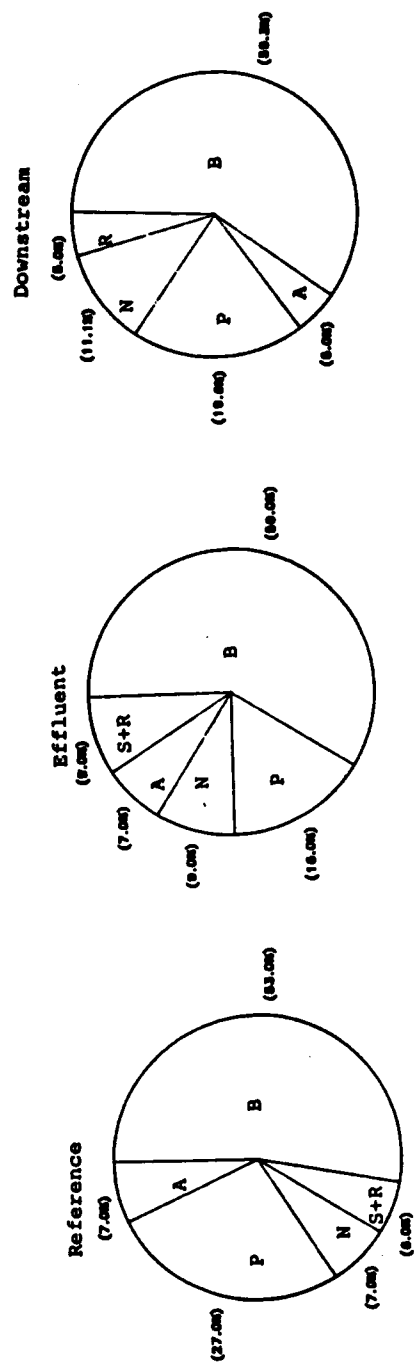


Figure 13. Changes in protozoan functional groups in a metals effluent.  
 B = bacterivore, P = photosynthesizer, A = algivore,  
 N = nonspecific consumer, R = raptor, S = saprotroph

An analysis of differences in the number of taxa and number of individuals in macroinvertebrate functional groups shows two trends: there were more scraper taxa colonizing the samplers at all stations at both sites; the percentage of individuals in the collector (filterer and gatherer) group shows a marked increase at both the thermal-organic stressed and metal-stressed stations (Figures 14 and 15). The increase is primarily attributable to the abundance of pollution-tolerant Orthoclaadiinae (Diptera) at those stations.



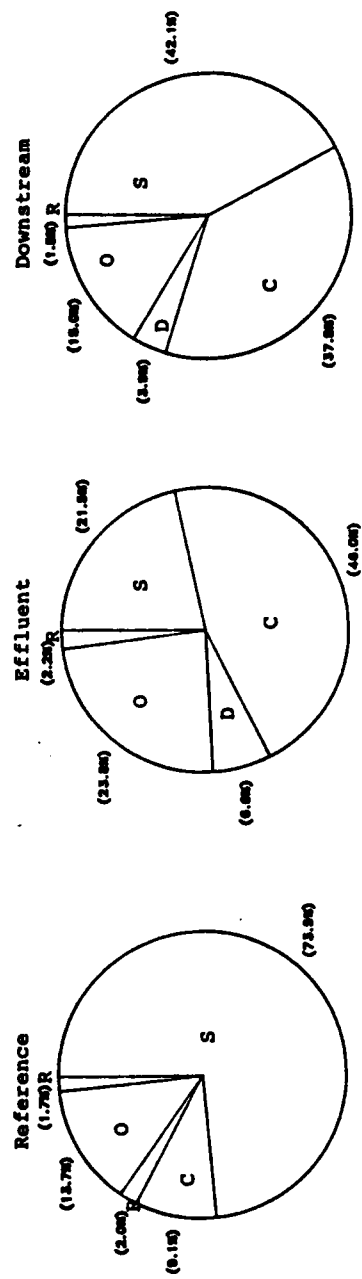


Figure 14. Changes in macroinvertebrate feeding groups in a thermal-organic effluent. S = scraper, C = collector, R = raptor, O = omnivore, D = detritivores.

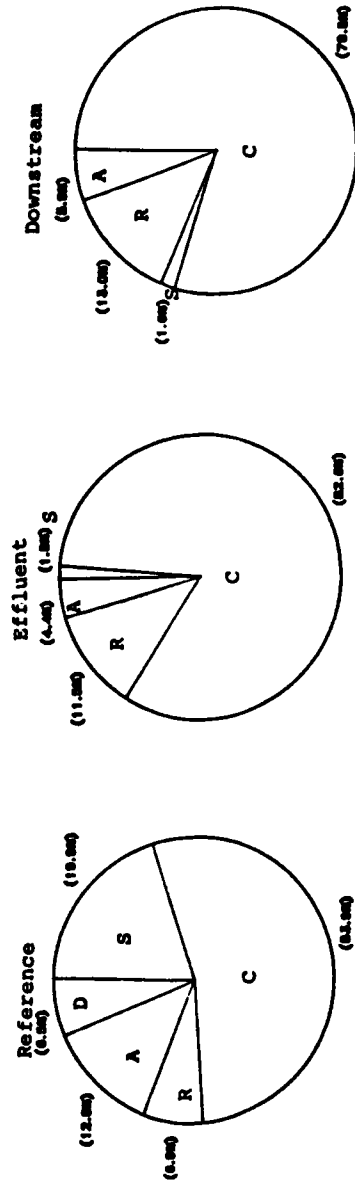


Figure 15. Changes in macroinvertebrate feeding groups in a metals effluent. S = scraper, C = collector, R = raptor, O = omnivore, A = algivore, D = detritivore.

## DISCUSSION

### COMPARISON OF COLONIZATION RATES

Protozoan and macroinvertebrate colonization dynamics were affected by pollution stress in both of the systems studied. The response of these two components of the aquatic community differed with respect to the parameters of the MacArthur-Wilson model. Protozoan colonization rate was accelerated in the thermal-organic effluent and downstream; this effect was not observed for macroinvertebrates. The highly enriched effluent contributed to the increased protozoan colonization rate in three ways. First, several authors have observed that protozoan colonization is faster in eutrophic waters (Kuhn, et al., 1980; Henebry and Cairns, 1985; Kuhn and Plafkin, 1977). Second, higher temperatures in the effluent facilitated colonization. Third, although the data provide a good fit to the model, the turbulent water near the effluent kept some of the community suspended, shortening the distance between the species pool and the PF unit islands. The thermal-organic effluent also supports a sewage-type microbial community; the similarity between the reference and impacted stations at equilibrium is probably due to the impact of domestic sewage effluents located several km upstream. Although domestic sewage effluents are chlorinated, resistant forms may survive and

flourish shortly downstream of such outfalls, where the chlorine dissipates rapidly but nitrogenous wastes persist.

The macroinvertebrate assemblages collected more slowly in the effluent and recovered somewhat downstream, but equilibrium numbers and community structural measures indicated that the established communities, whether protozoan or macroinvertebrate, were not different between the three stations.

The changes in protozoan and macroinvertebrate colonization dynamics in the metals effluent, as measured by  $S_{eq}$  and  $G$  were comparable to one another: the substrates at the downstream station collected fewer species of either group than the reference or effluent substrates collected. There were no significant differences in  $G$  for either protozoans or macroinvertebrates, making projections of  $S_{eq}$  from early colonization data similar for all stations. Thus, it is misleading to use  $G$  alone for water quality assessment, without also looking at the sample  $S_{eq}$ .

The uniformly-sized artificial substrates provide a standardized habitat among the three stations at the metals site, where the natural substrate sizes are so different, removing that potentially confounding factor from consideration. This fact is especially important because the low-complexity, low surface area sheets of rock at the downstream station may (albeit arguably) support fewer

species. In addition, the substrates at the downstream station were covered with a rusty brown sediment which was minimal at the effluent station and not seen at the reference station. This station is sufficiently far from the effluent for the oxidation states of the metals (especially Fe) to change to those less soluble at ambient pH and for chelation by dissolved organic materials to occur. The resultant precipitate or floc may increase the availability of the metals to the benthic organisms or physically coat the habitat making it unsuitable for colonization. Thus, the difference in species richness on artificial substrates at the downstream station may be attributable to a "chemical precipitation zone."

The MacArthur-Wilson model adequately described protozoan colonization at both sites, and macroinvertebrate colonization in all cases except for the reference station in the thermal-organic effluent. The poor fit in this last case may be the result of any or a combination of the three factors listed below. First, the variation between 'replicate' samples was large in proportion to the number of taxa collected, which was relatively small. Second, the samplers were placed on the stream bottom, where variability of artificial substrate samples is known to increase (Mason et al., 1973). However, the shallow streams precluded suspension of the substrates above the

bottom. The placement of the samplers in direct contact with the substrate may also prevent them from serving as true islands, although drift is the major means of macroinvertebrate colonization in streams (Minshall et al., 1983; Townsend and Hildrew, 1976). Thirdly, the lower number of taxa observed at the reference station as well as at the two effluent-impacted stations on the later sample dates supports the prediction of an interactive equilibrium community (MacArthur and Wilson, 1967). Peak numbers of species occur at the control station on sample day 8 and between 8 and 16 for the other two stations and decrease, then equilibrate. Removal of the interactive stage (35 and 44 d) data from the nonlinear regression improved the fit of the noninteractive model at all three stations and increased the predicted  $S_{eq}$  values.

Changes in colonization dynamics with respect to the MacArthur-Wilson model may be useful in assessing water quality. Whether one parameter, i.e.,  $G$  or  $S_{eq}$ , is more predictive remains to be clarified. Inspection of the equilibrium model indicates that  $G$  and  $S_{eq}$  are independent, but correlated. Early colonization data ( $S_t$ ) and the  $G$  value, used to project or predict  $S_{eq}$ , may result in an overestimation. Communities with a higher  $S_{eq}$  often have a lower  $G$  value, as  $G$  is essentially a measure of the proportion of  $S_{eq}$  collected per unit time. The organic and

metals effluent data indicate that  $G$  may be quite different among communities with no difference in  $S_{eq}$ , or similar in communities with great differences in equilibrium numbers, respectively. Using  $G$  as a criterion for water quality assessment would be misleading in either case. Differences in  $S_{eq}$  are more representative of conditions of the natural community which is presumed to be at equilibrium.

Macroinvertebrate communities, however, are notoriously heterogeneous, and the number and type of taxa per unit of habitat may vary widely. Community structural parameters point up such differences where they occur.

Time-course sampling of artificial substrates is still necessary, even if the equilibrium community is to be analyzed, to determine that an asymptote in the number of species has been reached. Based on the data presented here, two sampling dates are suggested in lotic environments, at 2 and 8-10 d for protozoans, and for macroinvertebrates, one a week to 10 d after placement of the substrates and the second about 14 d later. This appears to be more than adequate time for the control community to reach equilibrium. When reference and impacted stations are to be compared, it is important that the community collected at the reference station reach equilibrium; effluent-affected stations may take longer to equilibrate, indicating an impact. The time required to

reach equilibrium, or  $t_{100}$ , may also be a useful parameter. It is determined by observation, rather than calculation, and is thus independent of G. If the researcher prefers to analyze the equilibrium community at all sampling stations the  $t_{100}$  value is useful, but requires more extensive time-course sampling, such as was carried out in this study.

Traditional structural methods of assessing the community may be applied to data from artificial substrate colonization. This is more easily done with the macroinvertebrate data because total numbers, distribution of individuals among taxa, and species richness data are available in each sample. Protozoan artificial substrates, on the other hand, provide only species richness information. The PF units produced too large a sample for exhaustive analysis, rendering the data semiquantitative because only relative abundances may be estimated. Relative abundance estimates are both subjective, and affected by subsample bias.

The species diversity index for macroinvertebrate data at equilibrium indicated no difference between the three stations in the thermal-organic effluent; thus, in addition to having both the same number and kinds of taxa, the distribution of individuals among the taxa was similar. The diversity index does not indicate which species were numerically dominant, a feature allowed by manipulation of



the community similarity index (Pinkham and Pearson, 1976). The metals effluent provided species diversity indices which concur with the differences observed in the colonization data. Simuliidae and Chironomidae are numerically dominant at the effluent and downstream stations, far outweighing the contributions of taxa with fewer individuals. The difference between the effluent and reference stations is more obvious from this index than with any other means of assessment used in this study.

Community similarity indices provide additional information for both the protozoan and macroinvertebrate factions. Presence/absence data were used for both groups for consistency. By taking the identity of the separate species into account along with species richness, differences in community composition were highlighted. Species richness has an obvious effect on community similarity: the downstream station in the metals effluent receives a null match for every paired comparison involving the additional taxa colonizing at the other two stations. The difference between the three communities decreased at the end of the sampling as evidenced by both the protozoan and macroinvertebrate subcomponents; while the downstream samples became more similar, the difference in the number of taxa prevented them from being identical. Presence/absence data used for the community similarity

indices do not point out the dominance of any particular taxonomic group, although such a manipulation is possible if numbers of individuals is used.

Functional groups of protozoans have been defined by Pratt and Cairns (1985). For these groupings, feeding habits are determined at the genus level, except where differences between congeneric species are known. Changes in functional feeding groups of the protozoan assemblages may be more sensitive indicators of stress than macroinvertebrate feeding groups, since protozoans are in more intimate contact with the chemical environment (Cairns, 1982). Inorganic enrichment (i.e., the addition of nitrogen and phosphorus) will affect the autotrophic component of the protozoan community directly (Henebry and Cairns, 1984), and organic enrichment may alter the proportion of saprotrophic and detritus-feeding taxa in the protozoan assemblage. The decrease in the proportion of autotrophic protozoans collected at the effluent-impacted stations at both sites indicates that autotrophs are sensitive to the chemical conditions of the habitat.

Macroinvertebrate feeding groups are also affected by nutrient availability, since an increase in suspended particulate food in the thermal-organic effluent caused an increase in collectors at the impacted stations. Another factor causing an increase in collectors at both sites, and

particularly at the more obviously affected metals site, is the tolerance of the predominant Diptera including the Orthocladiinae, which are primarily collectors (Merritt and Cummins, 1974), to a variety of pollutants (REF). In the metals effluent there may not be an increase in filterable food, but there is probably a decrease in the number of interspecific competitors.

The founding premise for this study was that the various taxonomic levels of a community are expected to exhibit overall function which is comparable from one level to the next; this premise is a consideration in the development of ecological models designed to describe community function. The data collected for my study, which compares the functional response of two different levels of the community, indicates that this premise is not necessarily true. Protozoan and macroinvertebrate response to stress, in terms of changes in colonization rate, species richness, and functional groups, are not consistently comparable. Thus, while changes in community function are probably more pertinent than community structure in assessing the health of an impacted stream or pond (John Cairns, Jr., pers. comm.) it is unknown which, if any, taxonomic level of the community may function in a manner most representative of the entire community.

## CONCLUSIONS

Colonization dynamics of components of the aquatic community are affected by stress. Protozoan colonization dynamics do not respond in the same way as macroinvertebrate dynamics in organically enriched effluents and should not be substituted for macroinvertebrate colonization, particularly where a secondary protozoan source pool is suspected.

Early colonization data are not indicative of equilibrium conditions. The rate constant  $G$  may indicate a difference in accumulation rate, suggesting an impact, in cases where the equilibrium community is not seriously affected. The number of species at equilibrium may be significantly different at impacted stations which do not have a different  $G$  value. Equilibrium parameters provide the most information about the health of the community.

Structural descriptors of the equilibrium community are capable of indicating impact in stressed ecosystems. The community similarity index, which accounts for species identity as well as species richness and total abundance (when quantitative data are used) points out differences in evenness and species composition which result from environmental change. Functional group changes illustrate the effect of effluent impact even in cases where diversity indices show no difference, contributing to the strength of

an assessment.

Artificial substrate samplers for water quality assessment are best used as passive samplers of the equilibrium community. Short time-course sampling is recommended to establish the time to reach equilibrium, at least in the reference community. An interactive colonization stage may be reached at stations which colonize to equilibrium more quickly, if the researcher waits for all stations to reach equilibrium before analyzing community structure. The interactive-stage community is then likely to have a lower species richness and may also support a different community than those at the the noninteractive stage.

The assertion that a community functions similarly at all of its levels of organization is challenged by the data presented in this paper. Because this belief is fundamental to the development of several ecological theories, experimental evidence to support or negate it should be sought.

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## **APPENDICES**

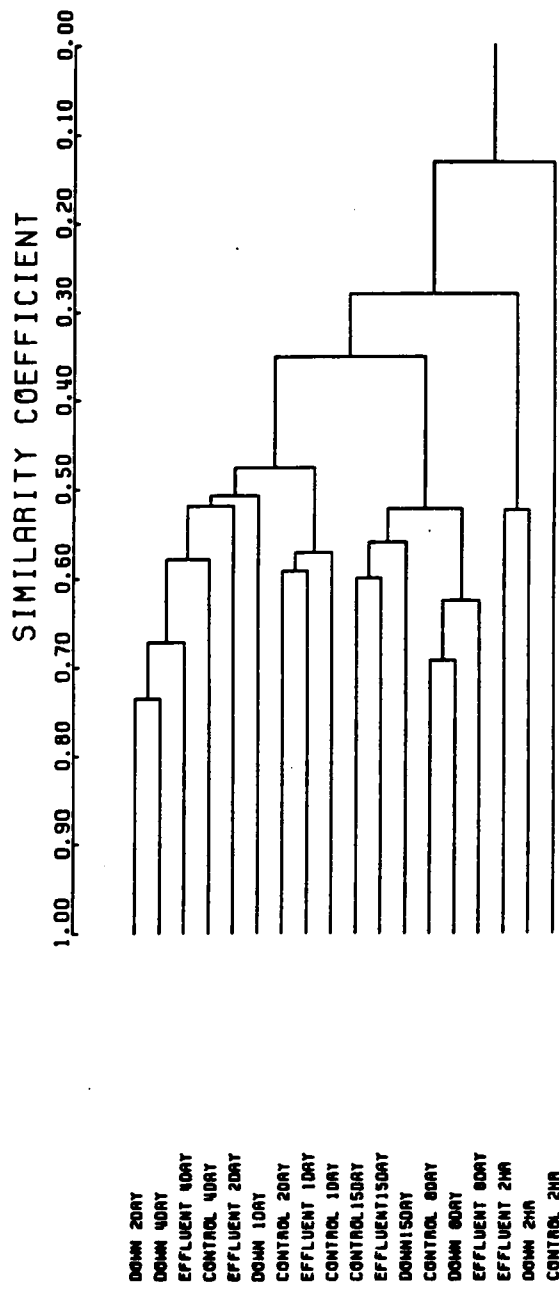


Figure A-1. Original dendrogram plots of protozoan community similarity in the thermal-organic effluent. A truncated figure, separated by date, appears in Figure 8.

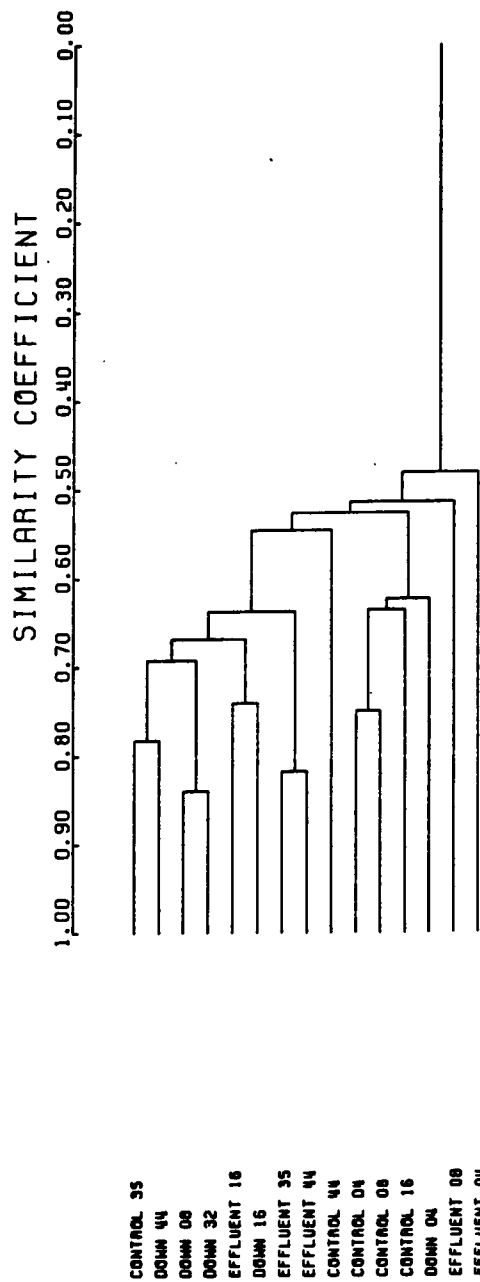


Figure A-2. Original dendrogram plot of macroinvertebrate community similarity in a thermal-organic effluent. A truncated figure, separated by sample date, appears in figure 9.

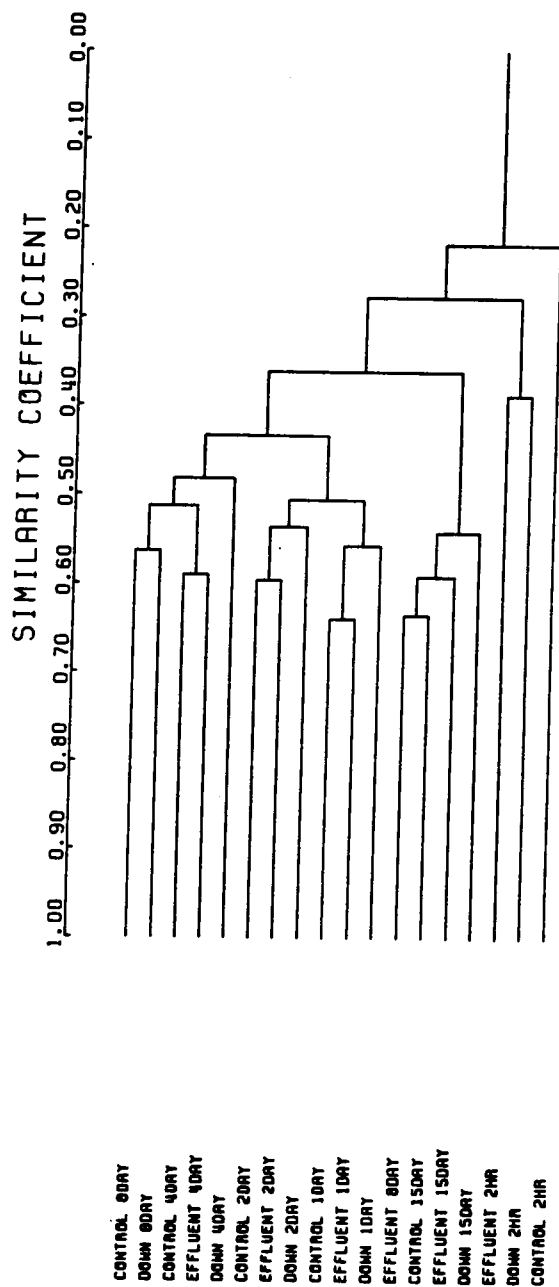


Figure A-3. Original dendrogram plot of protozoan community similarity in a metals effluent. A truncated version, separated by sample date, appears in Figure 10.

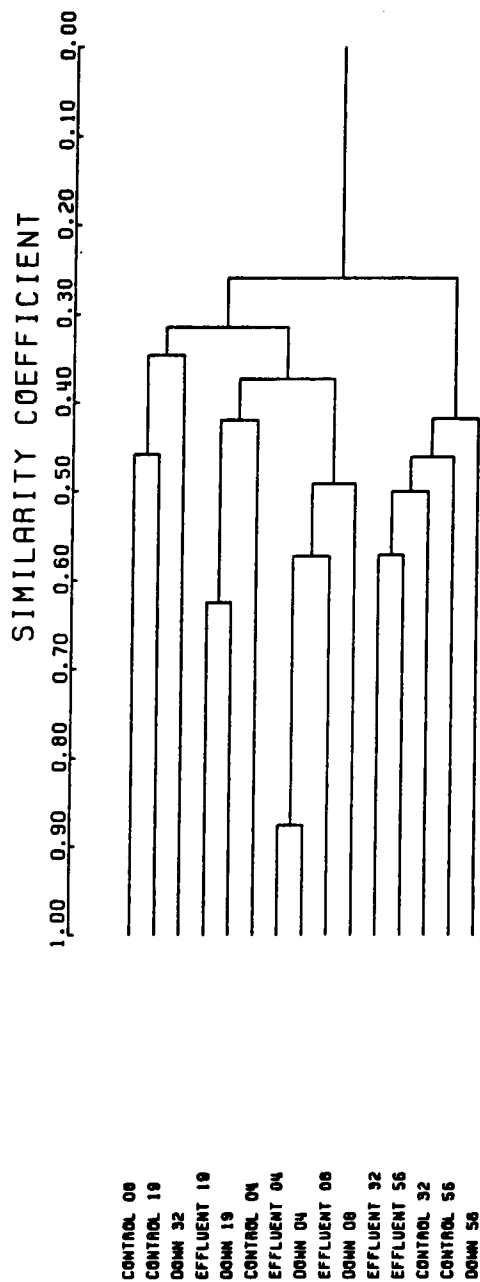


Figure A-4. Original dendrogram plot of macroinvertebrate community similarity in a metals effluent. A truncated version, separated by sample date, appears in Figure 11.

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