THE EFFECTS AND FATE OF LAMPRICIDE (TFM: 3 - TRIFLUORMETHYL - 4 - NITROPHENOL) IN MODEL STREAM COMMUNITIES

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ABSTRACT

THE EFFECTS AND FATE OF LAMPRICIDE (TFM: 3-TRIFLUORMETHYL-4-NITROPHENOL) IN MODEL STREAM COMMUNITIES

By

Alan Walter Maki

The effects of lampricide (TFM: 3-trifluormethyl-4-nitrophenol) on the structure and function of benthic communities were studied in a series of six replicated indoor model streams. The systems were considered as models of a typical woodland stream each consisting of a 13 foot pool section and a 13 foot riffle section. Artificial illumination was provided for each stream at a uniform surface intensity of $900^{\pm}$ 50 ft-ca.

A specially developed in situ stream respirometry system was used for measurements of net primary production and community respiration in pool and riffle communities. Pre-treatment levels of gross primary production varied from 10.7 $mgO_2hr^{-1}m^{-2}$ to 79.0 $mgO_2hr^{-1}m^{-2}$ through the course of the year and were suppressed by 25-50% during exposure to 9.0 mg/l TFM. Community respiration varied from 10.5 to 36.2 $mgO_2hr^{-1}m^{-2}$ and was increased 3-50% by TFM treatment. Calculated P/R ratios proved to be sensitive indicators of the influence of the toxicant.

No effects due to the lampricide exposure could be demonstrated on periphyton community structure including measurements of diatom cell density, diversity, equitability, biomass or species composition of filamentous green algae.

A statistically significant decrease in the total number of macroinvertebrates per unit area was demonstrated following treatment. Complete recovery to pre-treatment densities was observed within 2 to 3 months primarily through drift input. No treatment effect was determined from measurements of diversity, equitability, number of taxa or production rates of the macroinvertebrate community. A four-fold increase in drift rates of TFM-susceptible species was observed in the experimental channels during treatment.

Mean growth rates of experimental and control populations of young of the year brown trout, Salmo trutta varied from 1.709 to 3.676 mg gm^{-1} day^{-1} in replicated experiments and no significant difference due to the lampricide treatment was demonstrated.

The uptake of TFM residues employing ^{14}C-TFM was demonstrated to be basically an adsorption process. A two component curve consisting of a rapid initial uptake rate during the first two hours followed by a reduced linear rate for the remainder of the 24 hour exposure best describes the uptake curve for all 20 plant and animal species examined.

Macroinvertebrate species with soft, relatively permeable integuments accumulated significantly higher residue concentrations than those species with hard chitinized or calcareous exoskeletons. Rates of loss of TFM accumulations were correlated with water current and substrate associations. The mean half-life for riffle species was 17.8 hrs. and pool-dwelling species had a mean half-life of 140 hrs.

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LITERATURE REVIEW

History of the Sea Lamprey in the Great Lakes

The history of the expansion of the sea lamprey, Petromyzon marinus Linnaeus through the Great Lakes has been well documented (Hubbs and Pope, 1937; Shetter, 1949). Prior to 1921, the sea lamprey had been found only in Lake Ontario and its tributaries where it is apparently native (Dymond, 1922). Their attacks on local fish species there were noted at an early date (Gage, 1893; Surface, 1898, 1899).

Until relatively recent times the expansion of the sea lamprey through the remaining Great Lakes was halted by the natural barrier imposed by the Niagara Falls. It is generally accepted that the completion of the Welland Canal from Lake Ontario to Lake Erie in 1829 opened the route for the movement of the sea lamprey into the upper lakes. The construction of the Trent Waterway connecting Lake Ontario with the Georgian Bay of Lake Huron in 1918 opened another possible route of introduction (Applegate, 1950). During the next 25 years the record of the expansion through Lake Erie, Huron, Michigan, and Superior, in that order, was ascertained from singular fisherman's encounters and

documentation of spawning runs occurring in the tributari
The expansion was considered complete in 1948 with the first report of a sea lamprey from the Minnesota waters of Lake Superior. In 1947 and 1948 an inventory by the Michigan Department of Natural Resources indicated the presence of migrating sea lampreys or spawning activity in 92 Michigan streams in the drainages of Lakes Erie, Huron, Michigan and Superior.

Lamprey predation appears to be the cause of the decline of the lake trout fishery in Great Lakes waters through the 1940's and 1950's, and is probably the factor that is presently limiting abundance of spawning stocks in Minnesota, Wisconsin, and Michigan waters. This is indicated from an examination of the relation of wounding rates to length of trout (Pycha, 1970). The wounding rate for trout in the early 1960's averaged zero at about 13 inches, 20% at 20.5 inches and 30% at 22.5 inches. Very few fish survived past the length at which 25-30% of the fish were wounded which resulted in a very low spawning population. Summary data through 1972 have also shown the relationship of scarring frequency to total length is found in other species including chinook and coho salmon from Lake Huron and Michigan (Pycha, 1972).

The Development of TFM

The problem of controlling the sea lamprey in the Great Lakes has received considerable attention since their

impact on the commercial and sport fishery became apparent (Applegate and Moffet, 1955). Electromechanical wiers, traps and electrical fences were initially employed to block or destroy migrating adult sea lampreys. These devices, when installed in all known major spawning streams, provided an effective method of reducing the numbers of sea lampreys in each lake basin (Applegate, Smith and Nielsen, 1952; Erkkila, Smith and McLain, 1965).

Unfortunately, problems arising with the cleaning and maintenance of these structures particularly during spring runoff make these methods economically undesirable. Also, a control program based on the prevention of spawning has no effect on the substantial reserves of ammocoete larvae already existing in upstream areas. Since the larvae may spend as many as 5-15 years in the bottom muds of a stream before transforming (Howell, person. comm.), there exists the potential for continued production of high numbers of adult parasitic lampreys for several years after the installation of a 100% effective wier.

In light of these problems, research was initiated in the mid-1950's to find a more direct and immediate method for control of the sea lamprey. A detailed investigation of the life history of sea lamprey in Michigan revealed that the mean length of actual parasitic existence in the open waters of the lakes is only about 17 months. After this

parasitic stage, the sexually mature adults begin upstream spawning migrations in the winter months with the peak run occurring in late May and June. Spawning occurs in shallow riffles over a gravel and small rubble substrate with hatching approximately 20 days later. The newly hatched individuals (mean length approx. 8.3 mm) then burrow into soft silt banks and exist in relatively dense colonies with as many as 15 individuals per square foot in these backwaters and areas of low flow. The most vulnerable period in the life history of the lamprey appears to be this long, non-parasitic larval stage and the most direct attack would be the introduction of a toxicant to eliminate these larvae while they are concentrated in relatively high densities and relatively accessible areas of river bottom. The use of broad spectrum fish toxicants was undesirable because many of the lamprey spawning streams support substantial populations of other game fishes. Therefore it was decided to develop chemicals acutely toxic to larval lampreys at extremely low concentrations and non-toxic, at the same concentrations, to other fish species inhabiting the same natural environment (Applegate, et. al., 1957). The initial step in achieving this objective was the preliminary screening of a large and diverse series of predominantly organic chemicals. Static bioassays were employed as the test procedure and were designed to yield relative toxicity data at low levels within short time periods.

The screening tests revealed some compounds, at particular concentrations, to be more toxic to larval lampreys than to fishes. Among these substances was a group of mononitro-phenols consisting of several derivatives containing various amounts of the halogens, bromine, chlorine and fluorine. The differential toxicities of these mononitrophenols covered a sufficiently broad range of concentrations to permit their regulated application in natural streams to destroy lamprey larvae without damage to other fish. Requirements for a short treatment period were met by these compounds and at minimum effective concentrations of each of the candidate chemicals, all of the lamprey were killed in 16 hours. Two of the compounds killed all of the lampreys in less than 45 minutes without apparent harm to the game fish which remained exposed to these concentrations for 24 hours. Some of the compounds were also tested against several fish species in a running water raceway in which natural stream flow conditions were simulated. In these cases aqueous solutions of the sodium salts of each of these phenols were applied under conditions where continuous flow required their regulated metering into the stream during the entire test treatment period. Under these conditions, the toxic effects of the substances upon fishes seemed to be considerably less than the effects observed under laboratory-jar test conditions. Exposures to concentrations four times the

dose needed to kill 100% of the larval lampreys did not cause any evident harm to 12 different fish species present in the raceways (Applegate et. al., 1958).

Although all of these compounds are more toxic to lampreys than to most other aquatic organisms, certain ones are more desirable for practical applications because of their physical and chemical properties, ease of handling in the field, effectiveness at low concentrations, and cost. Of the compounds tested, TFM (3-trifluormethyl-4-nitrophenol) most closely met these requirements and was selected for development for field use (Applegate et. al., 1961).

Chemical and Physical Properties

TFM exists as a crystalline solid at room temperature, yellow to orange in color in its pure form, and light yellow-brown in technical grade preparations. It has a melting point of 76°C, molecular weight of 207 and ionization constant of 4.4×10^{-7} (DeBrouwer, 1930). It is sparingly soluble in water (0.498 gms TFM/100 gms HOH at 24.5°C) but highly soluble in most organic solvents. Aqueous solutions of TFM are acidic (pK 6.07 ± 0.03) and form phenolate salts in the presence of alkalis.

Phenolates of the alkali metals are weak bases, the free phenol is colorless in acid solution but deep yellow in base solution. The compound is highly stable and resistant to biochemical degradation. It is not detoxified by

any known natural process (Applegate et. al., 1958). Dawson, (1971) concluded that the toxicity of TFM to fish is not significantly reduced by short-term exposures to sunlight as an ultraviolet source.

TFM is selectively toxic among larval lampreys and fishes not only in the form of its free phenol but also as a sodium salt (Applegate et. al., 1958). The solubility of the sodium phenolate in water is much greater than that of the free phenol. Since the larvicide is best applied in the field as a water-miscible liquid formulation, only the phenolates are practical because their greater solubilities permit the preparation of more highly concentrated stock solutions. Certain of the mixed amine salts of TFM are as effective in their biological properties as the sodium salts. The amine salts have more desirable physical properties than the sodium salts, permitting commercial preparations of greater stock strength with better stability of the solutions when stored at low temperatures. Commercial formulations of the amine salts are used almost exclusively in the field treatments (Applegate et. al., 1961).

The TFM used as a lampricide in the United States is manufactured by the Farbewerke Hoechst Ag in Frankfurt, Germany, for the Great Lakes Fishery Commission. Technical and field grade formulations have the following composition:

Technical Grade: An aqueous formulation containing 82.4% TFM, used to formulate the field grade TFM (35%).

Field Grade: The field grade TFM (35%) is formulated with DMF (N,N-dimethyl formamide) in a highly basic solution.

Environmental Safety

Early field tests to determine the toxicity of TFM under natural stream conditions were conducted in the Mosquito, Silver, and Pancake Rivers in Michigan (Applegate et. al., 1961). Each application concentration was based on pretreatment toxicity tests conducted with water from the test stream. An application of 5.5 mg/l of TFM for 9 hours killed all lamprey larvae within 8 hours in the Mosquito River, and a concentration of 2.8 mg/l applied for 14 hours killed all lamprey larvae in the Silver River (U. S. Bur. of Comm. Fisheries, 1958). A range of 3 to 10 mg/l had been established for lamprey control in laboratory tests (Applegate and King, 1962).

A typical stream treatment involves conducting toxicity tests with actual stream water to determine the effective concentrations of the toxicant for each particular water quality. The term minimum lethal concentration is used to refer to the lowest concentration of TFM that kills 100% of the lamprey larvae in 9 hours or less, and the term maximum allowable concentration is used to refer to the highest concentration of TFM that does not kill more than 25% of the test fish in 18 to 24 hours. These two concentrations then define the concentration and time limits within which the

stream will be treated. The proper concentration of field grade TFM is then metered into the stream with calibrated pumps and the concentration is fortified at predetermined downstream locations to correct for dilution by tributaries and springs.

An experimental study was conducted in five streams tributary to Lake Superior and four tributary to Lake Michigan (Torblaa, 1968). Samples of the bottom fauna before and after treatment revealed that most groups of aquatic organisms were not adversely affected by exposure to lamprey larvicide. The total number of insects was less one week after treatment than before treatment, increased somewhat by 6 weeks after treatment and had returned to pretreatment levels one year after treatment. However, the sample sizes were small and the results may more accurately reflect inherent variability in substrate types than the effect of TFM.

A similar experimental design was employed by Haas (1970) to evaluate the effect of TFM on bottom fauna. Invertebrate community structure was unaffected by exposure to 1 mg/l but a treatment at 4 mg/l reduced the densities of all but two species examined. Small sample sizes and general variability in substrate type prevented an exact determination of the effects of TFM.

Exposure to a concentration of 10 mg/l TFM was fatal to 100% of exposed hydra, turbellarians, and blackfly

larvae, 99% of burrowing mayflies 89% of leeches and 50% of clams tested in static toxicity tests (Smith, 1967). Similar methods were used by Marking (1971) to determine the toxicity of TFM to two genera of aquatic invertebrates, snails (Physa sp.) and scuds (Gammarus sp.). The 96-hour LC_{50} for purified TFM was 1.29 mg/l for snails and 1.80 mg/l for scuds at 12°C.

TFM was tested by Marking (1971) for its toxicity to 11 species of cold and warm water fish. In standard static toxicity tests with all species, the LC_{50}'s ranged from about 1 to 6 mg/l of purified TFM. The toxicity of TFM was reduced by as much as a factor of 10 in hard water and by as much 59 at pH 9.5. In another investigation Dawson (1971) tested TFM against fingerling green sunfish in waters of various hardness buffered to pH's of 6.5, 7.5, 8.0 and 9.0. The compound was more toxic in softer waters than in harder water, especially at high pH values.

The toxicity of TFM is related to the amount of free phenol present since when TFM is exposed to low pH (6.8), it exists as a free phenol, but when exposed to a high pH, most of the TFM exists as a phenolate ion (Lennon, 1971). The relationship of toxicity and hardness was investigated by Johnson (1970), demonstrating that increased concentrations of divalent metal ions decrease the toxic action of TFM. Considering the wide range of hardness and pH that

may be encountered in Great Lakes tributaries, concentrations of 1 to 17 mg/l have proven lethal to exposed lamprey larvae (Dykstra and Lennon, 1966). An extensive review of the literature concerning safety and application of TFM was published by Schnick (1972).

Scope of Use of TFM

TFM was registered in the United States and Canada for use in lamprey control, based on research data developed through the U. S. Fish and Wildlife Service Laboratory at Hammond Bay, Michigan, and several experimental field trials in the early 1960's.

Following applications of the toxicant to streams harboring known populations of larval lampreys, populations in the lake were reduced by approximately 80% by 1962 and 90% by 1966. Treatment schedules were expanded to tributaries of Lakes Michigan and Huron with similar sharp declines in lamprey populations. The magnitude of this expansion is represented in Table 1. This summary of annual amounts of TFM applied to each of the upper Great Lakes' tributaries since 1958 and the total costs of U. S. and Canadian programs for sea lamprey control and research was tabulated from Annual Reports of the Great Lakes Fishery Commission (1960-1972). Through 1972, a total of 1,476,407 lbs. of TFM, expressed as active ingredient had been applied to Great Lakes tributaries at a total program cost in

TABLE 1.--Annual amounts of TFM applied to each of the upper Great Lakes' tributaries 1958-1972 and the total costs of U.S. and Canadian programs for sea lamprey control and research.

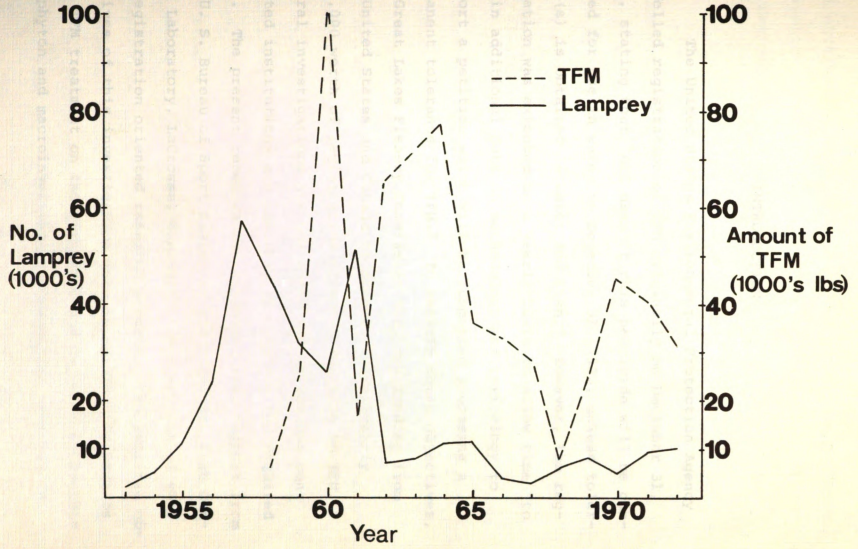
Year	Amount of TFM Used (lbs. active ingredient)				Lamprey Research & Control Program Costs U.S. & Canada*
	Lake Superior	Lake Michigan	Lake Huron	Lake Ontario	
1958	6,576				
1959	24,977				
1960	105,951	1,750	5,164		2,580,977
1961	17,920	24,689	37,651		2,723,428
1962	64,743	15,173	3,114		2,499,533
1963	71,529	25,125			2,412,927
1964	77,345	100,585			2,607,939
1965	36,458	62,843			2,571,294
1966	32,599	16,819	30,963		2,620,298
1967	28,409	31,124	40,101		2,801,490
1968	7,594	64,706	12,118		2,659,370
1969	24,960	42,255	23,711		2,662,199
1970	45,745	23,609	49,099		3,167,737
1971	40,030	37,134	63,523	18,319	3,369,912
1972	31,798	65,224	51,004	13,970	4,752,622
TOTALS	616,634	511,036	316,448	32,289	
GRAND TOTALS		1,476,407			$37,429,726

*Total costs of administration, research and application of TFM distributed among Great Lakes Fishery Commission, Fisheries Research Board of Canada, Department of Enviornment Canada, and U.S. Bureau of Sport Fisheries and Wildlife.

excess of $37.4 million. The impact of these treatments on spawning populations of sea lamprey in Lake Superior is represented in Figure 1, where the annual amounts of lampricide applied are plotted against the number of sea lamprey recovered from electrical barriers operated on eight tributaries of Lake Superior from 1958 to 1972. The graph accurately reflects the current status of lamprey stocks in the lake. Due to the non-persistent nature of TFM stream treatments, this method of control constitutes only a short-term stress on the population and must be repeated within particular tributaries every 3-5 years. Present plans of the Great Lakes Fishery Commission call for continued TFM treatment at these relatively high levels in order to maintain the present level of control.

100
80
1955
60
Year
65
1970

Figure 1.--Numbers of sea lamprey taken at electrical barriers operated in 8 tributaries of Lake Superior and the total amount of TFM applied to all tributaries of Lake Superior (1958-1972).


100
80
60
40
20
10
No. of Lamprey (1000's)
TFM
Lamprey
Amount of TFM (1000's lbs)
1955
60
65
1970
Year

INTRODUCTION

The United States Environmental Protection Agency cancelled registration of TFM originally on December 31, 1970, stating that "All uses of this pesticide will be cancelled for use in water on December 31, 1970, unless tolerance(s) is obtained in water and fish." However, the registration was extended on a yearly basis to allow time "to obtain additional data on methodology and toxicology to support a petition which will be submitted proposing a permanent tolerance for TFM." To satisfy these objectives, the Great Lakes Fishery Commission obtained funding from the United States and Canada to support approximately $600,000 worth of registration oriented research on TFM. Several investigations are now under way in various contracted institutions and agencies to generate the required data. The present research was conducted with support from the U. S. Bureau of Sport Fisheries and Wildlife, Fish Control Laboratory, LaCrosse, Wisconsin, as a section of this re-registration oriented research program. The specific objectives of this investigation were to examine the effects of a TFM treatment on the structure and function of benthic periphyton and macroinvertebrate communities existing in

model stream environments and to characterize the uptake and elimination rates of lampricide residues in several biotic components of these streams.

Experimental Approach

The effects of a particular environmental pollutant are generally assessed on a single organism existing in isolated aquaria or toxicity test chambers for set periods of time. An animal exists in nature as an integral part of the ecosystem both exerting effects and being affected by the interactions of the natural community. Rarely have investigators taken into account the influence of the interactions of community structure when assessing the effects of a toxicant on an animal (Ellis, 1968; Seim, 1970; Williams, 1969).

This community approach was used to examine the toxic influence of larval lampricide (TFM,-3-trifluormethyl-4-nitrophenol) on production and metabolism of replicated model stream communities. By imposing constraints on the physical and biological complexity of the model stream populations, the description of the community interactions under the influence of the toxicant are simplified. Knowledge of the numbers and kinds of species in the model streams and description of trophic level associations permits an accurate assessment of the impact of TFM on the productive capacity of the stream ecosystem. A series of replicated model streams allows for the examination, under controlled

conditions, of the effects of incremental changes in an environmental factor on ecological systems of convenient complexity and allows for the development of relevant predictions of the outcome of the effects of human activities on the biota of the system (Warren and Davis, 1971).

Artificial streams as models of small natural streams employing constraints on the complexity of communities have been used in this way by several investigators to observe interacting individuals and populations leading to a description of the operation of the community and existing trophic levels (Kevern and Ball, 1965; McIntire and Phinney, 1965; Davis and Warren, 1968).

This investigation was designed to simulate an environment where the toxicant is introduced into a stream allowing for colonization of the treated section by drift of upstream-dwelling organisms. Lamprey control rarely requires that TFM be introduced in the head waters of a stream since natural and manmade barriers effectively prevent adult lamprey migration and spawning in these upstream areas.

MATERIALS AND METHODS

The Model Streams

The site selected for the construction of the model streams was the Department of Natural Resources fish hatchery located in Mecosta Co., Paris, Michigan. The area has an abundance of small spring fed streams and offers a wide diversity of insect fauna for colonization of the model streams and a source of organisms for planned toxicity tests. The hatchery is located adjacent to Cheney Creek, a small stream of about .098 m^3/sec., arising from several springs approximately 1.45 km to the west. Paired cement rearing channels inside the hatchery were used as model streams. Each channel was 3.96 m long by 0.61 m wide and drained through a pair of 5.08 cm pipes into another downstream channel of equal dimensions. The replication possibilities offered by 13 of these channels afforded an excellent opportunity for conversion to model stream systems.

A cement head box and dam located about 320 m upstream on Cheney Creek maintained a constant water head which delivered water through a 30.58 cm underground pipe to the hatchery building. A steel rack and 4.76 mm mesh screen prevented most sticks and debris from clogging the

pipe but allowed for the passage of drifting insect larvae and particulate matter. Water was supplied to each pair of channels through four 3.17 cm iron pipes; water pressure was maintained by the change in elevation between channels and head box which created an 2.44 m head. The flow rate through each pair of channels remained constant throughout 1972 and 1973 at 200 l/min. Water supplied to each channel passed through the length of the model stream only once and was collected into common floor drains and discharged through a 25.40 cm pipe back into Cheney Creek downstream from the hatchery building. The standing volume of a single stream, including both riffle and pool sections, was approximately 550 liters. At a flow rate of 100 liters per minute per channel, the time for total water renewal rate was about 5.5 minutes.

The chemical characteristics of the water in the model streams are summarized in Table 2. Replicate water samples were taken during summer, fall and winter periods, preserved with HgCl and returned to the Water Chemistry Laboratory on campus for chemical analysis. The data show that the water quality remained relatively constant throughout the year with an average alkalinity of 179 mg/l $CaCO_3$, pH of 7.79, and average hardness of 211 mg/l. Water temperature reflected the spring origin of the water, never exceeding 13°C during summer months and dropping to 5°C in early December.

TABLE 2.--Water quality summary for model streams June-December, 1973.

Characteristic	Summer 27 June	Fall 21 September	Winter 19 December
Alkalinity (mg/l $CaCO_3$)	179	182	176
Ammonia (mg/l N)	0.07	---	---
Calcium (mg/l $CaCO_3$)	144	152	160
Carbon, Total (mg/l C)	38	38	42
Carbon, Organic	1	0	3
Chloride (mg/l Cl)	4.0	2.4	2.2
Specific conductance (micromhos at 25°C)	430	360	400
Hardness (mg/l $CaCO_3$)	214	214	204
Iron (mg/l Fe)	0.1	0.2	0.2
Nitrate (mg/l N)	1.05	1.32	1.57
Nitrite (ug/l N)	4.0	4.0	5.0
pH	7.92	7.45	8.0
Phosphorus, Total (mg/l P)	0.01	0.01	0.01
Sodium (mg/l Na)	2.0	3.0	2.3
Solids, Total (mg/l)	240	226	227
Sulfate (mg/l SO_4)	14	16	15

Six hatchery channels were utilized for the model stream communities providing for three complete systems each consisting of one control channel and an adjacent experimental channel. The upstream channels were designated pool communities and maintained at a 25.40 cm water depth. Colonization of these pool areas was begun in the fall of 1972 through input of organic matter and drifting insect larvae entering through the head box on Cheney Creek.

Lighting was supplied to the model streams in late April, 1973, in the form of 24 banks of four high intensity cool-white fluorescent lamps. The lamps were attached to sheets of .95 plywood painted white and supported by a superstructure of structural steel. The plywood sheets were attached by chains allowing for raising or lowering the lamps to adjust the surfact intensity of light. All lamps were placed to provide an intensity of 900 ± 50 ft-ca. at the water surface. This intensity was selected to allow for the colonization and growth of filamentous green algae on the substrate of the riffle communities. The illumination intensity was measured with a Weston Model 756 Sunlight Illumination Meter. All lights were wired through a relay box and electrical timer; the photoperiod was set to conform to natural day length and adjusted for seasonal variations.

In early May, natural stream substrate with associated flora and fauna was removed from Buckhorn Creek, a

similar spring-fed stream .40 km north of the hatchery and trucked to the model streams. The substrate, consisting of gravel and small rubble up to 10 cm diameter was added to each riffle section providing for a depth of 15.24 to 20.32 cm of substrate. Care was taken to insure a uniform distribution of particle sizes between streams. An eight week period was allotted for stabilization and growth of these riffle communities before experimentation was initiated in late June. By that time the streams had developed rich populations of diatoms and filamentous greens. Insect activity was evidenced by feeding of the species present and casebuilding behavior of the numerous species of Trichoptera. A complete listing of the flora and fauna that developed within the model system is presented in Table 3.

Determination of Treatment Concentration

A series of replicated toxicity tests were conducted with 7.62 to 10.16 cm rainbow trout obtained from hatchery stock at the Baldwin Rearing Station and larval silver lamprey, Ichthyomyzon unicuspis, collected by electroshocking mud banks of the South Branch of the Tobacco River at Clare, Mighigan. All specimens were brought to the lab and held in hatchery channels. The standard methods for streamside toxicity tests of Howell and Marquette (1962) were used to develop toxicity data for lampreys and to establish the level of protection for rainbow trout. Static toxicity

TABLE 3.--Taxonomic listing of flora and fauna of indoor model streams, Paris, Michigan 1973-1974.

Macroinvertebrates	Filamentous Green Algae	Diatoms
Gammarus pseudolimnaeus	*Stigeoclonium tenue*	*Tabellaria* sp.
Asellus militaris	*Spirogyra* sp.	*Cocconeis* sp.
	Cladophora sp.	*Diatoma* sp.
Plecoptera	*Rhizoclonium* sp.	*Achnanthes* sp.
Paracapnia sp.		*Nitzschia* sp.
Amphinemura varshava		*Meridion* sp.
Leuctra tenuis		*Fragilaria* sp.
Phasganophora sp.		*Synedra* sp.
Acroneuria lycorias		*Navicula* sp.
		Eunotia sp.
Ephemeroptera		*Pinnularia* sp.
Ephemerella lata		*Gyrosigma* sp.
Paraleptophlebia mollis		
Baetis sp.		
Trichoptera		
Hydropsyche sp.		
Cheumatopsyche sp.		
Goera calcarata		
Glossosoma sp.		
Neophylax sp.		
Limnephilus sp.		
Brachycentrus americanus		
Chimarra obscura		
Ochrotrichia sp.		
Diptera		
Simulium sp.		
Antocha sp.		
Chrysops sp.		
Prodiamesa sp.		
Diamesa sp.		
Cricotopus sp.		
Eukiefferiella sp.		
Tanytarsus sp.		
Chironomus sp.		
Miscellaneous		
Sialis sp.		
Stenelmis sp.		
Hydracarina sp.		
Physa sp.		
Lymnaea sp.		
Annelida		

tests were conducted in 25 liter glass aquaria placed in the hatchery channels. Ten liters of water were added and the proper concentration of field grade TFM was added. The material used was formulated in March, 1973, was labelled as 36% active ingredient and had a specific gravity of 1.212. Two animals of each species were tested in each aquarium with a single replicate for each test concentration. Water temperature was 58°C and pH 8.0. The entire test was replicated once and the results are presented in Table 4. Analysis of these data show a 10 hour Maximum Allowable Concentration (MAC_{25}) for rainbow trout to lie between 11.0 and 13.0 mg/l and a Minimum Lethal Concentration (MLC_{100}) for larval lamprey to lie between 3.0 and 3.5 mg/l. On the basis of these calculations, Cheney Creek water would be treated at 9.0 mg/l for lamprey control in actual field conditions. This concentration was determined as the level to be used in all tests and experiments to follow.

Model Stream Treatments

Stream treatment equipment was borrowed from the Bur. of Sport Fisheries and Wildlife Field Station at Ludington, Michigan. A 1:10 dilution of 1973 Field Grade TFM was made with water and pumped into the experimental channel of the model streams with an automotive fuel pump powered by a 12 volt battery at a flow rate of 28 ml/minute. Concentrations were continuously monitored by comparison with a standard

TABLE 4.--Time-to-death of larval lampreys, Ichthyomyzon unicuspis, and rainbow trout, Salmo gairdnerii, tested in several concentrations of field grade TFM. Figures represent total number of mortalities from two replicated tests during each time interval, a total of 8 individuals tested per concentration.

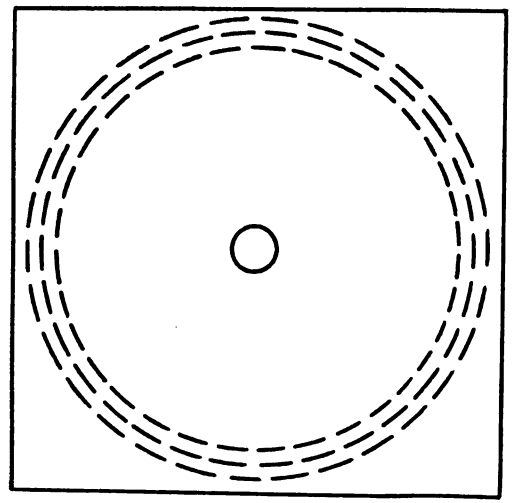
Concentration (mg/l)	Time Elapsed Hours	Larval Lamprey					Rainbow Trout				
		2	4	6	10	20	2	4	6	10	20
3.0		0	0	0	2	2	0	0	0	0	0
3.5		0	0	0	8		0	0	0	0	0
5.5		1	4	8			0	0	0	0	0
7.5		0	8				0	0	0	0	0
9.0		2	8				0	0	0	0	4
11.0		0	8				0	0	0	2	8
13.0		0	8				0	0	0	8	

curve developed for the Klett-Summerson Colorimeter based on the yellow color of TFM in an alkaline solution (Smith et. al., 1960). The experimental channel of model stream A was treated on September 5 at 9.0 mg/l for a period of 14 hours. The experimental channel of stream B was treated on November 1 at the same level also for 14 hours. Stream system C was reserved for a recirculating dose of ^{14}C-TFM to examine residue dynamics in various plant and animal components. Several experiments were conducted simultaneously in these model systems to examine and describe the impact of the toxicant on resident flora and fauna; these will be described in detail in the sections to follow.

Photosynthesis-Respiration Chamber

Rates of photosynthesis and respiration by intact benthic communities were monitored by the use of a modified photosynthesis-respiration (P/R) chamber as described by Cummins and King (1972 personal communication). The chamber consists of a circular plexiglass tray placed in the hatchery channels prior to colonization and addition of substrate and a tight-fitting circular top section which fits down around the buried bottom section sealing off the enclosed community (Figure 2). The bottoms were constructed of a 5.08 cm section of .63 cm plexiglass tubing of 27.94 cm internal diameter bonded to a flat 30.48 cm square of .63 cm plexiglass which served as the bottom of the chamber.

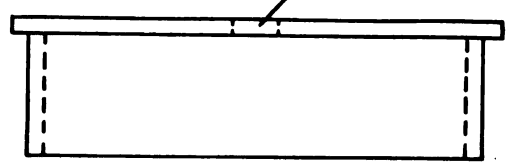
Figure 2.--Diagrammatic representation of photosynthesis-respiration (P/R) chamber.

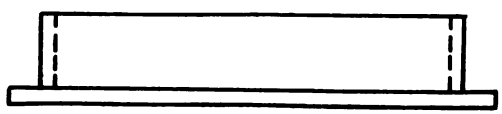


Top
View

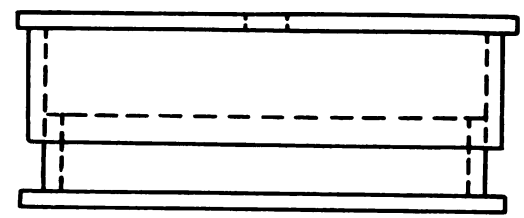
In situ Stream Metabolism Chamber

Oxygen Sensor Port





Assembled View



Thirty-six of these bottoms were placed in the control and experimental channels of Streams A, B, and C prior to colonization and addition of substrate. Top sections were similarly constructed fo a 7.62 cm section of tubing of 29.21 cm internal diameter also bonded to a 30.48 cm square of plexiglass sheet serving as the lid of the chamber. The lid was provided with a centrally located 3.81 cm port to accommodate a Yellow Springs Instruments Model 5420A Self-stirring B.O.D. Oxygen/Temperature Probe. The probe was connected to a YSI Model 54 Oxygen Meter designed to read directly in parts per million and compensated for temperature effects on both membrane permeability and oxygen solubility in water. A continuous record of oxygen concentration was provided by a Sargent-Welch Model SR Strip Chart Recorder.

Prior to an experiment, a pair of 1.27 x 17.78 cm rubber bands were stretched around the outside of a chamber bottom and covered with a light application of silicone stopcock grease. A chamber top was fitted down over the outside of the bottom counterpart and a water-tight seal was insured. Care was taken to evacuate all air bubbles through the center part prior to insertion and sealing of the oxygen sensor. The height, and consequently the volume, of the chamber was adjusted to equal the water depth of the stream outside the chamber by raising or lowering the top section. This was done to insure that the temperature

inside the chamber would remain equal to the ambient stream temperature thereby avoiding the effects of increased temperature on the metabolic rates of the enclosed organisms during the experiment. The chamber volumes varied from 3500 ml in the riffles to 4500 ml in the pools. The stirring rod of the BOD probe provided a continuous circular current within the confines of the P-R chamber to simulate natural stream flow.

An experiment with a single chamber was typically of 5 1/2 to 6 days duration to allow for two days of pre-treatment data, one complete day under exposure to 9.0 mg/l TFM, and two days of post-exposure data. Each chamber was opened, allowed to rinse with stream water and refilled at least every 24 hours to eliminate metabolic effects due to build-up of concentrations of ammonia or other waste products from the enclosed community. Several experiments particularly in the strongly heterotrophic pool communities required rinsing and refilling at intervals of 5 to 6 hours since oxygen concentrations would drop below 5 mg/l. Also during the summer months of peak standing biomass of Cladophora and Stigeoclonium tenue in the riffle communities, supersaturation with oxygen and the formation of oxygen bubbles in the chamber during photosynthesis required more frequent rinsing and refilling of the chamber.

Measurements of net photosynthesis were made during the daylight hours under constant 900 ft-ca. illumination

and measurements of community respiration were made on the same community after lights out (sunset). Two complete systems were run simultaneously, one in the autotrophic riffle communities and one in the heterotrophic pools. On the morning of the third day, after rinsing and refilling with stream water, each chamber was injected via a syringe through the rubber stopper with a pre-calculated dose of field-grade TFM to bring the concentration inside the chamber to 9.0 mg/l. The exposure continued for 24 hours to assess the effects of TFM on the entire daily photosynthetic and respiration rates. The following two mornings the chamber was rinsed and refilled with clear stream water for a two-day measurement of post-exposure metabolic rates. All rates were then calculated and compared from the calibrated strip-chart recorder paper.

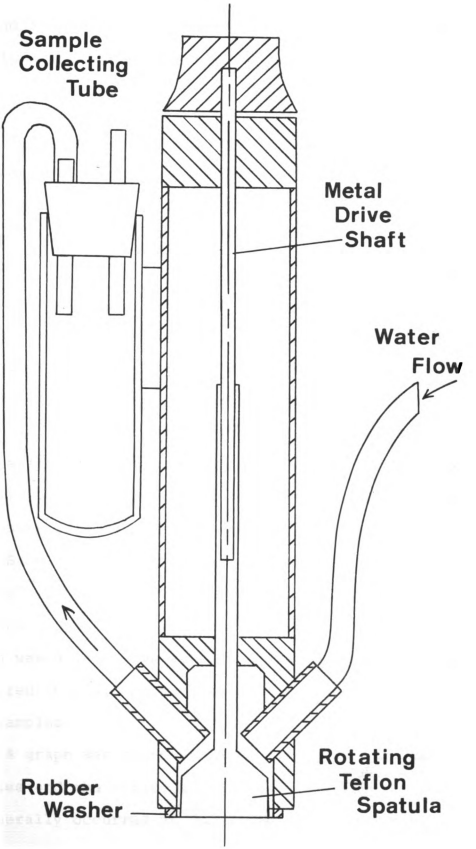
Periphyton Sampling

The species composition of the periphyton community was sampled by scrubbing the rubble in a P-R chamber and rinsing into 1 liter of water. Four 20 ml subsamples were taken, two for determination of diatom species composition and two for determination of biomass as ash-free dry weight of organic material. The diatoms were cleaned and mounted following a modification of Van der Werff (1955): The sample was placed into a 100 ml Berzelus tall form beaker and 25 ml of 30% hydrogen peroxide was added. This was allowed to

oxidize for 1 hour prior to the addition of 1 microspatual of potassium dichromate. After termination of the reaction, the beaker was filled with distilled water and allowed to settle overnight. The liquid was decanted, rinsed once more and allowed to settle for about 12 hours. The liquid was decanted to a 5 ml volume and the entire sample was stored in a 20 ml scintillation vial. A 0.5 ml subsample was placed on a heated coverslip, allowed to dry and inverted over a warmed slide with a drop of Hyrax mounting medium. Generic composition was determined by counting individuals in three transverse cross-sections of the slide under oil. Variation in early counts was assumed to be due to variability in subsampling a 20 ml volume from the original 1 liter sample, so a modified procedure was employed.

A periphyton sampler was developed by Cummins (1972 personal communication) which consistently removes a 1 cm^2 area of substrate through the action of a nylon spatula. The sample is forced up through a length of tygon tubing and collected in a 60 ml centrifuge tube by blowing a column of water through the apparatus with the mouth (Figure 3). This sampler was employed in all three model streams from August to December to characterize the generic composition of the diatom communities by scraping and combining three 5 cm^2 samples from each channel on a biweekly basis. The entire sample was then passed through the procedure outlined

Figure 3.--Cross-sectional diagram of stream periphyton sampler.


Sample
Collecting
Tube
Metal
Drive
Shaft
Water
Flow
Rotating
Teflon
Spatula
Rubber
Washer

above and it appeared that this technique yielded more consistently reproducible results.

Macroinvertebrates

The structure of the benthic macroinvertebrate community was determined from sample cores removed from both pool and riffle areas. A 45.72 cm length of 10.16 cm diameter plastic pipe was fitted with a rubber gasket on the bottom lip. This was then pushed down through the substrate to form a water-tight seal with the flat cement bottom of the model stream channels. The rubble and gravel substrate with associated flora and fauna was removed from the center of the core and the remaining water and fine matter was siphoned into a 0.5 mm mesh Nytex bag. The sample was rinsed of fine silt, filamentous green algae and all insects were picked live. All samples were preserved in 10% formalin and the substrate returned to the sample area.

Samples were similarly removed from the pool areas employing a 25.40 cm section of 5.08 cm diameter thin gauge metal pipe. By placing the hand over the top of the corer, the core was held in the tube by vacuum pressure and was transferred to a 0.5 mm mesh Nytex bag and treated as the riffle samples.

A graph was constructed of number species vs. number of samples and the inflection point of increase in new species generally occurred at three samples (Figure 4). Based

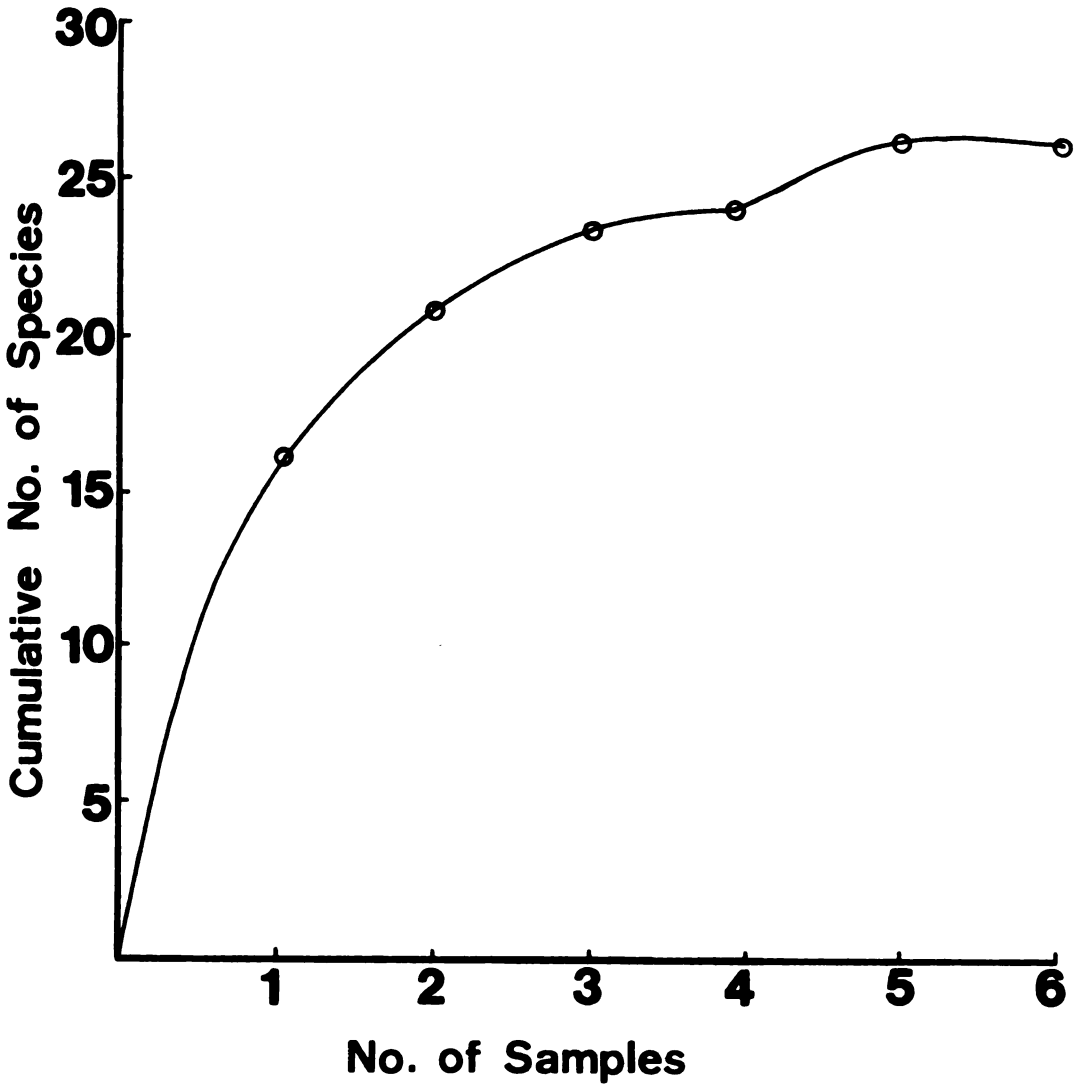


Figure 4.--Cumulative number of taxa obtained with increasing number of samples demonstrating inflection point.

on this data, three samples were selected as the number removed from a pool or riffle area at each sampling interval.

All samples were identified as near to species as possible and recorded on individual data sheets. A Digital PDP 1140 computer program was written to calculate the Shannon-Weiner diversity index, as described by Wilhm and Dorris (1968), employing sample data to estimate the actual population diversity. The formula is:

$$d = -\sum_{i=1}^{s} \frac{ni}{N} \log_2 \frac{ni}{N}$$

where N = the total number of individuals; ni = the number of individuals per taxa; s = the number of taxa; and d = an estimate of population diversity. The values of this index range from 0 to + infinity, however, they rarely exceed nine and usually fall between three and four (Wilhm, 1970). The three samples taken during each sampling period should yield an accurate estimate of the true population diversity (Wilhm and Dorris, 1968; Warren, 1971). This particular index was employed for the following general advantages: It is completely independent of sample size and accounts for relative abundance therefore is less affected by the absence of rare species which may be missed in sampling. It has no dimensions and is therefore independent of sampling units, i.e., biomass or numbers may be used to obtain similar values.

The index of species equitability, E, was calculated after MacArthur (1965). If all species are equally abundant, $d = \log_2 s$ and $s = 2^d$. The ratio 2 d/s, is a measure of species equitability or the relative evenness of distribution. Values range from a minimum of 0 to a maximum of 1.

After identification and counting of samples, estimates of biomass as ash-free dry weight of organic material were obtained on the total macroinvertebrate community and the filamentous green algae removed from the riffle areas with the 10.16 cm sample corer for Stream A. Samples were dried in a ventilated oven at 50°C for 48 hrs. prior to ashing in a muffle furnace at 500°C for 1 hour. The difference between pre- and post-muffle furnace weights represents biomass of organic material with no correction made for loss of water of hydration.

Estimates of production of two species of macroinvertebrates, a stonefly, Amphinemura varsharva and the amphipod, Gammarus pseudolimnaeus, were made using the Hamilton (1969) modification of the Hynes and Coleman (1968) technique. This method was used for its rapid deployment and relative accuracy (Waters and Crawford, 1973). It avoids the drawback of all other methods for production estimation, that of having to assign each individual of a particular population to a specific age class.

Fish Production

Two experiments were conducted to examine the effects of a 24 hr. exposure to 9.0 mg/l TFM on the growth rates and production of young-of-the-year brown trout, Salmo trutta. Sixty fish ranging in size from 60 to 100 mm were electroshocked from Buckhorn Creek in Paris, Michigan, on October 5 and held in a hatchery trough. Individuals were anesthetized with MS-222 and each fish was differentially fin clipped and total length, fork length and weight were recorded. Twelve fish were added to each control and experimental pool areas of Streams B and C 5 days prior to treatment. Growth was then determined from control and experimental pool fish in Streams B and C after 43 and 35 days respectively. Fish were collected from each channel by dip-net, identified by fin clips, measured and weighed.

Kinetics of TFM Residues

Several experiments were conducted to examine the dynamics of uptake and elimination of TFM in 20 plant and animal components of the model streams using uniformly ring-labelled TFM obtained through the Fish Control Laboratory at LaCrosse, Wisconsin, and synthesized by Mallinckrodt Chemical Co. of St. Louis, Mo. It was supplied in sealed glass ampules containing 0.125 mCi with specific activity of 3.66 mCi/mM, dissolved in benzene and stored under nitrogen. For all experiments, the benzene was evaporated under

a stream of nitrogen, the ^{14}C-TFM resuspended in acetone and an isotope dilution was made with 95% pure analytical grade TFM (Aldrich Chemical Co.). The total concentration of labelled and unlabelled TFM in the treatment standard was then 21.6 mg/ml acetone. Dilution by this method yields an approximate activity in the water at time zero of 15.0×10^6 to 20.0×10^6 cpm per liter.

Due to the hazards encountered in releasing large amounts of radioactive materials into the aquatic environment, all experiments concerning exposure and uptake of ^{14}C-TFM were conducted in completely closed, recirculating systems. The buried P-R chamber bottoms enclosing unexposed communities in both pool and riffle areas served as enclosures for the ^{14}C-TFM experiments. The community was enclosed by placing a 10.16 cm section of 30.48 cm ID. tubing over the chamber bottom and sealing with rubber bands similarly to the respirometry experiments. The chamber had no cover to permit access to sampling of enclosed plants and animals and the height of the top was adjusted to protrude about 1.27 cm above the water surface to insure no exchange of water. Current was simulated inside the chamber through the use of a variable speed laboratory stirring motor. The depth of water was measured, the volume calculated (between 3 and 4.5 liters) and treated with the above isotope dilution of ^{14}C-TFM to bring the total concentration of TFM to 9.0 mg/l. Samples of enclosed flora and fauna were then

removed at approximately 1, 3, 5, 8, 14, 20, and 24 hour intervals with forceps thoroughly rinsed in clear water, placed in labelled vials and immediately frozen. Five ml samples of water were also removed at the same time intervals and immediately frozen.

At the termination of the 24-hour exposure period, the ^{14}C-labelled water was siphoned into a carboy, the chamber top gently lifted, and the community was exposed to the TFM-free water of the model stream channel again. Continual sampling of this community then developed data on retention times and elimination rates of TFM for the various components of interest. This treatment procedure was followed on a bi-weekly basis from July to December, each time employing a new, previously unexposed community. Occasionally, if the species of interest was not present within the community in sufficient numbers to permit sampling without depletion, that species was stocked into the chamber just prior to the treatment. This was particularly true in the case of the aquatic macrophytes which did not naturally occur in the model streams. Six to eight components were sampled during a particular experiment and three to five replicates of each of the 20 components were tested during the year.

Samples were kept frozen generally for a one week period prior to analysis. All plant, animal and sediment samples were dried at 50^{o}C for 48 hrs., weight adjusted to

100-150 mg and combusted to $^{14}CO_2$ and water in a semi-automated Nuclear Chicago combustion apparatus. The $^{14}CO_2$ was taken up in 10 ml of monoethanolamine-methyl cellosolve, 1:2 (V/V). A 2 ml portion of this was radioassayed with a dual channel Nuclear Chicago Unilux I (Model 6850) liquid scintillation spectrometer in 15 ml of a toluene-methyl cellosolve, 2:1 (V/V), fluor mixture. A 2 ml aliquot of each water sample was radioassayed in 15 ml of a toluene-Triton X-100 fluor, 2:1, (V/V).

Periodic efficiency curves were established for the instrument employing a series of internally quenched standards and all sample counts were converted to actual distintegrations per minute and uCi values employing the channels ratio and efficiency curve.

The entire experimental channel of model stream C was treated with a recirculating dose of ^{14}C-TFM on November 13. Water flow through the channel was blocked by inserting rubber stoppers in the spouts and drains. Recirculation of water was provided by placing a large diaphragm pump in the downstream end of the riffle area and discharging into a 7.92 m length of 5.08 cm plastic pipe which ran back up to the beginning of the pool area. The pump volume was sufficient to pump slightly more than the 100 l/minute normal stream flow rate and air was constantly sucked into the pipe providing for reaeration during the day. The water temperature rose 3^{o}C during the treatment and was

considered nominal. Stream volume was calculated to be 500 to 525 liters and was treated with an isotope dilution of 1.0 mCi of ^{14}C-TFM and 4.500 gms of analytical grade TFM all in 10 ml acetone. This dilution gave approximately 2.6×10^6 counts min. $^{-1}$liter^{-1} at time zero and an actual concentration of 8.68 mg/l labelled and unlabelled TFM.

Following the 24 hour exposure period, the discharge from the pump was directed into a 984 liter metal container and water flow was immediately begun in the channel. In this way discharge of radiolabelled water from the channel was minimized. Sampling of plant and animal components for determination of uptake and elimination rates was done similarly to the recirculating chamber experiments.

The recovery of ^{14}C-TFM from the large volumes of water was facilitated through the use of a non-ionic polymeric adsorbent (Lech, 1971). The water was acidified to pH 4.0 with 0.1 N HCl and passed through a 7.62 x 60.96 cm column of Amberlite XAD-2 resin. The water effluent from the column was periodically radioassayed and no activity was observed. The column was washed with two volumes of distilled water and the ^{14}C-TFM was eluted with one volume of methanol. The methanol was removed in a rotary evaporator and the compound was resuspended in acetone.

RESULTS

Community Metabolism

An example of the calculations for community metabolism data during a 24 hour period are given in Table 5. The values for dissolved oxygen were taken directly from the continuous record of oxygen concentrations provided by the strip chart recorder. Negative values for oxygen production frequently were obtained in the strongly heterotrophic pool communities. For example, an oxygen concentration of 9.8 mg/l at sunrise may drop to 6.4 mg/l during the course of the day under full illumination resulting in a negative value for gross primary production. The rate of loss was calculated and compared with the rate of oxygen depletion for that same community during night hours. If this nighttime rate was greater than the daytime rate of loss, as was generally the case during the summer months, the difference between these day and night rates was interpreted to be due to the oxygen production by diatoms and filamentous green algae on the surface of the pool community and was recorded as daytime gross production. However, during the exposure to 9.0 mg/l TFM, there was generally no detectable difference between rates of oxygen depletion in the pools during

TABLE 5.--Sample calculations for a highly productive riffle community in Stream B on August 21-22. (After Whitworth and Lane, 1965.)

Time of Day	Dissolved Oxygen (mg/l)	Time Intervals
Sunset (Day 1)	16.0	11 hours of dark
Sunrise	10.8	13 hours of light
Sunset (Day 2)	15.4	

Calculation of community respiration:

$$\text{Respiration/hr.} = \frac{\text{sunset (1) - sunrise}}{\text{time interval (hrs.)}}$$

$$= \frac{16.0 - 10.8}{12} = .433 \text{ mg}0_2\text{liter}^{-1}\text{hr}^{-1}$$

Gross community respiration = $.433 \times 24$
$= 10.39 \text{ mg}0_2\text{liter}^{-1}\text{day}^{-1}$

Calculation of community oxygen production:

Daytime oxygen increase = sunset (2) - sunrise

= 15.4 - 7.0

= 8.4 mg/liter

Daytime respiration = respiration/hr. x day length

= .433 x 13 hrs. = 5.63 mg/liter

Gross primary production = oxygen increase + daytime respiration

= 8.4 + 5.63

$= 14.03 \text{ mg}0_2\text{liter}^{-1}\text{day}^{-1}$

day-time and night. In these instances the gross production was recorded as zero and the rate of oxygen depletion was recorded entirely as respiration. In these instances the P/R ratio was also recorded as zero.

The summary calculations of primary production, community respiration and P/R ratios for a 5 day experiment with a particular chamber located in Stream B riffle community are shown in Table 6, and presented graphically in Figures 5 and 6. These figures for late August are among the highest values for gross primary production achieved in the model streams during the year. The high production values of .950 gm 0_2 m^{-2} day^{-1} are probably due to the large standing crop of filamentous green algae, primarily Cladophora sp. and Stigeoclonium sp. that were obvious during that time of year. The respiration values for the riffles remained relatively constant throughout the year at about .630 gm 0_2 m^{-2} day^{-1}.

The characteristic influence of the 9.0 mg/l exposure to TFM is represented in the respirometry data for August 23. Under these conditions the rate of gross primary production has dropped to a low of 0.815 gm 0_2 m^{-2} day^{-1} and the respiration rate of the enclosed community shows an increase to .640 gm 0_2 m^{-2} day^{-1}. The depressed evolution of oxygen by the algal community reflects the algi-static effect of TFM as demonstrated in laboratory toxicity tests

TABLE 6.--Primary production, community respiration and P/R ratios for Stream B, August 21 through 24. Communities exposed to 9.0 mg/l TFM on August 23.

Date	Gross Production (gmO_2 m^{-2} day^{-1})	Respiration (gmO_2 m^{-2} day^{-1})	P/R Ratio
	Stream B Riffle Community		
21 Aug	.939	.667	1.41
22 Aug	.961	.635	1.51
23 Aug (exposed)	.815	.640	1.27
24 Aug	1.070	.625	1.71
	Stream B Pool Community		
21 Aug	.135	.560	0.24
22 Aug	.139	.547	0.25
23 Aug (exposed)	0.0	.779	0.0
24 Aug	.108	.651	0.17

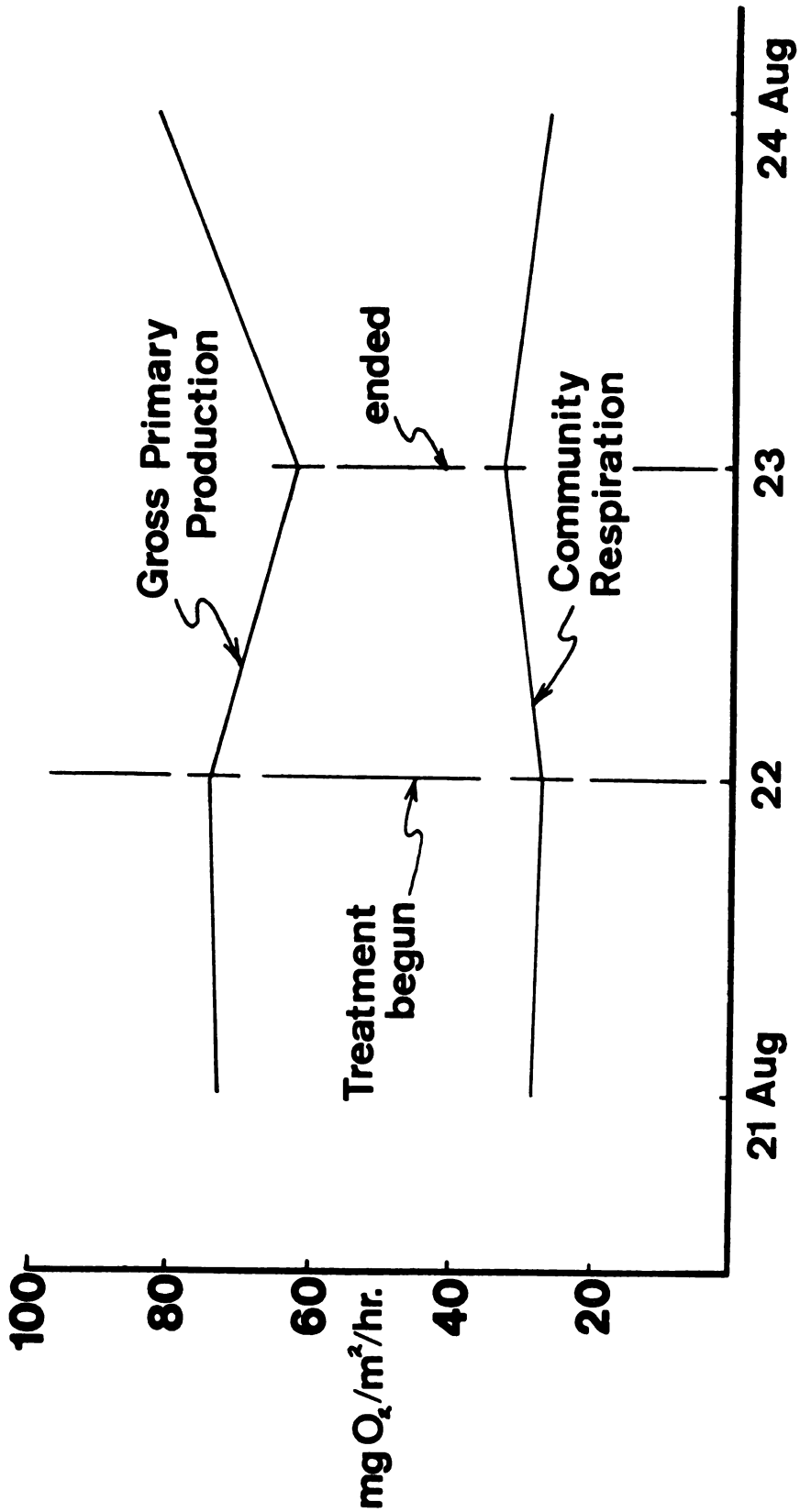


Figure 5.--Community metabolism in Stream B experimental riffle August 21-24.

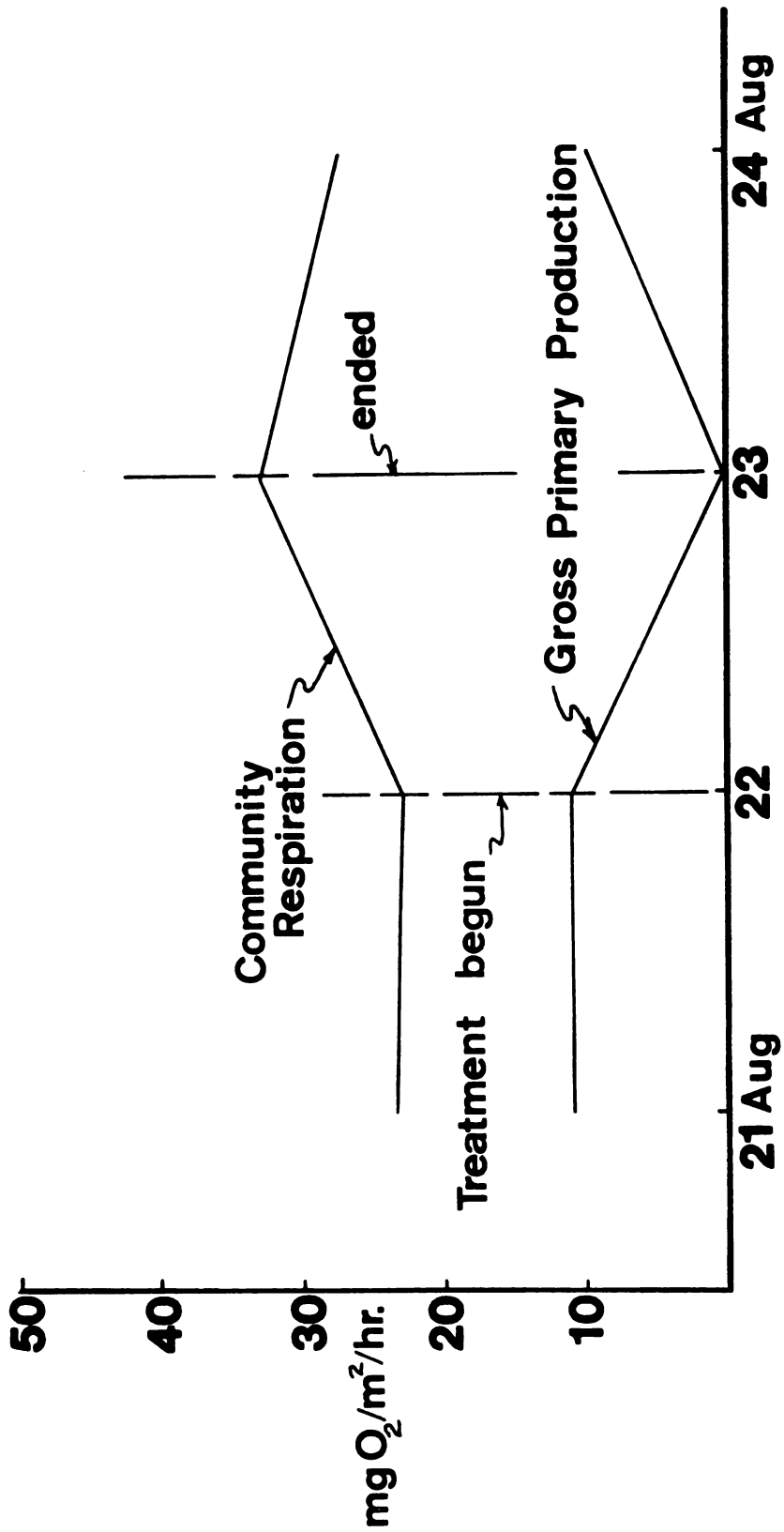


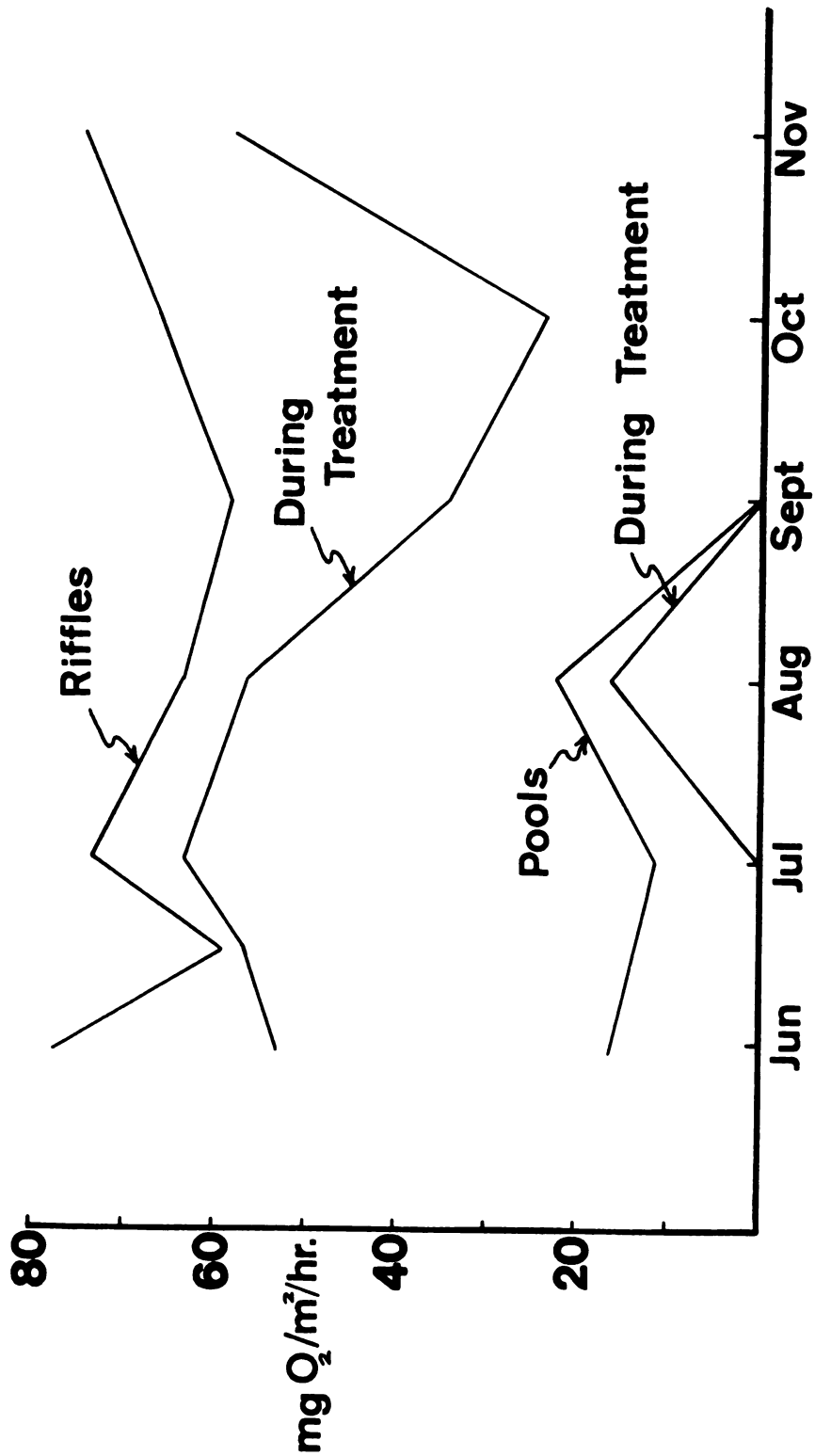
Figure 6.--Community metabolism in Stream B experimental pool during August 21-24.

(Maki et. al., 1974) and the increased respiration rates are believed to be primarily due to the response of the macroinvertebrates to the sublethal level of TFM. The increased respiration rate of invertebrates is recognized as a metabolic response to stress agents (Whitley and Sikora, 1970).

Rates of gross primary production, community respiration and P/R values obtained throughout the year are listed in the Appendix and presented graphically in Figures 7 and 8. The values for gross primary production in the riffles ranged from approximately 58.2 mg 0_2 $hr^{-1}m^{-2}$ to 79.0 mg 0_2 $hr^{-1}m^{-2}$ during the course of the experiments. Highest rates were recorded during the late summer and late fall periods which presumably were brought about by the increase in standing crop biomass of filamentous greens and attached diatoms respectively. The lowest production rates were recorded between these periods during the time when the communities were in a state of change, i.e., just developing green algae or greens sloughing off and diatoms becoming more obvious.

The effects of the 9.0 mg/l exposure of TFM on gross production rates also shown in Figure 7. For each experiment throughout the year, the rate of oxygen evolution was decreased. The amount of this departure presumably reflects the species composition of the primary producers. The gross production rate was depressed by about 5-10% during summer

Figure 7.--Rates of gross primary production recorded throughout 1973 under an illumination of 900 ft.-ca.


80
60
40
20
mg O_2/m^3/hr.
Jun
Jul
Aug
Sept
Oct
Nov
Riffles
During Treatment
Pools
During Treatment

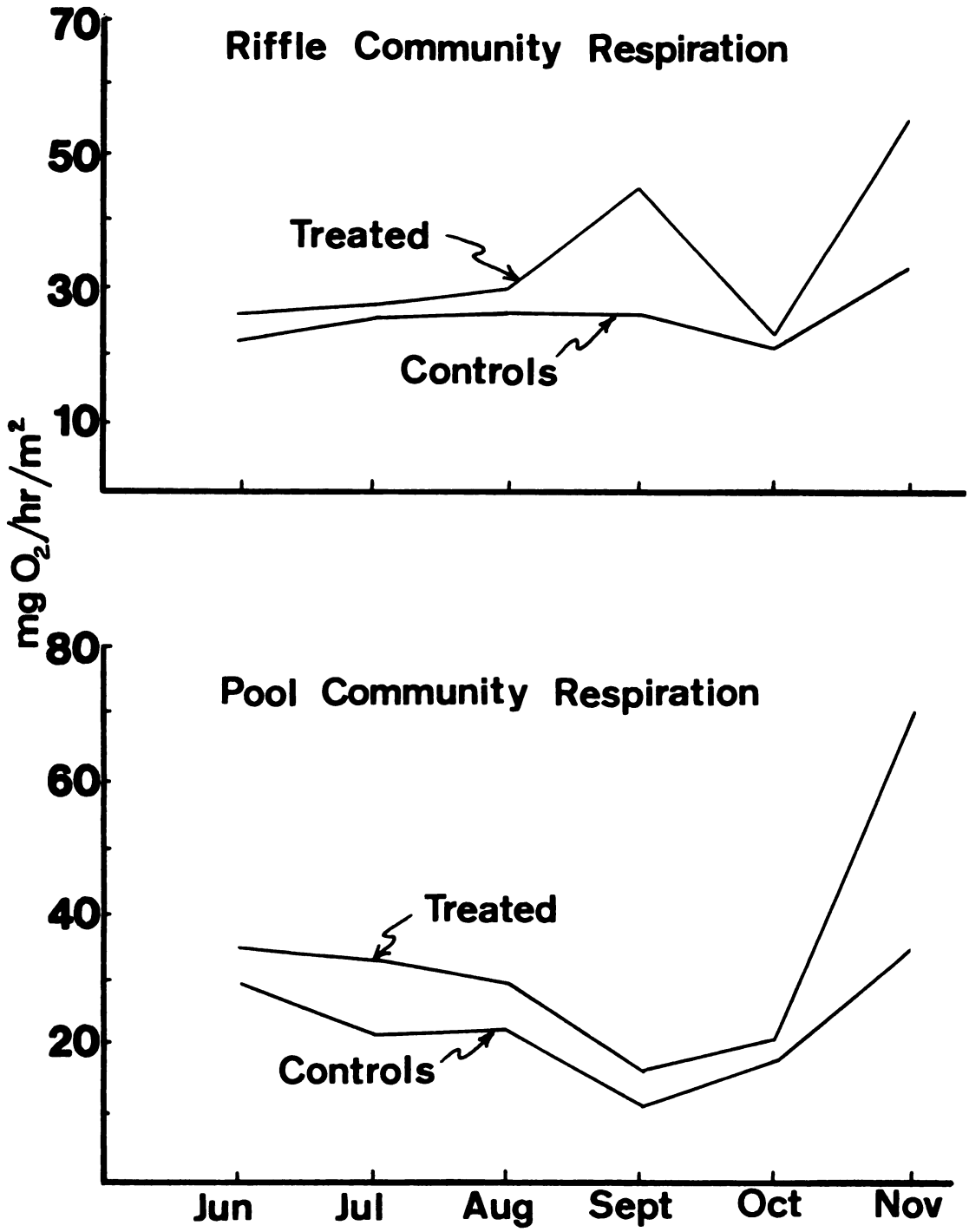


Figure 8.--Rates of community respiration observed in riffle and pool areas throughout 1973.

months when the producer community was dominated by filamentous greens, however, in the later fall months when the producer community was composed primarily of attached diatoms, the depression of oxygen production averaged 25-50%. In laboratory toxicity tests the diatoms were found to be more susceptible to TFM (EC 50:1.5-3.0 mg/l) than the green algae (EC 50 5.0-7.9 mg/l) (Maki et. al., 1974). The rates of gross primary production of the fall diatoms communities were obviously much more influenced by the TFM exposure than were the production rates of the summer filamentous green communities. The rates of gross primary production in the pretreatment controls were compared with the during treatment rates and found to be significantly different at the 0.01 level (Table 7).

The strongly heterotrophic nature of the pool communities throughout the year are shown in Figure 7. Values for gross primary production averaged 10.7 $mgO_2hr^{-1}m^{-2}$ to 22.5 $mgO_2hr^{-1}m^{-2}$ during the summer months and were primarily due to a low density of filamentous greens covering the surface of the organic detritus in the pools. As in the riffle communities, these populations disappeared from the model streams in early fall and positive values for oxygen production in the pools were not detected beyond this date. The response of these communities to the 9.0 mg/l exposure to TFM was also similar, showing a depression of oxygen evolution in all experiments. The depressed rate compared with

TABLE 7.--Results of Students' t-test of significant difference between annual mean values of community metabolism and standing crop data from control and treated dates.

Community Property	Means: Pre-Treatment Control	Means: Exposed to 9.0 mg/l TFM	Level of Significance (P)
Riffle Gross Primary Production ($mgO_2\ hr^{-1}\ m^{-2}$)	66.08	46.83	<0.01
Post-treatment		61.40	<1.0
Pool Gross Primary Production ($mgO_2\ hr^{-1}\ m^{-2}$)	26.48	11.84	<0.02
Post-treatment		21.64	<1.0
Riffle Respiration ($mgO_2\ hr^{-1}\ m^{-2}$)	26.03	34.74	<0.2
Post-treatment		23.41	<1.0
Pool Respiration ($mgO_2\ hr^{-1}\ m^{-2}$)	22.36	30.49	<0.2
Post-treatment		20.00	<1.0
Riffle P/R Ratio	1.36	0.79	<0.01
Post-treatment		1.38	<1.0
Pool P/R Ratio	0.39	0.15	<0.01
Post-treatment		0.29	<0.2
Stream A Diatom Density	1.37×10^9	2.12×10^9	<1.
Stream B Diatom Density	1.83×10^9	1.85×10^9	<1.
Total Biomass (ash free dry wt.)	16.61	17.32	<0.9
Percent Frequency of Occurrence:			
Spirogyra	21.11	19.44	<1.
Cladophora	60.00	62.22	<1.
Stigeoclonium	18.89	18.33	<1.

the pre-treatment control data was found to be highly significant at the 0.02 level (Table 7).

The temporary nature of the effects of TFM was demonstrated by the post-treatment experiments. Values for gross primary production in both the riffle and pool communities returned to pre-treatment rates within 24-36 hours post-exposure. No significant difference was found between these pre- and post-treatment rates (Table 7).

The influence of the TFM exposure on pool and riffle community respiration values as measured by the P/R chamber is shown in Figure 8. The pre-treatment respiration values for the riffle communities ranged from 20.3 to 32.4 $mg0_2hr^{-1}m^{-2}$ and increased to 22.0 to 60.7 $mg0_2hr^{-1}m^{-2}$ under the exposure to 9.0 mg/l TFM. This represents an increase of 3 to 50% due to the toxicant. Similar results were obtained in the pool communities where the high density of burrowing midge larvae was primarily responsible for the increased respiration values obtained in the presence of TFM. Normal values for respiration in the pools ranged from 10.5 to 36.2 $mg0_2hr^{-1}m^{-2}$ and were increased to 15.5 to 73.5 $mg0_2hr^{-1}m^{-2}$ by the exposure to TFM. Respiration results were generally more variable between the sampling dates presumably due to emergence of species during the year and unequal distribution of organisms between the chambers. The difference in mean riffle and pool respiration rates were significant at

only the 0.2 level (Table 7). Post-treatment experiments indicated a rapid recovery and return to pre-treatment respiration rates within 24-36 hours. No statistical difference was found between pre-treatment and post-treatment rates (Table 7).

The most sensitive indicator of the effects of TFM on community metabolism was the comparison of P/R ratios obtained on a pre- and post-treatment basis with those obtained under exposure to the 9.0 mg/l in the riffles (Figure 9). Since the action of TFM is to decrease oxygen production values and increase respiration values, the P/R ratio will naturally be subject to large changes in the presence of the toxicant. The mean ratios of pre- and post-treatment experiments varied from 1.06 to 1.78 while those ratios obtained under exposure to TFM varied from 0.5 to 1.27. In most experiments, the P/R ratio for the pool communities was reduced to zero and averaged approximately a 50% decrease in the remaining experiments. Analysis of the differences between pre-and post-treatment means and the during treatment means for both the riffle and pool communities proved the differences significant at the 0.01 level (Table 7).

Periphyton Community Structure

Densities of dominant genera of diatoms observed in samples removed from experimental and control channels of

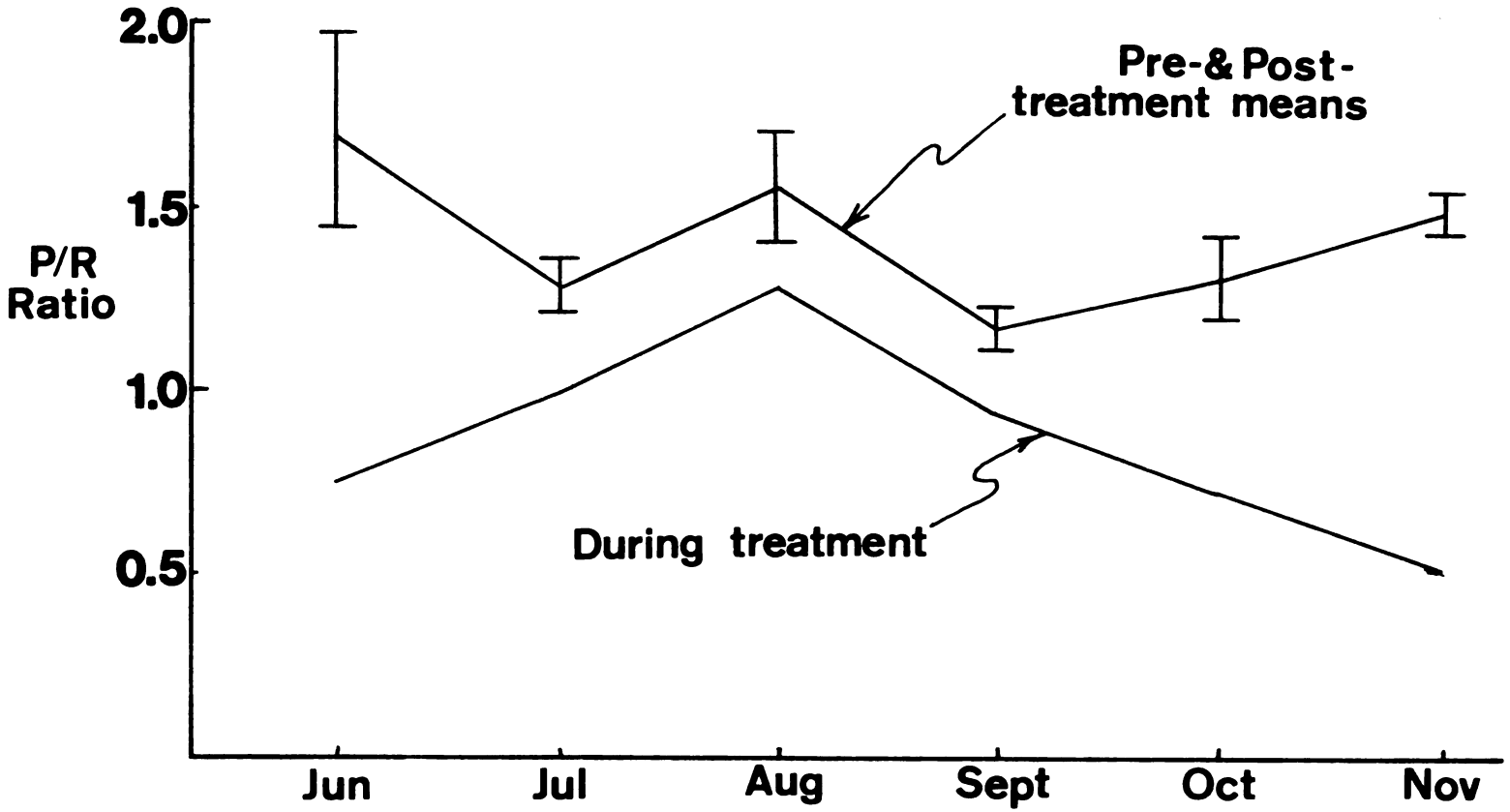


Figure 9.--Pre- and post-treatment mean P/R ratios for riffle communities compared with ratios obtained under exposure to 9.0 mg/l TFM.

Streams A and B are represented in Figures 10 and 11. Cell counts represent the total number of frustrules observed in three horizontal transects of a slide containing an aliquot of cells from a sample area of $5cm^2$. Correction factors were applied to compensate for the area of slide counted, aliquot of cells and finally to normalize the cell count to a square meter basis.

The data for both Streams A and B are quite similar, the general trend representing a drop in total number of individuals through the summer to a minimum density of about 1.20×10^9 cells/m^2 in the early fall months. A period of relatively rapid growth follows in late fall and early winter to reach a density of approximately 3.0×10^9 cells/m^2 by December. There is no significant difference in the total number of individuals between control and experimental channels. However, a slight decrease in the total density during the week immediately following the dose in the experimental channels may indicate a toxic effect on the relatively TFM-sensitive diatom community. As mentioned earlier, the 96 hr. LC_{50} for TFM in laboratory cultures of diatoms lies in the vicinity of 1.0 to 3.0 mg/l and it may be possible that the 9.0 mg/l exposure for 24 hours exerts a toxic influence on the exposed diatoms. This drop in total number of individuals occurring in only the experimental channels during the week following treatment, although not statistically

Figure 10.--Diatom cell densities in Stream A.

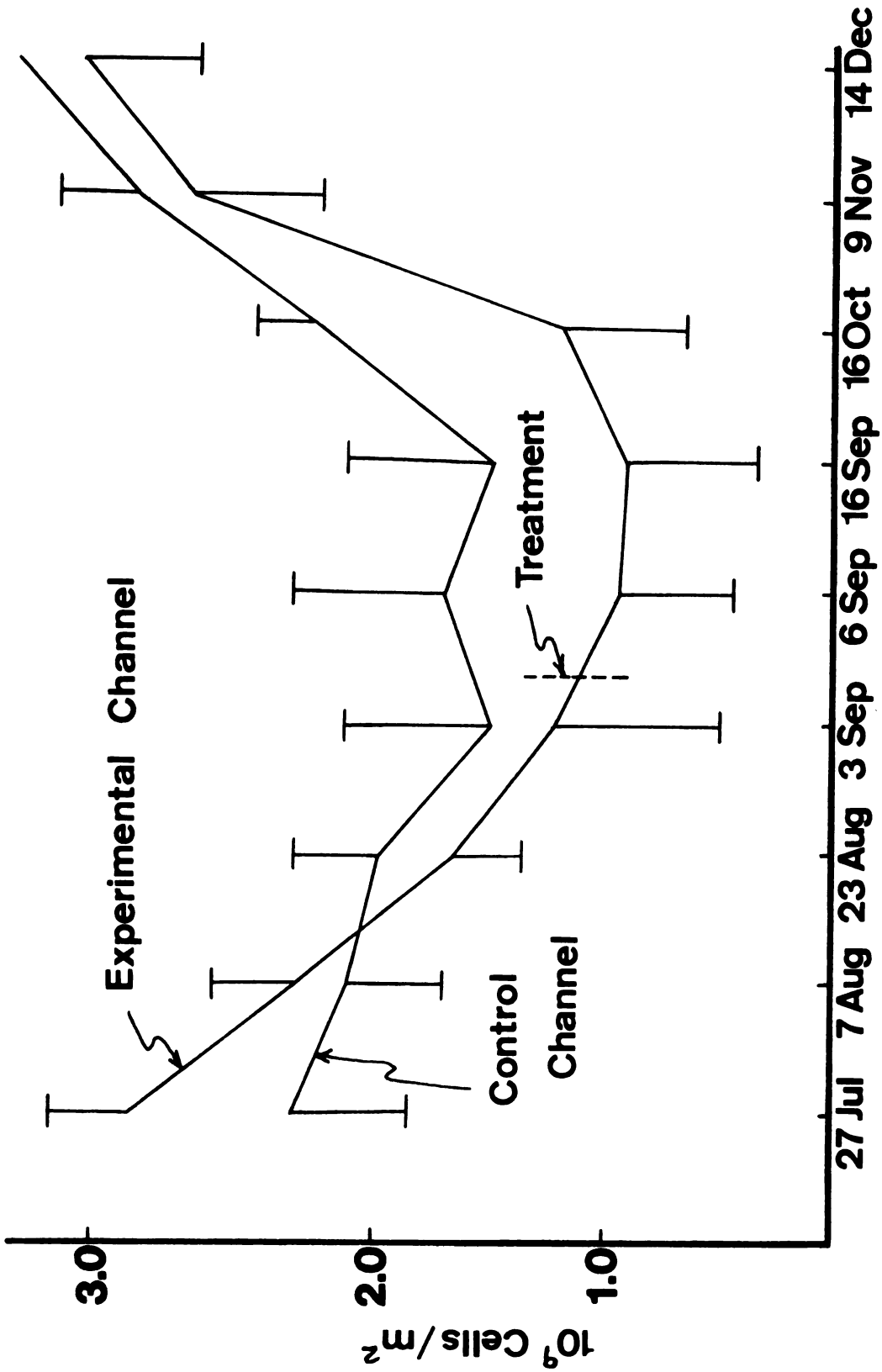
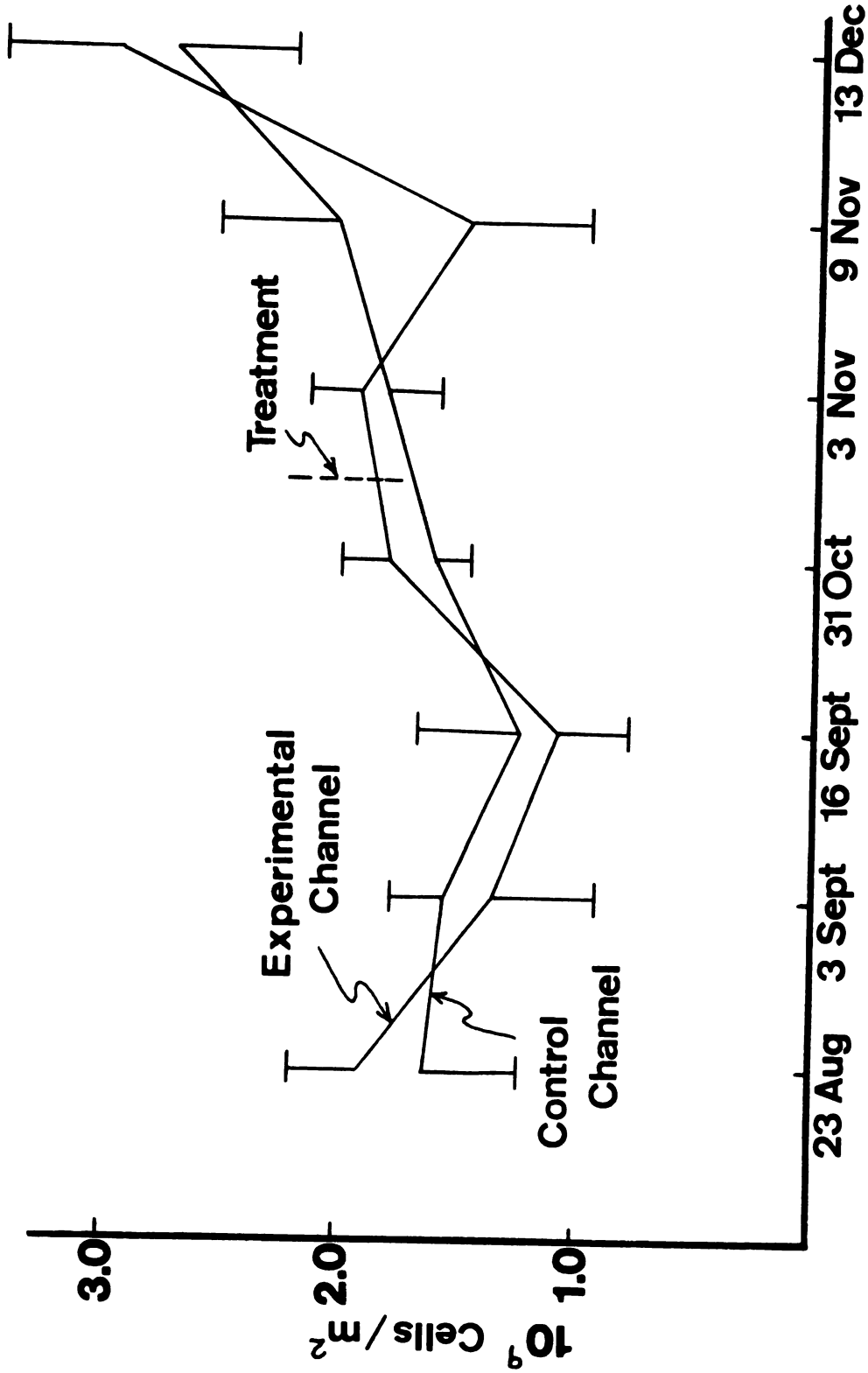

Experimental Channel
Treatment
Control Channel
3.0
2.0
1.0
10^9 Cells/m^2
27 Jul
7 Aug
23 Aug
3 Sep
6 Sep
16 Sep
16 Oct
9 Nov
14 Dec

Figure 11.--Diatom cell densities in Stream B.


3.0
2.0
1.0
10^9 Cells/m^2
Treatment
Experimental Channel
Control Channel
23 Aug
3 Sept
16 Sept
31 Oct
3 Nov
9 Nov
13 Dec

significant may reflect a temporary change in community structure brought about by exposure to TFM. At any rate, the effects are temporary and growth rapidly ensues to parallel or actually exceed densities found in the unexposed control channels (Figure 11). A t-test of the mean cell densities in control and experimental channels revealed no significant difference between these communities (Table 7).

The generic composition of the diatom flora of the riffle communities in Streams A and B were analyzed with the Shannon-Wiener diversity index (Wilhm and Dorris, 1968) and MacArthur's (1965) method for species equitability (Tables 8 and 9). Diversity values ranged from a low of 1.36 in the early fall to a high of 2.64 in early winter samples. Values for species equitability varied as the reciprocal of diversity with highest equitability values recorded in the early fall community indicating that the population existing at that time consisted of low densities of few different genera. The experimental channel of Stream A was treated with 9.0 mg/l TFM on September 4 and Stream B treated at the same level on November 1. Tables 8 and 9 demonstrate no significant difference in the diversity or equitability values calculated from samples removed from both control and experimental channels of each stream following the treatments.

The standing crop of filamentous green algae removed from Stream A riffle communities with the use of the 10.16 cm

TABLE 8.--Structure of diatom communities existing in Model Stream A control and experimental channels.

Date	14 July	27 July	23 Aug	18 Sept	19 Oct	27 Nov	Annual Mean ± 1 S.D.
Stream A Control							
Diversity	2.33	2.11	1.36	1.56	2.23	2.03	$1.93 \pm .388$
Equitability	.718	.720	.857	.985	.691	.817	$0.80 \pm .112$
Number of genera	7	7	5	6	10	9	7.33 ± 1.86
Stream A Experimental							
Diversity	2.60	2.21	2.05	2.15	2.64	2.10	$2.28 \pm .279$
Equitability	.777	.718	.828	.74	.777	.718	$0.76 \pm .043$
Number of genera	8	7	4	6	11	10	7.67 ± 2.58

TABLE 9.--Structure of diatom communities existing in Model Stream B control and experimental channels.

Date	23 Aug	3 Sept	16 Sept	31 Oct	3 Nov	9 Nov	13 Dec	Annual Mean $\pm$ 1 S.D.
				Stream B Control				
Diversity	1.42	1.39	1.62	1.93	2.18	2.30	2.24	$1.88 \pm .291$
Equitability	.887	.872	.931	.709	.681	.722	.787	$.798 \pm .105$
Number of genera	5	5	6	11	10	10	9	8.41 ± 2.17
				Stream B Experimental				
Diversity	1.33	1.28	1.57	1.85	2.24	2.31	2.43	$1.86 \pm .322$
Equitability	.791	.823	.896	.796	.711	.706	.768	$.784 \pm .096$
Number of genera	5	4	6	10	10	11	10	8.17 ± 2.16

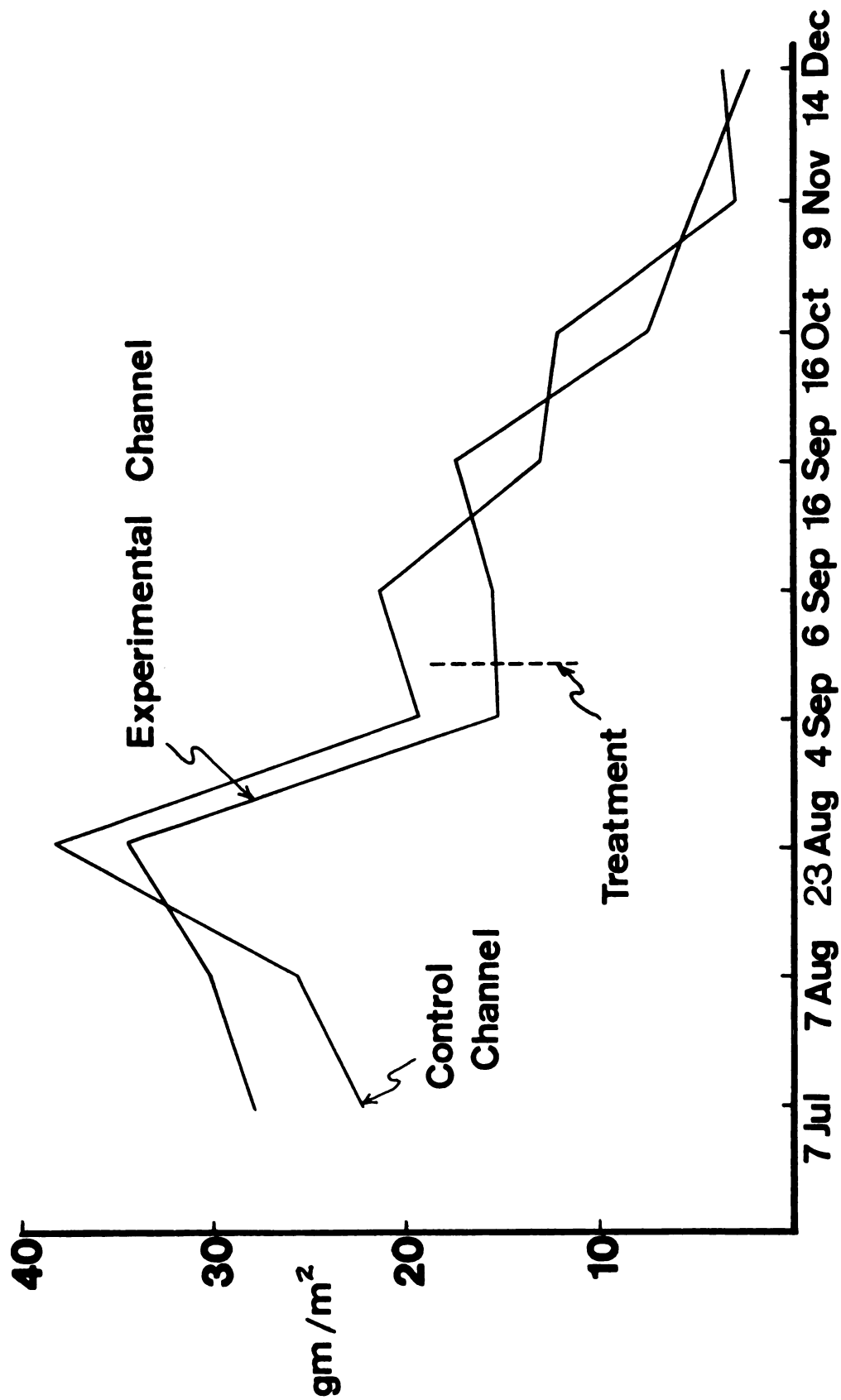
corer was analyzed in detail to determine any differences that may be due to the lampricide treatment. The samples were homogenized by hand shaking in the sample vial, two wet mounts of an aliquot of this homogenized material were made, and 15 random fields were examined to determine relative percent frequency of occurrence of the three dominant species. Table 10 gives the results for Spirogyra, Cladophora, and Stigeoclonium from experimental and control channels of Stream A. The major trend evident from the samples is a gradual succession throughout the year from a Spirogyra dominant community in early summer to a Cladophora dominant community in the early winter. The relative frequency of occurrence was not significantly different between control and experimental channels (Table 7).

The biomass of the filamentous green algal community is represented in Figure 12. The ash-free dry weights expressed as grams organic matter/m^2 are graphed for both experimental and control channels of Stream A. The peak biomass occurred in both channels during late August with a continuous decline in biomass occurring through the December samples. Analysis of these biomass figures demonstrated no statistical difference between control and exposed populations that could be caused by the lampricide (Table 7). This presumably could be predicted from the relative toxicity of TFM to green algae since most species have a 96 hr. LC_{50}

TABLE 10.--Approximate percent composition of the filamentous green algal flora of Stream A experimental and control channels determined from wet mounts of preserved material.

Date	Spirogyra		Cladophora		Stigeoclonium	
	Experimental	Control	Experimental	Control	Experimental	Control
10 July	80	80	20	20	0	0
7 Aug	30	40	40	40	30	20
23 Aug	10	10	50	60	40	30
3 Sept	10	5	60	60	30	35
Stream A Treatment						
6 Sept	20	10	60	70	20	20
16 Sept	10	10	90	80	0	10
16 Oct	0	0	100	90	0	10
9 Nov	10	10	50	60	40	30
13 Dec	20	10	70	80	10	10

Figure 12.--Biomass as ash-free dry weight of filamentous green algae from control and experimental channels of Stream A.


Experimental Channel
Control Channel
Treatment
gm/m²
40
30
20
10
7 Jul
7 Aug
23 Aug
4 Sep
6 Sep
16 Sep
16 Oct
9 Nov
14 Dec

of about 6-8 mg/l TFM, a 24 hr. exposure to 9.0 mg/l would be expected to have minimal effects.

Macroinvertebrates

There were 38 different taxa representing 14 orders of macroinvertebrates sampled from the total model stream systems. Substrate associations and current differences between riffle and pool communities played a key role in the distribution of species. The pool areas, with soft particulate organic matter and flow rate of about 0.5 ft./sec. were characterized by a heavy density of midge larvae, annelid worms, gastropod and pelecypod molluscs. Throughout the summer months the dominant member of the pool communities was the midge, Tanytarsus sp. often accounting for more than 90% of numbers and total biomass. This species emerged in mid-September and temporarily left the pool communities seemingly depauperate of fauna, however, the next generation soon grew to sufficient size to be retained by the mesh of the sample rinsing net and Tanytarsus sp. continued dominant in numbers throughout the late fall and early winter.

The riffle communities supported a much greater number of taxa due to the more diversified habitat of the gravel and rubble substrate (Table 11). No single species was particularly dominant as in the pool communities, instead, five or six species were generally common to all

TABLE 11.--Total number of taxa collected from each stream community throughout the sample year.

		Summer	Fall	Winter	Total
Stream A					
Experimental	Riffle	26	28	31	34
	Pool	8	11	12	14
Control	Riffle	27	27	30	32
	Pool	7	10	11	13
Stream B					
Experimental	Riffle	28	30	33	35
	Pool	9	11	10	14
Control	Riffle	28	27	29	32
	Pool	10	11	12	12
Stream C					
Experimental	Riffle	23	27	26	29
	Pool*	--	--	--	--
Control	Riffle	27	29	28	30
	Pool*	--	--	--	--

*Used for fish production experiments - no samples taken.

three streams each sharing equal prominence among samples. The amphipod, Gammarus pesudolimnaeus and a Nemourid stonefly, Amphinemura varsharva were common in all samples taken. Also abundant in most of the samples were the small mayfly, Baetis sp., isopods, Asellus militarus, trichopterans Glossosoma sp., Cheumatopsyche sp., Brachycentrus sp. and diamesian and orthoclad chironomids.

The variation between pool and riffle macroinvertebrate populations is clearly shown in Table 12 where the Shannon diversity index values and equitability are presented for the three streams on a seasonal basis. In all instances, the calculated values for diversity of the riffle communities exceeds the values calculated for the corresponding pool communities generally by 2 to 3 times. This result could be predicted from the species composition information since the pool communities average only about 14 taxa and the riffle areas averaged about 31 different taxa present, a higher value for the diversity index should be obtained from the riffle communities. The range of diversity values for riffle communities varies from 2.16 to 3.49 while the range of diversity for the pool communities is from 0.48 to 2.05. A comparison of the standard deviations of the annual means demonstrates that no significant difference exists between diversity values obtained from control and experimental channels of the same stream system and indeed only slight differences exist among similar communities of all three

TABLE 12.--Shannon diversity indices and equitability in terms of numbers of individuals from each community during each season and annual means.

		Summer		Fall		Winter		Annual Mean ± 1 S.D.	
		$\bar{D}$	E	$\bar{D}$	E	$\bar{D}$	E	$\bar{D}$	E
Stream A									
Experimental	(Riffle	2.16	0.47	2.63	0.49	2.70	0.64	2.68 (±.164)	0.52 (±.11)
	(Pool	0.91	0.41	1.80	0.61	0.72	0.36	1.47 (±.35)	0.52 (±.15)
Control	(Riffle	2.84	0.58	2.71	0.52	2.50	0.62	2.59 (±.23)	0.50 (±.14)
	(Pool	0.63	0.38	2.01	0.77	0.57	0.43	1.43 (±.53)	0.54 (±.16)
Stream B									
Experimental	(Riffle	2.91	0.49	2.93	0.53	2.93	0.68	3.00 (±.17)	0.59 (±.11)
	(Pool	2.05	0.83	1.89	0.72	0.48	0.34	1.38 (±.48)	0.51 (±.21)
Control	(Riffle	2.96	0.63	2.57	0.55	3.23	0.74	2.90 (±.26)	0.56 (±.08)
	(Pool	1.88	0.82	1.37	0.62	0.75	0.33	1.38 (±.68)	0.52 (±.21)
Stream C									
Experimental	Riffle	*		2.95	0.65	2.41	0.76	3.35 (±.21)	0.74 (±.05)
Control	Riffle	*		3.24	0.66	3.49	0.88	3.10 (±.18)	0.62 (±.05)

*No summer samples taken.

stream systems. A seasonal trend is evident in the diversity values indicating a slight increase in diversity as the sampling periods progress toward the winter months. This is explained by the fact that adult insects emerging primarily during the summer months in turn have deposited their eggs back into the model streams. These newly hatched larval forms in first and second instars in early fall were too small to be retained in the 0.5 mm mesh of the sample-rising net and were therefore not seen in the samples. As these individuals grew into later instars, they were effectively sampled and retained by the sampling gear and by their presence would serve to increase the calculated diversity values.

Values for species equitabilty ranged from 0.49 to 0.88 in the riffle communities and 0.33 to 0.83 in the pool communities and were not distinctly different between pool and riffle areas. The general trend seen in the annual means is for the species equitability to parallel the diversity value, i.e., lower in the pool communities and higher in the riffles. Since the fauna of the pools consists primarily of one dominant species, Tanytarsus sp., the species equitability is low. Conversely since the fauna of the riffle communities consists of several species of equal or near-equal prominence, the species equitability is higher.

The diversity and species equitability figures were analyzed on a pre-treatment and post-treatment basis to

characterize any significant influence of the 9.0 mg/l TFM exposure on the macroinvertebrate community structure. Mean values for diversity, equitability, number of species, and number of individuals per square meter were calculated from samples removed prior to treatment of each stream with lampricide and the same values were then calculated from samples removed after the exposure period. Riffle and pool sections of experimental and control channels of each stream were calculated separately, in this manner any changes in community structure occurring as a natural event would be observed in the untreated control channel thus avoiding the false assumption that the change was a function of the TFM treatment.

Stream A

Community Structure.--Table 13 represents the macroinvertebrate community structure of control and experimental channels of Stream A. The mean values for pre-treatment samples represent data from 10 July to 3 September; the 14-hr. treatment with TFM occurred on 4 September and the post-treatment sampling period covered the period to 12 March. No significant difference exists between the control and experimental communities prior to the treatment and the similarity remains throughout the post-treatment period. However, there is a reduction in the total number of individuals in all riffle and pool communities. Since this reduction was also observed in the control channel, it may be explained

TABLE 13.--Community structure of benthic macroinvertebrates from Stream A control and experimental channels.

	Stream A Control		Stream A Experimental	
	Riffle	Pool	Riffle	Pool
Pre-treatment				
Diversity	2.85±0.237*	0.66±0.050	2.53±0.513	0.716±0.167
Equitability	0.55±0.077	0.37±0.042	0.47±0.073	0.41 ±0.059
Number of Species	13.16±1.72	4.33±0.519	12.83±3.71	4.43 ±1.03
Number of Individuals/m^2	10629±1952	35801±6678	12423±2354	36145 ±7160
Post-treatment				
Diversity	2.59±0.237	1.43±0.528	2.68±0.164	1.47 ±0.350
Equitability	0.49±0.142	0.54±0.164	0.52±0.113	0.52 ±0.151
Number of Species	13.07±2.75	5.73±1.110	12.86±2.67	5.64 ±1.49
Number of Individuals/m^2	10072±1338	22528±3882	10234±1642	24470 ±4854

*Standard error of the mean.

by the natural emergence of resident species. The magnitude of this reduction in number of individuals is greater in the pool communities than the riffles due to the extensive emergence of Tanytarsus sp. from these pools.

Analysis of sample data on the basis of mean numbers may tend to mask any short-term effects present in the experimental channel. A standard t-test of the significance of difference between pre- and post-treatment community structure of the macroinvertebrates was conducted by combining all the data from samples removed up to 2 weeks post-treatment and comparing with pre-treatment levels. The reduction in fauna was found to be significant at the 0.05 level (Table 17) and more importantly, the reduction in fauna in the adjacent control channel was not significantly different for the same time period. However, this reduction in total number of individuals did not remain significant throughout the sample period. When the levels of pre-treatment abundance are compared with samples removed at 2 and 3 months following treatment, no difference can be shown between the samples (Table 17).

The reduction in total number of individuals in the pool communities was also compared between pre- and post-treatment samples employing the standard t-test. In this instance, the number of individuals lost from both control and experimental communities between pre-treatment and 2-week

post-treatment samples was significant at the 0.05 level (Figure 13). The post-treatment mean for the control channel was lower than that for the experimental channel indicating that the significant reduction was due to the mass emergence of Tanytarsus sp. occurring at that time. Figure 13 also represents the reduction in fauna between pre- and post-treatment samples from the riffle area of model Stream A. From the graph, one can see that the drop in total number of individuals in the experimental riffle is much sharper than in the control riffle but that recovery occurs within approximately 2 months due to input from drift and the recruitment of new individuals into the population. The degree of recovery increases the population density to above pre-treatment levels and exceeds the number of individuals in the control channel.

The biomass, expressed as ash-free weight of the entire macroinvertebrate community of Stream A was determined by ashing a series of samples from Stream A after the individuals had been sorted and identified. Figure 14 represents the results from experimental and control channels of model Stream A and indicates that the total macroinvertebrate biomass ranged from approximately 3.0 gms/m^2 in late summer to a peak of 19.0 gms/m^2 in March. Results agree relatively closely with the actual numbers of individuals shown in Figure 14 with the exception that the increase in

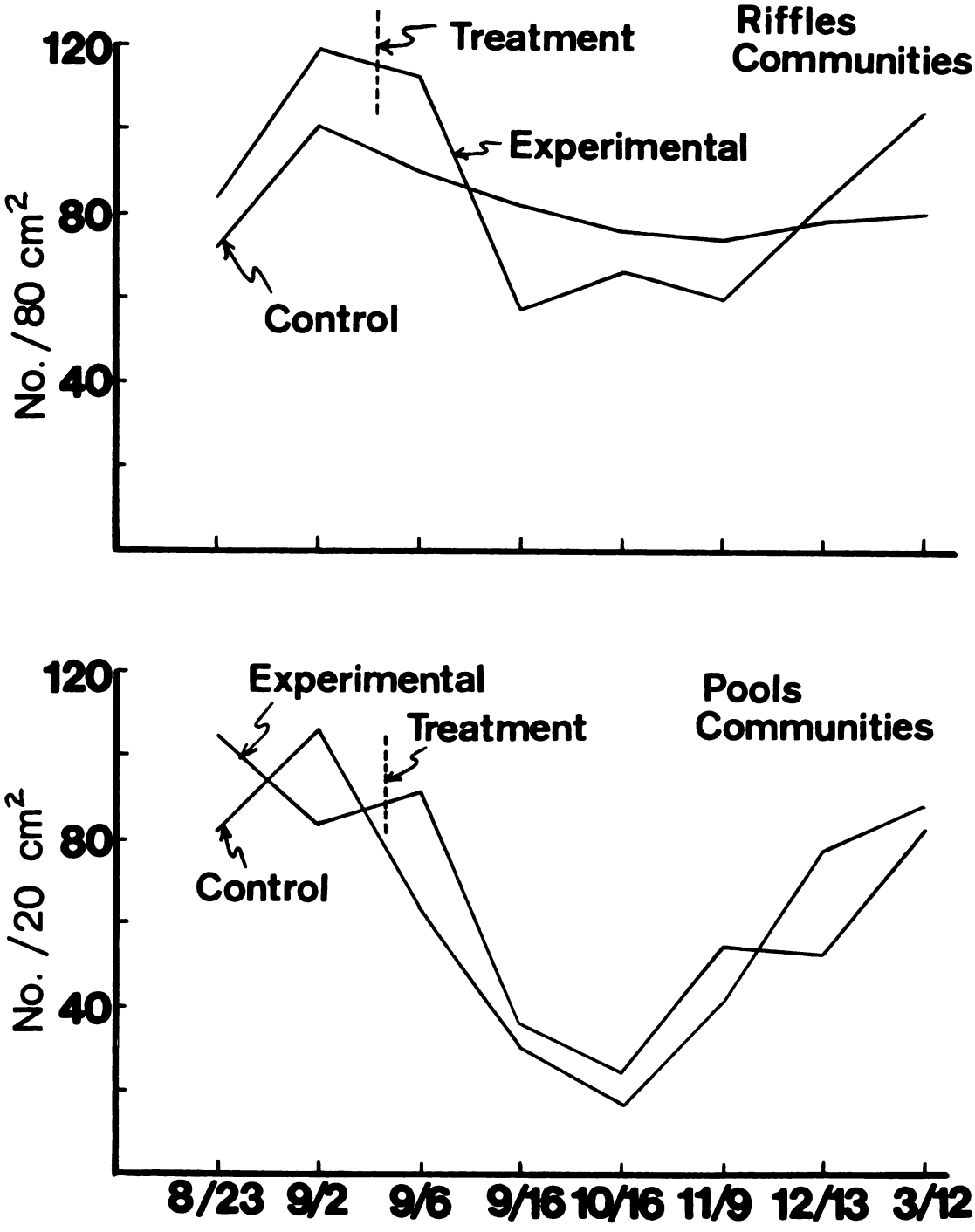


Figure 13.--Number of macroinvertebrate individuals per unit area of substrate in Stream A riffles and pools.

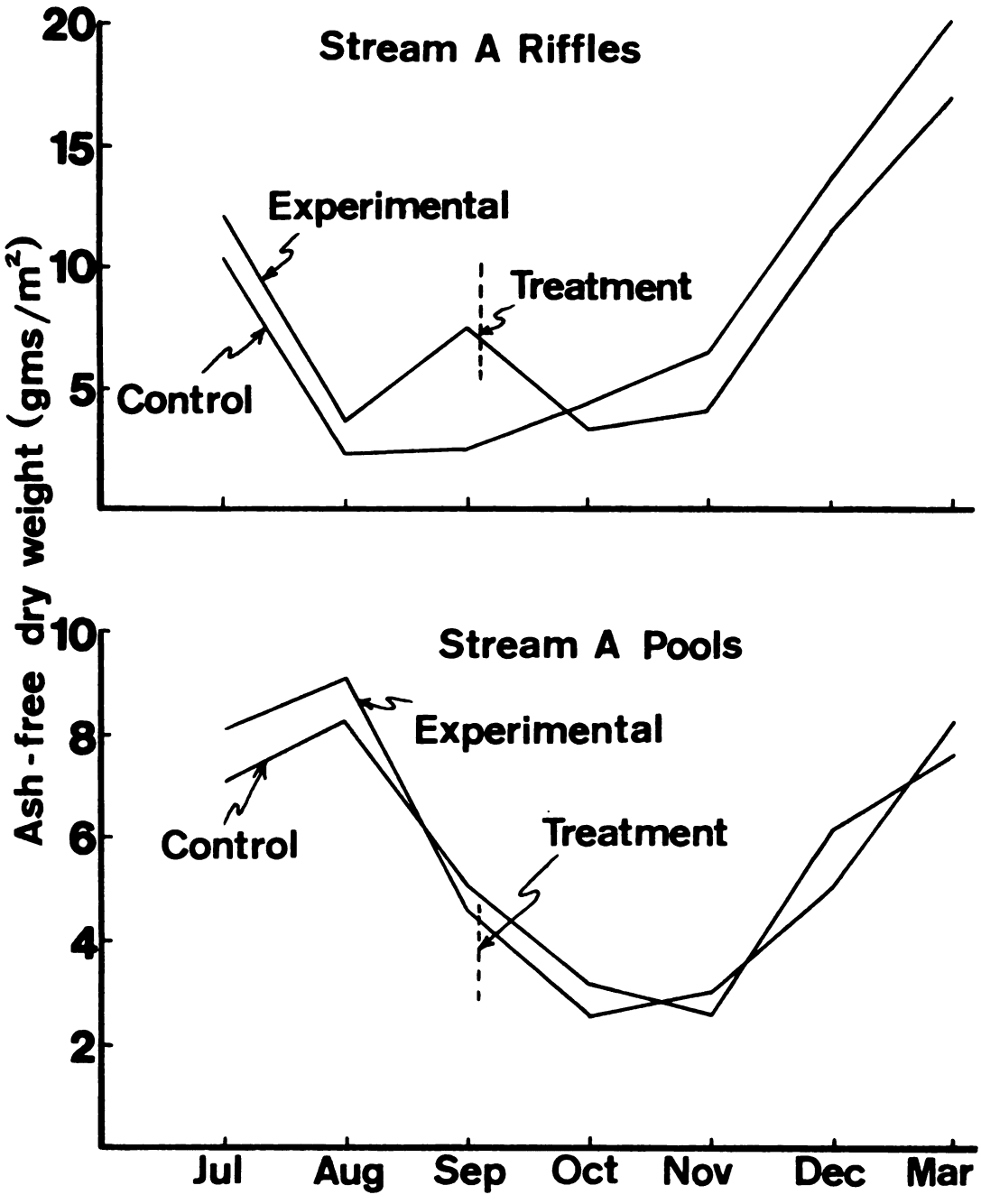
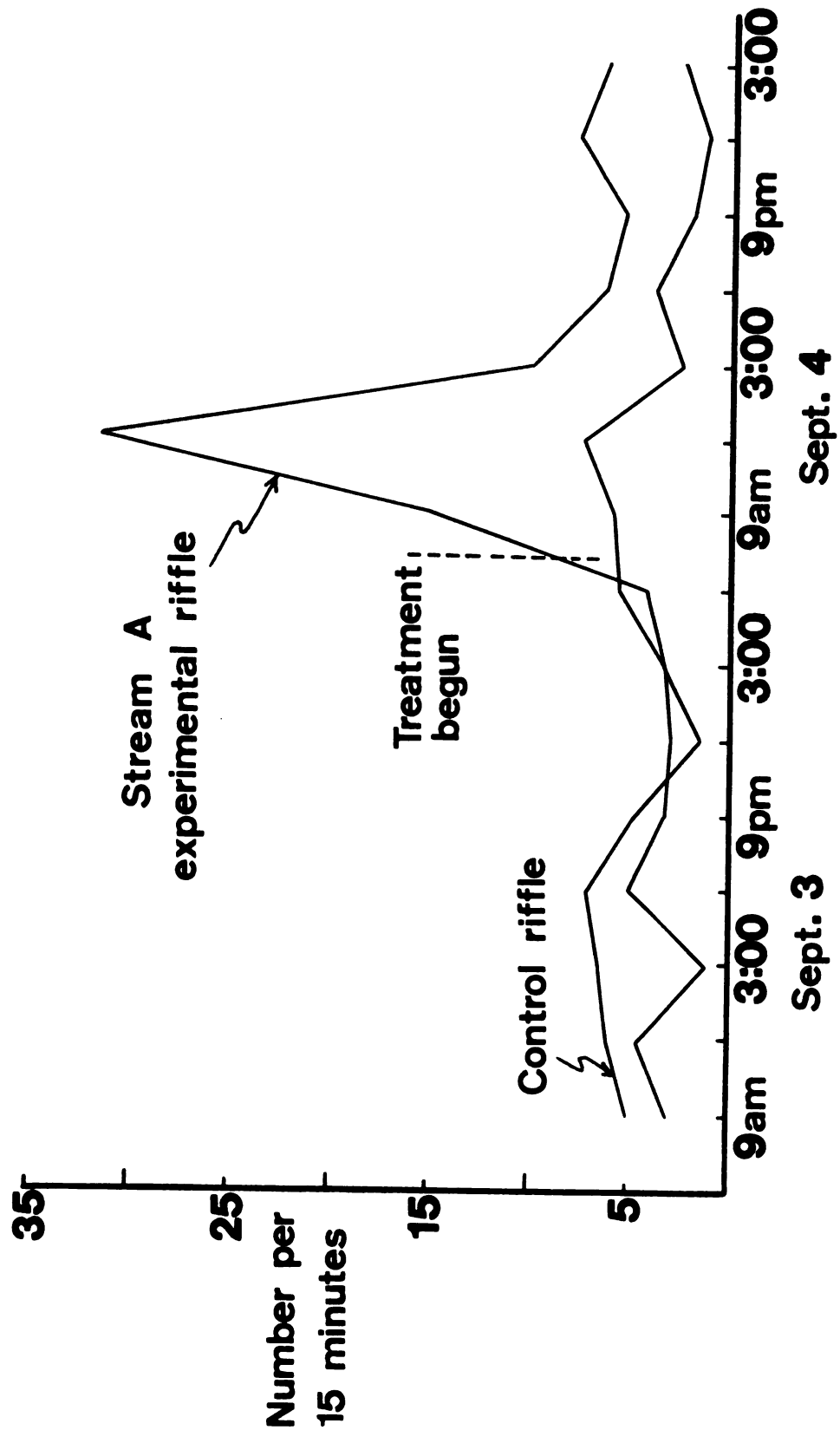


Figure 14.--Standing crop as ash-free dry weight of macroinvertebrates from Stream A.

number of individuals in the experimental riffle community does not result in an increase in total biomass to exceed control level.

Drift.--The most striking effect occurring in the macroinvertebrate community of Stream A during lampricide treatment was the significant increase in drift pulse recorded immediately after the initiation of treatment (Figure 15). A simultaneous record of the number of individuals drifting from both experimental and control channels was obtained by covering the stream outlets with a .186m^2 section of 0.5 mm nylon screen. Due to blockage by drifting particulate organic matter and insects, the nets were fished for 15 minute intervals every three hours and once every hour at the onset of treatment. A full 24 hour record of drifting individuals obtained on the day immediately prior to treatment shows both experimental and control channels have similar drift rates of approximately 1 to 5 individuals per sampling period. Curiously no significant increase in drifting individuals was recorded after sunset, the period of highest drift rates in natural streams. In samples taken immediately after the initiation of treatment, the drift numbers rise to a peak of 32 individuals per 15 minutes in the experimental channel while simultaneous samples taken in the adjacent control channel indicate no increase in drifting individuals over the previous days' estimates. The

Figure 15.--Simultaneous drift rates from Stream A experimental and control riffle channels during treatment with 9.0 mg/l TFM.


Stream A
experimental riffle
Treatment
begun
Control riffle
Number per
15 minutes
35
25
15
5
9am
3:00
9pm
3:00
9am
3:00
9pm
3:00
Sept. 3
Sept. 4

drift rate in the experimental channel remains higher than the control channel throughout the entire treatment period. The initial sharp increase in drift rates is the result of only a few of the more TFM susceptible species and only three genera constitute approximately 80% of the total number of the drift peak. These three genera are a trichopteran, Trentonius distinctus, the amphipod, G. pseudolimnaeus and a mayfly Baetis sp. The caddisfly is the single most abundant species in all drift measurements taken during treatment but was never found as a prominent component of drift in pre-treatment or control samples. Therefore, its presence and numerical abundance in the drift samples taken during treatment are brought about by exposure to the toxicant. Toxicity data developed for this species employing continuous flow proportional diluters indicates that the 96-hour LC_{50} for this species is approximately 1 to 2 mg/l TFM making this caddisfly one of the most susceptible of all invertebrate species tested.

Identical procedures were employed to characterize the effects of the toxicant on drift of macroinvertebrates leaving the pool areas. Similar results were obtained for these communities indicating a significantly higher drift rate leaving the experimental pool area during treatment with TFM (Figure 16). In this instance, the drift was composed of one species, the amphipod G. pseudolimnaeus. Since

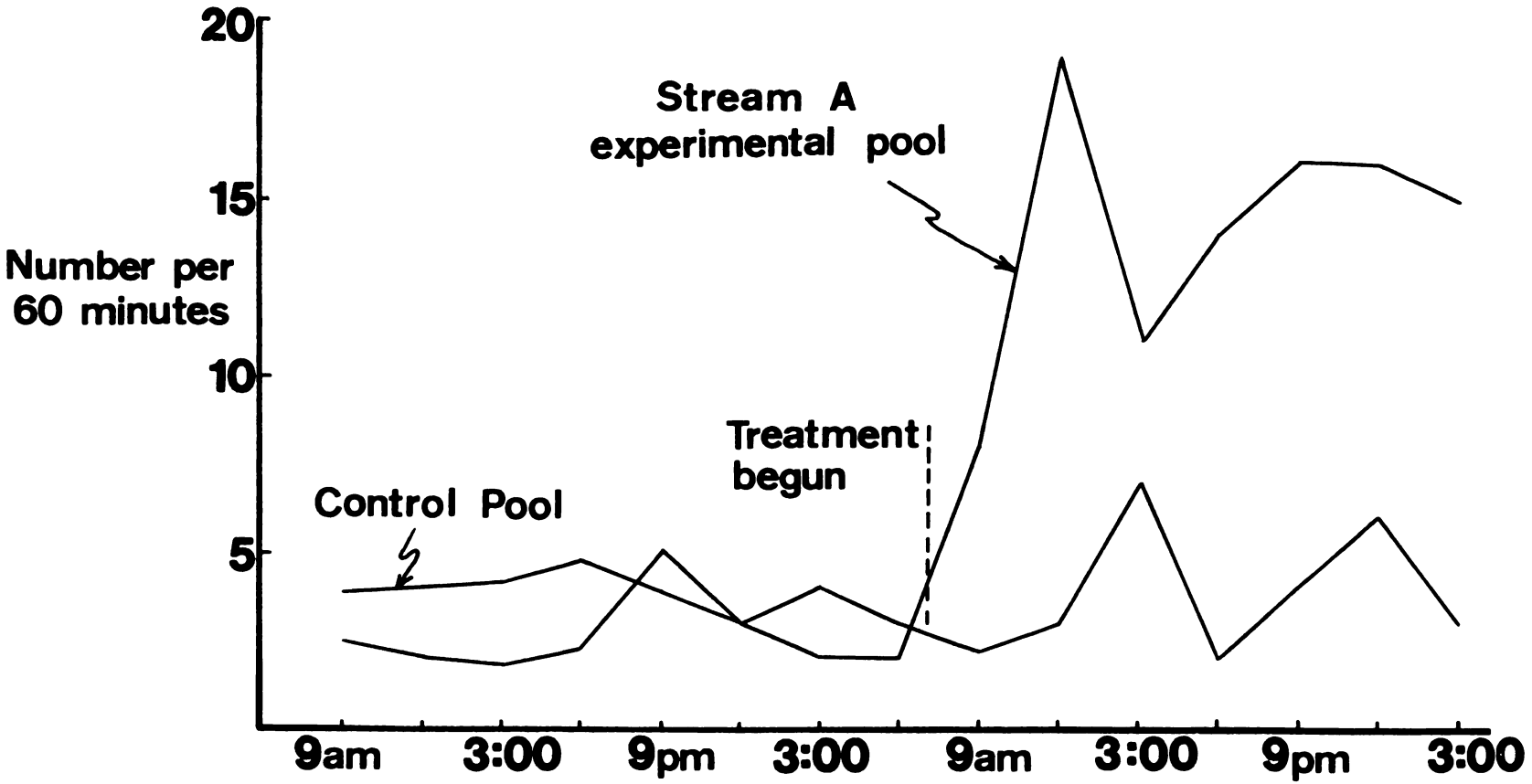


Figure 16.--Simultaneous drift rates from Stream A experimental and control pool channels during treatment with 9.0 mg/l TFM.

the water leaving the pool sections had very little particulate organic matter and since drift rates out of the pools were significantly lower than riffle areas, the nets were fished for a total of 60 minutes at each sampling period without problems due to clogging. Pre-treatment drift rates average 2 to 6 individuals per hour and the exposure to TFM increases this rate to approximately 12 to 19 individuals per hour.

Production.--The production figures for two species, A. varsharva and G. pseudolimnaeus in the control and experimental channels of Stream A are given in Table 14. The standing crop of the stonefly was significantly lower in the experimental channel than in the control channel throughout the entire sampling period. Average biomass throughout the year in the experimental channel was 2.77 gms/m^2 compared to 4.13 gms/m^2 in the control channel and calculated values for production varied correspondingly from 13.63 gms/m^2 in the experimental area to 17.89 gms/m^2 in the control. Cohort turnover ratios or the ratio of production to standing crop were 4.91 in the experimental and 4.33 in the control indicating that a proportionately greater amount of production per unit biomass occurred in the experimental channel than in the control. Obviously, no negative or adverse effect on the production of A. varsharva could be attributed

TABLE 14.--A comparison of standing crop, production and cohort turnover ratios for two species of macroinvertebrates from control and experimental channels of Stream A.

	Stream A Control Riffle			Stream A Experimental Riffle		
	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Cohort Turnover Ratio	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Cohort Turnover Ratio
			Amphinemura varsharva			
Pre-treatment	1.38	3.79	2.74	0.42	1.39	3.33
Post-treatment	4.49	15.73	3.49	3.45	16.12	4.68
Total (Sept-March)	4.13	17.89	4.33	2.77	13.63	4.91
			Gammarus pseudolimnaeus			
Pre-treatment	28.62	124.11	4.34	19.64	82.68	4.21
Post-treatment	16.61	68.58	4.13	30.05	133.57	4.45
Total (Sept-March)	19.74	82.42	4.18	27.85	124.55	4.47

to the TFM treatment since growth and production in the exposed community exceeded that recorded for the unexposed control.

Similar results were also obtained for the amphipod, G. pseudolimnaeus. Production values of 124.5 gm/m^2 and 82.42 gm/m^2 were recorded for standing crops of 27.8 mg/m^2 and 19.74 gm/m^2 in experimental and control communities respectively. The calculated cohort turnover ratios were then 4.47 for the experimental channel and 4.18 for the control indicating a greater amount of production per unit biomass occurred in the experimental channel. Therefore, the exposure to TFM has no negative effect on production of G. pseudolimnaeus and indeed, may have a stimulatory effect causing increased growth.

Stream B

Community Structure.--A replicated treatment employing the experimental and control channels of Stream B was carried out on November 1. The mean values of the various measurements of community structure are represented in Table 15. The pre-treatment numbers represent means of samples taken from 24 August to 1 November and the post-treatment samples cover the period from treatment to 12 March. The mean values were not significantly different in the experimental community treated with TFM. In all communities the mean number of individuals per square meter increased in

TABLE 15.--Community structure of benthic invertebrates in Stream B control and experimental channels.

	Stream B Control		Stream B Experimental	
	Riffle	Pool	Riffle	Pool
Pre-treatment				
Diversity	2.97±0.257*	1.88±0.364	3.09±0.261	1.93±0.176
Equitability	0.64±0.064	0.82±0.095	0.56±0.107	0.74±0.118
Number of Species	12.33±1.150	4.66±0.471	15.51±1.64	5.17±0.408
Number of Ind./m^2	10086±1916	19832±3411	12766±2808	14854±3139
Post-treatment				
Diversity	2.93±0.264	1.38±0.678	3.03±0.173	1.38±0.484
Equitability	0.56±0.078	0.53±0.213	0.59±0.111	0.51±0.201
Number of Species	13.77±2.59	5.55±1.33	13.89±1.450	5.44±0.527
Number of Ind./m^2	11125±2221	21089±4007	10279±2364	16433±3286

*Standard error of the mean.

the post-treatment samples which is the exact opposite effect observed in Stream A where the mean number of individuals was observed to decrease in all post-treatment samples. Because the effect was observed in both control and experimental channels, the increase in individuals was probably a function of the natural population dynamics rather than effects due to the toxicant.

The apparent discrepancy in results obtained between Stream A and B is best explained by considering the difference in timing of the two treatments. In Stream A, the pre-treatment samples were taken during the summer months when the streams had a greater number of individuals present. The post-treatment samples were taken during early fall when the summer and fall-emerging insects had left the system hence showing a slight decrease in total density of individuals in the streams. The pre-treatment samples for Stream B were taken during this time of low density, however, the post-treatment samples were taken throughout the early winter months when the following year's generation had grown to sufficient size to be retained in the sampling apparatus. Values for diversity, equitability and total number of taxa were consistent between pre- and post-treatment samples showing no significant variation due to the toxicant or natural events occurring within the stream.

Since comparison of the data on the basis of seasonal means may obscure immediate and short-term effects of the

toxicant on the macroinvertebrate community, the samples taken immediately before treatment and those taken two weeks post-treatment were also compared. In this instance, as in Stream A, the samples taken two weeks following treatment were found to contain significantly fewer individuals than those samples taken immediately prior to treatment. The difference in number of individuals in the control channels for the same time period showed no significant decrease and actually demonstrated an increase in total numbers attributed to random sample variation (Table 17).

Drift.--Similar to Stream A, an immediate increase in the number of individuals drifting from the experimental riffle channel was demonstrated following the initiation of treatment with 9.0 mg/l TFM (Figure 17). A simultaneous record of the number of individuals composing the drift in both control and experimental channels demonstrates that approximately four to six individuals are caught in the drift nets during a 10 minute period. However, immediately after the initiation of lampricide treatment, the drift rate reached about 33 individuals per 10 minute interval in the exposed channel and remained at one to five individuals throughout the entire time period in the adjacent control channel. During the entire treatment period the drift rate remained approximately eight individuals higher than simultaneous measurements of drift in the control. In this

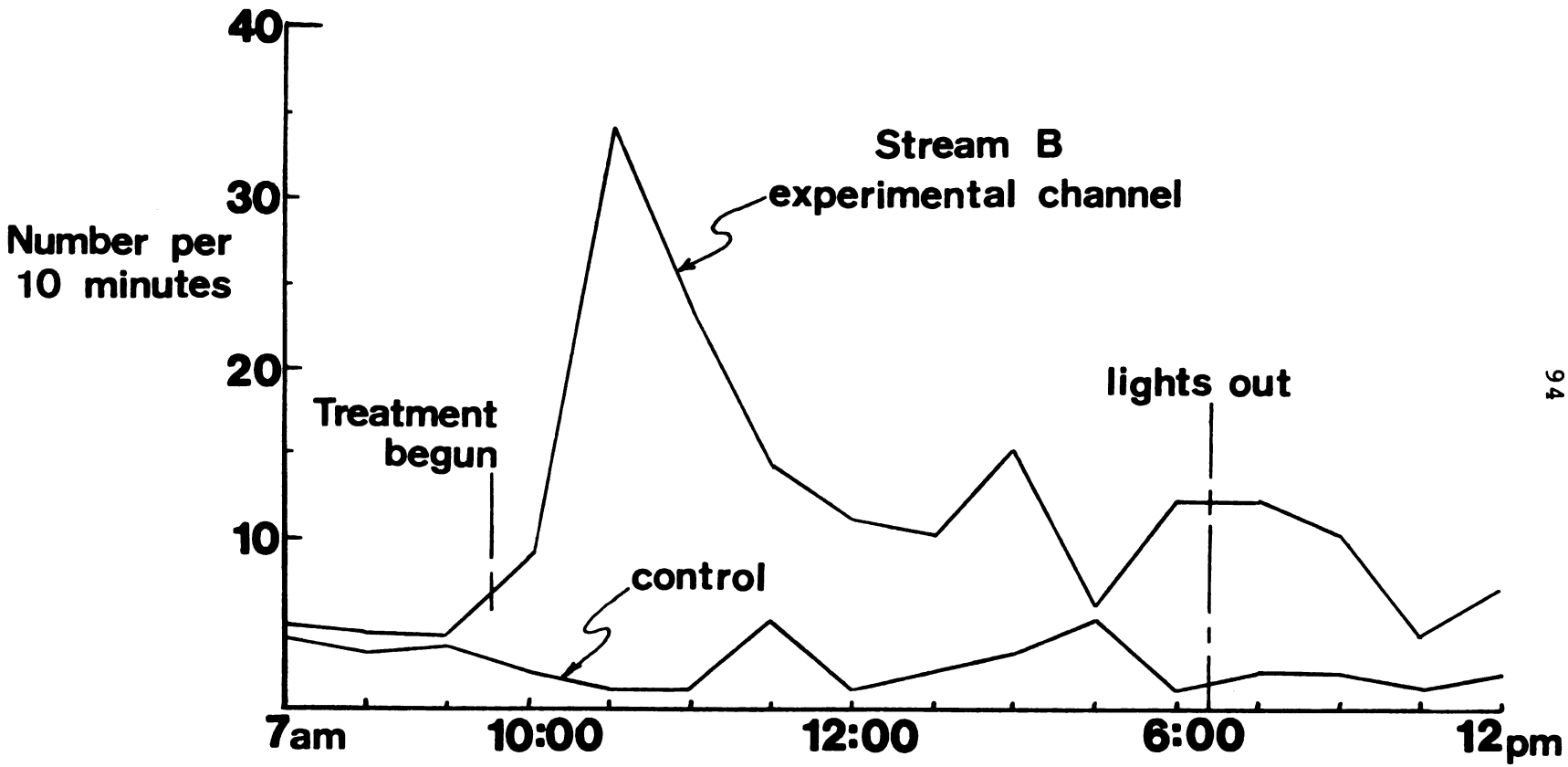


Figure 17.--Simultaneous drift rates from Stream B experimental and control riffle channels during treatment with 9.0 mg/l TFM.

instance, as in Stream A, no correlation of drift rate was observed with the onset of darkness. Trentonius distinctus and Baetis sp. made up 85% of the drift. No macroinvertebrate drift measurements were made on the pool communities because the ends of the pool areas had been screened to contain trout that had been stocked in these sections.

Production.--The production of the Nemourid stonefly, A. varsharva and the amphipod G. pseudolimnaeus were analyzed in detail (Table 16). The standing crop of the stonefly in the experimental riffle area was 4.19 gms/m^2 compared to 3.19 gms/m^2 in the control riffles. Production figures were also comparably higher in the experimental riffle at 16.22 gm/m^2 compared to 13.27 gmx/m^2. However, the turnover ratio of production to biomass was 3.87 in the experimental area and 4.15 in the control indicating that the control community produced more on a per unit biomass basis than did the exposed community. However, the magnitude of difference in turnover ratios, 0.28, is well within the error tolerance for this method of production estimate and does not necessarily represent a real difference.

Similar results were obtained for the amphipod, G. pseudolimnaeus with a standing crop of 19.95 gms/m^2 in the experimental area compared to 8.90 gms/m^2 in the control. The higher biomass in the experimental channel accounted for 84.68 gms/m^2 of production compared to 38.17 gms/m^2 in

TABLE 16.--Comparison of standing crop, production and cohort turnover ratios for two species of invertebrates from control and experimental channels of model Stream B.

	Stream B Control Riffle			Stream B Experimental Riffle		
	Standing Crop (gm wet wt/m^2)	Production (gm wet wt/m^2)	Cohort Turnover Ratio	Standing Crop (gm wet wt/m^2)	Production (gm wet wt/m^2)	Cohort Turnover Ratio
Amphinemura varsharva						
Pre-treatment	2.49	7.45	2.98	3.20	9.44	2.95
Post-treatment	1.50	6.67	4.44	2.05	7.76	3.78
Total (9 Sept 1973-12 Mar 1974)	3.19	13.27	4.15	4.19	16.22	3.87
Gammarus pseudolimnaeus						
Pre-treatment	10.91	44.38	4.07	35.09	151.47	4.32
Post-treatment	8.09	32.14	3.97	13.89	57.96	4.17
Total (9 Sept 1973-12 Mar 1974)	8.90	38.17	4.29	19.95	84.68	4.24

TABLE 17.--Results of Students' t-tests of significance of difference between pre- and post-treatment community structure of benthic macroinvertebrates.

Community Structure Tested	Means		Level of Significance (P)
	Pre-treatment Control	Exposed to 9.0 mg/l TFM	
Stream A Experimental			
Total Number of Individuals			
2 weeks post-treatment	100.67	64.00	<0.05
3 months post-treatment	100.67	91.83	<1.0
Stream A Pools - 2 weeks post-treatment			
Control	93.67	62.00	<0.05
Experimental	102.64	74.55	<0.05
Stream B Experimental Riffle			
2 weeks post-treatment	103.5	73.67	<0.05
3 months post-treatment	103.5	116.92	<1.0
Stream B Control Riffle			
2 weeks post-treatment	82.23	90.44	<1.0
Stream B Pools - 2 weeks post-treatment			
Control	54.86	71.01	<1.0
Experimental	40.22	42.77	<1.0
Stream A Riffle			
Ash-free dry weight of total macroinvertebrates	7.938	8.130	<1.0
Stream A Pool			
Ash-free dry weight of total macroinvertebrates	6.961	6.637	<1.0

the control community. Similar turnover ratios of 4.29 and 4.24 were obtained for the control and experimental areas indicating that essentially no difference exists between the two communities when production is expressed on a per unit biomass basis. Based on these results, 9.0 mg/l TFM is shown to have neither a stimulatory nor inhibitory effect on the long-term production of these two species of macroinvertebrates.

Since the amphipod, G. pseudolimnaeus was common in all core samples taken from control and experimental pool communities of both Streams A and B, an additional production estimate was calculated for these channels to determine if an effect due to the lampricide exposure was present. Production figures calculated for these four communities ranged from 46.44 gms/m^2 to 57.34 gms/m^2 and biomass estimates ranged from 9.70 gms/m^2 to 13.86 gms/m^2 (Table 18). Differences in the total turnover ratio for control and experimental channels of Stream B were 4.28 and 4.14 respectively, indicating no significant difference existed between these two communities when production was expressed on a per unit biomass basis. Differences were somewhat more pronounced between the control and experimental channels of Stream A where the turnover ratios were 4.53 and 5.13 respectively. This indicates that although there was a lower standing crop in the experimental area than in the control, the individuals present

TABLE 18.--Comparison of standing crop, production and turnover ratios for Gammarus pseudolimnaeus from control and experimental pool channels of model Streams A and B.

	Gammarus pseudolimnaeus					
	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Turnover Ratio	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Turnover Ratio
	Stream A Control Pool			Stream A Experimental Pool		
Pre-treatment	15.36	60.38	3.93	20.89	95.46	4.47
Post-treatment	8.80	33.98	3.86	7.07	38.48	5.44
Total (Sept-March)	10.26	46.44	4.53	9.70	49.82	5.13
	Stream B Control Pool			Stream B Experimental Pool		
Pre-treatment	18.22	80.27	4.41	18.33	71.15	3.88
Post-treatment	8.44	35.19	4.17	12.07	51.82	4.29
Total (Sept-March)	11.23	48.08	4.28	13.86	57.34	4.14

in the experimental channel produced a greater amount of biomass per unit weight of standing crop than did the control community over the same time period. This increase in turnover ratio is more likely due to the variability of sampling technique and inherent error of the production estimation technique rather than to any real difference brought about by the exposure to TFM.

Stream C

Community Structure.--The primary purpose of Stream C was for description of the dynamics of TFM residues in a stream system. The treatment was conducted with ^{14}C-TFM and exposure was done under recirculating conditions. Drifting individuals were not lost from the system, and therefore only minimal sampling was done to describe the effects of the toxicant on community structure. No samples were taken from the pool communities of Stream C since young brown trout were held in these communities for a replicate experiment concerning the effects of the toxicant on fish production.

The pre- and post-treatment effects of the recirculating treatment with ^{14}C-TFM on community diversity, equitability, number of taxa and number of individuals per square meter as shown in Table 19. Again in this instance, as with Streams A and B the standard error of the mean for the pretreatment samples overlaps the standard error for the

TABLE 19.--Community structure of benthic macroinvertebrates from Stream C control and experimental riffles.

	Stream C Control Riffle Community	Stream C Experimental Riffle Community
Pre-treatment		
Diversity	3.23 ± 0.268*	2.95 ± 0.154
Equitability	0.66 ± 0.050	0.65 ± 0.081
Number of Species	14.33 ± 3.06	12.30 ± 1.73
Number of Individuals/m^2	9785 ± 1484	9.98 ± 1145
Post-treatment		
Diversity	3.14 ± 0.562	3.35 ± 0.221
Equitability	0.62 ± 0.047	0.74 ± 0.054
Number of Species	13.83 ± 2.13	13.38 ± 1.94
Number of Individuals/m^2	9377 ± 1322	9308 ± 1452

*Standard error of the mean

post-treatment samples indicating that for all samples taken, essentially no difference can be detected due to the lampricide exposure. A slight rise was indicated in the mean number of individuals per square meter in the experimental channel following treatment but the increase is within the range of sampling error and does not indicate a real difference.

Production.--The production of the Nemourid stonefly, A. varsharva and amphipod, G. pseudolimnaeus were analyzed in detail to determine if any difference between control and experimental channel populations could be attributed to the effects of TFM. Both species were common in samples taken from Stream C but their density was significantly lower than in Streams A and B. Standing crop figures were 0.66 gms/m^2 and 0.63 gms/m^2 for the stonefly in Stream C experimental and control channels respectively. The production value for the control area was 2.32 gms/m^2 with a turnover ratio of 3.65 and was 2.30 gms/m^2 with a turnover ratio of 3.47 for the experimental area indicating no difference between the two communities with respect to the effects of the toxicant (Table 20).

The biomass and corresponding production figures for G. pseudolimnaeus were also significantly lower in both control and experimental channels of Model Stream C than in Streams A or B. The standing crop in Stream C experimental

TABLE 20.--A comparison of standing crop, production and cohort turnover ratios for two species of macroinvertebrates from control and experimental riffles of Stream C.

	Stream C Control Riffle			Stream C Experimental Riffle		
	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Cohort Turnover Ratio	Standing Crop (gms wet wt/m^2)	Production (gms wet wt/m^2)	Cohort Turnover Ratio
			Amphinemura varsharva			
Pre-treatment	0.71	1.59	2.25	0.47	1.21	2.58
Post-treatment	0.61	2.10	3.41	0.29	0.99	3.44
Total (Sept-March)	0.63	2.32	3.65	0.66	2.30	3.47
			Gammarus pseudolimnaeus			
Pre-treatment	15.72	55.48	3.53	1.85	6.19	3.35
Post-treatment	6.85	29.19	4.26	9.39	36.47	3.88
Total (Sept-March)	8.38	34.48	4.13	7.33	29.85	4.07

channel was 7.33 gms/m^2 with a production of 29.85 gms/m^2 while the standing crop in Stream C control channel was 8.23 gmx/m^2 with a standing crop of 34.48 gms/m^2. The corresponding turnover ratios for experimental and control communities were 4.13 and 4.07 respectively which was again well within the error of the method and represents no essential difference in production of G. pseudolimnaeus between control and exposed populations.

Fish Production

The results of the growth and production experiments with young-of-the-year brown trout, Salmo trutta in experimental and control pool areas of model Streams B and C are given in Table 21. The growth test in Stream B covered 43 days and the fish were initially stocked at a rate of 25.55 gms/m^2 in the control pool and 25.95 gms/m^2 in the adjacent experimental pool. A 1.27 cm mesh seine was suspended over these communities to insure that the fish would not escape. Also, the hatchery raceway screens were put in place at the end of the pool sections to prohibit emigration downstream into the riffle communities.

During the treatment, these young of the year fish were visibly affected by the toxicant. After approximately 8 hours of exposure the individual fish were observed to move from their protective and secretive habitats in the shadows of the pool to open, unprotective areas. Their

TABLE 21.--Growth and production of young-of-the-year brown trout, Salmo trutta in experimental and control channels of Streams B and C following an exposure to 9.0 mg/l TFM.

Stream Channel	Length of Experiment	Pre-treatment Biomass (gm/m^2)	Post-treatment Biomass (gm/m^2)	Production (gm/m^2)	Mean Growth Rate (mg/gm/day)
Stream B Control	43 days	25.55	27.43	1.877	1.709
Stream B Experimental	43 days	25.95	25.83*	-0.121	-0.108
				1.851**	1.795
Stream C Control	35 days	23.93	27.00	3.078	3.676
Stream C Experimental	35 days	17.15	19.06	1.903	3.170

*Weight loss due to death of two fish during TFM exposure.
**Production calculated on basis of survivors excluding mortalities.

behavior was lethargic and they were not visibly affected by movements of the observer. An increase in gill ventilation rates was observed for these individuals but the lethargic state and lack of fright response remained throughout the exposure. The two smallest individuals of 6.0 cm and 6.9 cm standard lengths and weights of 1.78 gm and 2.00 gm respectively died during the treatment presumably as a direct effect of the toxicant. The survivors had returned to normal secretive behavior patterns and normal gill ventilation rates by the following morning.

At the termination of the 43 day growth period the control pool had a post-treatment biomass of 27.43 gm/m^2 indicating a gross production of 1.89 gm/m^2 of fish. In the experimental pool, the loss of the two individuals due to mortality caused a net decline in post-treatment biomass to 25.83 gm/m^2 and a negative gross production of -0.121 gm/m^2. Results for the experimental pool fish were also calculated by subtracting the weight of the dead fish from the initial mean biomass in order to examine the growth rate of the survivors without the negative effect of the mortality losses. Calculating by this method yielded a gross production of 1.851 gms/m^2 for the survivors.

Due to the inherent variation in growth between individual fish these figures for mean production per unit area of stream bottom mean little for a comparative test.

For this reason the rate or amount of production per gram of fish flesh standing crop was calculated for experimental and control fish. The mean growth rate calculated by this method for the control fish was 1.709 mg gm^{-1} day^{-1} compared to a net decline of -0.108 mg gm^{-1} day^{-1} in the experimental pool. However, if the growth rate is calculated on the basis of the survivors alone, neglecting the two mortalities, a growth rate of 1.795 mg gm^{-1} day^{-1} is obtained. A test of the null hypothesis that the individual growth rates for survivors in the experimental pool were equal to growth rates obtained in the control pool indicated that the growth rates between communities were equal with 99% confidence (Table 22).

Similar results were obtained with the recirculating treatment of Stream C (Table 21). Fish were stocked into the control pool at a rate of 23.93 gm/m^2 and into the experimental pool at 17.15 gm/m^2. During the recirculating treatment with ^{14}C-TFM behavioral changes in the fish were also observed, however, no mortalities were recorded and all fish were recovered at the termination of the 35 day experiment. At this time, the post-treatment biomass in the control community had increased to 27.00 gms/m^2 indicating a gross production of 3.078 gm/m^2. The experimental fish biomass had risen to 19.06 gm/m^2 with a gross production of 1.903 gm/m^2. Calculation of growth rates on a per gram of standing crop basis indicates a rate of 3.676 mg gm^{-1} day^{-1} in the control pools and a rate of 3.170 mg gm^{-1} day^{-1}

TABLE 22.--A comparison of individual growth rates of brown trout, Salmo trutta, between control and experimental channels of Model Streams B and C.

Mean Growth Rates	Stream B	Stream C
Control	$X_1 = 1.709$ mg gm^{-1} day^{-1}	$X_3 = 3.676$ mg gm^{-1} day^{-1}
Experimental	$X_2 = 1.795$ mg gm^{-1} day^{-1}	$X_4 = 3.170$ mg gm^{-1} day^{-1}
	Statistical Hypotheses	
	HO: $X_1 = X_2$	HO: $X_3 = X_4$
	Hl: $X_1 \neq X_2$	Hl: $X_1 \neq X_2$
	$t = 0.3274$	$t = 0.7404$
	$t(.01,20) = 2.845$	$t(.01,20) = 2.845$

Conclusion: Accept HO: $X_1 = X_2$ and $X_3 = X_4$

in the experimental pool. A statistical comparison of the individual growth rates for control and experimental fish indicates that no difference exists between these communities with 99% significance (Table 22).

Kinetics of TFM in the Stream Community

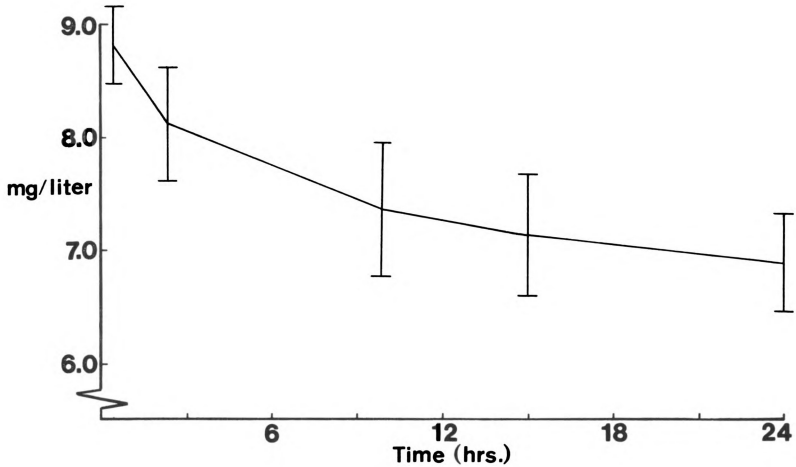
Uptake of TFM Residues

The uptake, bioconcentration and elimination rate of TFM was determined for 20 separate plant and animal components in nine replicated experiments employing ^{14}C-TFM in recirculating systems. All exposures were for 24 hours with an initial TFM concentration of 9.0 mg/l. Sample data for each species was averaged between the three to five replicates to add statistical significance to the mathematical descriptions of residue dynamics.

The uptake rate of TFM by all components of the stream community is expressed in Figure 18 by the reciprocal loss of TFM activity from water over the 24 hour exposure period. There is a rapid initial drop in concentration to about 8.0 mg/l within the first two hours following initiation of treatment. The slope of the loss of activity curve then levels off to about 6.9 mg/l after the entire 24 hour exposure period.

The graph accurately reflects the reciprocal behavior of the toxicant in the biotic components of the stream. An examination of the TFM concentrations in most of the

Figure 18.--Uptake of ^{14}C-TFM expressed as loss from water. Mean $\pm$ 1 S.D.


9.0
8.0
mg/liter
7.0
6.0
6
12
18
24
Time (hrs.)

species and components demonstrated a rapid uptake within the first 2 hours. This initial uptake would then correspond to the rapid loss of activity in the stream water occurring during the same time period. Following this initial surge, the uptake rates level off and increase at a linear rate throughout the duration of the exposure. Since there were generally only two samples taken during this initial rapid uptake component, accurate mathematical description of this portion of the curve was not possible.

The mathematical expression employed for the final characterization of uptake rate was a simple linear regression of ug TFM/gm tissue on a dry weight basis against the exposure time in hours. Calculation of the sample data by this method yields a Y-intercept value which is an accurate estimate of the tissue concentration reached at the end of the two hour initial rapid uptake period. The linear portion of the uptake curve is then accurately described by the regression equation of the form:

$$Y = a + b\ (X)$$

where: Y = concentration of total TFM residue in the organism expressed as ug per gm dry wt.

a = the y-intercept of regression

b = rate of uptake or slope of the regression

X = exposure time in hours.

The regression intercept, regression coefficient, sample standard deviation of the regression coefficient S_b,

and confidence intervals of the slope were calculated according to Steele and Torrie (1960). An example of these statistics for the amphipod, G. pseudolimnaeus is shown in Figure 19. The data are typical of the uptake dynamics of TFM in all of the biotic components examined. The two earliest data points taken at 1 and 3 hours indicate that the amphipod has a total body residue of 39.5 $\pm$ 5.6 µg/gm and 78.9 $\pm$ 21.7 µg/gm at these time intervals respectively. A simple linear regression of all of the data points throughout the 24 hour exposure indicates that the Y-intercept is 54.6 µg/gm with a positive slope of 4.77. The calculation of the Y-intercept value thus yields an accurate estimate of the total TFM residue at the termination of the 2 hour initial rapid uptake period and the equation for the straight line allows for accurate prediction and description of the secondary or reduced linear phase of uptake. The calculated value of the slope indicates that during this secondary phase, the amphipod concentrates a body residue of TFM at approximately 4.77 µg/gm body weight per hour and 95% confidence intervals for this slope was $\pm$1.126. The relatively high correlation coefficient of .94 indicates that the assumption of linearity in the relationship is valid. The equation can be employed to estimate the total residue concentration at any time during the 24 hour exposure by substituting the time of exposure for the value of X in the equation.

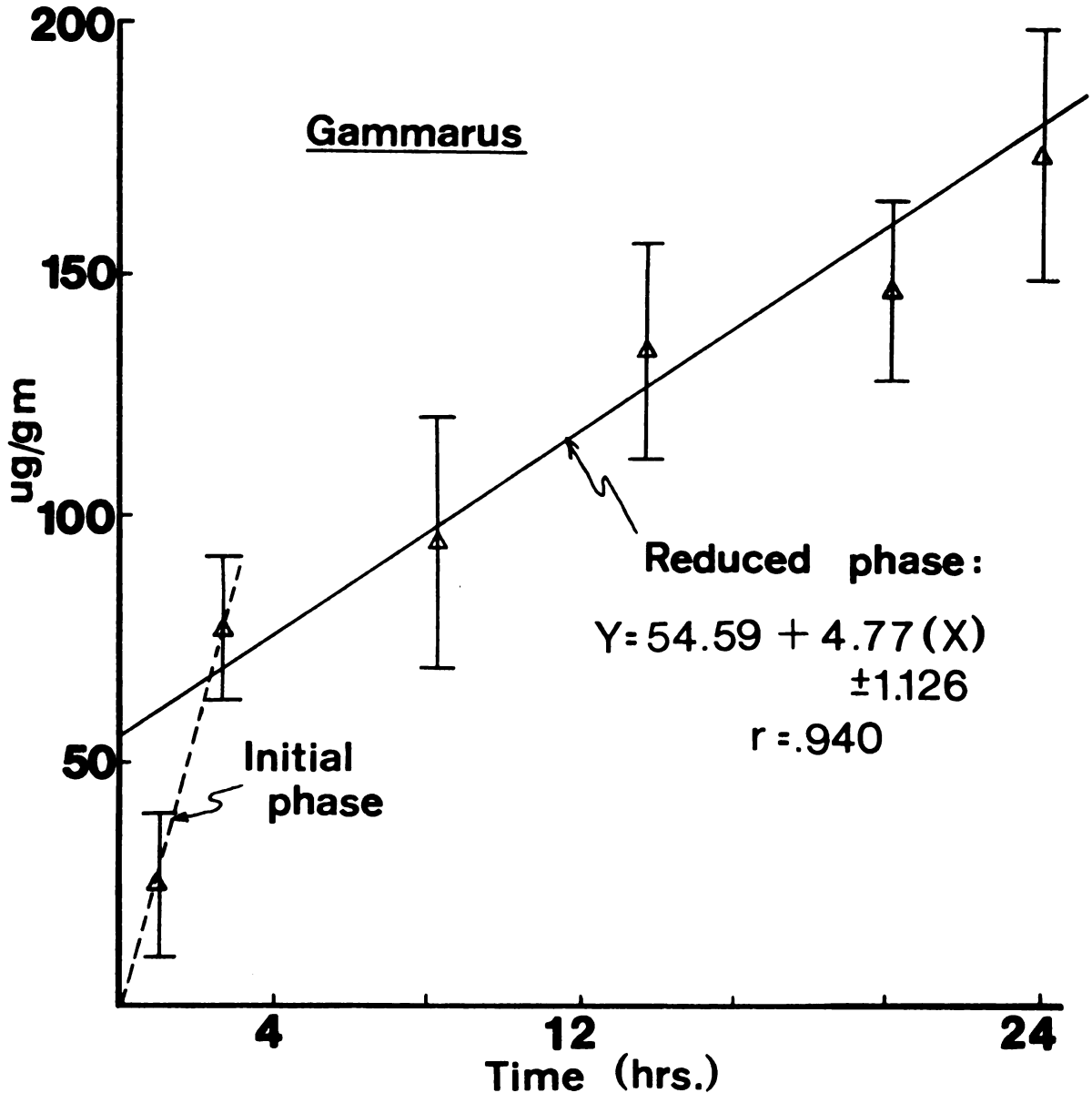


Figure 19.--The rate of uptake of ^{14}C-TFM by the amphipod, G. pseudolimnaeus during a 24 hour exposure to 9.0 mg/l TFM.

Table 23 lists the critical values of linear regressions for the remaining 19 plant and animal components examined during the year. Calculated values for the Y-intercept which estimate the total body burden at the end of the 2 hour initial rapid phase of uptake range from 1.603 µg/gm for young crayfish, Orconectes propinquus to a high of 221.994 µg/gm for annelid worms. Rates of uptake for animal components during the secondary linear phase range from 0.344 µg gm^{-1} hr^{-1} for the crayfish to 17.899 µg gm^{-1} hr^{-1} for the caddisfly larvae, Brachycentrus americanus. The general trend evident from these comparisons is that the rate of uptake and therefore the ultimate total body burden of TFM is directly related to the permeability of the test species' integument. Those species with relatively rigid sclerotinized exoskeletons or those with calcareous shells such as the clams and snails concentrate TFM at much slower rates and consequently the total TFM concentration at the termination of the 24 hour exposure period in these species is significantly lower than those species with relatively soft, membranous integuments. Large molluscs of the genus Anodonta were separated into three components and each was analyzed separately as part of an experiment to evaluate the mollusc as an indicator of environmental concentrations of TFM in a treated stream. Although many more clams were exposed and analyzed than for most of the other species, the span of the

TABLE 23.--Accumulation of total TFM residue in several plant and animal components exposed for 24 hrs. to a recirculating concentration of 9.0 mg/l TFM.

Species	Y-Intercept	Rate of Uptake (Slope)	95% Confidence Intervals of Slope	Correlation Coefficient
Gammarus pseudolimnaeus	54.597	4.774	± 1.126	.940
Asellus militaris	43.193	4.509	± 0.144	.981
Orconectes propinquus	1.603	0.344	± 0.144	.981
Anodonta sp. Foot	18.297	10.899	± 7.774	.824
Anodonta sp. Gills	45.115	7.831	± 6.570	.881
Anodonta sp. Viscera	32.397	9.054	± 7.044	.809
Pisidium sp.	34.966	3.634	± 1.395	.986
Physa sp.	16.989	2.729	± 0.447	.994
Hexagenia sp.	39.343	1.965	± 1.673	.793
Glossosoma sp.	114.543	7.976	± 2.307	.976
Limnephilus sp.	95.053	3.320	± 4.819	.987
Brachycentrus americanus	129.779	17.899	±10.327	.921
Brachycentrus cases only	189.258	27.939	±19.529	.987
Annelid worms	221.994	9.663	± 7.906	.868
Cladophora and *Stigeoclonium* (pool)	39.429	0.891	± 0.806	.969
Stigeoclonium (riffle)	43.951	2.587	± 1.242	.838
Ceratophyllum demersum	54.089	2.327	± 0.312	.964
Elodea canadensis	21.029	1.249	± 1.812	.991
Moss	27.282	0.482	± 0.551	.988
Aufwuchs (5 cm^2) (wet weight)	0.202	0.252	± 0.173	.973
Leaf discs (20 mm dia.)	43.609	1.784	± 0.563	.903
Sediment	14.476	0.866	± 0.771	.865

confidence limits on the uptake rates for the three components indicates the variability encountered between the individuals. This variation in residue concentrations was assumed to be explained by the variations in activity levels of the different clams during the exposure. Some individua;s were observed to be actively siphoning, others moving about with foot extended and still others tightly clamped shut with minimal siphoning activity during the period of exposure. These behavioral differences add great variability to the total residue concentrations and make the clam an unreliable indicator of environmental levels of TFM.

Brachycentrus americanus and its vegetative cases were analyzed separately to examine variations in total residues. After the samples were thawed the animals and cases were separated, dried and combusted as individual samples. The cases consistently produced high residue levels as did the animals. It is speculated that this high level in the case may be due to the microflora and microfauna associated with the case. However, 20 mm diameter leaf discs did not concentrate the extremely high residue levels observed for Brachycentrus cases. Since they had entered the stream during the previous fall, they were relatively well processed and probably did not support comparable communities of microflora.

Several plant components of the model streams were also examined and concentration ranges at the end of the

initial rapid uptake period ranged from 21.029 μg/gm for Elodea canadensis to 54.089 μg/gm for Ceratophyllum demersum in the pool communities. Rates of uptake during the secondary linear phase were generally slower than animal components ranging from 0.482 μg gm^{-1} hr^{-1} for stream moss to 2.587 μg gm^{-1} hr^{-1} for the filamentous green algae Cladophora and Stigeoclonium growing together. Uptake and accumulation by the combined Aufwuchs community was examined employing the periphyton sampler used for species composition determinations. A total of 5 cm^2 were removed from an exposed rock surface and concentrated onto a preweighed 25 mm dia. Millipore filter. The entire filter and sample were then combusted for analysis of total residue. Due to the variation in dry weight figures with such low total biomass the residue accumulations are expressed on a wet weight basis after air drying at room temperature for approximately 1 hour.

Elimination of TFM Residues

Numerous transformations were also attempted with elimination rate data for several representative components and it was determined that the best description of these data was obtained by a semi-log transformation of the time in hours. The actual concentrations of TFM as determined by radioassay for each component were regressed against the log time in hours. The majority of the total body burden

was eliminated rapidly, within the first day after exposure for most components. However, detectable residue levels were present in most of these components for 2 to 4 weeks following exposure. The use of the log time axis thus permitted an accurate description of the elimination curve with a linear equation. The equation for this relationship is of the general form:

$$Y = a + (-b) \ (\log X)$$

where: Y = concentration of total TFM residue in the organism expressed as ug per gm dry weight

a = the Y-intercept of regression or initial concentration in tissue at the initiation of the elimination period

b = rate of loss or slope of regression

log X = log of time in hours.

A graph of these data for a typical macroinvertebrate, G. pseudolimnaeus is shown in Figure 20. The value for the Y-intercept in the equation is 169.14 µg/gm and represents the initial concentration in the tissues of the amphipod following the 24 hour exposure period. The rate of loss indicated by the negative slope is 62.41 µg gm^{-1} $[\log (hr)]^{-1}$ with 95% confidence intervals of $\pm$0.253. The relatively high correlation coefficient of .97 indicates that the linear relationship is an accurate description of the rate of loss data. Calculation of the half-life, the time until 50% of the initial TFM residue is eliminated, was

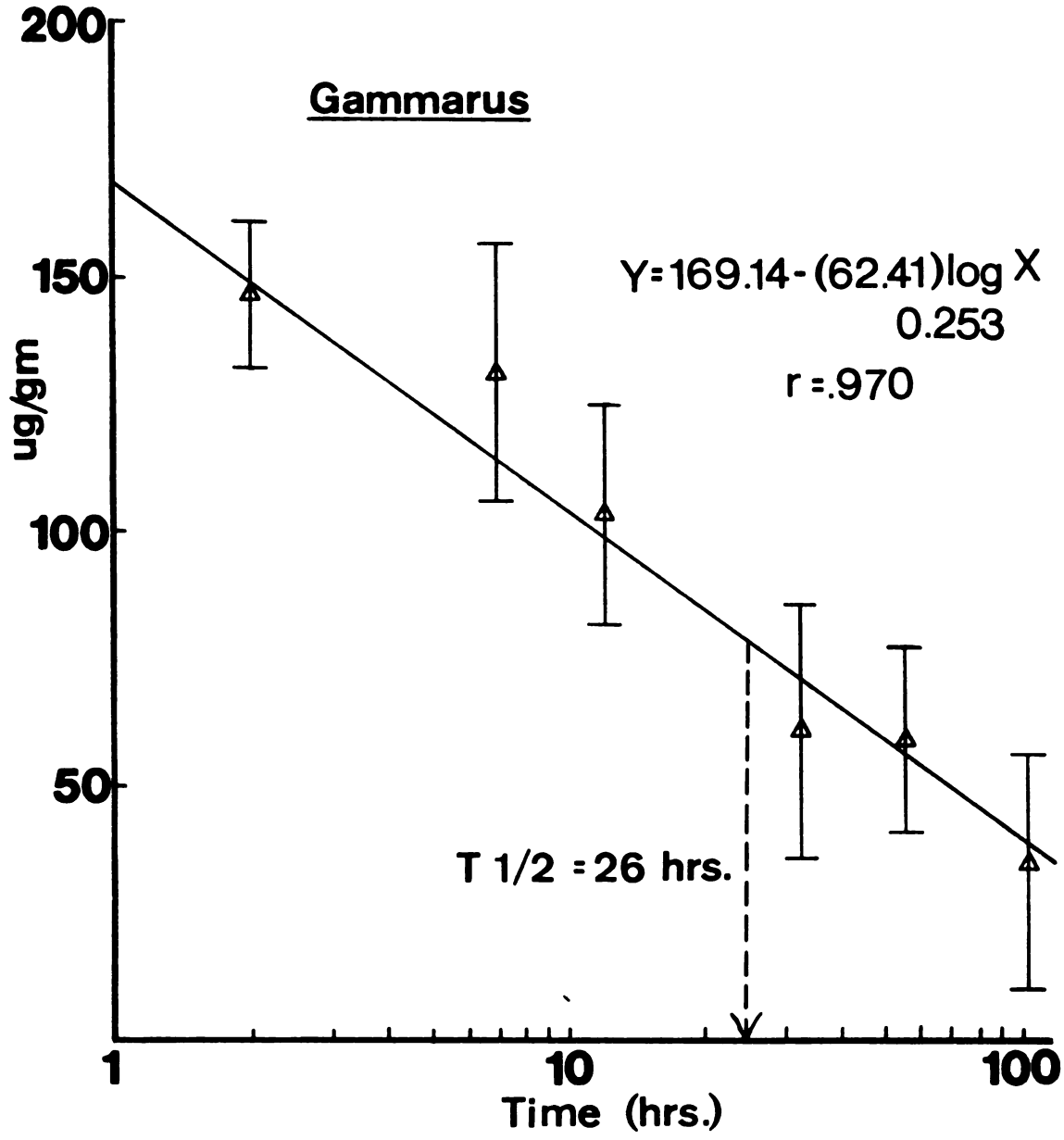


Figure 20.--The rate of elimination of ^{14}C-TFM by the amphipod, G. pseudolimnaeus following a 24 hr. exposure to 9.0 mg/l TFM.

done by substituting 50% of the initial body burden as µg/gm into the equation for Y and solving for X in hours. The half-life solution was checked for accuracy with the graph of the actual data. The half-life for G. pseudolimnaeus was found to be 26 hours.

The critical values for elimination data for the remaining plant and animal components are listed in Table 24. The half-life data of the animal components range from 6.52 hours for the crayfish, Orconectes to a high of 38.3 hours for Hexagenia. The half-life figures for the isopod, Asellus militaris and annelid worms were 194. hrs. and 3808 hrs. respectively. It is probable that the longer half-lives for these species reflect continued residue concentration from their close association and burrowing habits into the organic matter of the pool bottoms.

A strong correlation exists between the half-life data and the substrate associations of the test species, i.e., those species closely associated with the organic matter and sediment of the pool communities had significantly longer half-lives than those associated with the gravel and rubble substrate of the riffle communities. The mean half-life calculated for pool species was approximately 140 hours while the mean for the riffle-dwelling species was only 17.8 hours. A comparison of the filamentous green algae, Cladophora and Stigeoclonium sampled from pool and riffle

TABLE 24.--Half-life and rate of loss of total TFM residue from several plant and animal components following a 24 hr. exposure to 9.0 mg/l TFM.

Species	Initial Concentration in Tissue (ug/gm)	Half-life (hours)	Rate of Loss (slope)	95% Confidence Intervals of slope	Correlation Coefficient
Gammarus pseudolimnaeus	169.142	26.1	- [illegible].409	±0.253	.970
Asellus militaris (pool)	151.382	194.0	- [illegible]	±9.057	.984
Orconectes propinquus	9.856	6.5	- [illegible].933	±0.211	.885
Anodonta sp. Foot	279.776	26.9	- [illegible].687	±0.244	.600
Anodonta sp. Gills	232.944	22.6	- 14.[illegible]	±0.296	.670
Anodonta sp. [illegible]	249.272	22.6	- 11.538	±0.304	.644
Pisidium sp.	122.182	7.1	- [illegible].884	±0.844	.931
Physa sp.	82.479	23.2	- 14.[illegible]	±0.733	.893
Hexagenia sp. (pool)	86.481	38.3	- [illegible]	±1.851	.977
Glossosoma sp.	305.410	11.3	-[illegible].763	±0.999	.926
Limnephilus sp.	174.680	14.0	- 46.506	±0.699	.937
Brachycentrus [illegible]	558.361	19.3	-[illegible].030	±2.486	.957
Brachycentrus [illegible]	859.536	23.7	-[illegible].631	± .925	.965
Annelid worms	452.912	3808.0	- [illegible].524	±2.743	.899
Cladophora and Stigeoclonium (pool)	60.876	66.1	- 11.168	±0.878	.977
Cladophora and Stigeoclonium (riffle)	105.088	25.5	- 31.446	±0.884	.986
Ceratophyllum demersum	109.848	440.0	- 21.144	±1.869	.945
Elodea canadensis	50.976	52.4	- 16.[illegible]	±0.989	.961
Moss	38.568	14.2	- [illegible]	±0.302	.947
Aufwuchs (5 cm^2) (wet wt.)	6.250	24.5	- [illegible]	±0.034	.925
Leaf discs (20 mm. dia.)	86.429	11.0	- 21.522	±3.073	.997
Sediment	35.254	170.0	- [illegible].230	±1.895	.898

environments demonstrates this difference in half-lives between the two areas. The half-life calculated from data obtained by exposing and following the algae in the pool area is 66.1 hr. while the half-life calculated for the same species in the riffle area is only 25.6 hr.

These relatively rapid half-lives of residue elimination and substrate associations indicate that the uptake of TFM is largely an absorption phenomenum on the integument of the animal. The compound is relatively polar and water soluble and for this reason does not appear to be tightly bound in any significant concentrations and is rapidly eliminated upon exposure to TFM-free water.

DISCUSSION

Measurements of changes in the metabolic rate of stream and river communities have generally been used to characterize changes in the stream community brought about by nutrient inputs and cultural eutrophication (Odum, 1956; Cole, 1973). These changes are generally of a long-term nature and involve comparisons with similar upstream stations for control data. Several workers have employed laboratory model streams of various degrees of complexity to describe the metabolic responses of the benthic community to nutrient input or changes in the physical environment such as temperature or light intensity (Odum and Hoskins, 1957; Beyers, 1963; McIntire et. al., 1964; Kevern, 1962; McIntire and Phinney, 1965). However, very few investigations into the effects of toxicants on community metabolism of stream systems exists outside of work done at Oregon State with Kraft mill effluent (Williams, 1969).

Community Metabolism

The primary purpose of the community metabolism measurements in this study was to determine if respirometry measurements would provide a short-term assessment of the influence of TFM lampricide on the stream community. Since

the compound has been shown to be strongly algi-static at relatively low exposure levels (Maki et. al., 1974), it was hypothesized that chronic effects on algal metabolism may be produced during a short-term exposure. This hypothesis was also supported by the work of Huang and Gloyna (1968); who tested the effects of numerous phenolic compounds on photosynthetic oxygen production using a direct manometric technique, they found that halogenophenols exhibited greater destructive effects than the unsubstituted phenol and that introduction of a nitro group to phenol greatly decreased the chlorophyll content of exposed cultures. They found that the o-,m-, and p-nitrophenols reduced the rate of photosynthetic oxygen production by as much as 70% at relatively high exposure levels of 50 to 100 mg/l.

The values for gross primary production in the riffle communities of this study ranged from 58.2 $mgO_2hr^{-1}m^{-2}$ to 79.0 $mgO_2hr^{-1}m^{-2}$ during the course of the experiments which is considerably lower than the 189 to 434 $mgO_2hr^{-1}m^{-2}$ reported by McIntire and Phinney (1965) (Table 25). However, greater illumination intensities, warmer temperatures and extremely high standing crop figures for the primary producers in those streams makes comparisons invalid. Reese (1966), obtained measurements of primary production in non-enriched sections of a shallow woodland stream ranging from 52 to 200 $mgO_2hr^{-1}m^{-2}$. Since the intent of this study was

to model a small woodland stream, favorable comparisons can be made. Under an exposure to 9.0 mg/l TFM the gross primary production dropped from 5 to 50% of the pre-treatment rates in the riffle communities and was reduced to non-detectable levels in the pool areas.

Pre-treatment rates of community respiration ranged from 20.3 to 33.1 $mgO_2hr^{-1}m^{-2}$ in the riffle areas and from 10.2 to 54.0 $mgO_2hr^{-1}m^{-2}$ in the pool areas. These values are again lower than the range of 104 to 171 $mgO_2hr^{-1}m^{-2}$ reported by McIntire and Phinney also probably due to the lower standing crop of primary producers in this study. The values compare favorably with the range of 16 to 125 $mgO_2hr^{-1}m^{-2}$ determined by Williams (1969) and Reese (1966) for small woodland streams. Exposure of the communities in this investigation to 9.0 mg/l TFM consistently caused an increase in respiration rates of from 5 to 40% in the riffle areas to a range of 10 to 50% in the heterotrophic pool areas. This is undoubtedly a measure of the increased respiration rates of the enclosed macroinvertebrate community as a response to the sublethal stress of the toxicant exposure.

The P/R ratios calculated for the riffle communities in this study ranged from 0.92 to 2.07 and from 0.0 to 0.68 in the pool communities. The P/R ratio is an excellent measure of the degree of heterotrophy of a community,

TABLE 25.--A comparison of community metabolism measurements obtained in the present study with estimates from other laboratory and natural systems.

Citation	Location	Gross Production ($mgO_2\ hr^{-1}\ m^{-2}$)	Community Respiration ($mgO_2\ hr^{-1}\ m^{-2}$)	Daily P/R Ratio
This study	Model Streams			
Pre-treatment	Means Riffles	66.08	26.03	1.36
Pre-treatment	Means Pools	26.48	22.36	0.39
During treatment	Means Riffles	46.83	34.74	0.79
During treatment	Means Pools	11.84	30.49	0.15
Post-treatment	Means Riffles	61.40	23.41	1.38
Post-treatment	Means Pools	21.64	20.00	0.29
Reese (1966)	woodland stream	52	54.5	0.85
		203	120	0.47
Williams (1969)	laboratory stream	76-438	12-125	1.18-7.0
McIntire and Phinney (1965)	laboratory stream	189-434	104-171	1.3-2.5
Baumgardner (1966)	Skeleton Creek, Oklahoma	116.6-562	479-116	0.24-0.50
Odum (1956)	Silver Springs	660	117	2.9

generally the lower the ratios, the more strongly heterotrophic conditions as shown by the calculated ratios for the pool areas. The data again compare favorably with Reese (1966) where P/R ratios ranged from 0.16 to 0.85 and averages slightly lower than the P/R ratio of 2.5 calculated by McIntire and Phinney (1965). The P/R ratio was found to be the most sensitive indicator of the toxic influence of TFM where values calculated for communities under exposure to the toxicant dropped from 0.5 to 1.0 compared to pre-treatment ratios. In most instances the effects of the lampricide were sufficient to drive gross production in the pool communities to un-detectable levels which then produced a P/R ratio of zero.

The temporary nature of the toxic effects of TFM is demonstrated in the post-treatment data and should be emphasized. In all cases examined in both pool and riffle areas, the gross production, respiration, and P/R ratios measured within one or two days following the day of exposure had returned to pre-treatment levels with no significant differences detectable.

Periphyton Community Structure

The toxicity of TFM to various algal groups can be generally correlated with taxonomic divisions at the ordinal level. The values for 96 hr. EC-50's range from a mean of 2.2 mg/l for diatom species (Bacillariophyceae) to a mean of

4.8 mg/l for green algae (Chlorophyta) and a mean of 7.9 mg/l for blue-greens (Cyanophyta) (Maki, et. al., 1974). The values do not actually represent algicidal levels of the toxicant for, when these exposed cultures were filtered free of TFM and resuspended in lampricide-free growth media, growth ensued at normal control rates. Similar results were obtained with cultures exposed to concentrations as high as 30 mg/l. Therefore, the compound appears to exert an algi-static effect with most pronounced influence on the diatom species.

Several experiments were conducted during the course of the model stream treatments to determine if an effect on the species composition of the periphyton community could be demonstrated from a 24 hour exposure to 9.0 mg/l TFM. Seasonal densities in the standing crops of the diatoms in Streams A and B varied from a low of approximately 1.20 x 10^9 cells/m^2 during the early fall months to peaks of approximately 3.0 x 10^9 cells/m^2 during the early summer and early winter periods. With these strong variations, only through comparison with periphyton community structure in the adjacent control channel could any effects due to the TFM treatment be identified. Results between control and experimental riffle channels of Streams A and B agreed satisfactorily and the agreement is believed due to the accuracy and reproducibility of the special periphyton

sampler employed in the sampling of these communities. A statistical analysis of the species composition data for pre- and post-treatment sampling dates indicates that no difference in relative densities of the total diatom community can be attributed to the lampricide exposure. An analysis of the community structure of the diatoms in Streams A and B also demonstrated no difference between the generic diversity, equitability and total number of genera present in control and experimental channels on a pre- and post-treatment basis (Tables 8 and 9).

Since no effects on the structure of the diatom communities were demonstrated due to exposure to TFM, and since the diatoms appear to be the most sensitive of the algal groupings, predictably minimal effects due to the toxicant should be demonstrated on the filamentous green algal community. This hypothesis was tested on the green algal assemblage of Stream A by sampling to measure the standing crop as ash-free dry weight and a determination of the relative percent frequency of occurrence of the species present. The biomass of the total green algal community reached a peak in late summer at 38 gms/m^2 and dropped continuously to a low of about 3 gms/m^2 in mid-December. Control and experimental riffle populations dropped at approximately the same rates and no difference could be attributed to the TFM exposure of September 4. Similarly, there were no effects on

the relative frequency of occurrence of the three dominant greens, Spirogyra, Cladophora and Stigeoclonium although a gradual change in succession from a Spirogyra-dominant to a Cladophora-dominant community was observed. Since this succession also occurred in the adjacent control channel, the influence of TFM was insignificant.

Macroinvertebrate Community Structure

The taxonomic composition and general community structure of the macroinvertebrates in all three model stream systems was directly related to flow rate and substrate type differences between the pool and riffle communities. Numbers of taxa were highest in riffle areas ranging from 23 to 25 taxa and from 7 to 14 taxa in the pool areas with abundance, measured as total number of individuals per unit area of stream bottom, measuring 2 to 3 times higher in the pools than riffles. This decrease in total number of taxa and increase in number of individuals produced equal differences in the calculated values of the Shannon diversity index for pre-treatment samples, generally 2 to 3 times lower in the pool areas than the riffles.

Several concurrent measurements on the community structure and population dynamics of the macroinvertebrates existing in the model streams were made in the attempt to accurately characterize the effects of a lampricide treatment on the exposed communities. Much effort was expended

to accurately measure the species diversity in all riffle and pool communities on a pre- and post-treatment basis generally since diversity indices have been proposed as sensitive indicators of environmental change in aquatic systems (Wilhm and Dorris, 1968; Hooper, 1969). The theoretical major regulators of species diversity are stability, predictability, and rigor of the environment (Paulson and Culver, 1969). Communities with high species diversity are characterized as highly predictable with low temporal variability (Slobodkin and Sanders, 1969). If TFM were to exert a selective toxicity against certain species or species complex and eliminate them from the model streams a comparison of diversity indices between pre- and post-treatment samples should reflect the difference in community structure. Calculated values for diversity in all three streams indicated no variation, either increase or decrease, that could be correlated with the lampricide exposure.

It appears that most severe effects of an introduction of a toxicant to a stream system is the immediate reduction in macroinvertebrate density. Reductions in the bottom-living insects ranging from 100% to 10% have been reported following forest spraying operations (Ide, 1968). The insect survivors are mainly tolerant egg, pupal or diapausing larval stages (Hynes and Williams, 1962). The most sensitive indicator of macroinvertebrate community change

in this study and in the two previous field studies of the effects of TFM (Torblaa, 1968; Haas, 1971) was the decline in total number of individuals per unit area of stream bottom. Although lack of replications and variability in substrate types sampled add a high degree of sampling error to the results Torblaa (1968) determined that approximately 77% of the invertebrate taxa present were reduced in abundance one week following treatment. Haas (1971) demonstrated similar effects with an approximate reduction of 90% of invertebrate taxa examined.

An approximate reduction of 25 to 30% in density of the total macroinvertebrate population occurred in the riffle areas of both Streams A and B following exposure of these communities to 9.0 mg/l TFM as determined from samples taken 2 weeks after treatment. Although reductions in the macroinvertebrate fauna were also observed in Stream A control riffle, the drop was more severe in the experimental channel and was determined significant at 95% confidence. A similar drop did not occur in the control channel of Stream B but an increase was actually recorded lending additional significance to the decrease observed following treatment of the experimental riffle of Stream B.

A decrease in the total number of individuals in the pool fauna was also observed in post-treatment samples of Stream A but since an equal decrease occurred simultaneously

in the control channel no significance due to the lampricide was implied. The drop in total individuals was explained by the natural hatching and emergence of the resident population of the midge Tanytarsus which occurred during the two weeks following treatment. In the post-treatment samples of Stream B pool areas taken 2 weeks following the November 1 treatment, an actual increase in total number of individuals was demonstrated due to the growth of early instar midge larvae to sufficient size to be retained by the 0.5 mm sampling net.

A sharp increase in the frequency of drifting individuals has been reported by several investigators as an immediate response to the introduction of a toxicant to a stream system. Hoffman and Surber (1945) reported significant increases in the drift rates of immature Ephemeroptera and Trichoptera following DDT spraying in West Virginia. Similarly, Hoffman and Drooz (1953) demonstrated the sharp decline in abundance of Trichoptera larvae as a result of drift following a DDT application. Immediate increases in the drift rates of the total benthic fauna have been demonstrated as a response due to the toxicants rotenone and Zectran (Binns, 1967; Gibson and Chapman, 1972).

Similar increases in drift rates of the macroinvertebrate fauna were demonstrated within 15 minutes following the introduction of TFM to model Streams A and B.

Increases of 5 times the number of individuals taken in simultaneous samples of the adjacent control channel were recorded in the experimental riffle. However, only three of the more TFM -susceptible species made up approximately 80% of this initial drift pulse, Trentonius distinctus, Gammarus pseudolimnaeus and Baetis sp. and the rate rapidly dropped to within 2 times the control channel drift rate within 3 hours following initiation of treatment. The rate then remained at approximately 1.5 to 2 times the control rate throughout the remainder of the treatments. This increase in drift pulse during the treatment is proposed as a major route of egress of the macroinvertebrate fauna which is reflected in the observed decrease in density of post-treatment fauna. This factor along with direct toxicant-induced mortality of the more susceptible species are the major causative agents bringing about the reduction of fauna.

Waters (1964) demonstrated that drift of benthic fauna is also a major mechanism in bringing about the recolonization of artificially disturbed sections of stream substrate and that this recolonization can occur in relatively short time intervals. He found that the mayfly Baetis sp. returned to 100% of its former density within 4 days following artificial removal. Torblaa (1968) reported that insect abundance exceeded pretreatment densities within six weeks following TFM treatment. Similar results reported in this study from post-treatment samples indicate that drift probably in combination with growth of young instars into

effective sampling size brought about the full recovery of stream populations within 3 months following treatment.

Production estimates were made for two species of macroinvertebrates common to all three streams, Amphinemura varsharva and Gammarus pseudolimnaeus in an attempt to determine if a long-term chronic effect of the TFM treatment could be demonstrated on these species. The Hynes and Coleamn (1968) technique was used for its relative simplicity and accuracy. Waters (1973) has demonstrated that results obtained with this method are slightly higher but within about 15% of estimates obtained by removal-summation, instantaneous growth and the Allen curve methods. Although one of the implied assumptions is that the species be univoltine which applies to the stonefly but not to the amphipod, the data were calculated for both species from control and experimental channels to gain comparative figures for the two populations. In any case, the method should yield accurate estimates of standing crops for both species.

The data demonstrated that the populations were comparable between control and experimental channels of the same stream system but that larger differences in standing crops, and therefore total production, existed between Streams A, B, and C. The standing crop figures for Amphinemura varsharva varied from a low of 0.66 gms/m^2 in

Stream C to a high of 4.13 gms/m^2 in Stream A with production values of 2.30 gms/m^2 and 17.89 gms/m^2 respectively. The figures do not represent entire annual production since the estimates were calculated from sample data taken between September when the insects were 1 and 2 mm in length through March when they had attained a total length of 8 and 9 mm. Therefore, the figures do not estimate entire annual production and should more accurately be termed cohort production over the sampling interval.

Calculated results for the amphipod show wide variations between Streams A, B, and C as a result of their multivoltine nature and the presence of staggered generations present during each sampling period. Standing crops vary from 7.33 gm/m^2 in Stream C to 27.85 mgs/m^2 in Stream A resulting in production figures of 29.85 gms/m^2 and 124.55 gms/m^2 respectively.

Calculation of turnover ratios which reflect the quality of production on a per unit biomass basis showed no consistent pattern between control and experimental channels. The ratio was higher than controls in the experimental channels. The ratio was higher than controls in the experimental channels of Stream A and lower than controls in Stream B and C. However, variations were slight, generally of the order of 0.25, and undoubtedly lie within the experimental error of the technique.

Therefore, similarly to the community metabolism data, the temporary nature of the effects due to TFM treatment should be emphasized. The most severe effect demonstrated on the macroinvertebrate community was the temporary reduction to 25 or 30% of the pre-treatment density observed in the experimental channels. Effects were of a short-term nature and no significant alteration of the environment occurred to prevent relatively rapid recolonization of the treated channels through drift input.

Fish Production

Fish production in various aquatic systems has been correlated with standing crop of benthic macroinvertebrates in numerous investigations (Allen, 1951; Ellis and Gowing, 1957; Davis and Warren, 1965). Differential responses in production rates of fish populations exposed to toxicants have been reported as a reflection of variation in standing crops of food organisms. Elson (1967) demonstrated significant decreases in production of young salmon in the Miramichi River following DDT application. The reduction in standing crop and production was attributed to sharp declines in fish food organisms and direct toxicant-induced mortalities of the fish. Exposure of the tropical fish Cichlasoma bimaculatum to pentachlorophenol caused an increased food consumption which increased production to equal that of unexposed

controls (Chapman, 1965). Assessment of the effects of a toxicant on a fish population by estimations of growth present an excellent opportunity to examine the total impact of the toxicant on the fish's behavioral and physiological responses because growth and production reflect an integration of these effects.

The effects of TFM treatment on the short-term production of control and experimental populations of young of the year brown trout, Salmo trutta were determined during the treatments of Streams B and C and there was no significant difference between mean growth rates of the control and experimental fish for either stream.

Kinetics of TFM in the Stream Community

Several investigations regarding the ultimate fate of TFM residues in the aquatic environment have been conducted. Most of the early investigations suffered from the lack of good quantitative methodology to detect lampricide residues from field and laboratory samples. A streamside bioassay unit was described by Howell and Marquette (1962) employing colorimetric methods to determine concentrations in natural stream waters. The method is useful for determining the quantity of TFM present in natural waters which possess conflicting background colors (Smith et. al., 1960; Ebel, 1962). These colorimetric measurements, however, are limited to solutions containing about 0.1 mg/l or greater

from concentrated samples. Compounds other than the lampricide also may be concentrated to such a degree that the resulting background color obscures the determination (Shapiro 1957, 1958). Daniels et. al., (1965) investigated several analytical techniques including adsorption onto activated carbon, ion exchange resins and activated carbon. These methods were generally considered ineffective for detection of TFM residues from samples of fish, bottom sediments and water (Billy et. al., 1965; Smith, 1966). A satisfactory gas chromatographic procedure has since been developed (Allen and Sills, 1971) which is capable of detecting TFM residues concentrations in fish and other biological tissues of 0.01 ug/gm.

Several investigations have been conducted employing these various methods to characterize the environmental fate of TFM residues. Kempe (1973) employed model systems in 500 cc Erlenmeyer flasks with water and natural lake sediments to determine the rate of loss of TFM. He demonstrated the progressive decline of TFM to undetectable levels within 4 weeks following initial exposure; and the systems became nontoxic to see lamprey and goldfish. He concluded that TFM is degraded to nontoxic metabolites by microorganisms that live in sediment water systems. The fate of TFM in rainbow trout was examined by Lech and Costrini (1972) during studies of the in vivo and in vitro metabolism of the toxicant. TFM was found to be reduced in vitro by nitroreductase to 3-trifluormethyl-4-aminophenol.

The formation of TFM glucuronide was demonstrated *in vitro* utilizing liver extracts and the major metabolite of the compound *in vivo* also appeared to be TFM glucuronide.

The use of uniformly labelled ^{14}C-TFM and liquid scintillation techniques greatly simplified the analysis and quantification of lampricide residues in the several components examined during the course of this study. The uptake and accumulation rates are unlike those reported for chlorinated hydrocarbon pesticides (Rudd, 1964; Woodwell, 1967; Reinert, 1972) and the differences are believed due to the relative polarity and water solubility of TFM compared to these compounds. The graph of the uptake rates of TFM were similar for all plant and animal components examined and can best be described as consisting of two separate components, the first being a rapid initial rate probably corresponding to simple adsorption of the polar compound on the surface of the test species and the second or reduced phase of uptake corresponding to incorporation of the compound into internal tissues. The uptake rates were analyzed for 24 hr. periods in all experiments to allow for comparisons with actual field treatments of 9.0 mg/l. Although this period is longer than most stream treatments which are generally of the order of 14 to 18 hours, the experiments were standardized at 24 hours to allow a complete day and night cycle under exposure.

Rates of uptake and therefore the total body burden of TFM residues at the termination of the 24 hour exposure were strongly correlated with the epidermal structure of the animal components. It was demonstrated that those animals with relatively well chitinized or calcareous exoskeletons adsorbed lower quantities of TFM during both the initial and secondary phases of uptake. This type of behavior would then lend support to the hypothesis that the uptake of the lampricide is primarily an adsorption phenomena since those animals with relatively soft and water permeable integuments accumulated extremely high residues compared to the relatively impermeable crayfish, clams and snails. These data also correlate well with the acute toxicity data for the compound. The 96 hour LC_{50} values of the compound are similarly correlated with the epidermal structure of the test species, i.e., those with the relatively soft exoskeletons have lower LC_{50} values than do species with rigid integuments exposed under identical conditions (Maki, unpublished data).

Additional support for the explanation of uptake and accumulation on the basis of adsorption is offered from examination of the elimination rates of the compound from these animal components. Significant reductions in total body burdens begin immediately following the termination of exposure and introduction of clear stream water. Values for half-life of residue concentrations range from 6.5 to 38 hrs. and are also correlated with the epidermal structure of the

test species. Those species with relatively rigid exoskeletons, offering little possibility for adsorption have shorter half-lives than do the species with softer integuments. These rapid rates of loss and half-lives of a matter of hours also suggest that TFM is rather loosely bound or adsorbed on the surface of the animals and rapidly becomes water soluble upon termination of exposure.

The amount of water passing over the surface of the test animal also appears to have an influence on the elimination rate. A comparison of half-lives between riffle-dwelling and pool-dwelling species demonstrates means of 17.8 hrs. and 140 hrs. respectively. The longer half-lives determined for annelid worms and those species in close association with the rich organic substrate of the pool communities does not rule out the possibility of metabolism and biotransformation of TFM residues by associated microflora and microfauna. Ancillary experiments with solvent extracts of sediment cores and subsequent analysis by thin-layer radiochromatography in various solvent systems indicates that TFM residues become tightly bound to a component of the sediment and that this is a relatively large and polar molecule. However, results are inconclusive at this time and further description would be speculation.

SUMMARY

1. A series of six replicated model streams were established in indoor hatchery channels, stocked with natural stream substrate and associated fauna, provided with artificial illumination and allowed to colonize for 2 to 6 months prior to experimentation.

2. In situ measurements of net primary production and community respiration were made on these streams employing a specially developed respirometry system. The effects of a 24 hr. exposure to 9.0 mg/l of TFM indicated a 25-50% suppression in rates of gross primary production and a 3-50% increase in rates of community respiration in riffle and pool areas. Post-treatment measurements of community metabolism indicated rates had returned to pre-treatment levels within 1 or 2 days following exposure.

3. Description of periphyton community structure was determined from pre- and post-treatment samples taken from the model streams. No permanent effects on diatom cell density, diversity, equitability, biomass of filamentous green algae or species composition of the green algae could be demonstrated from a 14 hr. exposure to 9.0 mg/l TFM.

4. Minimal effects due to the lampricide were detected in the community structure of benthic macroinvertebrates. A significant decrease in the total number of individuals was detected from samples removed up to 2 weeks following exposure. However, this difference was of a temporary nature and exposed communities had increased to pre-treatment densities within 2 to 3 months following exposure. No effects due to the lampricide could be demonstrated on diversity, equitability, number of taxa or production rates of macroinvertebrates.

5. Increases of up to 4 times the control rate of macroinvertebrate drift were demonstrated in experimental channels during exposure to the lampricide.

6. No significant differences were detected in mean growth rates of young of the year brown trout, Salmo trutta between control and experimental populations exposed to 9.0 mg/l of TFM.

7. The uptake and accumulation of TFM residues by several plant and animal components was described as basically an adsorption phenomena. The two component uptake curve consists of a rapid initial phase followed by a reduced linear phase. Macroinvertebrate species with soft, relatively permeable integuments accumulated higher body burdens than those species with hard chitinized or calcareous exoskeletons.

8. Elimination of accumulated TFM concentrations occurred rapidly in the plant and animal components examined. Riffle-dwelling species had a mean half-life of 17.8 hrs. and pool-dwelling species had a mean half-life of 140 hrs. indicating a strong correlation between rates of loss and water current-substrate associations.

CONCLUSIONS AND RECOMMENDATIONS

The underlying theme to the investigations conducted with typical woodland stream flora and fauna during the course of this study must stress the temporary nature of the effects of TFM. No permanent deleterious effect on stream metabolism, production or taxonomic composition could be demonstrated by the methods employed in this research.

Recommendations: In consideration of the above, the following recommendations concerning the continued use of TFM are indicated. The effects of the toxicant on long-term genetic changes of macroinvertebrate taxa and direct effects on the composition of the microbial community were not monitored and remain a possibility for continued research. Mortalities of experimental fish during treatment indicate the further need for toxicity information concerning fish. Toxicant concentrations may appear sublethal in simple toxicity tests but exposed fish in the natural stream may accumulate lethal levels from ingestion of invertebrate individuals and associated residue concentrations in addition to residues obtained from the water. The wide range of toxicity values influenced by water quality

variations, in particular hardness and pH, indicate that levels for protection of aquatic species must consider local water quality. Also, treatments must not include head water reaches of stream systems thereby insuring a continued source of drifting species to re-populate downstream treated sections. Assuming field treatments with TFM continue to be preceded by a careful series of bioassays to determine minimally effective concentrations of the toxicant for each watershed, minimal deleterious effects on stream flora and fauna seems assured.

However, chemical treatment for pest control is probably never the ultimate method of eradication. This point is underlined from a consideration of the present methods of sea lamprey control with TFM. The compound has been extensively used throughout the Greak Lakes drainage for approximately 14 years with results indicating significant suppression of lamprey stocks rather than total eradication. The continual potential exists for the rapid increases in lamprey populations should the annual stress of TFM treatments be eliminated. The use of TFM is highly efficient in terms of the numbers of individual lamprey destroyed per treatment when the densities of lamprey populations are high but becomes progressively less effective on a per treatment basis when the populations are low (Hanson, 1970). The continued existence of this residual spawning populations in the lakes is sufficient

to provide for a continual annual crop of larval lampreys in successive years following a stream treatment. For this reason, major lamprey spawning streams must be re-treated every 3 to 5 years for the foreseeable future. The need for an additional ancillary or integrated approach to the control of lamprey populations is evident. However, at present there is no substitute that combines the relative effectiveness and economy of TFM. Research programs are currently underway in both Canada and the United States to develop an integrated method of control. The use of pathogens, parasites, predators, and non-parasitic competitor releases, combined with the release of sterile males all hold promise toward the development of a future integrated control program for sea lamprey.

APPENDIX

TABLE A-1.--Daily values of community metabolism demonstrating effects of a 9.0 mg/l TFM exposure.

Date		Gross Primary Production ($gmO_2/m^2/day$)	Gross Primary Production ($mg/hr/m^2$)	Respiration ($gmO_2/m^2/day$)	Respiration ($mg/hr/m^2$)	P/R Ratio
July 10-14 RIFFLE (14 hr. daylength)						
7/10		1.008	77.5	.488	20.3	2.07
7/11	treated	.684	52.6	.636	26.3	1.08
7/12	treated	.469	36.1	.636	26.5	0.74
7/13		.540	41.5	.449	18.7	1.20
7/14		.817	62.8	.459	19.1	1.78
July 24-27 RIFFLE (13 hr. daylength)						
7/24		.775	59.6	.603	25.1	1.29
7/25		.762	58.6	.620	25.8	1.23
7/26	treated	.739	56.8	.752	31.3	0.98
7/27		.832	64.0	.643	26.8	1.29
July 24-27 POOL (13 hr. daylength)						
7/24		.201	15.5	.752	31.3	0.27
7/25		.210	16.2	.686	28.6	0.31
7/26	treated	.0	0	.811	33.8	0
7/27		.188	14.5	.727	30.3	0.26
August 21-24 RIFFLE (13 hr. daylength)						
8/21		.939	72.2	.667	27.8	1.41
8/22		.961	73.9	.635	26.5	1.51
8/23	treated	.815	62.7	.640	26.7	1.27
8/24		1.070	82.3	.625	26.0	1.71
August 21-24 POOL (13 hr. daylength)						
8/21		.135	10.4	.560	23.3	0.24
8/22		.139	10.7	.547	22.8	0.25
8/23	treated	.0	0	.779	32.5	0
8/24		.108	8.3	.651	27.1	0.17
September 3-6 POOL (12 hr. daylength)						
9/3		.271	22.6	.400	16.7	0.68
9/4		.257	21.4	.388	16.2	0.66
9/5	treated	.200	16.7	.565	23.5	0.35
9/6		.107	8.9	.555	23.1	0.19

TABLE A-1.--(Continued).

Date		Gross Primary Production ($gmO_2/m^2/day$)	Gross Primary Production ($mg/hr/m^2$)	Respiration ($gmo_2/m^2/day$)	Respiration ($mg/hr/m^2$)	P/R Ratio
September 18-21 RIFFLE (12 hr. daylength)						
9/18		.743	61.9	.662	27.6	1.12
9/19		.747	62.3	.637	26.5	1.17
9/20	treated	.680	56.7	.733	30.5	0.93
9/21		.789	65.8	.669	27.9	1.18
September 18-21 POOL (12 hr. daylength)						
9/18		.311	25.9	.616	25.7	0.50
9/19		.262	21.8	.586	24.4	0.45
9/20	treated	.194	16.2	.808	33.7	0.24
9/21		0.78	6.5	.483	20.1	0.16
October 14-19 RIFFLE (12 hr. daylength)						
10/14		.679	56.6	.566	23.6	1.20
10/15		.720	60.0	.682	28.4	1.06
10/16		.778	64.8	1.091	45.5	0.71
10/17		.405	33.8	.249	10.4	1.63
10/18		.301	25.1	.241	10.0	1.25
10/19		.479	39.9	.364	15.2	1.32
October 15-18 POOL (12 hr. daylength)						
10/15		0	0	.261	10.9	0
10/16		0	0	.245	10.2	0
10/17	treated	0	0	.372	15.5	0
10/18		0	0	.298	12.4	0
October 29-November 1 RIFFLE (11 hr. daylength)						
10/29		.755	68.6	.506	21.1	1.49
10/30		.741	67.4	.519	21.6	1.43
10/31	treated	.262	23.8	.527	22.0	0.50
11/1		.664	27.7	.407	19.6	1.41
October 29-November 1 POOL (11 hr. daylength)						
10/29		0	0	.461	19.2	0
10/30		0	0	.434	18.1	0
10/31	treated	0	0	.489	20.4	0
11/1		0	0	.176	7.3	0

TABLE A-1.--(Continued).

Date	Gross Primary Production ($gmO_2/m^2/day$)	Gross Primary Production ($mg/hr/m^2$)	Respiration ($gmO_2/m^2/day$)	Respiration ($mg/hr/m^2$)	P/R Ratio
November 13-16 POOL (11 hr. daylength)					
11/13	0	0	.479	20.0	0
11/14 treated	0	0	.468	19.5	0
11/15	0	0	.480	20.0	0
November 26-30 RIFFLE (10.5 hr. daylength)					
11/26			.776	32.3	
11/27	.742	71.4	.781	32.5	0.95
11/28	.733	70.9	.794	33.1	0.92
11/29 treated	.608	57.9	1.457	60.7	0.42
11/30	.917	87.3	.702	29.3	1.31
November 26-30 POOL (10.5 hr. daylength)					
11/26	0	0	.799	33.3	0
11/27	0	0	.868	36.2	0.61
11/28	0	0	1.296	54.0	0.50
11/29 treated	0	0	1.763	73.5	0
11/30	0	0	1.102	45.9	0.67

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