THE RESPONSE OF AQUATIC INSECT COMMUNITIES AND CAGED *In situ* ASIATIC CLAMS (*Corbicula fluminea*) TO DECHLORINATED MUNICIPAL EFFLUENT IN THE TRINITY RIVER IN NORTH TEXAS.

THESIS

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Sandra T. Spon, B.S.
Denton, Texas
December, 1994

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Dischargers to the Trinity River in North Texas were required to dechlorinate their effluents in 1990-91. Field surveys were conducted above and below an outfall to determine the response of resident immature insects and caged *in situ* juvenile Asiatic clams to chlorinated and dechlorinated effluent. Within six months after dechlorination began, insect community composition and *C. fluminea* survival significantly improved at stations below the outfall. Significantly lower clam growth within one mile below the dechlorinated effluent indicated the presence of non-chlorine toxicants. Effects from chlorinated and dechlorinated effluent exposure were comparable between *Ceriodaphnia dubia* lab tests and *in situ C. fluminea*.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER 1

INTRODUCTION

BACKGROUND OF STUDY

Regulations

Chlorine has been documented in many studies as a toxicant to numerous aquatic plant and animal species (Brooks and Seegert, 1973; Brungs, 1973; Arthur, et al., 1975; EPA, 1984). AQUIRE, a U.S. Environmental Protection Agency database, lists over 40 references pertaining to the acute toxicity of chlorine to aquatic species (EPA, 1994). Chlorinated effluents discharged by wastewater treatment plants have caused major impacts to aquatic communities located below the outfall (Osborne and Davies, 1987; Dickson, et al., 1989). Based on the mandate in the Clean Water Act (CWA, 1970) that prohibits the discharge of toxic materials in toxic amounts, the U.S. Environmental Protection Agency (EPA) and the Texas Water Commission (TWC) in 1990 issued a requirement that all major wastewater treatment plants in the North Texas region begin dechlorinating their effluents prior to discharge. In response to this new initiative, the Institute of Applied

Sciences at the University of North Texas (UNT) developed a cooperative agreement with the U.S. EPA to study the response of aquatic biota to dechlorinated effluent discharged from the Village Creek Wastewater Treatment Plant in Fort Worth, Texas.

Village Creek Wastewater Treatment Plant

The Village Creek Treatment Plant began operations in 1958 as a 5 million gallon per day (MGD) facility that serviced the East Fort Worth area. The plant expanded in 1965, 1972, and 1980 to eventually reach a capacity of 96 MGD and a service area that included Fort Worth and surrounding cities. In 1987, the plant was further upgraded to 120 MGD and was able to treat wastewater generated by 750,000 people and several industries located in 23 communities in the Fort Worth area (Fort Worth Water Department, 1988). The Trinity River Dechlorination Study was conducted during the 120 MGD discharge regime.

The Village Creek plant is an advanced secondary treatment facility that uses a conventional activated sludge process. The activated sludge consists of microorganisms capable of processing organic matter in the wastewater. Microbes are separated from the treated wastewater prior to effluent filtration. Proper disinfection of filtered wastewater is accomplished with chlorine exposure for a 20-minute contact time in retention basins. After chlorination,

the effluent is dechlorinated with sulfur dioxide prior to discharge into the Trinity River (Fort Worth Water Department, 1988). Dechlorination officially began at the Village Creek facility in January 1991.

The effluent limitations required by the National Pollution Discharge Elimination System (NPDES) permit for the Village Creek facility are as follows: pH = 6.0 - 9.0; Biochemical Oxygen Demand (BOD) = 10 mg/l; Total Suspended Solids (TSS) = 15 mg/l; Dissolved Oxygen (DO) = 6.0 mg/l in summer and 4.0 mg/l in winter; Ammonia (N) = 2.0 mg/l from June through November and 5.0 mg/l from December through May; and minimum chlorine residual (Cl_2) = 1.0 mg/l (Fort Worth Water Department, 1988).

Pre-dechlorination Baseline Study

The University of North Texas conducted a field and laboratory assessment of the Trinity River in August, September, and October 1990 prior to effluent dechlorination at the Village Creek facility. The effluent contained approximately 1.0 mg/l of total residual chlorine during the baseline study. Sampling stations were established upstream and downstream from the discharge point and the following activities were completed: 1) Fish, benthic macro-invertebrates, zooplankton, phytoplankton, and periphyton were collected; 2) *In situ* tests were performed with caged *Corbicula fluminea*, *Pimephales promelas*, and *Chironomus*

larvae; 3) Colonized periphytometers and macroinvertebrate artificial substrates were transferred from reference stations to stations below the discharge; 4) Ambient toxicity tests with *P. promelas*, *Ceriodaphnia dubia*, and Microtox®; 5) Physical and chemical analyses of water and sediment; 6) Sediment toxicity tests with midge larvae, cladocerans, and microbial assays were conducted by Dr. Allen Burton at Wright State University.

Preliminary results of the pre-dechlorination study documented that chlorine residuals were a major cause of observed ambient toxicity to *C. dubia* and *P. promelas* in laboratory tests. Both test organisms were able to survive in ambient water samples after they were dechlorinated in the laboratory with sulfur dioxide; however, *C. dubia* productivity was still impacted (R. Guinn, pers communication, 1991). The baseline study also confirmed that the study area selected in the Village Creek vicinity was accessible and adequate for sampling activities. It was verified that no other major point source discharges existed upstream from the study area. The baseline assessment provided a pre-dechlorination database for comparison with data collected after chlorine was removed from the effluent.

OBJECTIVES OF RESEARCH

The Trinity River dechlorination study was designed to:

1) Determine the aquatic community response before and after chlorine is removed from the Village Creek effluent. (How does chlorinated effluent effect the community? Will dechlorination have a positive effect on most members of the aquatic community that inhabit the river downstream from the outfall?)

2) Monitor the recovery pattern of biota included in the study area during a two year dechlorination period. (Will recolonization of biota to areas downstream from the treatment plant vary with time, species, and/or distance below the outfall?)

3) Compare the responses of resident biota (i.e., *C. fluminea* and benthos) and traditional lab test organisms (i.e., *C. dubia* and *P. promelas*). (What is the relationship between the responses of lab and field organisms exposed to chlorinated and dechlorinated effluent?)

4) Document the ecological changes that result from a regulatory action imposed by the EPA. (What is the real world outcome of the EPA requirement to dechlorinate effluents prior to discharge into the environment?)

The scope of my research concentrates on the following components of the Trinity River study:

1) The analysis of benthic macroinvertebrate

colonization of artificial substrate samplers placed in the Trinity River water column. Community composition and density were the assessment endpoints.

2) The analysis of the response of juvenile Asiatic clams (*C. fluminea*) placed in the Trinity River for use as biomonitors of ambient toxicity. Shell growth and survival were the assessment endpoints.

TRINITY RIVER CHARACTERISTICS

Physical Description

The West Fork of the Trinity River originates in Archer County, Texas and merges with the Elm Fork in Dallas County to form the main stem of the Trinity River. The West Fork is part of the Upper Trinity River Basin that drains the Fort Worth-Dallas metroplex area. The study area begins at the east end of Fort Worth, goes through Arlington, and ends just east of the Grand Prairie city boundary. Lake Worth and Benbrook Lake are two reservoirs that help regulate the flow in the vicinity of the West Fork study area.

Geology of the upper basin consists of older Cretaceous sedimentary formations of sand, shale, and limestone. Runoff and leaching from the formations contribute to high mineral concentrations and water hardness. Land use surrounding the study area includes urban, residential, industrial, cropland, pastureland, and grassland (Dickson et al., 1989).

In the study area, the main channel width ranges from approximately 15 m to 125 m and depth varies from 50 cm to over 200 cm. River flows can range from 0.2 to 91.8 m^3/s (mean = 4.4 m^3/s) in the summer (July/August/September, 1990-1992), and from 0.6 to 996.9 m^3/s (mean = 79.2 m^3/s) in the spring (March/April/May, 1990-1992). Annual mean flows can range from 5.4 m^3/s (1991) to 54.7 m^3/s (1990) and 58.6 m^3/s (1992) (U.S. Geological Survey, Austin, Texas, pers. communication, 1993). The river banks are typically steep and covered with a dense canopy of deciduous trees (i.e., oak, elm, cedar, pecan) and shrubs.

Historical Impacts

The Trinity River was an intermittent stream prior to becoming a regulated river channel where flow is now controlled by over 28 reservoirs. The first dams were built on the Trinity in the upper basin in the late 1800's. The Trinity River drainage has been used as a dumping grounds for untreated wastewater and sewage since the establishment of the Dallas-Fort Worth urban area in the 1860's. The advent of state and federal water pollution controls in the 1960's and 1970's prompted managers to develop clean-up plans for the Trinity basin. As a result of these pollution control efforts, ambient water quality of the Trinity River has improved in the Dallas-Fort Worth area over the past twenty years. For example, biochemical oxygen demand has

decreased from 44 mg/l in 1977 to <10 mg/l in 1991 and dissolved oxygen has increased from approximately 3 mg/l in 1977 to 8 mg/l in 1991. Ammonia levels have decreased in the Village Creek effluent from approximately 3 mg/l in 1984 to <0.5 mg/l in 1990. Fish kills were once a common event in the Trinity River but no major kills have been reported since 1986. These water quality improvements in the Trinity River can be primarily attributed to successful upgrades to the wastewater treatment process (Dickson et al., 1989; Brush and Promise, 1992; Plummer, 1992).

Water Quality

The twenty mile study area is located in water quality segments 806 and 841. Sampling Stations 1 and 2 are located within segment 806 which extends from Lake Worth Dam to just upstream of the confluence with Village Creek. The Village Creek facility and sampling Stations 3 through 7 are located within segment 841 which extends from Village Creek to a point immediately upstream of the confluence with the Elm Fork Trinity River in Dallas (Texas Water Commission, 1991; Plummer, 1992). The Trinity River receives treated municipal effluent and non-point source runoff from urban and agricultural areas. The river flow in the study area below the Village Creek facility is typically 80 - 90% effluent.

The following water quality criteria (effective July 10, 1991) have been established for Segments 806 and 841,

respectively: 100 and 175 mg chloride/l; 100 and 175 mg sulfate/l; 500 and 850 mg total dissolved solids/l; 5.0 and 4.0 mg dissolved oxygen/l; 200 and 200 fecal coliforms/100 ml; 6.5 - 9.0 and 6.5 - 9.0 pH range; and 33.9°C (93°F) and 35°C (95°F) ambient water temperature. In addition, when river flows are less than 2.3 m^3/s (<80 ft^3/s) at a West Fork gaging station, dissolved oxygen must be at least 2.5 mg/l in Segment 841. (The chloride, sulfate, and solids criteria are expressed as annual averages that should not be exceeded while the coliform criteria is based on a thirty-day geometric mean). Segment 806 has been classified as high quality for aquatic life while segment 841 provides intermediate quality for aquatic life. Both segments are classified as water quality limited (Texas Water Commission, 1991).

Aquatic life designations reflect the differences in water quality criteria between the two segments, the lack of major point sources in Segment 806, and the presence of the Village Creek Wastewater Treatment Plant in Segment 841. In their ecological survey of the upper and lower Trinity River basin, Dickson et al., (1989) found diversity in fish and macroinvertebrate communities to be high in Segment 806 and intermediate to high in Segment 841.

The 'water quality limited' classification for both segments indicates that point and/or nonpoint source pollution prevents either segment from attaining higher

ambient water quality. Some water quality concerns have been identified in Segments 806 and 841. Concern for the following toxicants exists for both segments: fecal coliforms, dissolved oxygen, cadmium, copper, lead, zinc, chlordane (in fish tissue), heptachlor, hexachloro-cyclopentadiene, and lindane. In addition, Segment 806 may contain mercury, diazinon, DDE, chlordane, endrin, and PCB's while Segment 841 may be effected by phosphorus, inorganic nitrogen, arsenic, and aldrin (Plummer, 1992).

CHEMISTRY OF CHLORINE AND DECHLORINATION

Chlorine Species in Effluent Disinfection

Municipal effluent at the Village Creek wastewater facility is disinfected with chlorine gas dissolved in water. By-products of chlorination include free and combined chlorine. Free chlorine consists of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-). Free chlorine is a strong oxidizing agent which easily combines with ammonia to form monochloramine (NH_2Cl) and dichloramine ($NHCl_2$).

The primary path of degradation for free chlorine is photolysis. However, free chlorine tends to exist for only a short period of time in natural waters because it can rapidly oxidize inorganic compounds (i.e., ammonia). Dichloramine is an unstable molecule that is quickly oxidized to nitrogen gas and nitrate. Monochloramine is more

residual in the environment compared to free chlorine and dichloramine because it is a weaker oxidizing agent and forms a more stable molecule. (Johnson, 1978; Morris, 1978).

Toxicity of Chlorine to Biota

Several studies have documented the impacts of chlorine toxicity to aquatic organisms. Factors that affect chlorine toxicity in freshwater include temperature, pH, presence of inorganic and organic compounds, pollutants, time of application, concentration, and exposure period (Brungs 1973; Brooks and Seegert, 1978; Cairns et al., 1990). Hypochlorous acid and monochloroamine tend to be the most toxic forms of chlorine affecting aquatic life. Hypochlorous acid is slightly more toxic but less persistent than monochloroamine (Hellawell, 1986).

Twenty years ago, Brungs (1973) recommended that total residual chlorine (TRC) levels should not exceed 0.01 mg TRC/l for more resistant organisms and 0.002 mg TRC/l for the protection of most aquatic biota. These criteria for chlorine were intended for areas that received a continuous flow of chlorinated effluents. The current ambient water quality criteria for chlorine is 0.011 mg TRC/l for a 4-day maximum average and 0.019 mg TRC/l for a 1-hour maximum average (EPA, 1984).

Based on a review of over twenty studies on the effects of chlorine toxicity to biota, Brungs (1973) concluded that

salmonids were the most sensitive to chlorine followed by invertebrates, warmwater fish, snails, and crayfish. Results from Arthur et al., (1975) helped support Brung's assertions about sensitivity differences between organisms to chlorine exposure. In Arthur's study, seven fish and six invertebrate species were exposed to chlorinated effluent in short-term (7-day) toxicity tests. Brook trout (*Salvelinus fontinalis*) and Coho salmon (*Oncorhynchus kisutch*) were more sensitive than fathead minnow (*Pimephales promelas*), white sucker (*Catostomus commersoni*), walleye (*Stizostedion vitreum*) yellow perch (*Perca flavescens*), and largemouth bass (*Micropterus salmoides*). An amphipod (*Gammarus pseudolimnaeus*) was the most sensitive invertebrate compared to a stonefly (*Pteronarcys* spp.), caddisfly (*Hydropsyche* spp.), crayfish (*Orconectes virilis*), and two snails (*Campeloma decisum* and *Physa integra*). LC_{50} concentrations of TRC ranged from 0.08 to 0.26 mg/l for fish and 0.21 to >0.81 mg/l for invertebrates. In long term life-cycle tests, survival and reproduction were impacted for *P. promelas*, *G. pseudolimnaeus,* and *Daphnia magna*. *D. magna* was the least tolerant (NOEC = 0.002-0.004 mg TRC/l) followed by *G. pseudolimnaeus* (NOEC = 0.012 mg TRC/l), and *P. promelas* (NOEC = 0.014 mg TRC/l) (Arthur et al., 1975). Results from a field study by Szal et al., (1991), showed that *in situ* fathead minnows were impacted by chlorinated effluent discharged into a receiving stream. LC_{50} concentrations of

total residual chlorine ranged from 0.17 to 0.34 mg TRC/l and NOEC's ranged from 0.03 to 0.23 mg TRC/l.

Chloramine toxicity to *P. promelas* and *G. pseudolimnaeus* was documented by Arthur and Eaton (1971) in long term tests. The chloramines included mono-, di, and other chloramines and exposure period was 21 weeks for the minnows and 15 weeks for the amphipods. *P. promelas* was more tolerant to chloramines (NOEC = 0.0165 mg total chloramines (TCA)/l) compared to *G. pseudolimnaeus* (NOEC = 0.0034 mg TCA/l). In Taylor's (1993) study, free chlorines (hypochlorite ion and hypochlorous acid) were more toxic than chloramines to *Ceriodaphnia dubia*. In 24-hour continuous-flow tests, LC50's were 0.005 mg HOCL/l and 0.006 mg OCl/l and increased for chloramines to 0.016 mg NH_2Cl/l and 0.027 mg $NHCl_2$/l. In static tests, LC50's increased for free chlorine (0.08 mg OCl/l and 0.14 mg HOCL/l) and decreased for chloramines (<0.02 mg/l for NH_2Cl/l or $NHCl_2$/l).

The effects of sub-lethal concentrations of total residual chlorine and monochloramine on *Corbicula fluminea* were assessed by Sappington (1987, as cited by Doherty, 1990). *C. fluminea* experienced significant decreases in glycogen levels and siphoning rates after exposure to 0.30 mg TRC/l for 30 days in artificial streams. In contrast to the artificial stream results, *in situ* clams did not show significant differences in glycogen levels when transplanted

to field sites receiving 0.30 to 0.40 mg TRC/l chlorinated discharge. Exposure of *C. fluminea* to 0.73 mg NH_2Cl/l in artificial streams produced significant reductions in glycogen and tissue water content after 7 days and a complete cessation of siphoning after 30 days.

Dechlorination with Sulfur Dioxide

Chlorine toxicity to fish and invertebrates can be significantly reduced or completely removed by dechlorination with sulfur dioxide (Brungs, 1973). Sulfur dioxide is the most popular method of dechlorination because it is extremely soluble, quickly reduces residual chlorine, and can be dispensed with the same equipment used for chlorination. Sulfur dioxide can be metered into chlorinated effluents as either a gas or liquid. In the dechlorination reaction, SO_2 quickly converts positive chlorine atoms from free and combined chlorine into negative chloride atoms. When dissolved in water, sulfur dioxide forms a weak solution of sulfurous acid. Free chlorine reacts with the sulfurous acid and forms sulfuric and hydrochloric acid. Monochloramine reacts with sulfurous acid and forms NH_4HSO_4 and hydrochloric acid (White 1972).

MACROINVERTEBRATE COMMUNITY

Ecology

As secondary producers, aquatic insects function as processors of organic matter (i.e., algae, zooplankton, and insects) into forms which are available to other aquatic organisms. Insects also constitute a prey base for other insects and fish.

In streams and lakes, aquatic insects perform several roles within the nutrient cycle. The ingestion and conversion of detritus by benthos results in biochemical changes; microbial activity is also increased by creating a larger surface area of particulate organic matter from the ingested detritus. Macroinvertebrates can translocate nutrients from the sediment to the water column via digestive activities. Burrowing mayflies and chironomids are the major benthos involved in bioturbation. Benthic insects are capable of the uptake and excretion of phosphorous at the sediment-water interface. Phosphorous is often a limiting nutrient in freshwater systems. Burrowing actions of benthos provide a pathway for interstial nutrient-rich water to enter the water column and oxygen-rich surface water to enter the sediment (Merritt, et al. 1984).

Benthic organisms are involved in the mineralization and recycling of organic matter from allochthonous and autochthonous sources. They are an important link between

primary producers and tertiary consumers in the trophic sequence. Benthic larvae are a major food source for fish (Lind, 1985).

Value as Biomonitors

Benthic macroinvertebrates are the aquatic group most often used for biomonitoring purposes in water quality studies (Hawkes, 1979; Hellawell, 1986; Rosenberg and Resh, 1993). The following attributes of aquatic insects are recognized as being advantageous in a biomonitoring program (Rosenberg and Resh, and citations within, 1993): 1) aquatic insects are present in all types of aquatic systems and habitats and are potentially exposed to toxicants in the water or sediment; 2) macroinvertebrates are comprised of numerous species that have a wide range of responses to environmental stressors (i.e., tolerant to sensitive); 3) their relatively sedentary existence allows for the examination of spatial patterns that occur within impacted areas; 4) the long life cycles of insects (compared to other aquatic organisms) allows for the temporal analysis of changes caused by environmental impacts.

Because they possess the four characteristics outlined above, macroinvertebrates lend themselves to being continuous monitors of water quality through various pollution regimes (i.e., intermittent or constant discharges; variable concentrations of single or multiple

toxicants). Though they are rather sessile creatures, aquatic insects are able to leave an area though drift when pollution reaches intolerant levels. The presence or absence of certain species of macroinvertebrates can indicate the type or degree of impact that has occurred in an area (Roback, 1974; Hawkes, 1979; Hellawell, 1986).

The use of aquatic insects as biomonitors has become more feasible over the years because of the following technical developments (Rosenberg and Resh, and citations within, 1993): 1) simple and inexpensive sampling equipment is available; 2) the taxonomy of many groups has been worked out and identification keys are readily available; 3) several data analysis methods (i.e., biotic and diversity indices) have been developed for community level biomonitoring; 4) the tolerance/sensitivity of many common taxa to various types of pollution has been evaluated; and 5) macroinvertebrates are suitable in experimental approaches to biomonitoring.

Several researchers have successfully used macroinvertebrates as biomonitors of water pollution. Whitehurst and Lindsey (1990) selected benthic macro-invertebrates for use in their study of organic pollution in lotic habitats. Their choice was based on the capacity of benthic insects to respond quickly to environmental changes and reveal impacts through community abundance and diversity. Dickson et al., (1989) determined community

composition and density of benthic macroinvertebrates at sites located near wastewater treatment outfalls in the Trinity River. The poor condition of benthic communities below the discharges indicated polluted conditions in the environment.

Macroinvertebrate community response has been compared to responses of single-species lab organisms and microcosms. Pontasch, et al. (1989) found that the response of benthic communities to a complex effluent were similar between the field and microcosms. In a study by Birge, et al. (1989), embryo-larval tests with fathead minnows correlated well with species richness of insect communities located downstream from a secondary municipal wastewater treatment plant.

The relationship between ambient toxicity and instream response was assessed by Dickson, et al. (1992) by applying canonical correlation analyses to results from three studies (Birge's and Dickson's studies discussed above and data from EPA's Complex Effluent Toxicity Testing Program). The instream variables of benthic and fish richness were selected by the correlation analysis to have the greatest influence on establishing a significant relationship with the toxicological variables. In data from an EPA study of Kanawha River, West Virginia, benthic macroinvertebrate richness was significantly correlated (R^2=0.75) with the ambient toxicity variables of *C. dubia* neonate production

and *P. promelas* dry weight. A similar relationship (R^2=0.94) was found between fish richness, evenness, and biotic index in the Trinity River and ambient toxicity variables of *C. dubia* neonate production, *P. promelas* dry weight and survival, and Microtox light loss (Dickson, et al. 1992).

Corbicula fluminea BIOLOGY

Introduction into United States

The freshwater Asiatic clam (*Corbicula fluminea*) was accidently introduced from Southeast Asia to the northwest coast of the United States in 1938 (Burch, 1944, as cited in Aldridge and McMahon, 1978). The range of the clam has expanded from the Columbia River in Washington, south to Baja, California and east to Florida (Sinclair, 1971). *C. fluminea* was first noticed in the upper Trinity River drainage in North Texas in the early 1970's when clam shells were discovered in Tarrant County reservoirs. Clams were probably brought into the upper Trinity drainage from remote sources. Live bait shops from Arkansas and Louisiana typically transport large amounts of water with minnows and it is possible that *C. fluminea* was present in this water. Another likely source of Asiatic clams was from dispersion by migratory birds (Britton and Murphy, 1977).

Numerous observations and studies have documented *C. fluminea*'s tendency to clog irrigation canals and intake

pipes into industrial and public water facilities. Research has been conducted on the life cycle dynamics of this exotic species in an attempt to control its population, reduce its economic impact, and determine its influence on indigenous mollusk communities (Sinclair, 1971; Britton, 1977; Britton, 1986).

Reproduction

C. fluminea is primarily hermaphroditic (Sinclair and Isom, 1963; Sinclair, 1971; Kraemer et al., 1986) but is also capable of self-fertilization. Mucous from adult clams appears to be the mechanism by which cross-fertilization occurs. Sperm-filled mucous strands from one clam are released and connected via the siphons of a neighboring clam. Oogenesis occurs throughout the year but slows down in January and February. Spermatogenesis is seasonal and influenced by water temperature (Kraemer et al., 1986).

As a species, *C. fluminea* is able to maximize the number of offspring produced in a year by employing a reproductive strategy of cross and/or self-fertilization depending on seasonal variations (Kraemer et al., 1986). In the fall, both sexual and asexual reproduction occurs (Kraemer et al., 1986) and the highest spates of young clams are produced (Coldiron, 1975; Aldridge and McMahon, 1977; Kraemer et al., 1986). Self-fertilization is most likely to occur in the fall when decreasing water temperatures (<17°C)

prompt a decline in spermatogenesis.

Embryogenesis

The *C. fluminea* embryo is incubated within the marsupial gills of a parent clam. The egg initially develops into a barrel-shaped trochophore that is able to swim, via it's cilia, within the adult's gills. The trochophore undergoes metamorphosis to the veliger larvae which consists of a velum (flange-like membrane), rudimentary valves and a straight hinge. During the pediveliger stage the foot begins to develop. In the final juvenile stage the velum disappears, statocysts are differentiated, the foot, gut, and valves are well-developed, and the ciliary surface increases on the gills (Kraemer et al., 1986). The juvenile is usually the developmental stage which is spawned by the adult although earlier stages may also be released (Kraemer et al., 1986; Doherty et al., 1987). The shell valves of a juvenile are typically 0.20 mm in length when released from the adult into the environment (Sinclair and Isom, 1963; Sinclair, 1971; Aldridge and McMahon, 1978; Kraemer et al., 1986; Mattice and Wright, 1986). Soon after being released the planktonic juvenile becomes benthic with the aid of a byssus (Sinclair and Isom, 1963; Sinclair, 1971; Kraemer et al., 1986).

Sexual Maturity

C. fluminea juveniles become reproductively active at various ages and sizes depending on environmental conditions (i.e., water temperature) and the season when they were spawned. If a clam is spawned in the spring or summer, it can potentially reproduce before it is two months old. Shell length size at sexual maturity varies from 10 mm (Aldridge and McMahon, 1978) to 18 mm (Coldiron, 1975). Adult clams are capable of producing thousands of juveniles during each reproductive season. Estimates can range from approximately 85 to 600 juveniles/adult/day or a total release of 400,000 juveniles/m^2 in the spring spawning season and 25 to 740 juveniles/adult/day or a total release of 750,000 juveniles/m^2 during the fall spawning season (Aldridge and McMahon, 1978).

Environmental Influence on Reproduction

The *C. fluminea* population typically exhibits reproductive peaks during the spring/early summer and in the fall (Coldiron, 1975; Aldridge and McMahon, 1978; Mattice and Wright, 1986; McMahon and Williams, 1986). Two studies conducted in Texas waters showed that water temperature influences the timing of these peaks. Coldiron (1975) found that reproduction can be initiated in the spring when temperatures are above 13°C. Clams with incubating larvae were abundant when temperatures were consistently in the low

twenties during late May. Reproductive activities were gradually suppressed when temperatures climbed above 25°C in the summer. Aldridge and McMahon (1978) observed a similar trend with spawning activities. Spawning began in late April when water temperatures rose above 19°C and ceased at 32°C. Spawning resumed in late August when temperatures fell below 32°C and ended in early December when temperatures dropped below 13°C.

Life Span

The life span of *C. fluminea* ranges from 1.5 years to 3 (Aldridge and McMahon, 1978; McMahon and Williams, 1986) or 4 years (Eng, 1979, as cited in McMahon and Williams, 1986). The following annual mortality rates were determined for three age classes in a North Texas population of *C. fluminea*: 98% mortality during first year of life; 69% mortality during second year; and 97% mortality during third year (McMahon and Williams, 1986).

Growth Pattern

There is an inverse relationship between *C. fluminea* growth rate (of shell or tissue) and an individual's shell size (Aldridge and McMahon, 1978; Britton et al., 1979; Buttner and Heidinger, 1980; Doherty, et al., 1990). *C. fluminea* exhibits the highest rate of shell growth during its juvenile stage. Shell length (anterior to posterior

distance) is typically the variable used to determine shell growth. An example of this type of growth pattern was illustrated by a North Texas population of *C. fluminea* where growth rate was compared among four size classes (McMahon and Williams, 1986):

initial length =	5 mm:	growth =	0.18 mm/day	(5.4 mm/month)
"	10 mm:	"	0.14 mm/day	(4.2 mm/month)
"	20 mm:	"	0.07 mm/day	(2.1 mm/month)
"	30 mm:	"	0.02 mm/day	(0.8 mm/month)

Shell growth rates can vary with environmental conditions such as temperature. Growth appears to be initiated when water temperatures rise above 14°C (Eng, 1979, as cited in McMahon and Williams, 1986) or 15°C (McMahon and Williams, 1986). In North Texas waters, maximum growth can occur between 25°C and 30°C (McMahon and Williams, 1986). Mattice and Wright (1986) suggested the optimum temperature for growth was approximately 25°C while Foe and Knight (1986) found that temperatures between 18°C and 20°C produced the greatest shell growth. In North Texas waters, *C. fluminea* shell growth is greatly reduced from November to early April (Britton et al., 1979; McMahon and Williams, 1986).

Shell growth rates decrease drastically when *C. fluminea* becomes reproductively active. This decline may be caused by an inhibition of water flow across the adult's gills because of clam larvae incubating on the gills (see embryogenesis section above). A smaller amount of water flow

across the gills translates into less food being filtered and ingested by the adult (Mattice and Wright, 1986).

Feeding Mechanism and Diet

C. fluminea is primarily a nonselective filter-feeder but is also capable of deposit feeding. Deposit-feeding probably occurs in highly organic substrate habitat or when suspended particle concentrations are inadequate (Way, et al. 1990).

Food particle size and concentration can have a significant effect on clam filtration rates. Based on lab experiments by Way, et al. (1990), filtration rates were highest (100 ml/hour) with suspended particles (3 x 5 um) collected from the same river where *C. fluminea* test organisms inhabited. However, *C. fluminea* was also able to filter the following sizes of latex microspheres at rates between 60 - 81 ml/hour: 1 um, 2 um, 5 um, and 16 um. The clams showed non-discriminant selection of particles when filtration rates were the same for 5 um microspheres and 5 um *Chlorella*. An inverse relationship existed between suspended particle concentration and filtration rate. Filtration rates increased from 66 ml/h to 145 ml/h as concentration decreased from 11 mg particles/l to 4 mg particles/l. The gill saturation point suggested for *C. fluminea* ranges from 15 - 20 mg particles/l.

A tri-algal diet of equal proportions of *Ankistrodesmus*, *Chlorella*, and *Chlamydomonas* (total concentration of 10^5 cells/ml) produced positive growth in lab feeding experiments with *C. fluminea*. Particle size of the algae mixture was not determined (Foe and Knight, 1986).

Ecology

The habitat preferred by *C. fluminea* is fine, well-oxygenated sand substrates (Belanger et al., 1985). Only small numbers of clams tend to occur in highly organic and peat sediments. *C. fluminea* populations will decrease in areas where silt begins to accumulate. The round shell shape and small siphon size of *C. fluminea* may hinder the clam from extending it's siphons above a silt layer to reach the more oxygenated surface (Belanger et al., 1990).

Predators of *C. fluminea* include fish, waterfowl, racoon, crayfish, flatworm, (Sinclair and Isom, 1963) and muskrat (Buttner and Heidinger, 1980).

Value as Biomonitor

C. fluminea can be a suitable organism for *in situ* biomonitoring in drainages where it is already established. Several characteristics of the life cycle of *C. fluminea* encourage its use as a freshwater mollusk biomonitor. *C. fluminea* has the capacity to bioaccumulate a variety of toxicants at sublethal levels. Behavioral, physiological,

and life cycle responses of *C. fluminea* can indicate toxicity in the environment (Doherty, 1990). The clams are easily available in most creek and river drainages because of their prolific nature and widespread range. Short-term (i.e., 30 days) growth experiments can be conducted with juveniles because of their fast growth rates. Reproductive responses can be monitored within a relatively short time period because *C. fluminea* reaches sexual maturity at a young age and has a high reproductive potential.

C. fluminea has been successfully used as an *in situ* biomonitor in the Trinity River in Texas. Shell growth of juvenile clams was shown to be a sensitive indicator of ambient toxicity in waters below discharges from wastewater treatment plants (Dickson, et al., 1989).

Doherty (1990) reviewed the literature for lab and field studies that exposed *C. fluminea* to metals, trace elements, pesticides, organic and thermal pollution, and oxidizing and reducing agents. Various *C. fluminea* response endpoints used in these experiments included the following: shell and tissue growth, enzyme activity, lipids, glycogen, reproductive behavior, siphoning behavior, and tissue and shell concentrations. Some of Doherty's findings are summarized below.

When exposed to trace elements and metals in the water, *C. fluminea* was able to accumulate and concentrate these toxicants at levels that were significantly higher than

ambient concentrations. The clams proved to be good candidates for biomonitoring because they did not suffer lethal effects despite heavy body burdens. When exposed to sediments containing trace elements and metals, however, *C. fluminea* did not bioaccumulate toxicants to any significant extent. *C. fluminea* appears to bioaccumulate lipid soluble and residual organic compounds (i.e., organochlorines) found in pesticides. The pesticides are bioavailable to the clams from either the water column or the sediments. Thermal stressors and a diverse range of toxicants (i.e., ammonia, asbestos, and chlorine) can produce negative responses in *C. fluminea* (Doherty 1990).

CHAPTER 2

MATERIALS AND METHODS

SAMPLING STATIONS AND SCHEDULE

Seven sampling stations were established within a twenty river mile study area in the West Fork of the Trinity River. Two stations (1 and 2) were upstream from the Village Creek Wastewater Treatment Plant and five stations (3 through 7) were downstream from the plant (Figure 1). Stations 1 through 7, respectively, were approximately located at the following river miles: 509.1, 506.8, 505.8, 505.2, 500.7, 498.0, and 489.1 (Table 1). The treatment plant was located at River Mile 505.9, immediately upstream from Station 3. Upstream Stations 1 and 2 served as reference sites for the stations located below the effluent discharge. The greatest impacts from chlorinated effluent were expected at Stations 3 and 4 while a zone of recovery was anticipated at Stations 5 and 6. Station 7 was potentially a downstream reference site that received little or no influence from the effluent.

A pre-dechlorination baseline study was completed in the summer of 1990 and a two year dechlorination study was

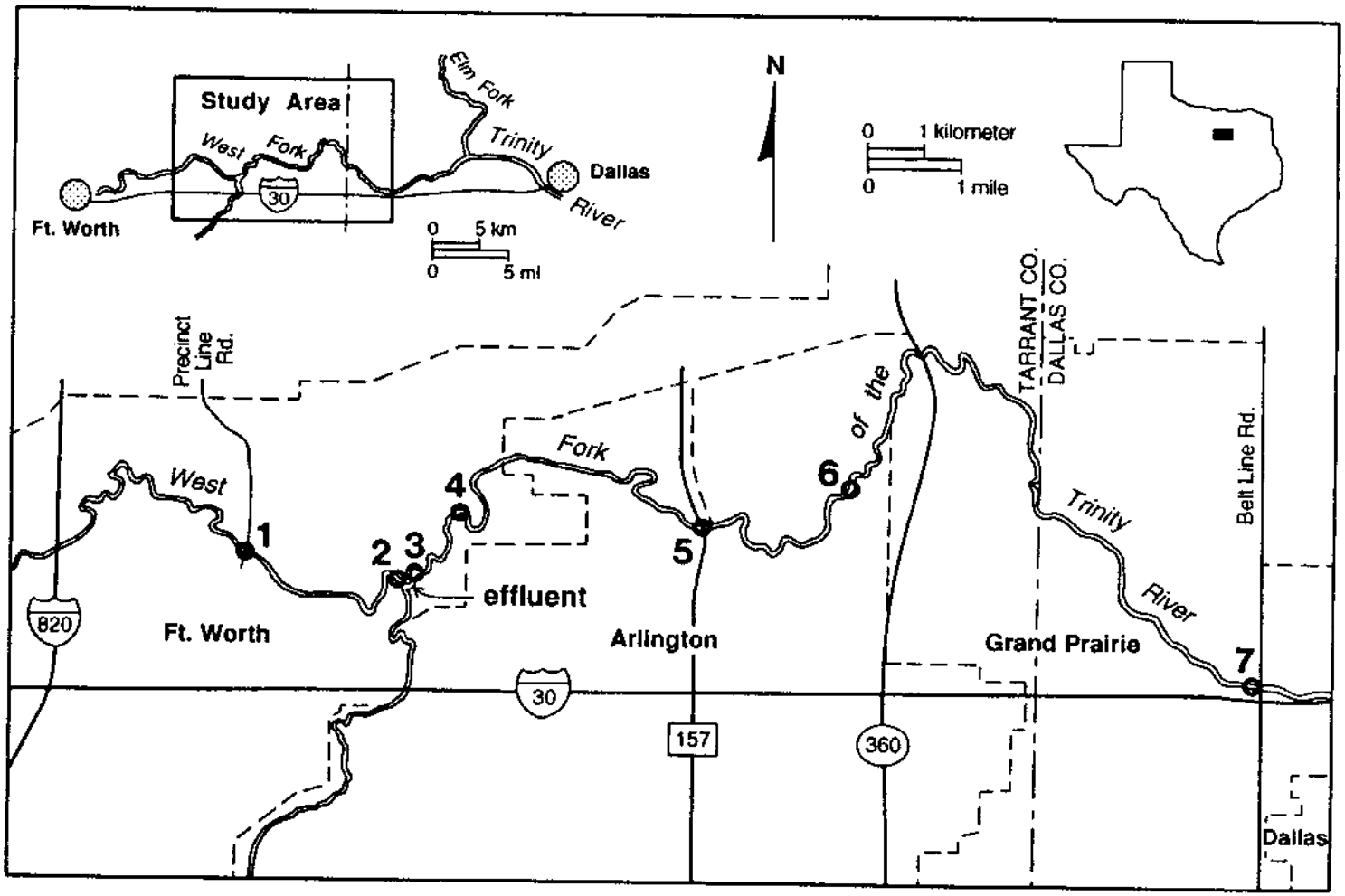

Figure 1. Location of the Village Creek Wastewater Treatment Plant outfall and seven sampling stations (1 and 2 upstream and 3, 4, 5, 6 and 7 downstream of the effluent) in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

Station	River Mile[1]	Location[1]
1	509.1	Precinct Line Road, Old Randol Mill 3.2 miles above treatment plant
2	506.8	Upstream of Village Creek 0.9 miles above treatment plant
--	505.9	Effluent outfall from Village Creek Municipal Wastewater Treatment Plant
3	505.8	Immediately downstream from outfall 0.1 miles below treatment plant
4	505.2	Arlington-Bedford Road 0.7 miles below treatment plant
5	500.7	Texas State Highway 157 bridge 5.2 miles below treatment plant
6	498.0	Forestwood Drive residential area 7.9 miles below treatment plant
7	489.1	West Beltline Road 16.8 miles below treatment plant

[1] - River miles and locations were determined from the following sources: a) data from the Trinity River Authority of Texas, Arlington, Texas; b) used digitizer on topographic maps to measure distances between stations (University of North Texas); c) city map of Arlington, Texas.

Table 1. River mile and location of seven sampling stations established in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

	Macroinvertebrate	Asiatic Clam
1990[1]		
8/23 - 9/26	X	X
1991[2]		
5/27 - 6/27	X	--
7/19 - 8/16	--	X
9/14 - 10/16	--	X
1992[2]		
4/16 - 5/13	X	--
8/4 - 9/1	X	X
9/12 - 10/10	--	X

[1] = Pre-dechlorination
[2] = Dechlorination
X = sampled for this date
-- = no sample for this date

Table 2. Sampling schedule for macroinvertebrates and Asiatic clams for the Trinity River dechlorination study, West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

conducted in the spring and summer of 1991 and 1992. Analyses were conducted on four macroinvertebrate samples (spring and summer) and five Asiatic clam (*C. fluminea*) growth experiments (summer and early fall) (Table 2).

MACROINVERTEBRATE SURVEY

Aquatic insects were collected from the Trinity River water column with a multiple-plate artificial substrate sampler (Hester and Dendy, 1962). For each sampling period, twenty-one artificial substrates (3 substrates/station) were constructed from standard low grade 1/8" (3 mm) hardboard cut into 3" x 3" (76 x 76 mm) square plates and 1" x 1" (25 x 25 mm) spacers (Figure 2). Fourteen plates and twenty-two spacers were arranged on a 5/16" x 6" (8 x 152 mm) bolt with a hole drilled 1/4" (6 mm) from the end of the bolt for a hitch pin attachment. Plates (pl) and spacers (sp) were arranged in the following order: 1pl - 1sp - 1pl - 1sp - 1pl - 1sp - 1pl - 2sp -1 pl - 2sp - 1pl - 3sp - 1pl - 2sp - 1pl - 3sp - 1pl - 2sp -1pl - 2sp - 1pl - 1sp - 1pl - 1sp - 1pl - 1sp. The first seven plates were placed smooth side down while the second seven were placed rough side down. The hardboard was secured on the bolt with a nut and smooth washer. To leach out chemicals used in the hardboard manufacturing process, the assembled substrate samplers were soaked in dechlorinated water for approximately two weeks

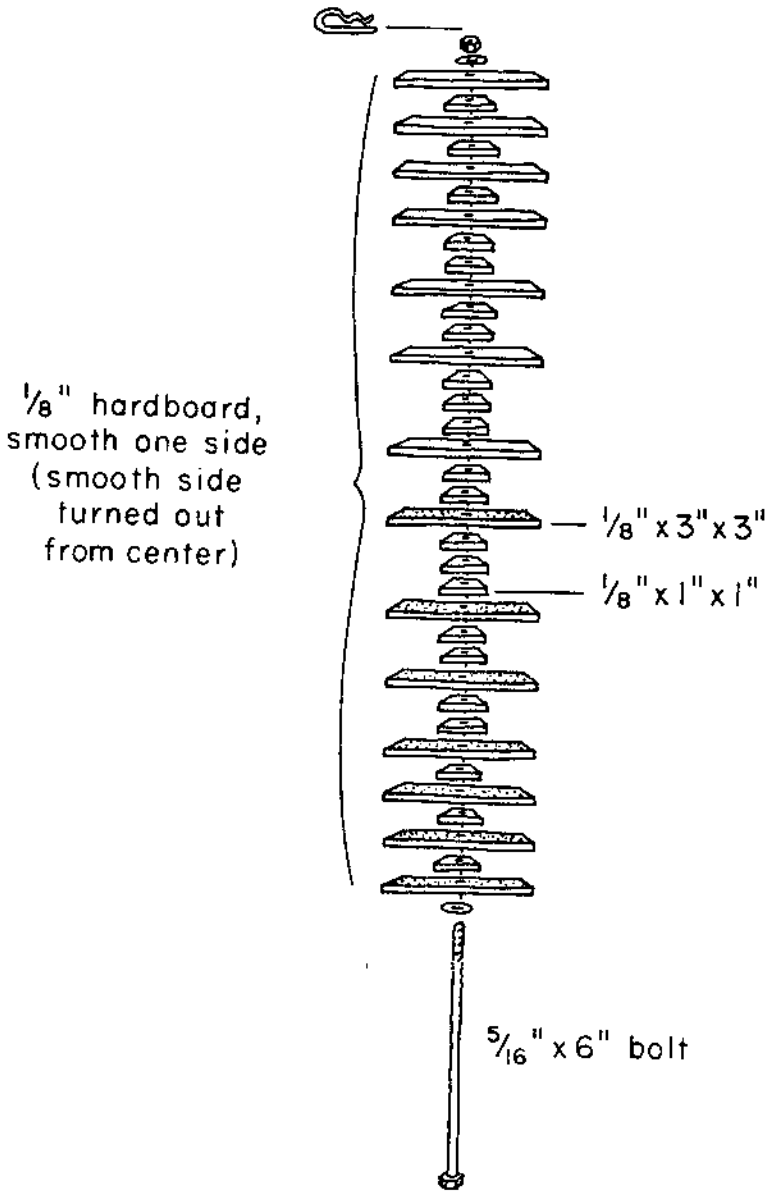

Figure 2. Multiple-plate artificial substrate sampler used to collect macroinvertebrates in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas (Drawing by Gerald Blow, UNT).

(water renewed weekly) and exposed to sunlight for two weeks. The soaking regime also softened the hardboard and created a more hospitable material for insect colonization than unsoaked/stiff hardboard.

During the 1990 and 1991 sample periods, the artificial substrates were hung from 1" x 1" x 18" (25 x 25 x 457 mm) angle iron with a hitch pin and attached to a 6' (2 m) metal fence post with a u-bolt (Figure 3). The fence posts were driven approximately 1.5'(45 cm) into the river substrate with a post-pounder; the angle iron/artificial substrates were situated 6" (15 cm) above the bottom. In 1992, a new artificial substrate holder was designed to facilitate the retrieval of samplers and clam cages during periods of high flows in the Trinity River. The impetus for a new design occurred when all macroinvertebrate substrates and *C. fluminea* cages were lost during floods in the North Texas region in October 1991, when the river flow increased from 0.71 m^3/sec on October 25th to 317.18 m^3/sec two days later. Trinity River levels rose well above the tops of the fence posts and made safe retrieval of the samplers impossible. The clam cages had been placed in the river channel in areas which became inaccessible after the river rose.

A major flaw in the previous holder was the technique of securing substrates/cages into the river substrate (the insect samplers were secured with a fence post while the clam cages were secured with rebar). High water conditions

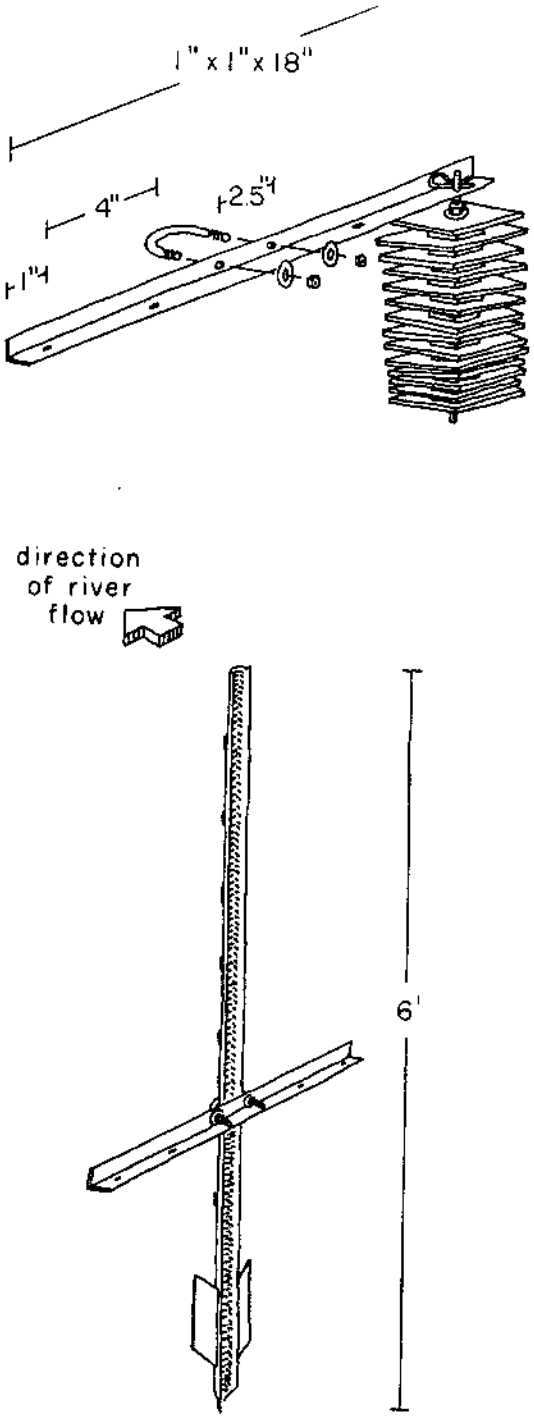

Figure 3. Sampling method used for macroinvertebrate collection in the 1990 and 1991 field season in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas (Drawing by Gerald Blow, UNT).

invariably hindered the retrieval of the sampling gear because of fast currents, floating debris, and difficulty in locating the substrates/cages which were sometimes two meters or more underwater. The new cage holder was designed with the following objectives in mind: 1) eliminate the need to stake substrates/cages into the river bottom (i.e., one could retrieve samplers from shore without physically wading into the river); 2) ability to withstand flood flows without being washed away or buried; 3) minimize vandalism; and 4) affordability, ease of construction, and user friendly in the field.

The new cage holder consisted of 1" x 1" x 21" (25 x 25 x 530 mm) angle iron welded into a one foot high (30 cm) triangle with 5/16" (8 mm) holes drilled into the top for the attachment of samplers (Figure 4). As in the 1990-91 season, the artificial substrates were suspended 6" (15 cm) above the river bottom. To decrease the propensity of the holder to be flipped over by water currents, 1" x 1" x 21" (25 x 25 x 530 mm) angle iron stabilizer bars or "feet" were bolted onto each corner of the triangular frame with 1/4" x 1" (6 x 25 mm) bolts and lock washers. The triangle was anchored to the river bank with airline control cable (diameter of at least 3/32" or 2.5 mm) attached to a 2' (61 cm) metal tie-down stake with a bottom auger bend. Flexible rubber tubing was used to protect the cable from friction with the angle iron. The cable was fastened to the triangle

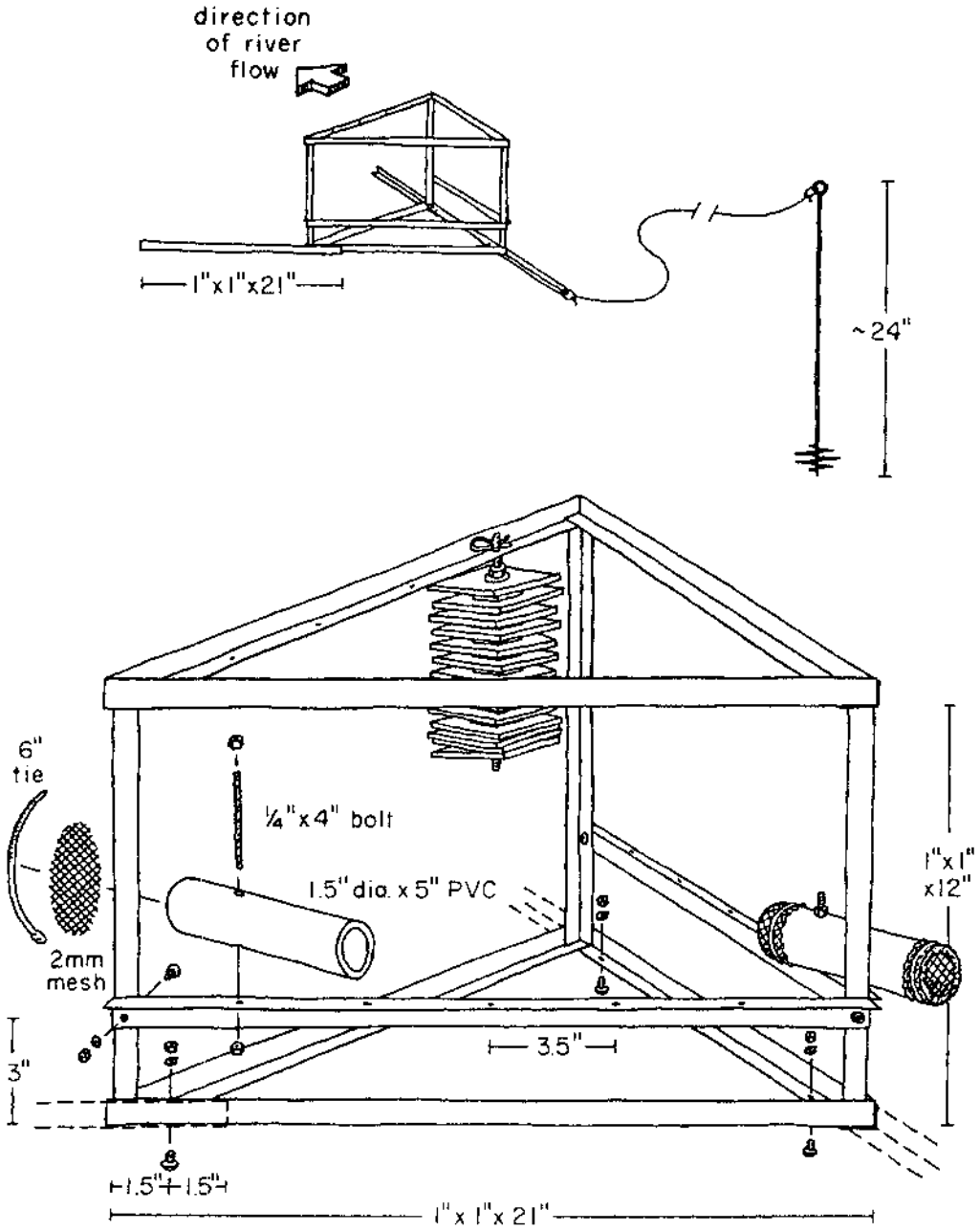

Figure 4. Cage holder used in the 1992 field season to collect macroinvertebrates (artificial substrates) and conduct *C. fluminea* growth studies (pipe cages) in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas (Drawing by Gerald Blow, UNT; cage holder welded by Johnny Laird, UNT).

frame with 3/8" (9 mm) wire rope clips. Clam cages were mounted on the triangle frame with side angle iron bars (see *C. fluminea* section).

The tie-down stake was placed in a location where it was accessible during high flow conditions. Steep banks were preferable because a greater height above the river could be attained over a shorter distance. Steep banks were also preferable because less cable was required. Generally, eight to ten meters of cable were needed to secure the triangles from the river to the bank. Under normal flow conditions, a person could simply stand in the river and set the holder on the substrate. During high flows, the holders could be pulled out of the water by using the cable anchored on the bank.

Artificial substrate samplers were allowed to colonize in the Trinity River for approximately 28 days. The samplers were set in areas with fairly slow currents (i.e., eddy currents) to facilitate colonization. Upon retrieval from the river, the samplers were placed into 2-3 liter plastic containers and immediately preserved in Kahles solution. The colonized samplers were scraped in the laboratory with a knife or piece of hardboard, rinsed into a 180 um (80 series) sieve with a sink sprayer hose, and preserved in 70% ethanol. Rose bengal stain was added to each sample two or more days prior to sorting to help distinguish aquatic organisms from debris (Dickson et al., 1989). A sample was

rinsed through the following sieve sizes to make the sorting process easier: 1000 um (18 series); 600 um (30 series); 420 um (40 series); 250 um (60 series); and 180 um (80 series). Organisms and debris from each sieve were rinsed into petri dishes with 70% ethanol and sorted with forceps under a compound microscope (7 - 10 magnification) into major taxonomic groups. Aquatic organisms were stored in 8-10 ml glass vials and sorted material was stored in 250 ml plastic bottles.

The following taxonomic keys were used to identify immature macroinvertebrates to the family and genus levels: Merritt and Cummins (1984), Pennack (1978), U.S. EPA (1978), Wiggins (1977), Edmunds et al., (1976), Bednarik and McCafferty (1979), and Ward and Whipple (1959). Ephemeroptera, Odonata, Trichoptera, Megaloptera, Coleoptera, and Diptera (except for chironomidae) were identified to family and (when possible) to genus and/or species. Chironomids were identified to the subfamily or tribe level. Oligochaetes were not reported because this study focused on the aquatic insect community.

Macroinvertebrate abundance was based on whole organisms (entire head and body) and heads with partial bodies that were identifiable to family or genus. Density was calculated as the number of organisms/m^2. The surface area of an artificial substrate was 0.1626 square meters.

Taxa richness was determined by the number of different taxa/substrate at the order, family, genus and/or species level. For example, early instars identified only to the family level (i.e., Hydropsychidae) were considered one taxa; later instars from the same family that were identified to a lower level were treated as another taxa (i.e., *Hydropsyche* spp.).

Species diversity was determined from the Brillouin diversity index (Brower and Zar, 1990) :

$$H = \left(\log_2 \frac{N!}{n_1!\ n_2!\ n_3! \ldots n_i!} \right) / N$$

where: N = total # of individuals for a given station
n = # of individuals in the ith taxa for a given station

Evenness was measured by the following formula (Pielou, 1975):

$$E = \frac{H}{\log_2 (S)}$$

where: H = Brillouin diversity index for a given station
S = total # of species for a given station

Diversity and evenness were computed by the Multivariate Statistics Package (MVSP) (Kovack, 1986). The indices were calculated for each replicate at a given station; mean diversity and evenness values were then calculated from the replicate values. The Brillouin diversity index ranges from 0 to 3.5 and evenness ranges from 0 to 1.0.

Community similarity was measured with the Percent Similarity (PS) index (Bray and Curtis, 1957; Brower, et al., 1990):

$$PS_{jk} = 2 \epsilon \frac{[\ \min\ (A_{ij}\ ,\ A_{ik})\ ]}{[\ (A_{ij} + A_{ik})\ ]}$$

where: i = species
j and k = samples
A = abundance of species i in samples j and k
min = lowest percentage for the species i

The PS values were calculated for all possible combinations of station pairs for each sampling date. Similarity values range from 0 (no similarity) to 1.0 (complete similarity).

A dendrogram was produced by applying the UPMGA (unweighted pair group method using arithmetic averages) (Sneath and Sokal, 1973) cluster analysis on the PS values. In the results section, dendrograms and associated bar graphs of community composition (Diptera, Trichoptera, Ephemeroptera, and other taxa) are provided for each sample date in the study.

Historically, interpretation of the results from cluster analysis of community data has been hampered by a lack of statistical power. It is difficult to judge whether a cluster reflects a true difference between communities (i.e., statistically significant) or an artificial difference (i.e., random variability within a community).

True clusters would be expected to be relatively the same over several samples, while artificial clusters would tend to vary highly with each sample. To help overcome the statistical deficiency in cluster analysis interpretation, the nonparametric "bootstrap" technique was used to assess the statistical significance of each cluster linkage (Nemec and Brinkhurst, 1988).

The bootstrap method works by generating new data sets from the original data by selecting, with replacement, species abundance data from samples in a cluster; the new data set is the same size of the original data. The amount of variability that occurs within and between samples is determined by the bootstrap resampling effort. The "within" variability is used as the standard of comparison for defining the statistical significance of cluster linkages. The hypotheses for the bootstrap technique are defined as follows: null (H_o) = two clusters are considered similar enough to represent a single community (the linkage should be accepted); alternative (H_a) = two clusters are not considered similar enough to represent a single community (each cluster represents a different community and the linkage should be rejected); alpha level = 0.05 (Nemec and Brinkhurst, 1988). The similarity coefficient calculations, cluster linkages, and 'bootstrapping' simulations (bootstrap simulations was applied to each cluster linkage) were conducted with the SIGTREE statistical package (Nemec and

Brinkhurst, 1988).

In the macroinvertebrate results section, station clusters that are statistically the same community are identified with labels of the same letter (i.e., a-a), while statistically unrelated clusters are noted by different letters (i.e., a-b). Bray-Curtis similarity coefficients and the probabilities associated with each cluster linkage are tabulated for each sample date.

Corbicula fluminea GROWTH

Juvenile *Corbicula fluminea* were collected from Clear Creek, a tributary to the Elm Fork of the Trinity River and approximately 65 kilometers (40 miles) north of the study area. Clear Creek was selected as a source for *C. fluminea* for three reasons: 1) the creek did not receive any point source discharges; 2) juvenile clams were usually abundant at the site; and 3) a desire to use clams which were resident to the Trinity River drainage. Since the clams in Clear Creek had not been previously exposed to municipal effluent, they represented a relatively "clean" population for use as biomonitors in the Trinity River study.

Juvenile *C. fluminea* were collected from Clear Creek approximately 24 to 36 hours prior to placement into the Trinity River. The mechanics of clam collection involved scooping sand/gravel substrate from depths of 1 to 15 cm

with a shovel, sieve, or plastic bucket and pouring the substrate through 1 - 3 mm mesh sieves. Two to three hundred clams approximately 3.0 - 7.0 mm in shell length (anterior to posterior) were selected from the sifting process and transported with Clear Creek water in 1 liter plastic containers to the University of North Texas (UNT) biology lab. From the pool of clams collected at Clear Creek, 75 clams were randomly chosen for the Trinity River cages (10/station; 5 extra). Clams were within 2 mm or less of one another for each sample period (see Initial Length section).

Juvenile *C. fluminea* were measured in the following manner: 1) vernier calipers were used to measure shell length (greatest anterior to posterior distance) and shell width (greatest distance between dorsal (umbo) and ventral margin) to either 0.1 mm (manual caliper) or 0.01 mm (battery operated digital caliper); and 2) a digital top-loading balance was used to record whole-shell and body weight to 0.001 gram. Each individual clam was placed into a 25 ml plastic container, covered with 2 mm mesh screen, fastened with rubber bands, and labeled for identification. The enclosed clams were set into a cooler with Clear Creek water (maintained between 20 - 25° C), two aerators, and an aquarium heater on the evening prior to the *in situ* test in the Trinity River.

At each Trinity River station, juvenile clams were placed into 1.5" x 5" (38 x 127 mm) pvc-plastic pipe flow-

through cages. The cages were covered on each end with 2 mm fiberglass mesh screens fastened with plastic cable ties. Each cage was labeled for identification of individual clams. One clam was placed in each pipe cage and a total of ten clams were placed at each station.

During the 1990 and 1991 field season, 5 pipe cages were mounted on one 1.5" x 3.5" x 13.5" (38 x 90 x 340 mm) wood block with 1/4" x 4" (6 x 100 mm) bolts and smooth washers (Figure 5). This wood stand was nailed into 1.5" x 3.5" x 4" (38 x 90 x 100 mm) wood legs located at each end. The purpose of the wood legs was to help prevent sediment accumulation by elevating the clam cages 7.5 cm (3 inches) above the substrate. The wood stand was staked at each end into the river bottom with 1/2" x 2' (13 mm x 61 cm) metal rebar welded into an L-shape. The cages were further anchored into the shore or substrate with rope and wooden stakes. Because the cages had to be staked into the substrate, site selection was dependent on water level and type of substrate. Generally, cages were placed in water depths of 1 meter or less and on fairly level substrate consisting of sand, gravel, and/or silt.

In the 1992 field season, the clam cage was modified to accommodate high river flows (see Macroinvertebrate section). A 1" x 1" x 21" (25 x 25 x 530 mm) angle iron bar was attached 7.5 cm from the bottom of the cage holder on two sides with 1/4" x 1" (6 x 25 mm) bolts and lock washers

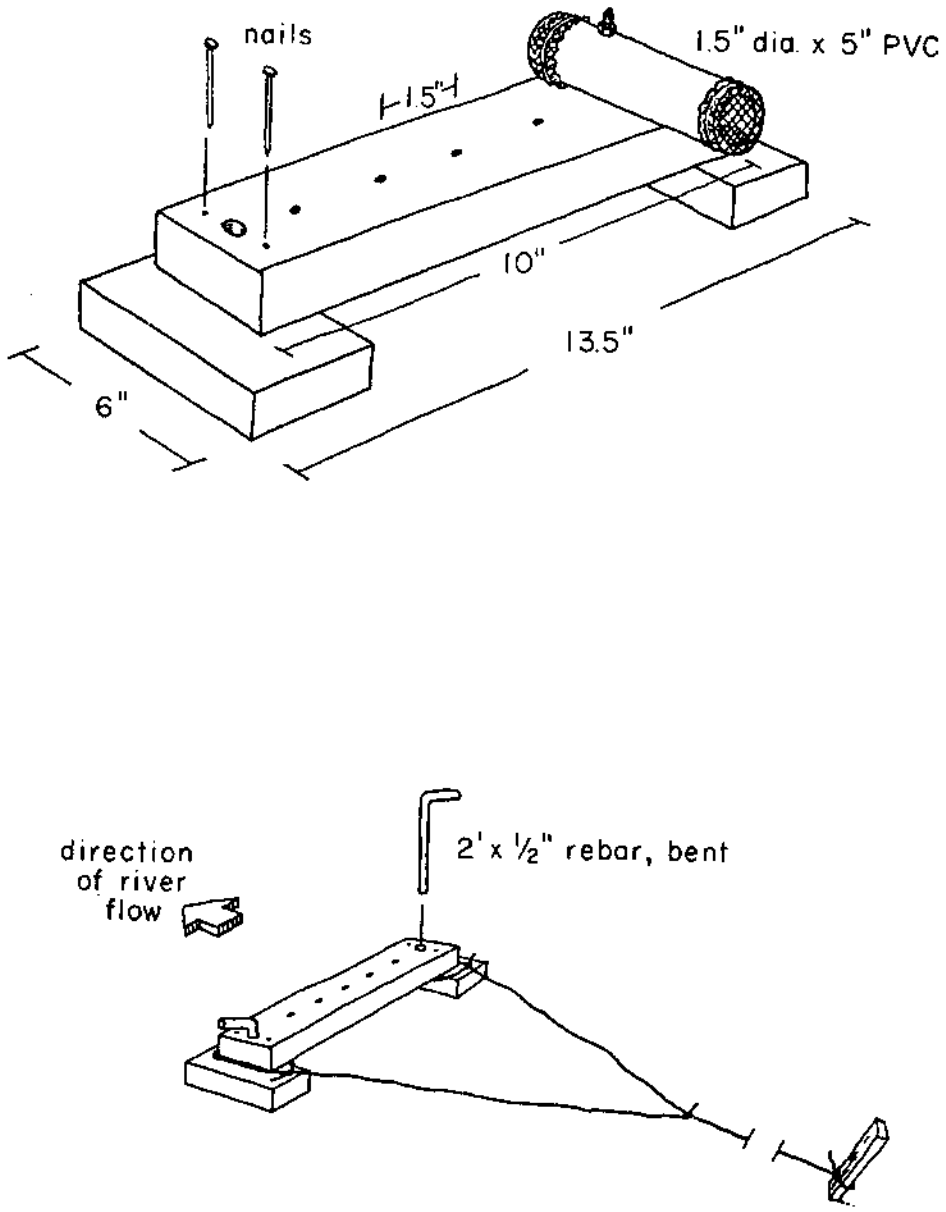

Figure 5. Sampling method used in the 1990 and 1991 field season for *C. fluminea* growth studies in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas (Drawing by Gerald Blow, UNT).

(Figure 4). The pipe cages were secured on the bars with 1/4" x 3" (6 x 76 mm) bolts and arranged parallel to the stream channel.

Juvenile *C. fluminea* were exposed at each station in the Trinity River for approximately 28 days. Upon retrieval, the clams were immediately checked for survival and then placed into 25 ml plastic containers in a water cooler. Clams were transported from the field within six hours to a UNT lab. Length, width, and weight measurements on each clam were completed within 24 hours of retrieval from the river. Measured clams were stored in plastic bags and frozen.

Shell length (anterior to posterior) gain was the variable used to monitor clam growth. Mean shell lengths from each station were compared for each sample period using the TOXSTAT statistical package (Gulley et al., 1989). Tukey's multiple range test was used on parametric data and Dunn's multiple comparison test was used on non-parametric data. Bonferroni (Dunn) T-Test (SAS, 1989) was used to analyze the non-parametric growth data from the 1990 pre-dechlorination *in situ* test. In the clam results section for growth, stations labeled with the same letter were statistically equal (i.e., a-a), while stations that were significantly different from one another do not share the same letter (i.e., a-b). *C. fluminea* survival data was analyzed with the Fisher's exact test (Gulley et al., 1989). A significance level of 0.05 was used in all analyses.

In the 1990 *in situ* tests, juvenile clams were measured for weight (whole-shell and body) and shell width (dorsal to ventral) but not shell length (anterior to posterior). To compare dechlorination growth data with the 1990 pre-dechlorination baseline data, a linear regression model was used to predict the lengths of juvenile clams used in 1990 (Length (mm) = 0.389 + 1.129 Width (mm); R^2 = 0.993) (SAS, 1989). The model was based on empirical data collected on the lengths and widths of 216 individuals of *C. fluminea* ranging from 3.1 to 14.6 mm in shell length (anterior to posterior). All individuals were collected from Clear Creek.

Initial Shell Length and Growth Potential

Shell growth rates of juvenile *C. fluminea* are known to be inversely proportional to shell size (Aldridge and McMahon, 1978; McMahon and Williams, 1986). To reduce bias in potential shell growth for the clams in the Trinity River study, it was necessary for initial shell sizes to be similar among all stations. Knowledge of initial shell sizes can help discern whether reduced clam growth at a station was caused by a stressor or by significantly larger initial sizes placed at one station compared to other stations. If there was no significant difference between initial shell sizes (i.e., all stations had juvenile shell lengths ranging from 3.0 to 4.5 mm), growth potential should be comparable among all stations.

Figure 6 and Tables 3 & 4 depict the initial shell lengths of juvenile *C. fluminea* used for *in situ* tests in the Trinity River. For each sample date, juvenile clams at each station were not significantly different from all other stations prior to placement in the Trinity River (1 = 2 = 3 = 4 = 5 = 6 = 7; Tukey's Multiple Comparison). Initial shell lengths ranged from 3.01 mm to 7.05 mm. The maximum difference between individual clam lengths within a particular test date ranged from 1.0 mm (Sept 1991) to 3.0 mm (July 1991). It was not possible to use the same exact shell sizes for each sample date because of variability in juvenile *C. fluminea* populations in the field at the time of collection.

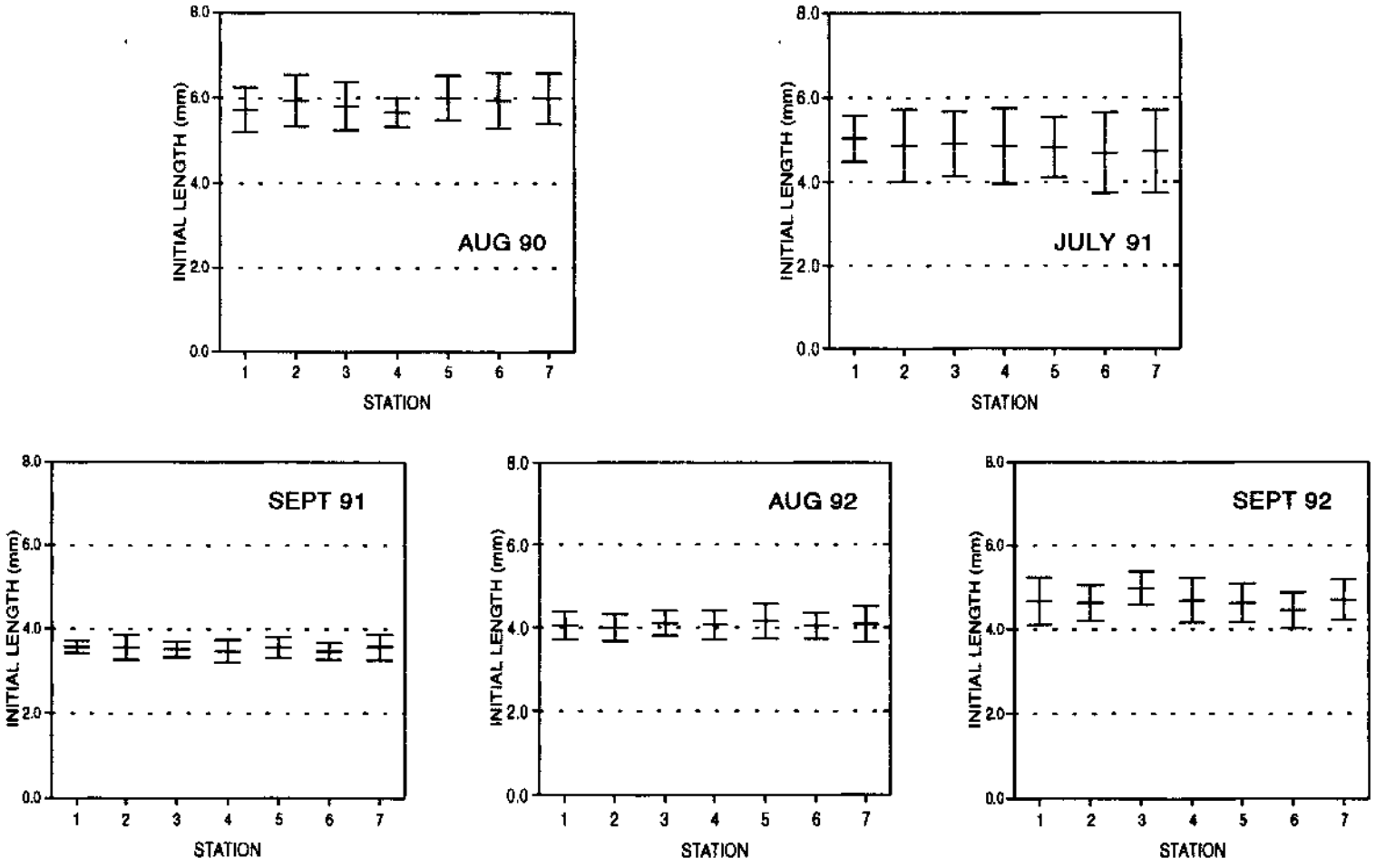

Figure 6. Initial shell lengths (mm) (mean +/- 1 standard deviation) of juvenile C. fluminea before they were placed in cages for in situ tests conducted in 1990, 1991, and 1992 in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

STATION	1	2	3	4	5	6	7
Aug. '90							
MIN	5.24	5.24	5.02	5.13	5.13	5.02	5.36
MAX	6.82	7.05	6.94	6.15	6.82	6.94	7.05
MEAN	5.73	5.94	5.81	5.66	6.01	5.94	5.99
SD	0.528	0.602	0.568	0.329	0.515	0.651	0.590
July '91							
MIN	4.10	3.70	3.70	3.60	3.90	3.10	3.20
MAX	6.00	6.10	6.10	6.30	6.20	6.20	6.10
MEAN	5.03	4.86	4.91	4.85	4.82	4.69	4.72
SD	0.548	0.853	0.767	0.892	0.715	0.962	0.981
Sept. '91							
MIN	3.41	3.04	3.22	3.09	3.16	3.08	3.01
MAX	3.82	3.94	3.78	3.95	3.96	3.70	3.92
MEAN	3.57	3.56	3.52	3.47	3.56	3.47	3.57
SD	0.150	0.295	0.184	0.267	0.251	0.202	0.303

SD = standard deviation

Table 3. Descriptive statistics on initial shell lengths (mm) of juvenile *C. fluminea* before they were placed in cages for *in situ* tests conducted in 1990 and 1991 in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

STATION	1	2	3	4	5	6	7
Aug. '92							
MIN	3.62	3.41	3.80	3.63	3.55	3.62	3.51
MAX	4.75	4.31	4.72	4.66	4.77	4.57	4.89
MEAN	4.06	3.99	4.11	4.07	4.16	4.05	4.09
SD	0.341	0.325	0.296	0.344	0.417	0.310	0.431
Sept. '92							
MIN	4.03	3.97	4.34	3.97	4.00	3.99	4.06
MAX	5.48	5.28	5.47	5.40	5.37	5.17	5.48
MEAN	4.67	4.63	4.98	4.69	4.63	4.45	4.70
SD	0.563	0.434	0.390	0.534	0.454	0.424	0.486

SD = standard deviation

Table 4. Descriptive statistics on initial shell lengths (mm) of juvenile *C. fluminea* before they were placed in cages for *in situ* tests conducted in 1992 in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

CHAPTER 3

RESPONSE OF MACROINVERTEBRATE COMMUNITY

INTRODUCTION

In the following chapter, the response of immature macroinvertebrates to chlorinated and dechlorinated effluent is discussed for each sample date. The spatial and temporal trends of macroinvertebrate colonization are described for the dechlorination recovery phase.

Macroinvertebrate artificial substrates were exposed in the Trinity River for approximately one month on four different sample dates. A baseline pre-dechlorination sample was completed in August/September 1990. Three dechlorination samples were conducted over a two year span: 1) May/June 1991; 2) April/May 1992; and 3) August 1992.

Based on density, the main aquatic insect orders represented throughout the study were Diptera, Trichoptera, and Ephemeroptera. These three groups occupied approximately 98% of the entire macroinvertebrate community for any given sample period. Additional orders included Odonata, Megaloptera, and Coleoptera. In the data presented, these orders were combined under the group 'Other'. Table 5 lists

Order: EPHEMEROPTERA

Family: Baetidae
Baetis spp. (Leach 1815)
Baetis longipalpus (Morihara & McCafferty 1979)
Family: Heptageniidae
Stenacron spp. (Jensen 1974)
Stenonema spp. (Traver 1933)
Stenonema integrum (McDunnough 1924)
Stenonema femoratum (Say 1823)
Family: Isonychiidae
Isonychia spp. (Eaton 1871)
Family: Tricorythidae
Tricorythodes spp. (Ulmer 1920)
Family: Caenidae
Caenis spp. (Stephans 1835)
Family: Leptophlebiidae
Choroterpes spp. (Eaton 1881)
Choroterpes mexicanus (Allen)

Order: ODONATA

Sub-order: Anisoptera
Family: Gomphidae
Erpetogomphus spp. (Selys 1858)

Sub-order: Zygoptera
Family: Calopterygidae
Hetaerina spp. (Hagen 1854)
Family: Coenagrionidae
Argia spp. (Rambur 1842)
Chromagrion spp. (Needam 1903)

Order: MEGALOPTERA

Family: Corydalidae
Corydalus cornutus (Linnaeus 1763)

Table 5. List of aquatic insects collected from artificial substrates exposed in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas for the dechlorination study conducted in 1990 - 1992.

Order: TRICHOPTERA

Family: Hydropsychidae
Hydropsyche spp. (Pictet 1836)
Hydropsyche orris (Ross 1938)
Hydropsyche simulans (Ross 1938)
Potamyia flava (Hagen 1861)
Cheumatopsyche spp. (Wallengren 1891)
Family: Polycentropodidae
Cyrnellus fraternus (Banks 1913)
Family: Hydroptilidae
Hydroptila spp. (Dalman 1819)
Hydroptila ajax
Neotrichia spp. (Norton 1905)
Family: Leptoceridae
Nectopsyche spp. (Muller 1879)

Order: COLEOPTERA

Family: Hydrophilidae
Berosus spp. (Leach 1817)
Family: Elmidae
Stenelmis spp. (Dufour 1835)
Heterelmis spp. (Sharp 1882)

Order: DIPTERA

Family: Ceratopogonidae
Bezzia spp.
Family: Simulidae
Family: Chironomidae
Sub-family: Tanypodinae
Orthocladiinae
Chironominae
Chironomini
Tanytarsini
Family: Empididae
Hemerodromia spp. (Meigen 1822)

Table 5 (continued). List of aquatic insects collected from artificial substrates exposed in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas for the dechlorination study conducted in 1990 - 1992.

all taxa collected during the study.

RESULTS

Pre-dechlorination - Late Summer 1990

Community Composition

Total immature macroinvertebrate densities were approximately 14,000 individuals/m^2 each at reference Stations 1 and 2 and ranged between 6,000 - 9,000 individuals/m^2 for downstream Stations 3, 4, and 7 (Figure 7). A large increase in density occurred at Stations 5 and 6 (>30,000 individuals/m^2 at each station). The following chlorine concentrations (TRC = total residual chlorine) were present in water samples collected in August and September: Station 3 - 1.04 mg TRC/l; Station 4 - 0.45 mg TRC/l; Station 5 - 0.14 mg TRC/l; Station 6 - 0.08 mg TRC/l; and Station 7 - 0.02 mg TRC/l.

The upstream reference Stations 1 and 2 exhibited greater overall diversity than the downstream stations. Diptera (primarily the Chironomidae family) occupied a smaller percentage of the community at Stations 1 and 2 (48% and 77%, respectively) compared to downstream stations. At stations below the effluent, dipterans always accounted for at least 86% of the total community. At Stations 3 and 4, Diptera totaled 99.0% and 99.2%, respectively.

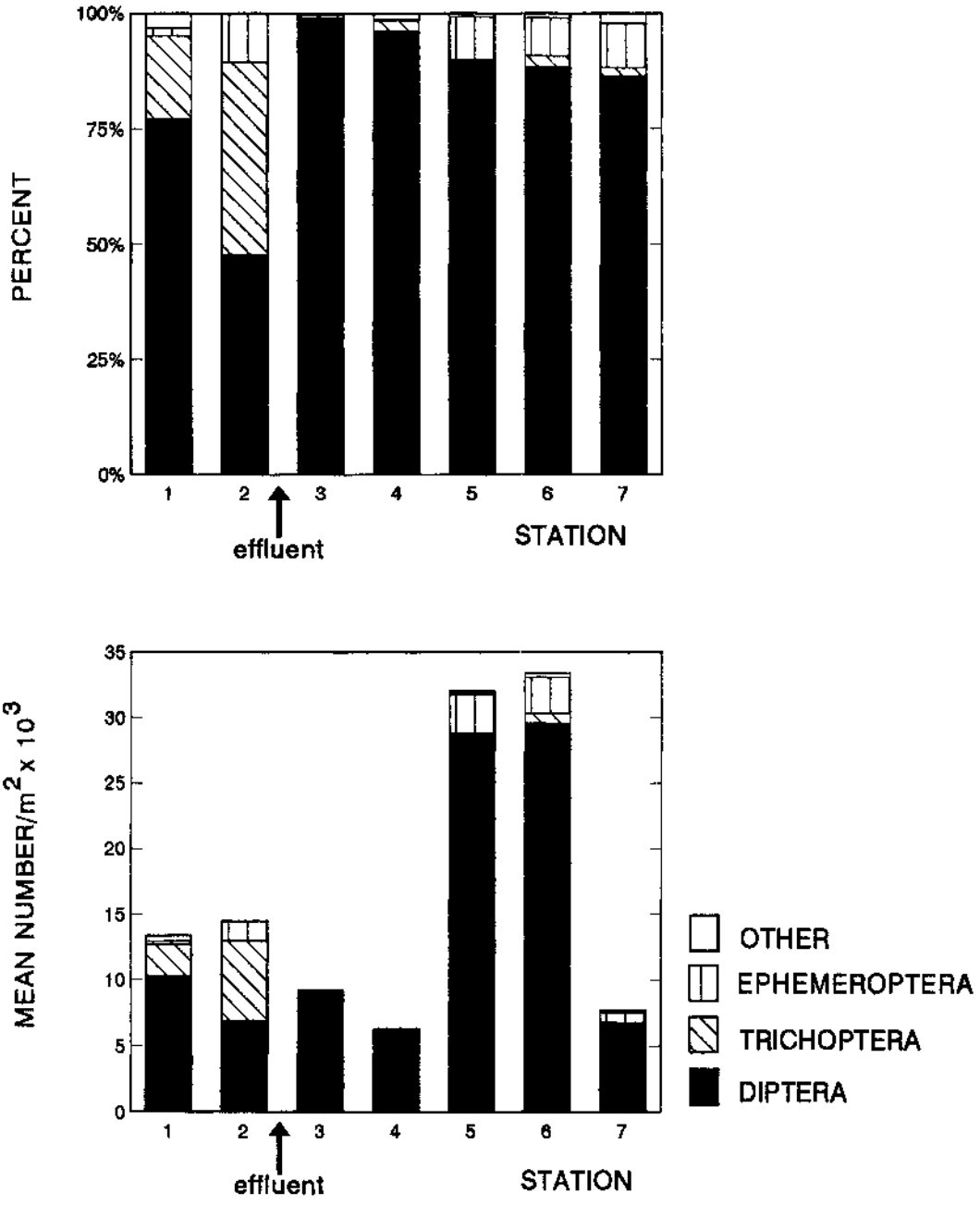

Figure 7. Community composition (%) and total mean density of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

Trichoptera were more abundant at Stations 1 and 2 (2,500 and 6,000 individuals/m^2, respectively) compared to all downstream stations. Caddisflies were virtually absent from Stations 3, 4, 5, and 7 (<155 individuals/m^2 each station). Ephemeroptera occurred in higher numbers at reference Station 2 (1,500 individuals/m^2) than at Stations 1, 3, 4, and 7 (<800 individuals/m^2 each station). Mayfly communities were most impacted at Stations 3 and 4 (within 1 mile below the outfall) and most abundant at Stations 5 and 6 (5 and 8 miles below outfall, respectively). The largest numbers of other taxa (odonates, coleopterans, and megalopterans) occurred at Station 1 (420 individuals/m^2) and the lowest densities (<25 individuals/m^2) at Station 2 and downstream Station 3.

Diptera

Overall densities were similar for Stations 1, 2, 3, 4, and 7 (range of 6,000 - 10,000 individuals/m^2) (Figure 8). Large increases were observed at Stations 5 and 6 where abundance peaked at almost 30,000 individuals/m^2. Diptera was dominated by the Chironominae subfamily (87% of Diptera collected) at all stations. The subfamilies Tanypodinae and Orthocladiinae were subdominant (5.4% and 7% of Chironomidae collected, respectively). Approximately twice as many Tanypodinae occurred at reference Stations 1 and 2 compared to Stations 3 and 4 below the outfall. At Station 5,

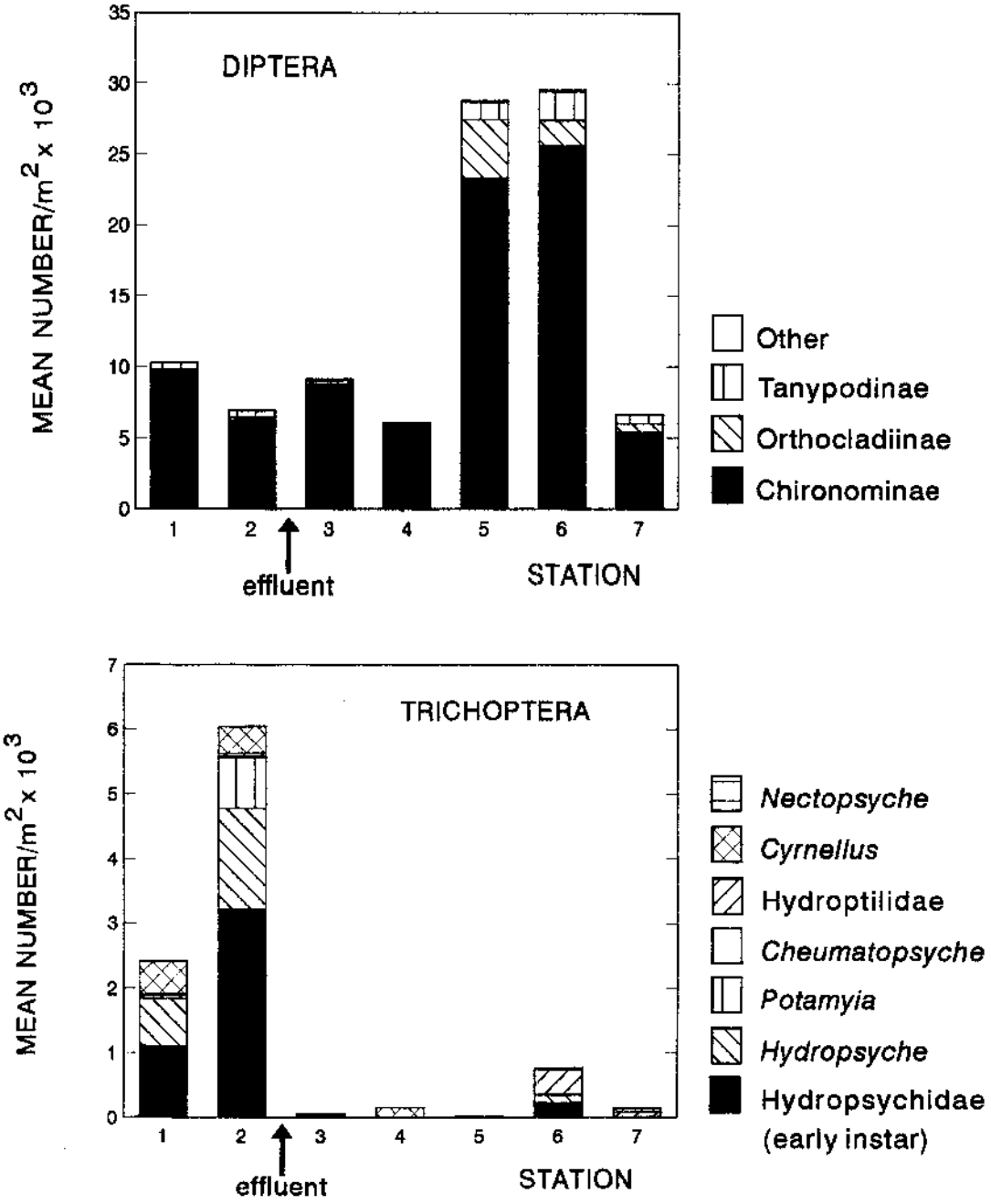

Figure 8. Mean density and taxa composition of Diptera (top) and Trichoptera (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

tanypods occurred in lower densities than orthoclads while the two subfamilies were almost equal at Station 6 and 7. Other Diptera included Empididae (*Hemerodromia* spp.), Ceratopogonidae, and Simulidae and densities ranged from 20 to 100 individuals/m^2 at each station.

Trichoptera

Caddisflies were present in small numbers at all stations below the effluent compared to reference Stations 1 and 2 (Figure 8). Total densities were approximately twice as high at Station 2 (6,000 individuals/m^2) compared to Station 1. Station 6 supported the largest caddisfly community (800 individuals/m^2) downstream from the effluent.

Early instars from the filter-feeding Hydropsychidae family were the dominant group of caddisflies at Stations 1 and 2. *Hydropsyche* spp. was the most common genus found at both stations while *Cheumatopsyche* spp. was the least common hydropsychid. *Potamyia flava* occurred in higher numbers at Station 2 (800 individuals/m^2) than Station 1 (50 individuals/m^2). *Cyrnellus fraternus* occurred about equally at Stations 1 and 2 and was the only caddisfly that colonized Station 4. *C. fraternus* also occurred in small numbers (<60 individuals/m^2) at Stations 3, 5, 6, and 7. Hydroptilidae (*Hydroptila* spp. and *Neotrichia* spp.) was most abundant at Station 6 (400 individuals/m^2) compared to all other stations. Hydroptilids were absent from Stations 4 & 5.

Ephemeroptera

Mayfly densities were largest at downstream Stations 5 and 6 (2,800 and 3,000 individuals/m^2, respectively) and successively decreased at Stations 2, 7, and 1 (Figure 9). Density at Station 2 (1,500 individuals/m^2) was approximately five times higher than Station 1. Mayflies were virtually absent (<20 individuals/m^2) from Stations 3 and 4 closest to the outfall.

At the reference stations, *Tricorythodes* spp. was the dominant taxa at Station 1 (54%) while baetids (48%) and tricorythids/caenids (41%) were about equally represented at Station 2. Downstream Stations 5, 6, and 7 were dominated by *Tricorythodes* spp. and Tricorythidae/Caenidae early instar complex. *Stenonema integrum* and early instars of Heptageniidae were present at all stations and occurred in densities less than 200 individuals/m^2/station. Baetidae were supported at all stations except Stations 3 and 4. A few *Isonychia* spp. (<15 individuals/m^2) were found at Stations 1, 2, and 6. *Choroterpes* spp. (Leptoplebiidae) was incidental at Station 2.

Other Taxa

Members of other taxa were most abundant at Station 1 (420 individuals/m^2) and least common (< 25 individuals/m^2) at Station 2 above and Station 3 below the outfall (Figure 9). At downstream stations, other taxa were smallest

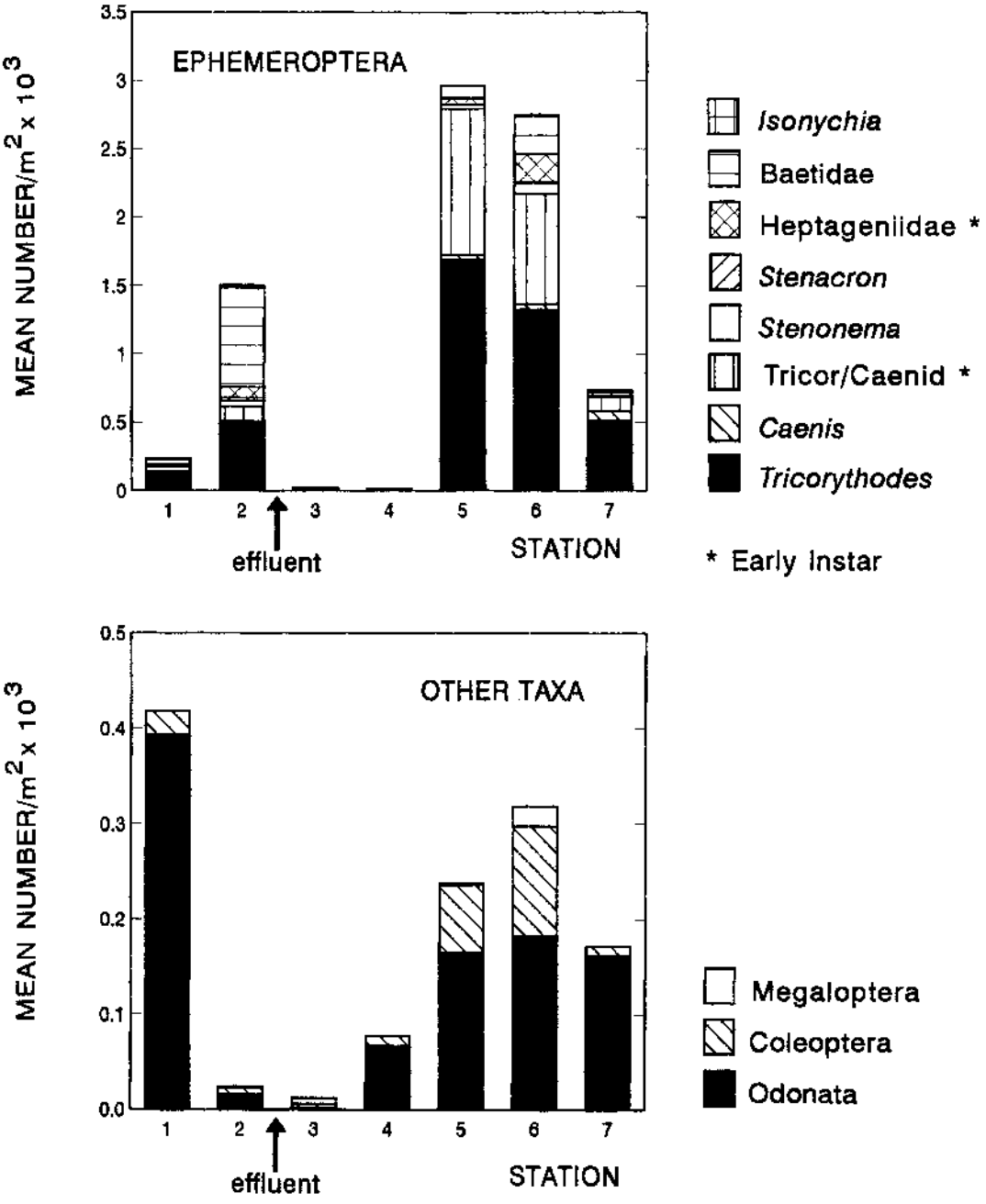

Figure 9. Mean density and taxa composition of Ephemeroptera (top) and other taxa (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

in number at Station 3, gradually increased with increasing distance from the outfall at Stations 4, 5, and 6, and declined slightly at Station 7. *Argia* spp. damselflies (Odonata:Zygoptera) were highest at Station 1 (400 individuals/m^2), followed by Stations 5, 6, 7, and 4. *Hetaerina* spp. was incidental at Station 6. Coleoptera (*Stenelmis* spp., *Heterelmis* spp., and *Berosus* spp.) were most common at Stations 5 and 6. The megalopteran predator, *Corydalus cornutus*, was present at Stations 2, 3, 5 and 6.

Community Indices

Similarity matrix values ranged from 0.38 between Stations 1 and 5 to 0.89 between Stations 5 and 6 (Table). Community composition at Stations 5 and 6 was significantly different from all other stations ($p=0.044$) based on the Bray-Curtis similarity analysis (Figure 10). Stations 1, 2, 3, 4, and 7 were not significantly different from each other ($p > 0.25$).

Linkage	Clusters Linked	Similarity	Probability
1	Station 5 & 6	0.888	0.2530
2	Station 4 & 7	0.826	0.5830
3	Station 1 & 3	0.814	0.5710
4	Station 1 & 4	0.676	0.4680
5	Station 1 & 2	0.609	0.3720
6	Station 1 & 5	0.384	0.0440

Table 6. The similarity value and probability of each cluster linkage calculated for the macroinvertebrate communities collected during August/September 1990, before dechlorination began.

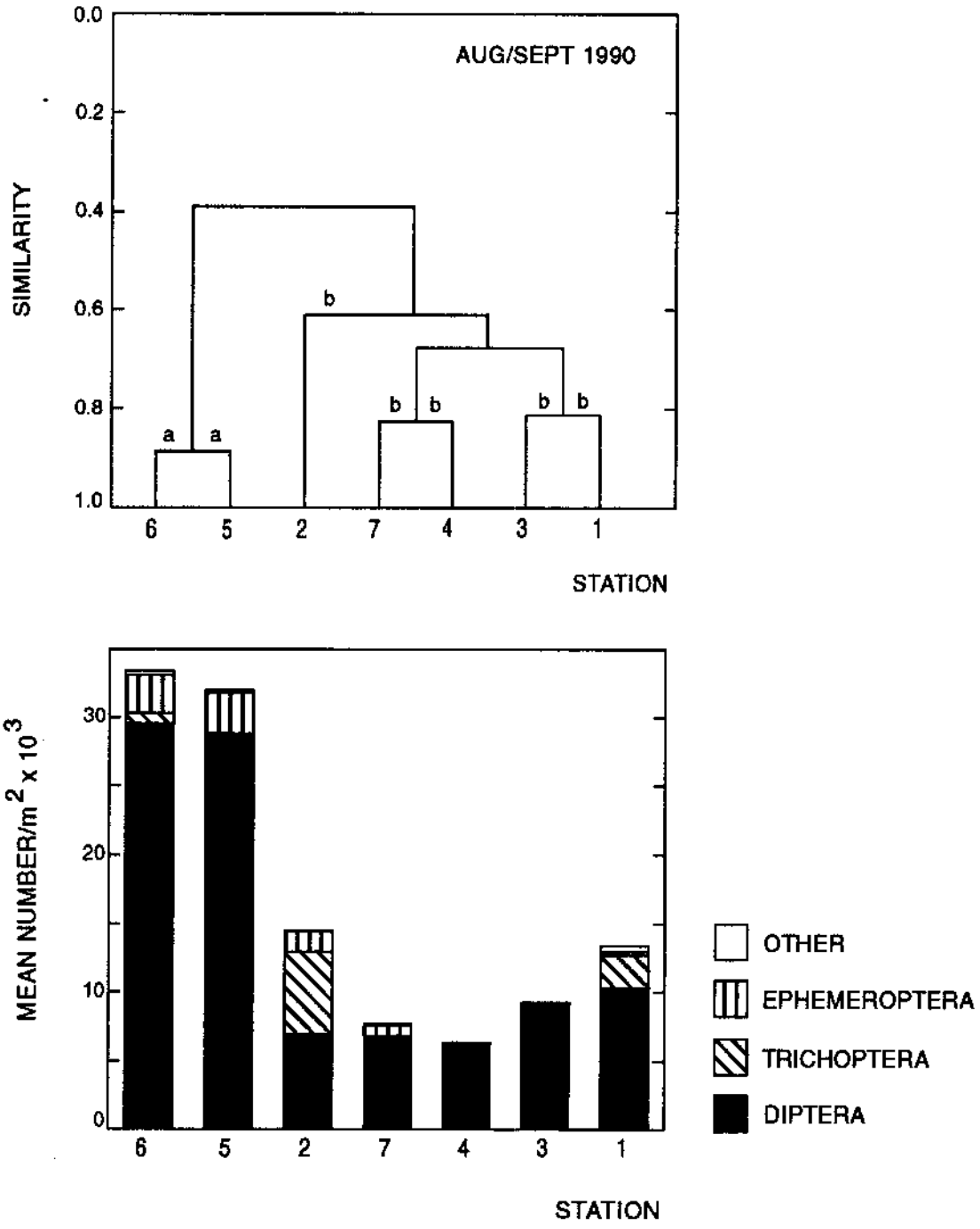

Figure 10. Bray-Curtis similarity index (top) of the macroinvertebrate community (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

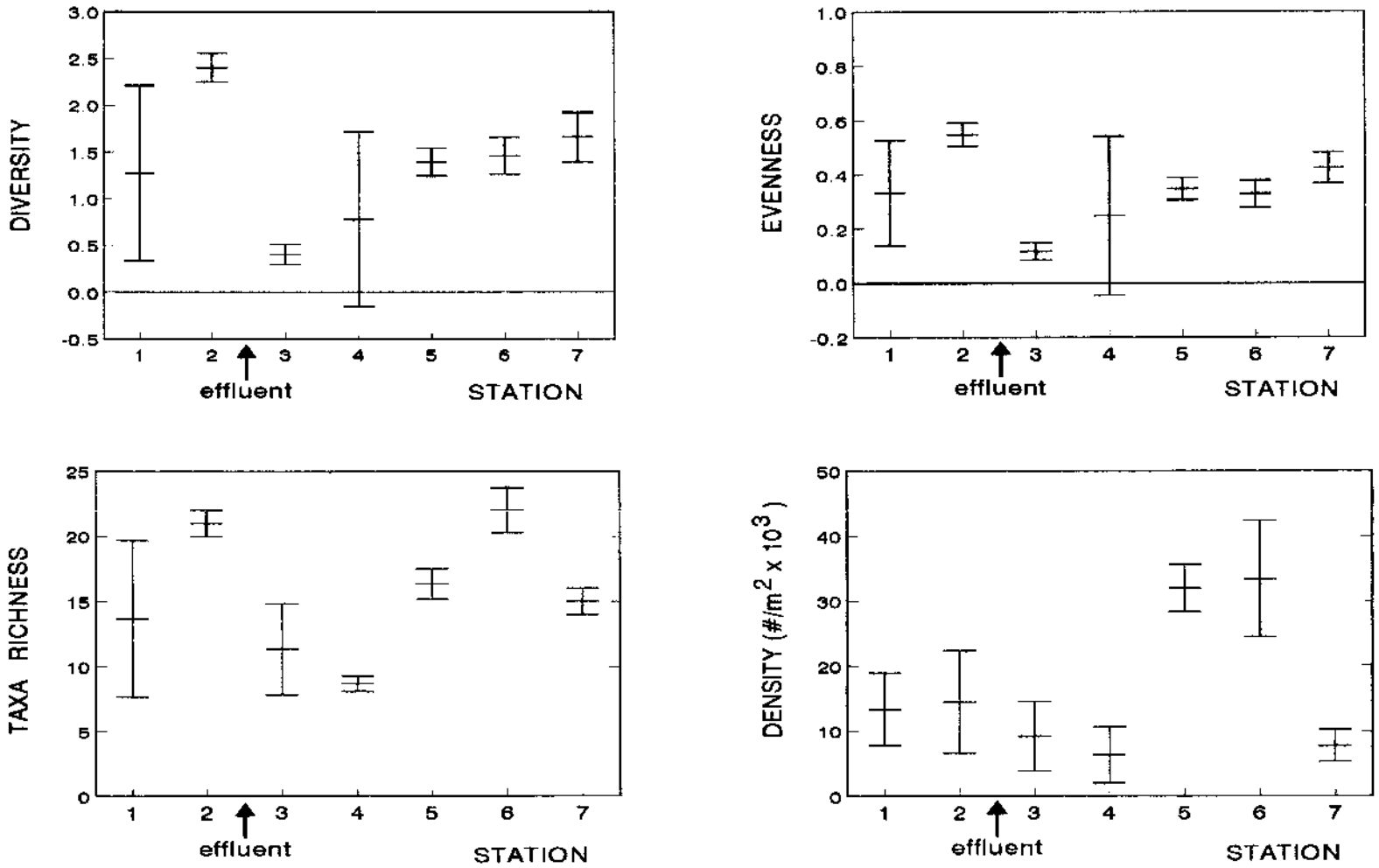

Figure 11. Community indices (mean +/- 1 standard deviation) of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

STATION	1	2	3	4	5	6	7
DIVERSITY							
MEAN	1.274	2.405	0.403	0.783	1.396	1.460	1.661
SD	0.939	0.153	0.107	0.934	0.149	0.198	0.264
CV	73.7%	6.3%	26.5%	119.2%	10.7%	13.6%	15.9%
EVENNESS							
MEAN	0.333	0.548	0.118	0.249	0.347	0.328	0.424
SD	0.194	0.043	0.032	0.293	0.041	0.050	0.057
CV	58.4%	7.9%	27.1%	117.9%	11.8%	15.3%	13.5%
RICHNESS							
MEAN	13.7	21.0	11.3	8.7	16.3	22.0	15.0
SD	6.0	1.0	3.5	0.6	1.2	1.7	1.0
CV	44.1%	4.8%	31.0%	6.7%	7.1%	7.9%	6.7%
DENSITY[1]							
MEAN	13370.2	14501.8	9225.1	6344.8	32011.1	33390.7	7718.3
SD	5543.5	7868.6	5333.4	4290.8	3650.6	8924.2	2437.3
CV	41.5%	54.2%	57.8%	67.6%	11.4%	26.7%	31.6%

SD = Standard Deviation; CV = Coefficient of Variation; [1] individuals/meter^2

Table 7. Descriptive statistics on immature macroinvertebrate communities collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

Brillouin diversity index ranged from 0.40 at Station 3 downstream to 2.41 at Station 2 upstream of the effluent (Figure 11 and Table 7). Diversity values gradually increased downstream from the effluent from 0.78 - 1.66 at Stations 4, 5, 6, and 7. Variability was extremely wide at Stations 1 and 4 where coefficients of variation were 74% and 119%, respectively. Variation ranged from 6% to 27% at all other stations. Evenness values were lowest at Station 3 (E=0.12) and highest at Station 2 (E=0.55). Coefficients of variation were between 8% - 30% at Stations 2, 3, 5, 6, and 7. Variation increased at Stations 1 (cv=58%) and 4 (cv=118%).

Stations 2 and 6 had the highest number of taxa with 21 and 22, respectively. The fewest number of taxa were found at Stations 3 and 4 (<12 taxa/station). Variation was less than ten percent at all stations except Station 1 (cv=44%) and 3 (cv=31%). Total macroinvertebrate density was similar at Stations 1, 2, 3, 4, and 7 and significantly higher at Stations 5 and 6. Coefficients of variation ranged from 11% at Station 5 to almost 70% at Station 4.

Dechlorination - Early Summer 1991

Community Composition

Macroinvertebrate density was between 4,000 and 6,000 individuals/m^2 for upstream reference Station 2 and downstream Stations 4, 5, 6, and 7 (Figure 12). Reference

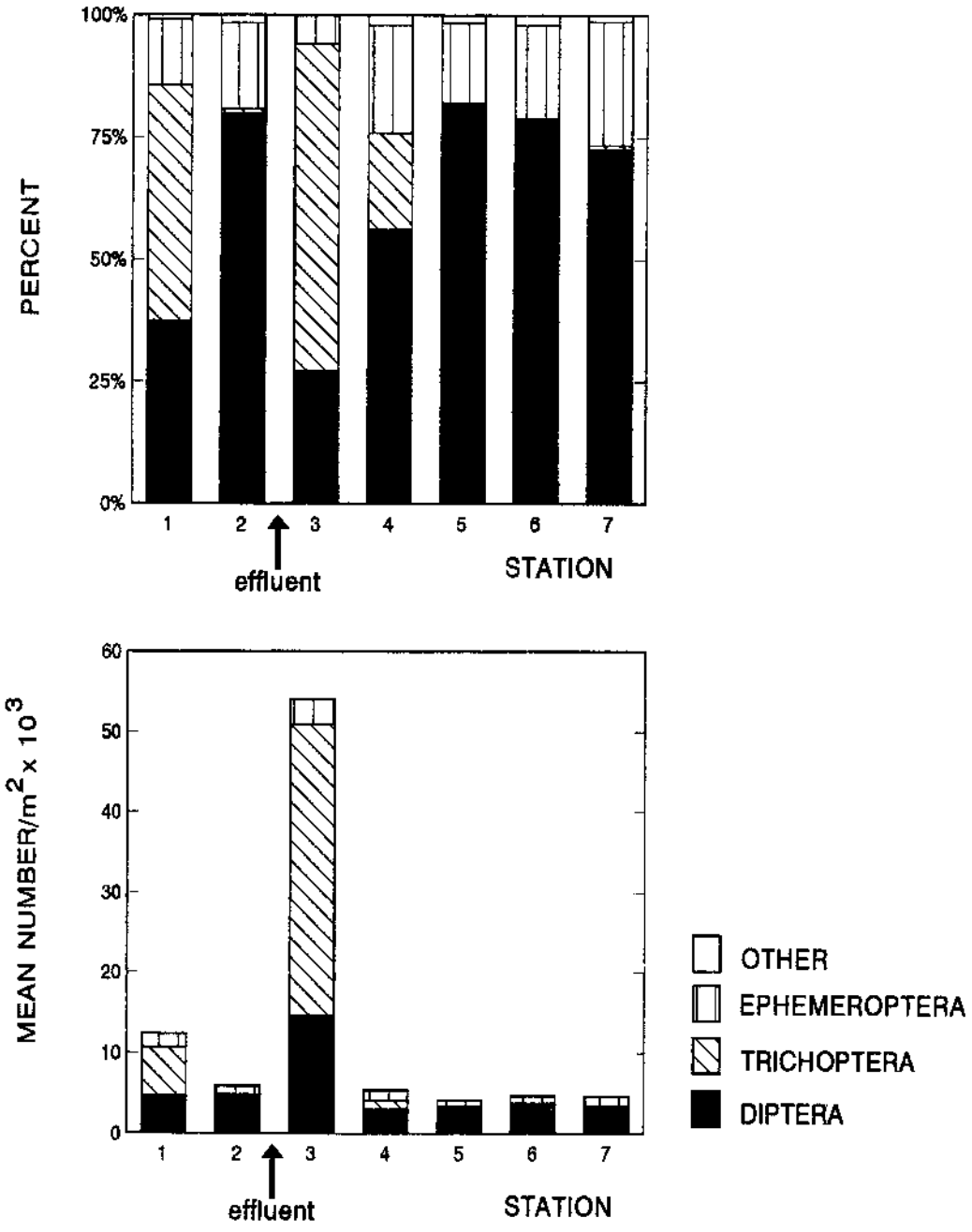

Figure 12. Community composition (%) and total mean density of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

Station 1 supported approximately 12,000 individuals/m^2. At Station 3, directly below the effluent, density was over four times higher (54,000 individuals/m^2) than Station 1.

Diptera (primarily Chironomidae) was the dominant taxa collected at Stations 2, 4, 5, 6, and 7. Chironomids occupied from 56% to 82% of the community at these stations. Trichoptera was the most abundant order at Stations 1 (48%) and 3 (67%). Caddisflies only occurred in substantial numbers at Stations 1 and 3 (6,000 and 36,000 individuals/m^2, respectively). Small numbers of caddisflies were present at Station 4 (1,000 individuals/m^2) and they were virtually absent from upstream Station 2 and downstream Stations 5, 6, and 7. Ephemeroptera density ranged from 700 to 1,700 individuals/m^2 at Stations 1, 2, 4, 5, 6, and 7. Mayflies were most abundant at Station 3 (3,100 individuals/m^2) below the outfall. Other taxa (odonates, coleopterans, and megalopterans) occupied less than one percent of the community at each station.

Diptera

Dipteran densities ranged from 3,000 to 5,000 individuals/m^2 for all stations except Station 3, where the population sharply increased to 15,000 individuals/m^2 (Figure 13). Chironominae was the majority subfamily at all stations (45% to 67%) and was most dominant at Station 3 (82%). The Tanypodinae population was approximately half the

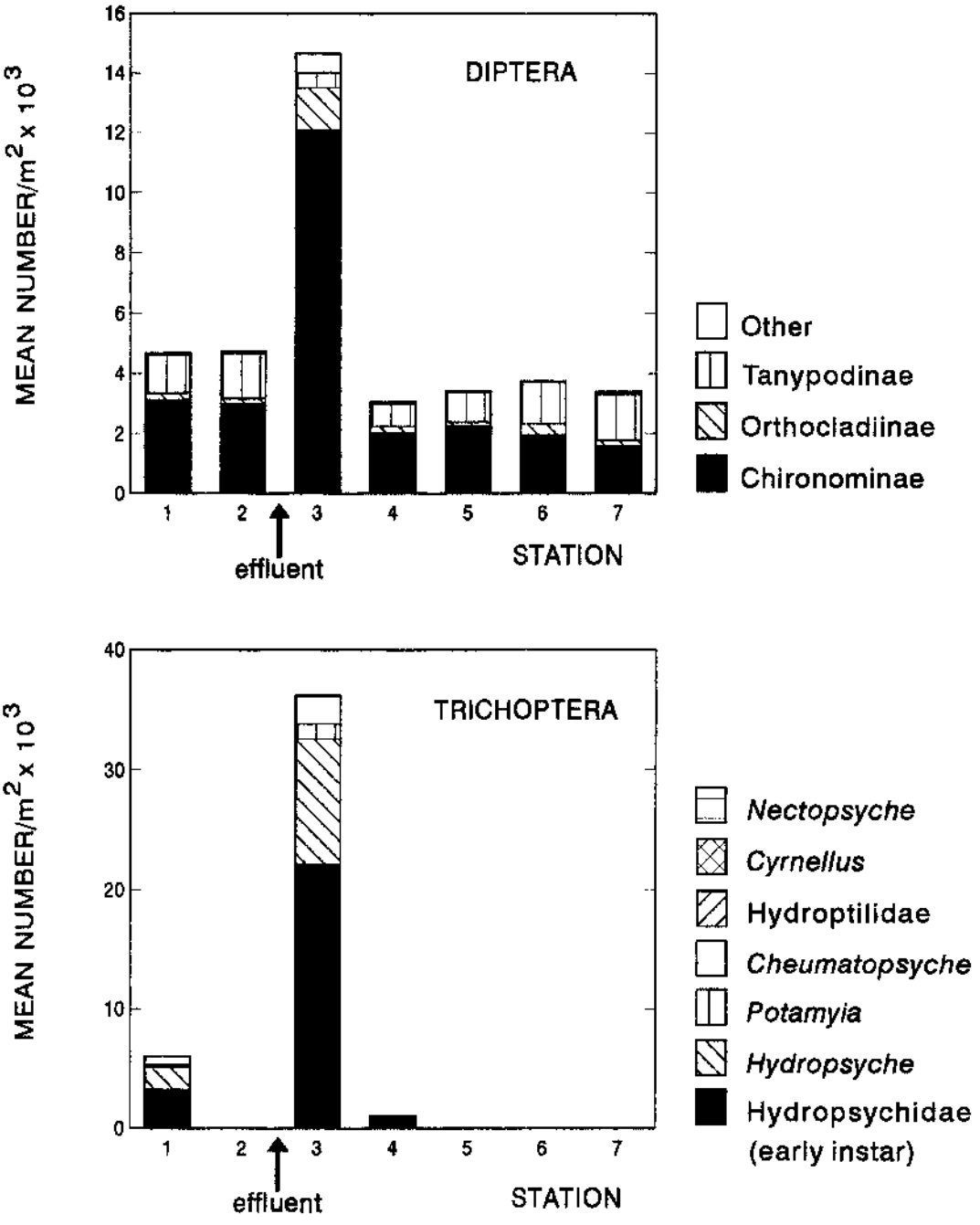

Figure 13. Mean density and taxa composition of Diptera (top) and Trichoptera (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

size of the Chironominae population at Stations 1, 2, 4, 5, and 6 while both taxa were equal at Station 7. Tanypodinae was present in comparable densities (1,000 - 1,500 individuals/m^2) at all stations except Stations 3 and 4 where the smallest populations occurred (500 and 750 individuals/m^2, respectively). Orthocladiinae was the least common midge at all stations except Station 3 where orthoclads were more abundant than tanypods. *Hemerodromia* spp. was the major taxa found in the 'Other' category and it was most abundant at Station 3 (600 individuals/m^2). Ceratopogonidae was present at all stations and Simulidae was found only at Station 3.

Trichoptera

An explosion of caddisflies (36,000 individuals/m^2) occurred at Station 3 directly below the outfall (Figure 13). Reference Station 1 supported 6,000 individuals/m^2 and density at Station 4 was 1,000 individuals/m^2. Caddisflies were virtually absent (< 60 individuals/m^2) from the remaining stations (upstream Station 2 and Stations 5, 6, and 7 below the outfall).

Trichopteran community composition was similar at reference Station 1 and Station 3 closest to the effluent. Approximately 60% of each station was dominated by early instars of Hydropsychidae. Of the three species found at Stations 1 and 3, *Hydropsyche* spp. was the most abundant

(30% each) followed by *Cheumatopsyche* spp.(12% and 6%, respectively), and *Potamyia flava* (4% each). At Station 4, hydropsychid early instars were present in similar proportions (58%) as Stations 1 and 3, but *P. flava* was the most abundant species (20%) followed by equal numbers of *Hydropsyche* spp. and *Cheumatopsyche* spp. At least one hydropsychid species was represented at Stations 2, 5, 6, and 7. *Cyrnellus fraternus* was present at all stations while Hydroptilidae (*Hydroptila* spp. and *Neotrichia* spp.) occurred only at Stations 3, 4, and 7. Both taxa groups were represented by densities less than 60 individuals/m^2 at each station. *Nectopsyche* spp. (Leptoceridae) was incidental at Stations 2, 4, and 7.

Ephemeroptera

Mayflies were most abundant directly below the effluent at Station 3 (3,000 individuals/m^2) and least common at Station 5 (700 individuals/m^2) (Figure 14). Densities ranged from approximately 1,000 to 1,700 individuals/m^2 for Stations 1, 2, 4, 6, and 7. Mayfly communities at Stations 1, 2, 4, 5, 6, and 7 were comprised primarily of members from the Tricorythidae and Heptageniidae families. At upstream Station 2, *Tricorythodes* spp. was the dominant mayfly species (400 individuals/m^2 each) and *Stenonema integrum* was subdominant (200 individuals/m^2 each). *Tricorythodes* spp. and *S. integrum* densities were within 100

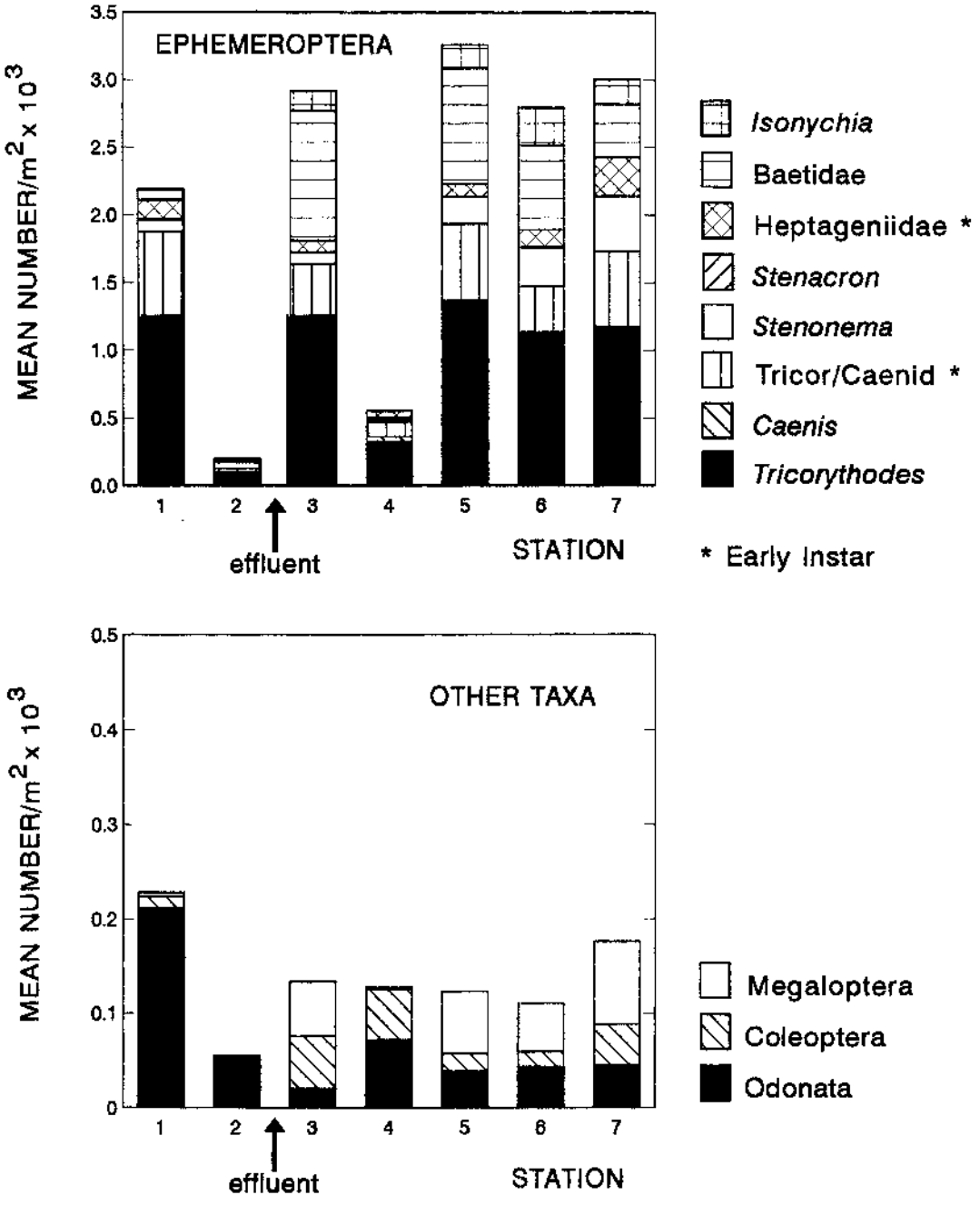

Figure 14. Mean density and taxa composition of Ephemeroptera (top) and other taxa (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

individuals/m² of each other at Stations 1, 4, 5, 6, and 7. Early instars of Heptageniidae occupied a larger segment of the community compared to early instars of Tricorythidae - Caenidae. Baetidae was found in small numbers (<80 individuals/m²) at Stations 1, 4, 5, 6, and 7 and was absent from Station 2.

At Station 3, Baetidae was the dominant mayfly (1,300 individuals/m²) and *Tricorythodes* spp. was second in abundance (600 individuals/m²). *S. integrum*, early instars of Heptageniidae, and early instars of Tricorythidae-Caenidae were each present in approximately equal numbers (300 individuals/m² each). The filter-feeding mayfly, *Isonychia* spp., was most common at Station 3 (400 individuals/m²), uncommon (<20 individuals/m²) at Stations 1 and 6, and absent from Stations 2, 4, 5, and 7. *Caenis* spp. was found at all stations except Station 6 and *Stenacron* spp. was present at all stations except Station 3. *Caenis* spp. or *Stenacron* spp. never exceeded 20 individuals/m² at any station. *Choroterpes* spp. (Leptophlebiidae) was found only at Station 6.

Other Taxa

Other taxa occupied less than two percent of the substrate community at each station (Figure 14). Total densities ranged from 60 to 100 individuals/m²/station. *Argia* spp. damselfly (Odonata:Zygoptera) density ranged from

50 to 100 individuals/m^2 for all stations except Station 3 where only 8 individuals/m^2 were present. A few *Chromagrion* spp. were found at Station 7. *Corydalus cornutus* (Megaloptera) was only present at Stations 1 and 3. Coleoptera (Elmidae) occurred at all stations in densities less than 20 individuals/m^2/station.

Community Indices

Similarity values ranged from 0.88 between Stations 6 and 7 to 0.17 between Stations 1 and 3 (Table 8). Upstream reference Station 2 and downstream Stations 4, 5, 6, and 7 had statistically similar communities ($p > 0.33$) based on the Bray-Curtis similarity analysis (Figure 15). Reference Station 1 was different from all other stations ($p=0.006$). Station 3, directly below the effluent, was also significantly different from all other stations ($p=0.001$).

Linkage	Clusters Linked	Similarity	Probability
1	Station 6 & 7	0.879	0.535
2	Station 2 & 6	0.828	0.550
3	Station 2 & 5	0.808	0.577
4	Station 2 & 4	0.746	0.330
5	Station 1 & 2	0.541	0.006
6	Station 1 & 3	0.171	0.001

Table 8. The similarity value and probability of each cluster linkage calculated for the macroinvertebrate communities collected during May/June 1991, approximately six months after dechlorination began.

Brillouin diversity index values ranged from 2.15 to 2.53 for Stations 2, 3, 5, 6, and 7 (Figure 16 and Table 9).

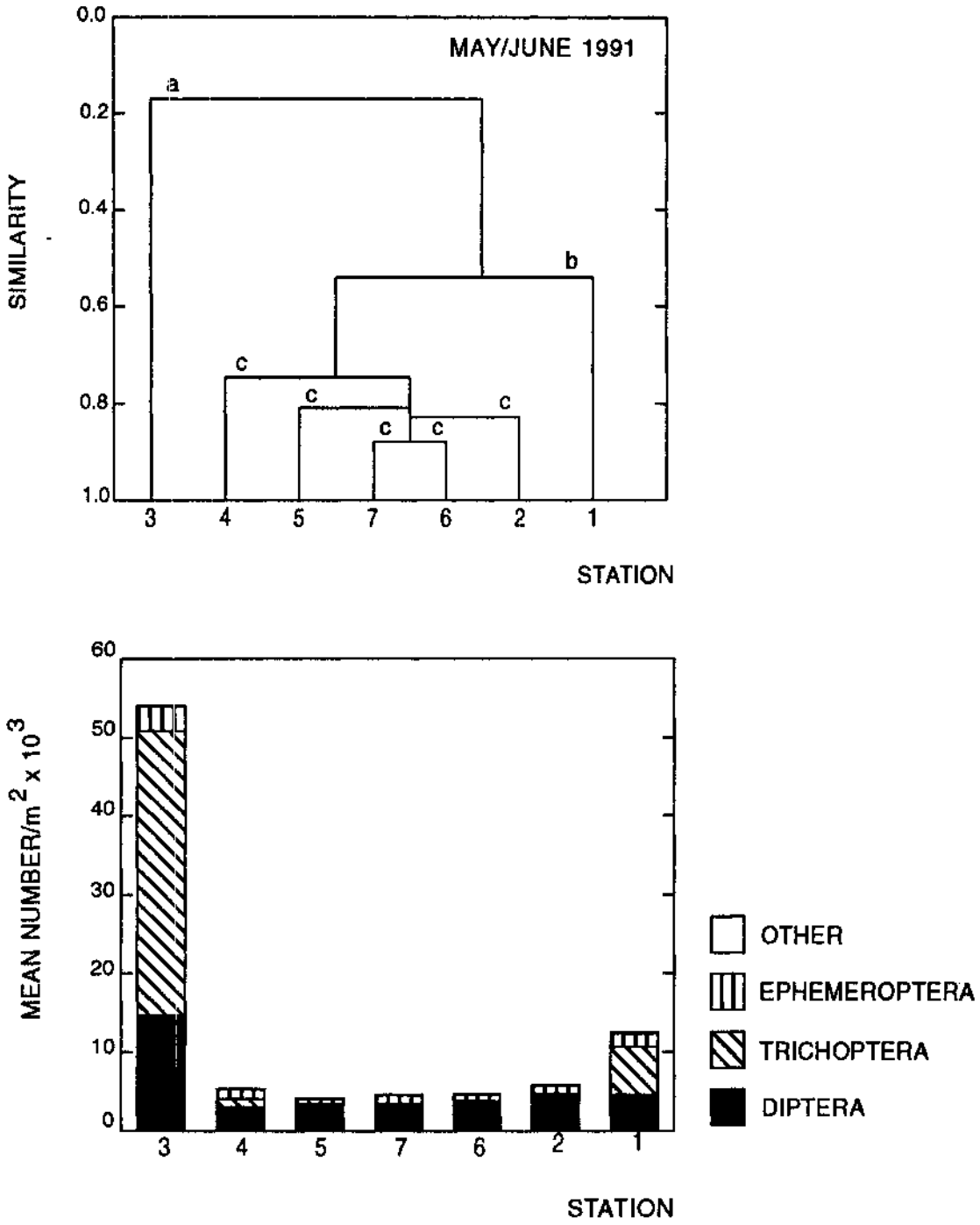

Figure 15. Bray-Curtis similarity index (top) of the macroinvertebrate community (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

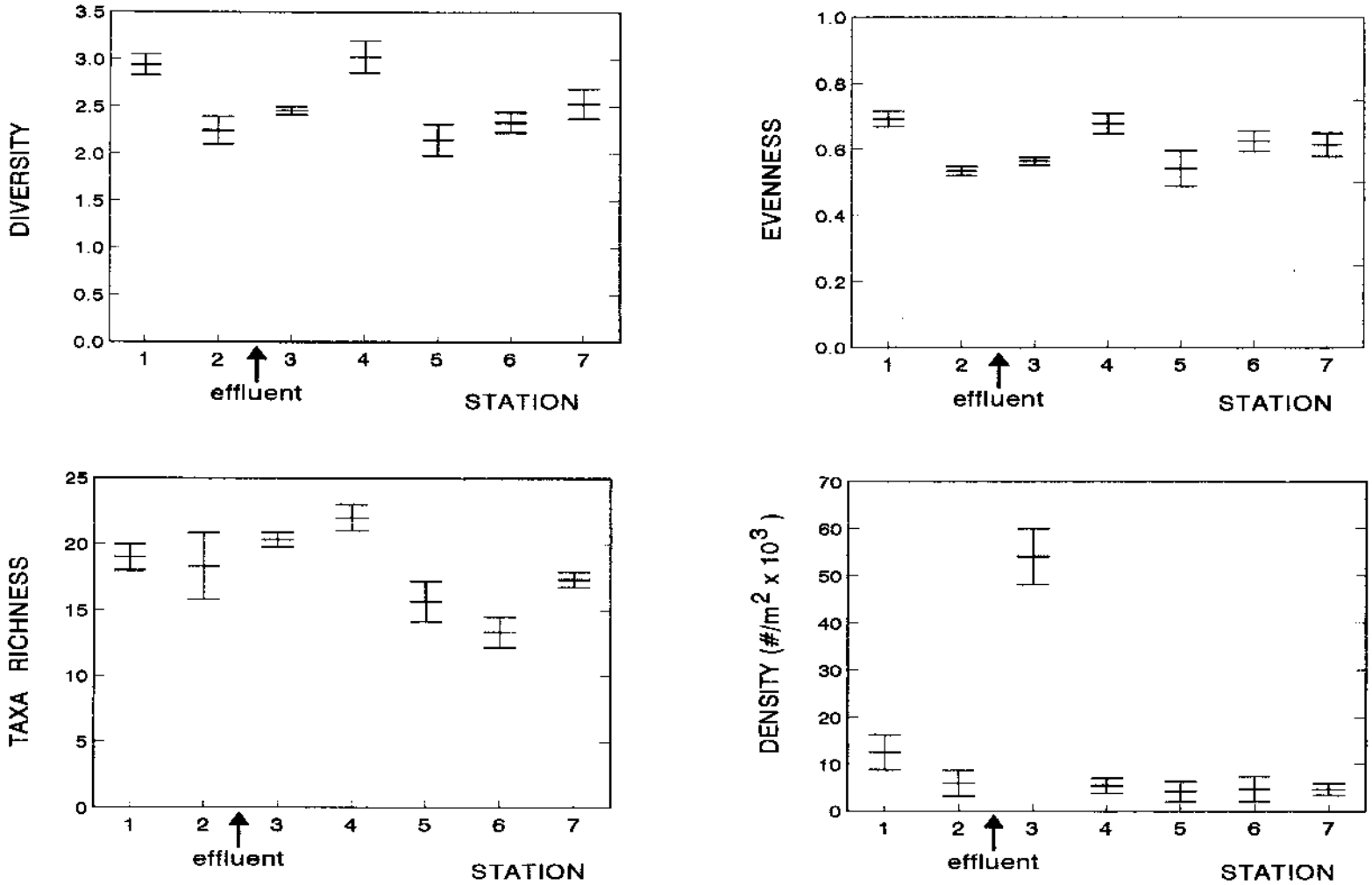

Figure 16. Community indices (mean +/- 1 standard deviation) of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

STATION	1	2	3	4	5	6	7
DIVERSITY							
MEAN	2.941	2.243	2.454	3.026	2.148	2.333	2.531
SD	0.111	0.147	0.042	0.168	0.166	0.108	0.158
CV	3.8%	6.6%	1.7%	5.5%	7.7%	4.6%	6.3%
EVENNESS							
MEAN	0.693	0.535	0.565	0.679	0.543	0.625	0.615
SD	0.023	0.014	0.012	0.031	0.054	0.031	0.035
CV	3.4%	2.7%	2.1%	4.5%	10.0%	4.9%	5.7%
RICHNESS							
MEAN	19.0	18.3	20.3	22.0	15.7	13.3	17.3
SD	1.0	2.5	0.6	1.0	1.5	1.2	0.6
CV	5.3%	13.7%	2.8%	4.5%	9.8%	8.7%	3.3%
DENSITY[1]							
MEAN	12474.4	5928.7	54057.0	5408.0	4167.7	4752.0	4655.6
SD	3696.4	2773.8	5902.9	1630.1	2185.6	2649.1	1210.0
CV	29.6%	46.8%	10.9%	30.1%	52.4%	55.7%	26.0%

SD = Standard Deviation; CV = Coefficient of Variation; [1] individuals/meter^2

Table 9. Descriptive statistics on immature macroinvertebrate communities collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during May/June 1991, approximately six months after dechlorination began.

Diversity increased to 2.94 and 3.03 for Stations 1 and 4, respectively. Coefficients of variation ranged from 4.0% to 8.0 % for all stations except Station 3 where it narrowed to 1.7%. Evenness values ranged between 0.54 to 0.64 for Stations 2, 3, 5, 6, and 7. Evenness increased slightly at Stations 1 and 4 to approximately 0.69. Coefficients of variation were lowest at Station 3 (2.1%) and highest at Station 5 (10.0%). Richness ranged from 13.0 to 17.0 taxa present at Stations 5, 6, and 7. The average number of species was higher (18.0 - 22.0) at Stations 1, 2, 3, and 4. Coefficients of variation were between 3.0% and 14.0%.

Mean density was between 4,000 - 13,000 individuals/m^2 for all stations except at Station 3 below the outfall, where density increased to over 50,000 individuals/m^2. Coefficients of variation ranged from 25% to 55% for all stations except Station 3 where variability decreased to 11%.

Dechlorination - Spring 1992

Macroinvertebrate colonization at three river stations was potentially impacted by several events. Artificial substrates were vandalized at Stations 4 and 6. Substrates at Station 4 were pulled out of the river during the first week of colonization and subsequently were left *in situ* for three weeks (instead of four). The substrates were stolen at Station 6 and no data exists for this sample date. Due to a

sudden drop in river depth, substrates at Station 3 were above the water surface for approximately 24-48 hours at the end of the third week of colonization.

Community Composition

Total larva and nymph macroinvertebrate densities were extremely variable for this sample date (Figure 17). Upstream Stations 1 and 2 each supported approximately 10,000 individuals/m^2 while downstream Stations 3 and 5 contained 30,000 and 20,000 individuals/m^2, respectively. The lowest densities occurred at Station 4 (3,000 individuals/m^2) and Station 7 (6,000 individuals/m^2).

Diptera (primarily Chironomidae) dominated all stations below the outfall, accounting for 61% (Station 3) to 97% (Station 4) of the benthic community on artificial substrates. At upstream Stations 1 and 2, Trichoptera (49% and 46%, respectively) and Diptera (43% and 49%, respectively) were represented in approximately equal proportions. Caddisflies were present in similar densities (approximately 4,500 individuals/m^2) at Stations 1, 2, and 5 while a large increase occurred at Station 3 below the outfall (11,300 individuals/m^2) and reduced numbers were found at Stations 4 and 7. Ephemeroptera occurred in similar densities (approximately 500 individuals/m^2) at Stations 1, 5, and 7 and was most abundant at Station 2 (900 individuals/m^2). The smallest mayfly populations (<200

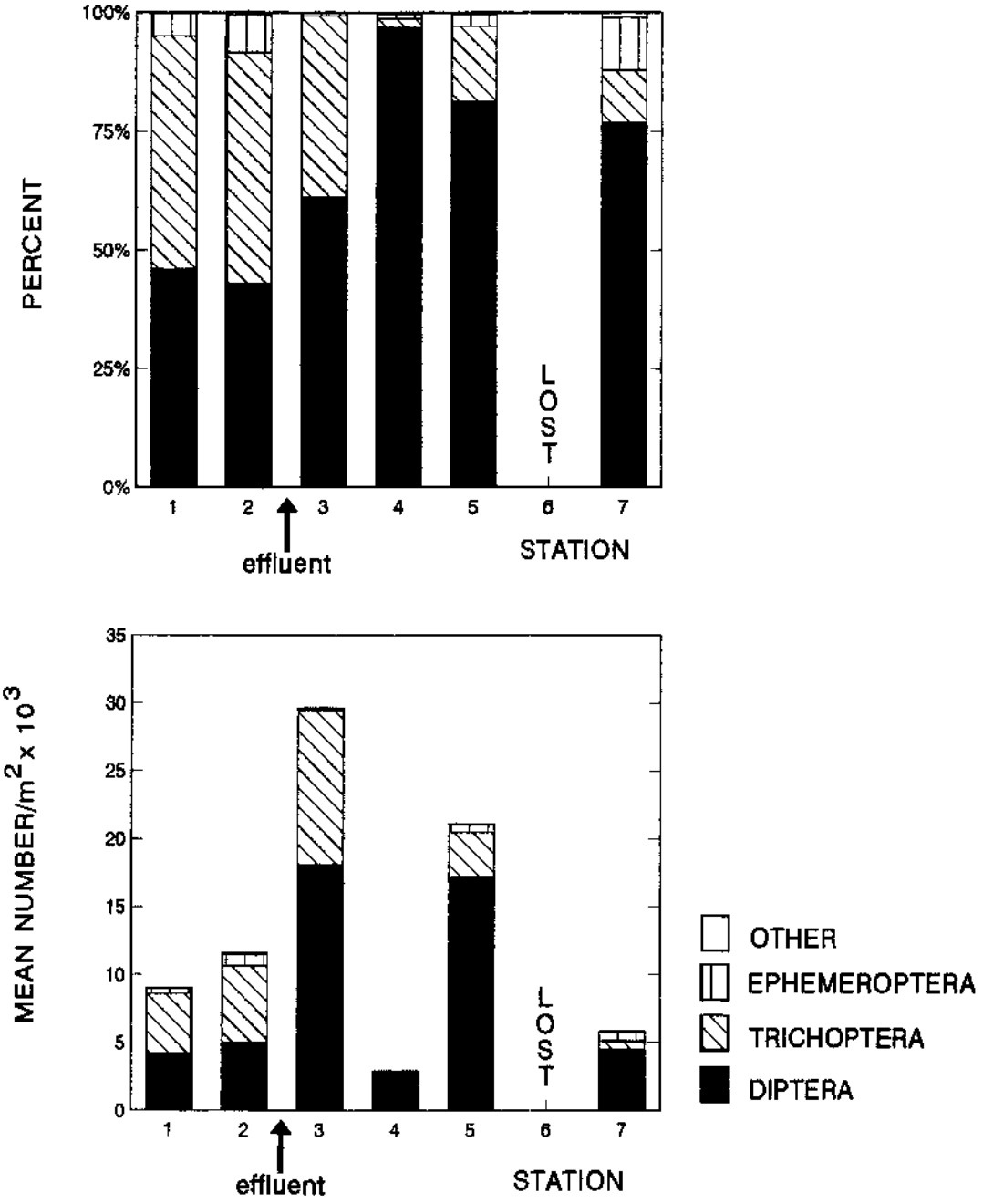

Figure 17. Community composition (%) and total mean density of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

individuals/m²) occurred at Stations 3 and 4 below the effluent. Other taxa (odonates, megalopterans, and coleopterans) occupied less than one percent of the substrate community at each station.

Diptera

Dipteran abundance was between 3,000 to 5,000 individuals/m² at upstream reference Stations 1 and 2, and downstream Stations 4 and 7 (Figure 18). At Stations 3 and 5, the Diptera population increased to approximately 18,000 individuals/m². Chironominae was the most abundant group (at least 55%) at Station 2 above and Stations 3, 4, and 5 below the outfall. Equal proportions of Chironominae and Tanypodinae were represented at Stations 1 and 7. Chironominae abundance was approximately 2,200 individuals/m² at all stations except Stations 3 and 5 where density increased to 10,000 individuals/m². Tanypodinae densities ranged from 1,300 to 1,800 individuals/m² for each station except Station 4 (500 individuals/m²). Orthocladiinae was the least common subfamily at all stations (<700 individuals/m²), except Stations 3 and 5 where orthoclads were five and two times more abundant, respectively, than tanypods. Other diptera (*Hemerodromia* spp., Ceratopogonidae and/or Simulidae) were represented in small numbers (<120 individuals/m² for each taxa) at all stations except Station 4 where none of these diptera were found.

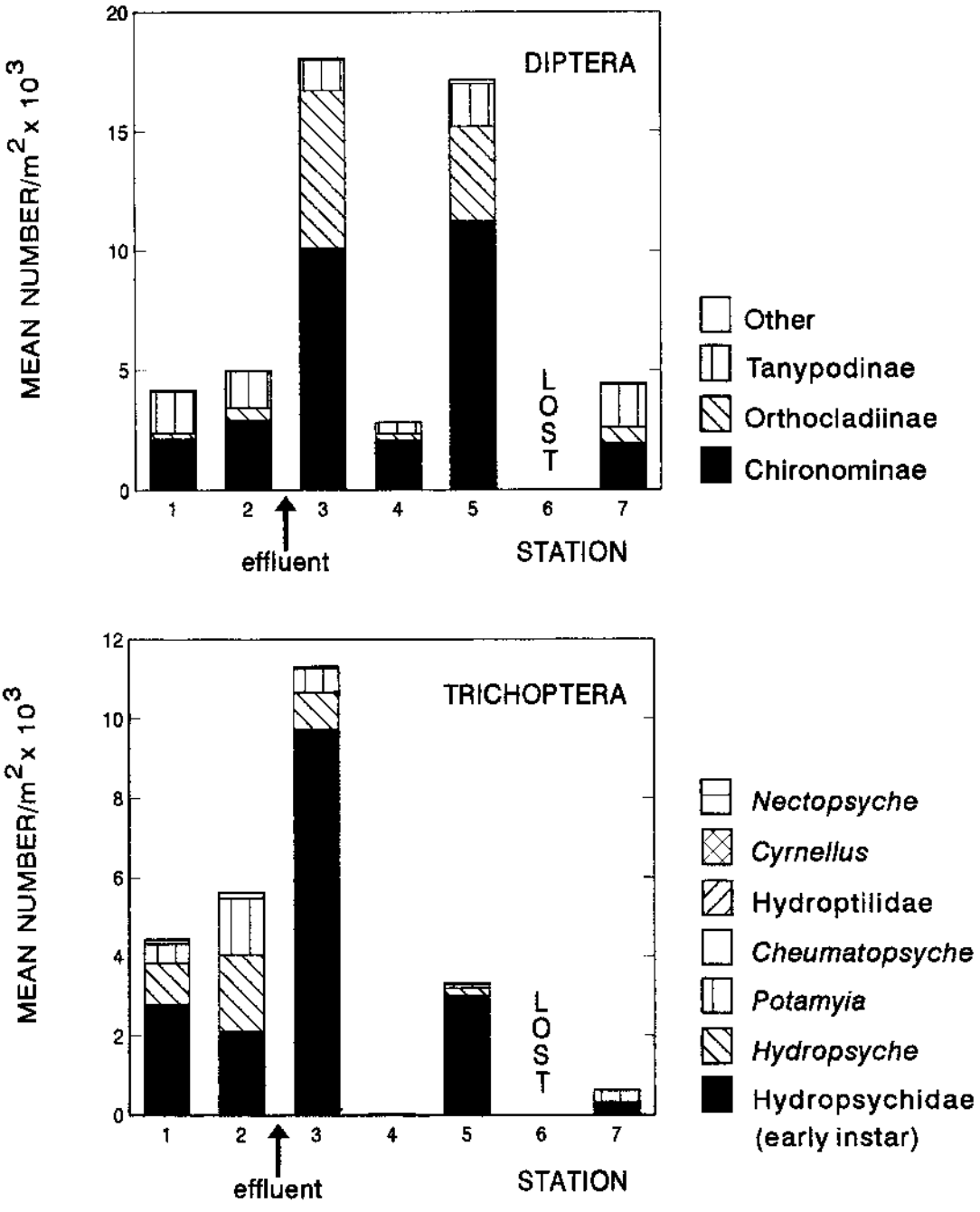

Figure 18. Mean density and taxa composition of Diptera (top) and Trichoptera (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

Trichoptera

Caddisfly abundance was between 3,000 and 6,000 individuals/m^2 at Stations 1 and 2 upstream and Station 5 downstream (Figure 18). Density dramatically increased to approximately 12,000 individuals/m^2 at Station 3 directly below the outfall. A sharp decline occurred in the caddisfly population at Station 7 (700 individuals/m^2) and they were virtually absent from Station 4 (50 individuals/m^2).

The dominant taxonomic group (at least 60%) at Stations 1, 3, 4, and 5 was the hydropsychid early instars. Station 3 supported the largest population of early instars (10,000 individuals/m^2). Equal proportions of hydropsychid early instars and *Hydropsyche* spp. occurred at Station 2 while early instars and *P. flava* were represented equally at Station 7. *Hydropsyche* spp. and *P. flava* were present in similar densities (1,000 and 500 individuals/m^2, respectively) and proportions at Station 1 above and Station 3 below the effluent; both genera were approximately twice as abundant at Station 2 and decreased substantially (<200 individuals/^{2} each taxa) at Station 5. *Cheumatopsyche* spp. was most abundant at Stations 1 and 2 (120 individuals/m^2), and declined at all stations downstream from the effluent (<30 individuals/m^2 at each station). *C. fraternus* was present at all stations except Station 7. Hydroptilidae (*Hydroptila* spp. and *Neotrichia* spp.) occurred at Stations 1, 3, 5, and 7. *Nectopsyche* spp. (Leptoceridae) was found at

Stations 2, 3, and 7. All three groups were present in small numbers (<30 individuals/m²) at each station.

Ephemeroptera

Mayfly density was between 400-600 individuals/m² at Stations 1, 5, and 7 and was most abundant at reference Station 2 (1,000 individuals/m²) (Figure 19). The smallest populations (<170 individuals/m²) occurred at Stations 3 and 4. The mayfly community at all stations was dominated by the following three families: Tricorythidae, Caenidae, and Heptageniidae. Reference Stations 1 and 2 supported the largest populations of *Tricorythodes* spp. (250 and 400 individuals/m², respectively) compared to downstream stations (<130 individuals/m² each station). Populations of Tricorythidae/Caenidae early instars were comparable (50 individuals/m²) between all stations except Station 4 (5 individuals/m²). Density of Heptageniidae early instars was higher at Stations 2, 5, and 7 (200 individuals/m²) compared to Stations 1 and 3 (50 individuals/m²). Equal proportions of *S. integrum* and Heptageniidae early instars were represented at Stations 1, 2, and 7 while at Station 5, *S. integrum* was approximately one-third the size of the early instar population. Small populations (<30 individuals/m² each) of *Caenis* spp., *Stenacron* spp., Baetidae, and *Isonychia* spp. were found at all stations.

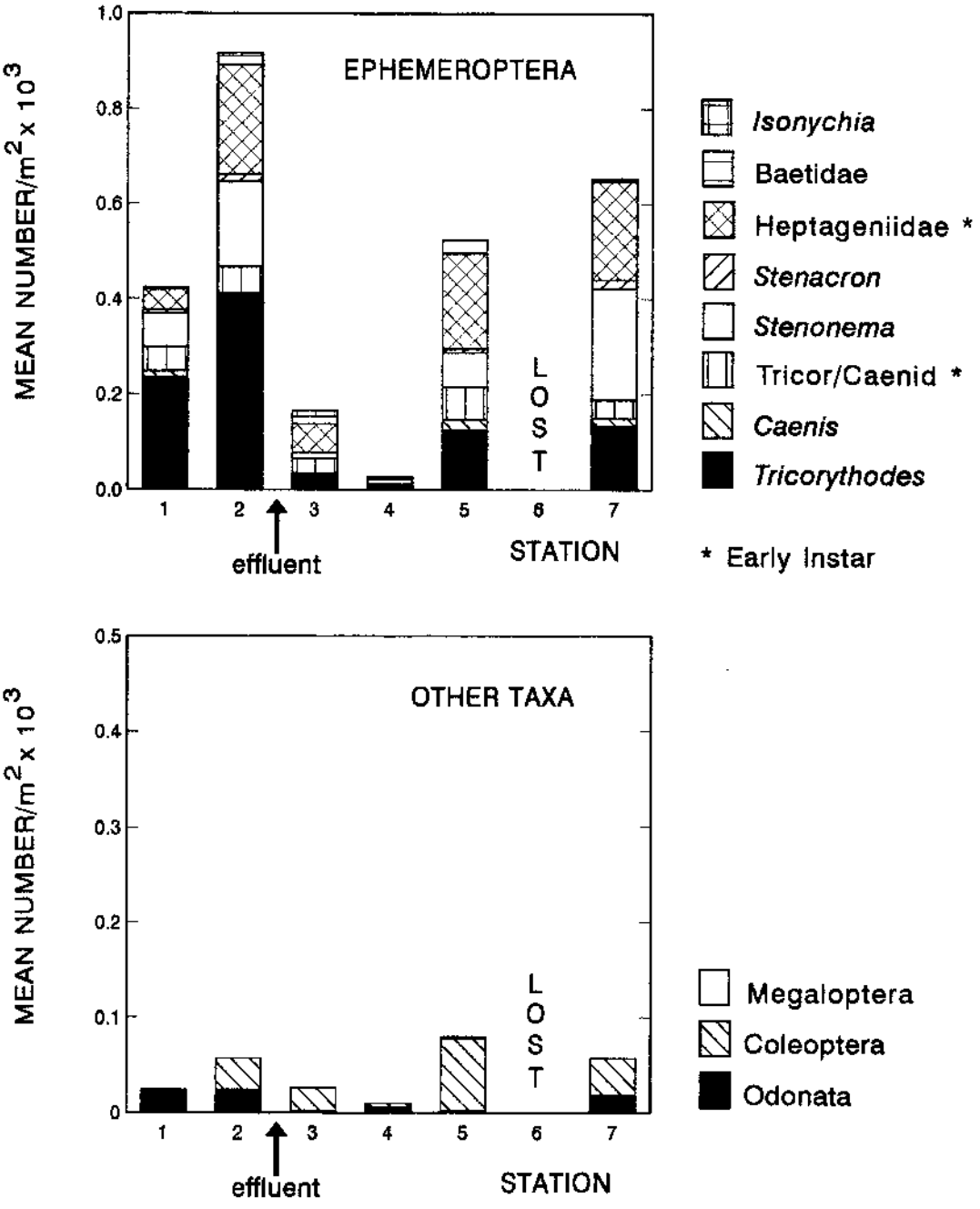

Figure 19. Mean density and taxa composition of Ephemeroptera (top) and other taxa (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

Other Taxa

Combined densities of Odonata, Coleoptera, and Megaloptera were less than 100 individuals/m^2 for all stations (Figure 19). Density of *Argia* spp. damselflies was similar at Stations 1, 2, and 7 (20 individuals/m^2) and at Stations 3, 4, and 5 (4 individuals/m^2). A dragonfly (*Erpetogomphus* spp.) was present at reference Station 2. *C. cornutus* (Megaloptera) was found only at Station 5. Elmidae beetles occurred at all stations except Station 1 and were most abundant at Station 5. Hydrophilidae beetles were found at Station 3.

Community Indices

Similarity matrix values were highest between Stations 1 and 2 (S=0.785; p=0.154) and lowest between Stations 1 and 3 (S=0.362; p=0.190) (Figure 20 and Table 10). Upstream Stations 1 and 2 were considered statistically similar communities by the Bray-Curtis analysis (S=0.785; p=0.154). All other stations were significantly different from each other (p<0.045).

Linkage	Clusters Linked	Similarity	Probability
1	Station 1 & 2	0.785	0.154
2	Station 3 & 5	0.748	0.027
3	Station 1 & 7	0.634	0.045
4	Station 1 & 4	0.498	0.020
5	Station 1 & 3	0.362	0.019

Table 10. The similarity value and probability of each cluster linkage calculated for the macroinvertebrate communities collected during April/May 1992, approximately 18 months after dechlorination began.

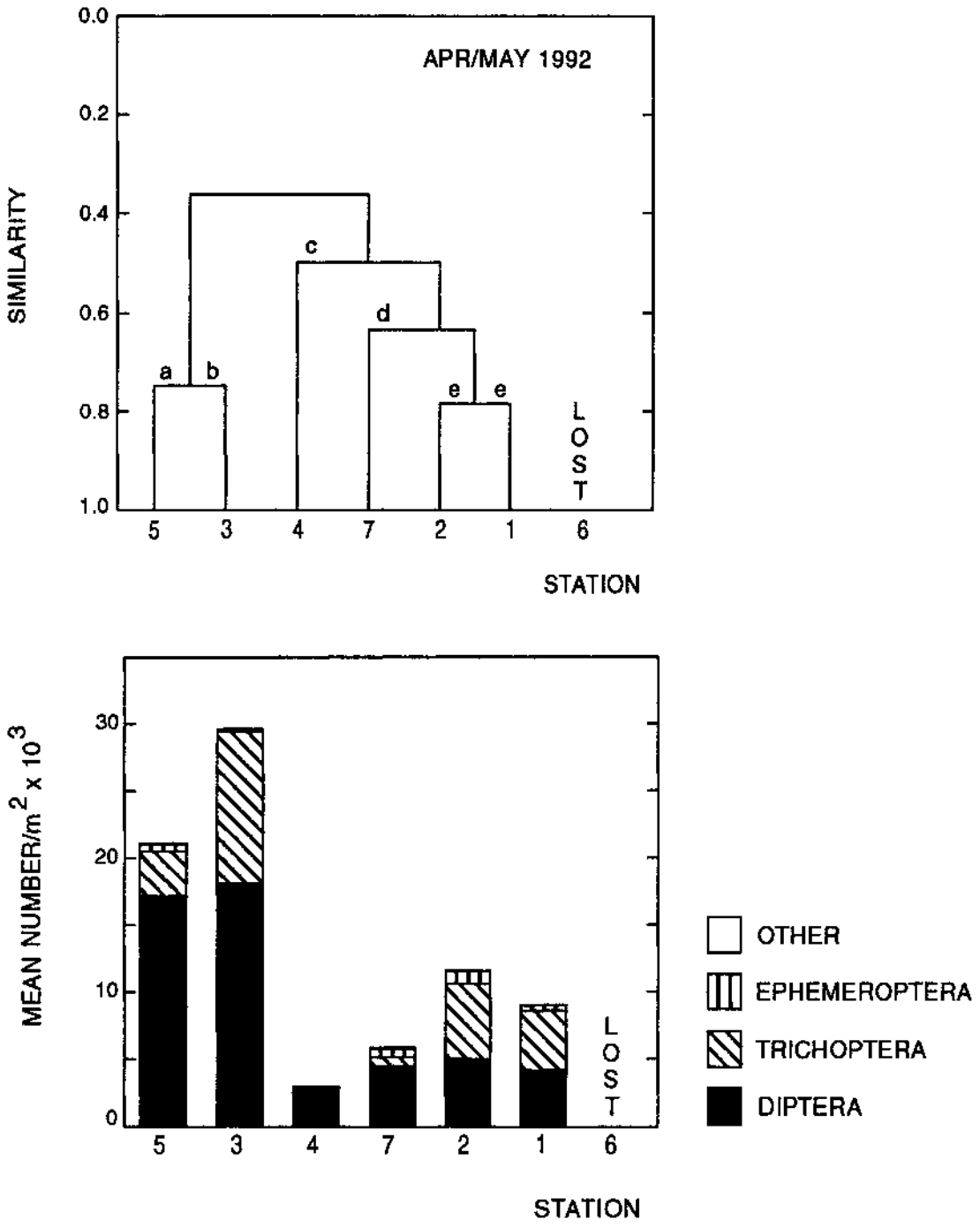

Figure 20. Bray-Curtis similarity index (top) of the macroinvertebrate community (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

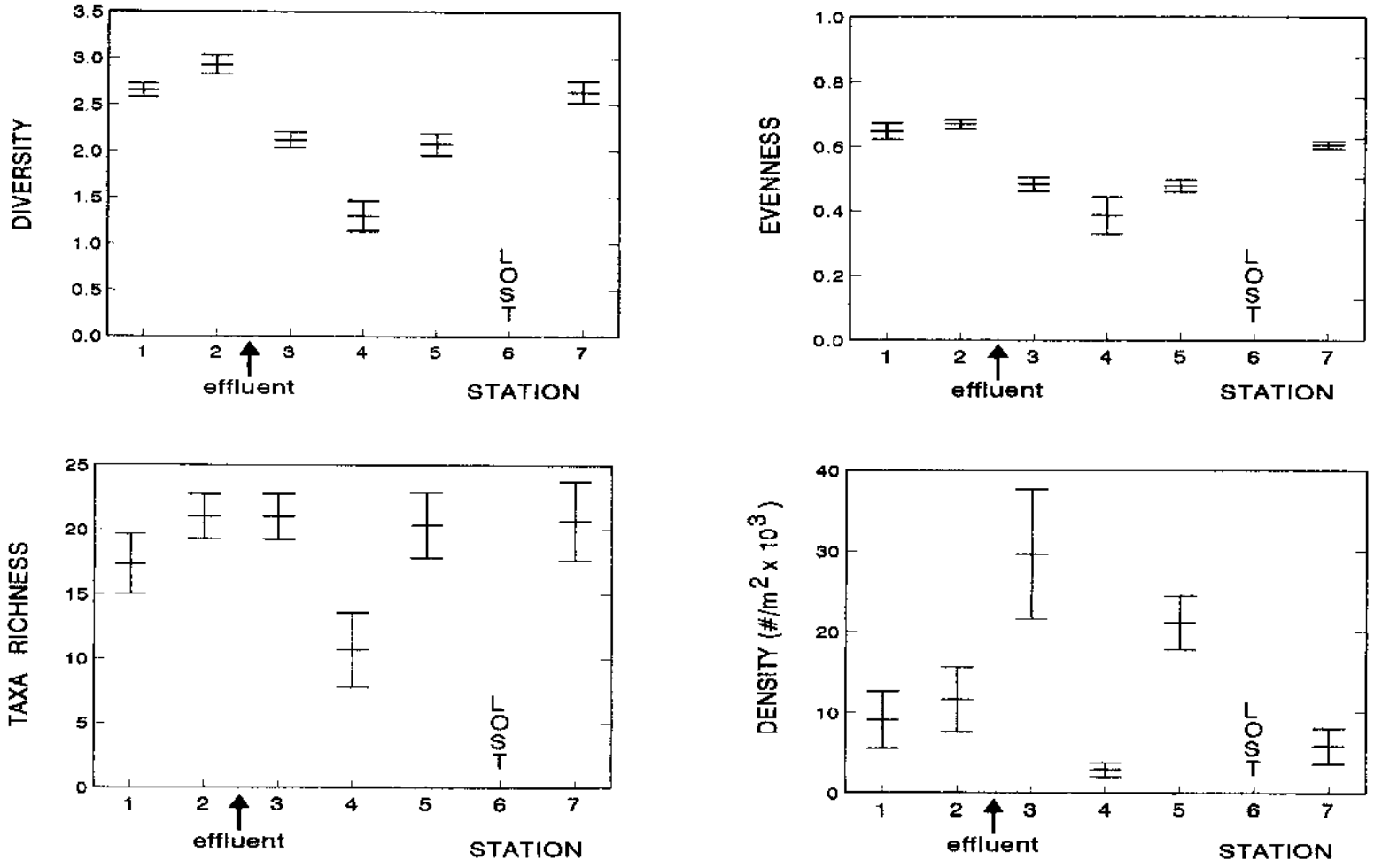

Figure 21. Community indices (mean +/- 1 standard deviation) of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

STATION	1	2	3	4	5	6 *	7
DIVERSITY							
MEAN	2.650	2.932	2.119	1.298	2.073	---	2.637
SD	0.074	0.101	0.086	0.161	0.119	---	0.117
CV	2.8%	3.4%	4.0%	12.4%	5.7%	---	4.4%
EVENNESS							
MEAN	0.646	0.668	0.483	0.386	0.478	---	0.605
SD	0.026	0.014	0.021	0.057	0.018	---	0.012
CV	4.0%	2.1%	4.4%	14.9%	3.7%	---	2.0%
RICHNESS							
MEAN	17.3	21.0	21.0	10.7	20.3	---	20.7
SD	2.3	1.7	1.7	2.9	2.5	---	3.1
CV	13.3%	8.2%	8.2%	27.1%	12.4%	---	14.8%
DENSITY [1]							
MEAN	9038.5	11592.9	29608.4	2898.7	21113.2	---	5820.0
SD	3552.5	4010.1	8028.6	874.2	3353.0	---	2221.1
CV	39.3%	34.6%	27.1%	30.2%	15.9%	---	38.2%

SD=Standard Deviation; CV=Coefficient of Variation; [1] individuals/meter^2; * = lost

Table 11. Descriptive statistics on immature macroinvertebrate communities collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during April/May 1992, approximately 18 months after dechlorination began.

Brillouin diversity index values ranged from 1.30 (Station 4) to 2.93 (Station 2) (Figure 21 and Table 11). Diversity at Stations 1, 2, and 7 was between 2.60 and 2.90 while Stations 3 and 5 were each approximately 2.10. Coefficients of variation were between 3.0% to 6.0% for all stations except Station 4 (12.4%). Evenness values were lowest at Stations 3, 4, and 5 (0.38 to 0.48) and increased at Stations 1, 2, and 7 (0.60 to 0.70). Coefficients of variation were between 2.0% to 5.0% for all stations except Station 4 (15%). Taxa richness ranged from 17.3 to 21.0 at all stations except Station 4 where it dropped to 10.7. Coefficients of variation were lowest at Stations 2 and 3 (8.2%) and highest at Station 4 (27.1%).

Densities ranged from approximately 3,000 individuals/m^2 (Station 4) to 30,000 individuals/m^2 (Station 3). Upstream Stations 1 and 2 were between 9,000 - 12,000 individuals/m^2 while downstream Stations 3 and 5 ranged from 20,000 to 30,000 individuals/m^2. Coefficients of variation ranged from 27% to 40% for all stations except Station 5 (16%).

Dechlorination - Late Summer 1992

Macroinvertebrate colonization was impacted at Station 4 by a physical disturbance (temporary dam) that occurred midway through the sample period.

Community Composition

Total immature macroinvertebrate densities were between 13,000 - 20,000 individuals/m^2 at Stations 1, 5, 6 and 7 (Figure 22). Density increased to 30,000 individuals/m^2 at Station 3 directly below the effluent. Upstream Station 2 and downstream Station 4 were colonized by 7,000 and 4,000 individuals/m^2, respectively.

Trichoptera was the dominant taxonomic group (50% - 60%) at Stations 3, 5, 6, and 7 while Diptera was the most common taxa at Stations 1 (50%), 2 (90%), and 4 (80%). Ephemeroptera comprised 10% to 20% of the benthic community at all stations except Station 2 (3%). Station 3 contained the highest density of dipterans and caddisflies (8,500 and 18,000 individuals/m^2, respectively). Stations 2 and 4 supported the smallest populations of caddisflies and mayflies. Density of other taxa (odonates, megalopterans, and coleopterans) was less than 200 individuals/m^2 at each station.

Diptera

Diptera abundance was approximately 6,500 individuals/m^2 at upstream reference Stations 1 and 2 and increased to 8,500 individuals/m^2 at Station 3 below the effluent (Figure 23). Density at downstream Stations 4, 5, 6, and 7 ranged from 3,000 - 5,000 individuals/m^2 at each station. The Chironominae subfamily dominated over 65% of

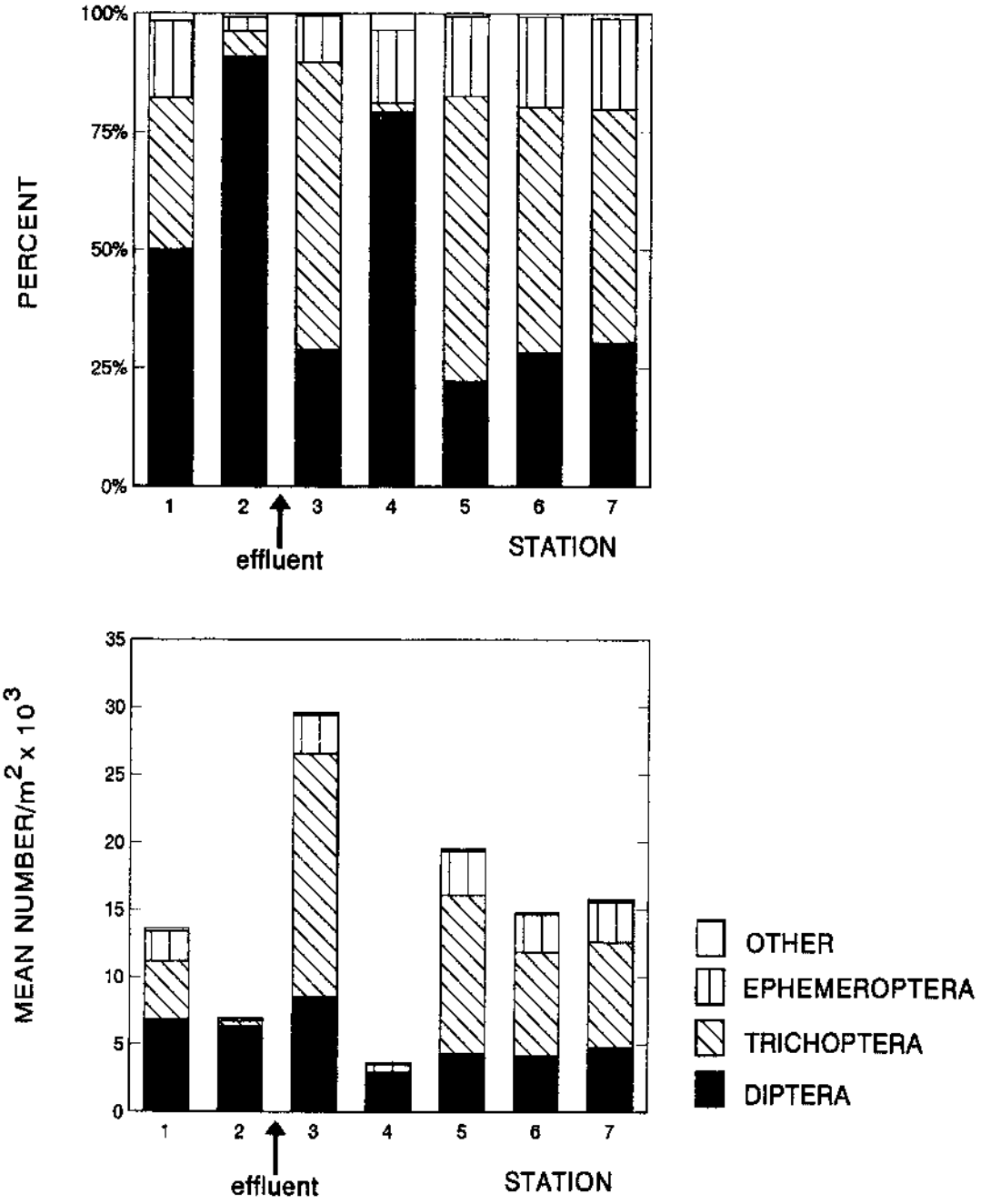

Figure 22. Community composition (%) and total mean density of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

the dipteran community at all stations and was most common at Station 3 (5,500 individuals/m^2). Reference Stations 1 and 2 supported the largest Tanypodinae populations (1,200 and 1,500 individuals/m^2, respectively) compared to all other stations (<600 individuals/m^2 each station). Tanypods were two to three times more abundant than Orthocladiinae at Stations 1 and 2 while the opposite composition was true at downstream Stations 3, 5, and 6. At Station 7, tanypods and orthoclads were equally represented. The Orthocladiinae population was most abundant at Station 3 (2,400 individuals/m^2) and ranged between 500 - 900 individuals/m^2) at all other stations except Station 4 (<50 individuals/m^2). Other dipterans, *Hemerodromia* spp. and/or Ceratopogonidae, occurred in small numbers (<30 individuals/m^2) at all stations.

Trichoptera

Caddisfly densities were highly variable between stations (Figure 23). Reference Station 1 supported ten times more caddis (4,500 individuals/m^2) than reference Station 2. The largest trichopteran community (18,000 individuals/m^2) occurred at Station 3 below the outfall. Densities decreased further downstream to 12,000 individuals/m^2 at Station 5 and 8,000 individuals/m^2 at Stations 6 and 7. Caddisflies were virtually absent from Station 4.

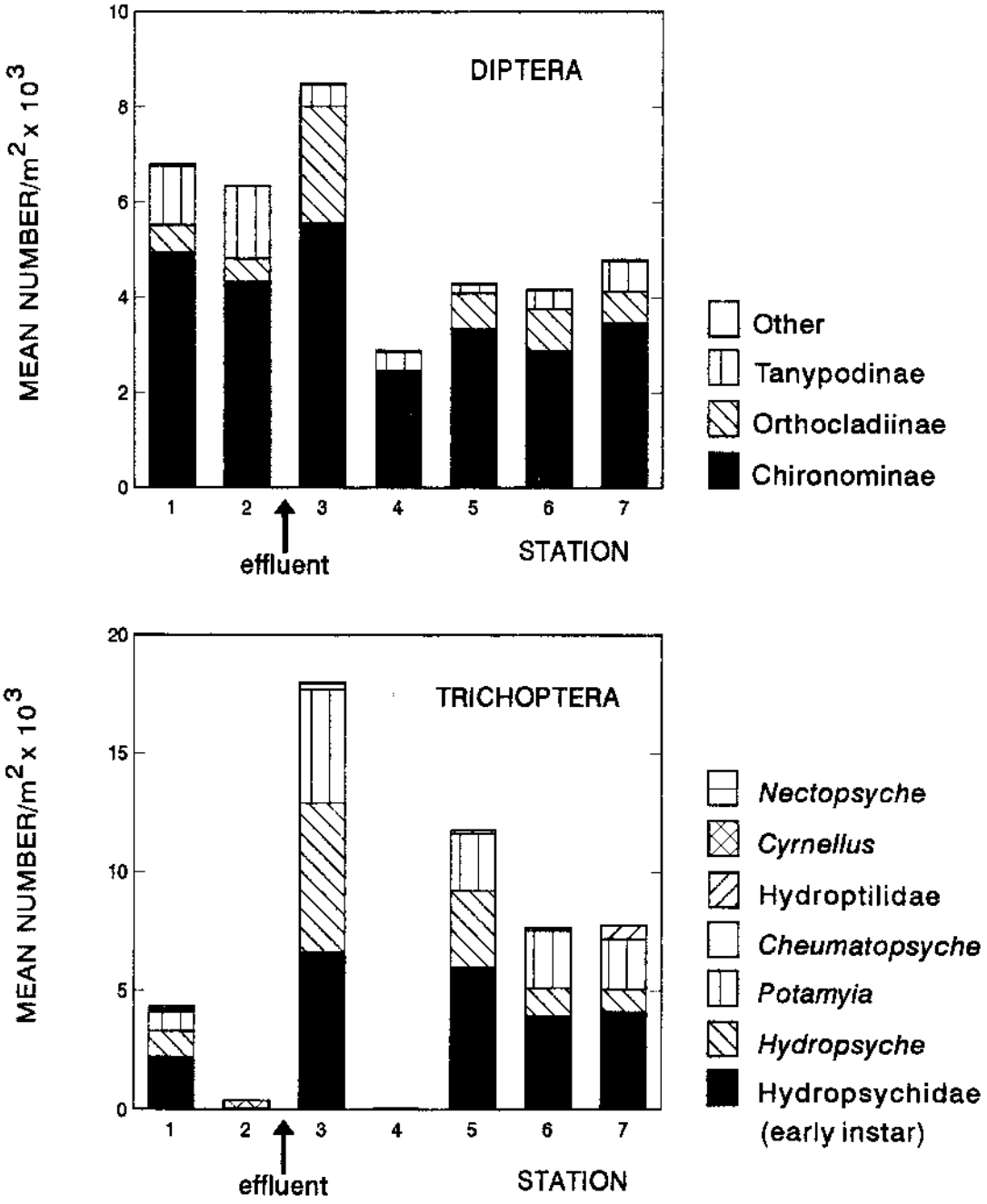

Figure 23. Mean density and taxa composition of Diptera (top) and Trichoptera (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

Hydropsychid early instars occupied over half the caddisfly community at Stations 1, 4, 5, 6, and 7 and forty percent at Station 3. *C. fraternus* dominated the small caddis population at Station 2. Hydropsychid early instar density was three times higher at Stations 3 and 5 compared to reference Station 1 (2,200 individuals/m^2). *Hydropsyche* spp. and *P. flava* were represented almost equally at Stations 1, 3, and 5 while *P. flava* was twice as common as *Hydropsyche* spp. at Stations 6 and 7. *P. flava* had similar densities (2,300 individuals/m^2) at downstream Stations 5, 6, and 7. *Cheumatopsyche* spp. had the smallest density of all hydropsychids (<250 individuals/m^2) and was present at all stations except Stations 2 and 4. Station 3 supported the largest populations for all three hydropsychid genera. Small populations of Hydroptilidae (*Hydroptila* spp. and *Neotrichia* spp.) and *C. fraternus* occurred at all stations. Hydroptilidae was most abundant at Station 7 (600 individuals/m^2) while *C. fraternus* was most common at upstream Stations 1 and 2 (100 and 300 individuals/m^2, respectively).

Ephemeroptera

Total mayfly densities ranged from 2,000 to 3,000 individuals/m^2 at each station except Stations 2 and 4 where numbers decreased to less than 600 individuals/m^2 (Figure 24). All stations were dominated by the combined density of

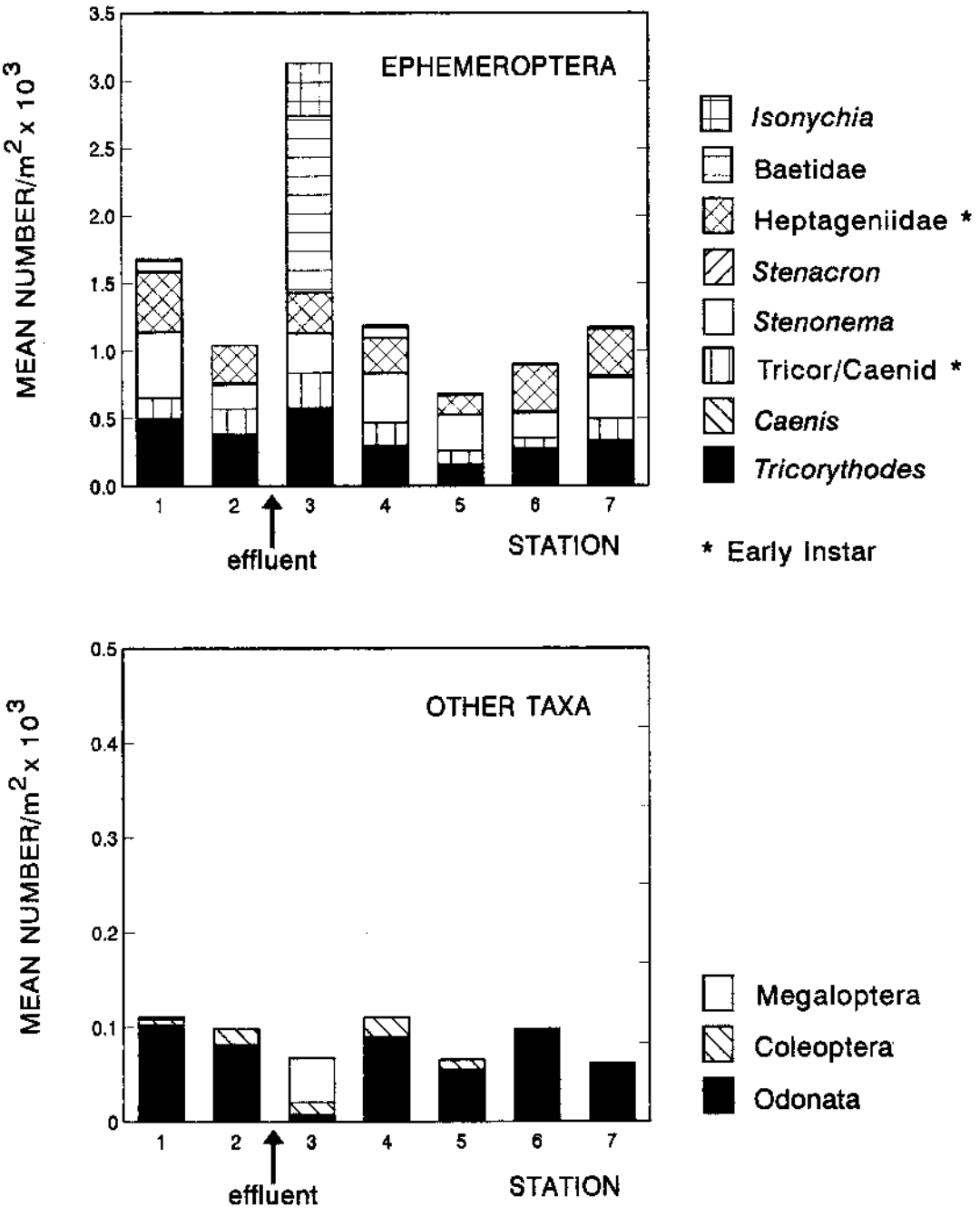

Figure 24. Mean density and taxa composition of Ephemeroptera (top) and other taxa (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

Tricorythodes spp. (1,100 - 1,400 individuals/m^2) and Tricorythidae/Caenidae early instars (350 - 650 individuals/m^2). Baetidae was subdominant at Stations 3, 5, and 6 while Heptageniidae taxa were subdominant at Stations 1, 2, 4, and 7. The population of Heptageniidae early instars was largest at Station 7 (300 individuals/m^2) compared to all other stations. *S. integrum* abundance was two to four times higher at Stations 5, 6, and 7 compared to Stations 1 and 3. Reference Station 2 supported the smallest densities of each mayfly taxa. *Caenis* spp. was present at all stations except Station 3 while *Stenacron* spp. occurred at all stations except Stations 5 and 7. Both taxa occurred in small numbers (<40 individuals/m^2) when present.

Other Taxa

All three orders occurred in densities less than 300 individuals/m^2 at each station (Figure 24). *Argia* spp. (Odonata:Zygoptera) was most abundant at Station 1 (200 individuals/m^2) and least common at Station 3. Damselfly densities were similar at Stations 2, 4, 5, 6, and 7 (50 individuals/m^2). *C. cornutus* (Megaloptera) was found in comparable numbers at all downstream stations except Station 4 and few occurred at upstream reference Stations 1 and 2. Elmidae riffle beetles (primarily *Stenelmis* spp. and *Heterelmis* spp.) were present at all stations except Station 2. *Berosus* spp. (Hydrophilidae) was found only at Station 3

below the effluent.

Community Indices

Similarity values ranged from 0.896 between Stations 6 and 7 to 0.366 between Stations 1 and 2 (Table 12 and Figure 25). Based on the Bray-Curtis analysis, Stations 2 and 4 were similar communities (p=0.057) and Stations 1, 3, 5, 6, and 7 were clustered together (p>0.136).

Linkage	Clusters Linked	Similarity	Probability
1	Station 6 & 7	0.896	0.623
2	Station 5 & 6	0.819	0.614
3	Station 1 & 5	0.687	0.208
4	Station 1 & 3	0.648	0.136
5	Station 2 & 4	0.592	0.057
6	Station 1 & 2	0.366	0.019

Table 12. The similarity value and probability of each cluster linkage calculated for the macroinvertebrate communities collected during August 1992, approximately 22 months after dechlorination began.

Brillouin diversity index values ranged from 2.83 to 3.12 at upstream Station 1 and downstream Stations 3, 5, 6, and 7 (Figure 26 and Table 13). Diversity decreased at Stations 2 and 4 to 1.91 and 2.03, respectively. Coefficients of variation were between 1.3% - 4.0% at all stations except Station 4 (cv=7.3%). The evenness parameter exhibited a similar pattern as diversity with higher values at all stations except Stations 2 and 4. Evenness ranged from 0.66 to 0.71 at Stations 1, 3, 5, 6, and 7 and dropped

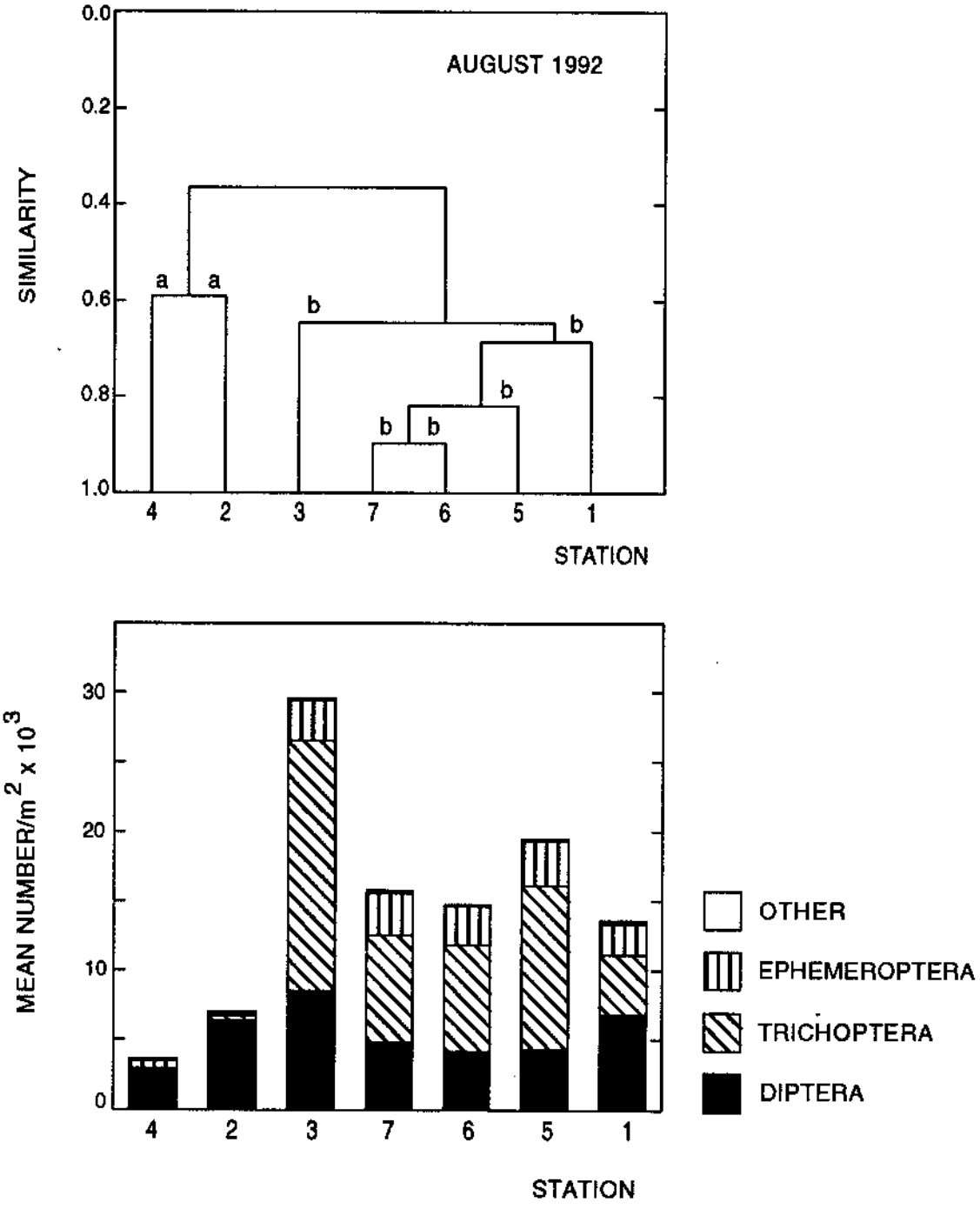

Figure 25. Bray-Curtis similarity index (top) of the macroinvertebrate community (bottom) collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

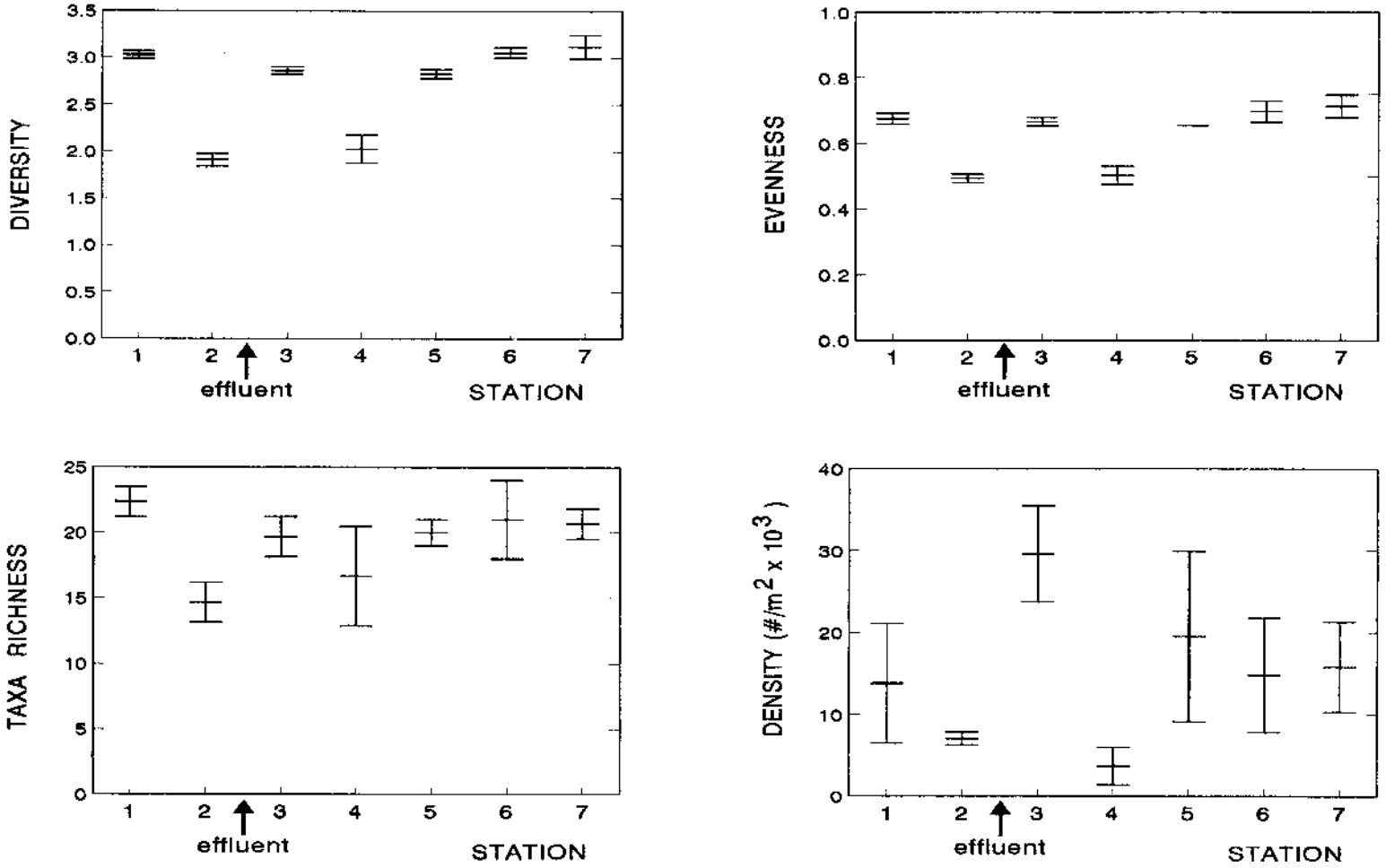

Figure 26. Community indices (mean +/- 1 standard deviation) of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

STATION	1	2	3	4	5	6	7
DIVERSITY							
MEAN	3.029	1.912	2.863	2.028	2.831	3.054	3.118
SD	0.041	0.069	0.040	0.148	0.046	0.055	0.124
CV	1.3%	3.6%	1.4%	7.3%	1.6%	1.8%	4.0%
EVENNESS							
MEAN	0.676	0.494	0.667	0.503	0.655	0.698	0.714
SD	0.016	0.014	0.012	0.028	0.0003	0.032	0.034
CV	2.4%	2.7%	1.8%	5.5%	0.1%	4.6%	4.8%
RICHNESS							
MEAN	22.3	14.7	19.7	16.7	20.0	21.0	20.7
SD	1.2	1.5	1.5	3.8	1.0	3.0	1.2
CV	5.2%	10.4%	7.8%	22.7%	5.0%	14.3%	5.6%
DENSITY [1]							
MEAN	13790.5	7039.8	29583.8	3710.5	19503.9	14780.6	15795.4
SD	7339.4	784.7	5872.0	2279.5	10374.6	6985.8	5475.2
CV	54.0%	11.2%	19.9%	62.6%	53.3%	47.4%	34.8%

SD = Standard Deviation; CV = Coefficient of Variation; [1] individuals/meter^2

Table 13. Descriptive statistics on immature macroinvertebrate communities collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately 22 months after dechlorination began.

to 0.50 at Stations 2 and 4. Coefficients of variation were less than 6% for all stations. Taxa richness ranged between 19.7 to 22.3 for Stations 1, 3, 5, 6, and 7. Mean taxa numbers decreased to 14.7 at Station 2 and 16.7 at Station 4. Variation was wider for the taxa richness endpoint compared to diversity and evenness. Coefficients of variation ranged from 5.0% to 22.7%.

Total density was highest at Station 3 below the outfall (30,000 individuals/m^2) and lowest at Station 2 above the outfall (7,000 individuals/m^2). Densities ranged from 14,000 to 20,000 individuals/m^2 at the remaining stations. Variation was between 35% - 65% for all stations except Stations 2 (cv=11.2%) and 3 (cv=19.9%).

Pre-dechlorination and Dechlorination Comparison

In the following section, immature macroinvertebrates collected before dechlorination in August 1990 are compared to those collected in August 1992, almost two years after dechlorination began. During the August 1992 sample, Station 4 was impacted by dam construction that occurred 20 meters upstream from the artificial substrates.

Community Composition

During the August 1990 sample, the most common taxa at reference Stations 1 and 2 was Diptera (77% and 48%, respectively), followed by Trichoptera (18% and 42%,

respectively), and Ephemeroptera (2% and 10%, respectively) (Figure 27). In the August 1992 sample, Diptera, Trichoptera, and Ephemeroptera colonized 50%, 30%, and 15% of the community, respectively, at Station 1 and 90%, 6%, and 5%, respectively, at Station 2.

During pre-dechlorination, all stations below the effluent were dominated by Diptera (85% to 99%), followed by Ephemeroptera (0.2% to 10%) and Trichoptera (0% to 3%). In the dechlorination sample at Stations 3, 5, 6, and 7, caddisflies dominated 50% to 60% of the insect community, dipterans were sub-dominant with 22% to 30%, and mayflies accounted for 10% to 20%. At Station 4, dipterans were the most abundant taxa (78%), followed by mayflies (15%) and caddisflies (2%).

Diptera

Chironomidae occupied approximately 99% of the dipterans collected at all stations during both pre-dechlorination and dechlorination samples (Figure 28). During pre-dechlorination, chironomid density was between 6,000 to 10,000 individuals/m^2 at all stations except for downstream Stations 5 and 6 where almost 30,000 individuals/m^2 were present. In the dechlorination sample, 6,500 to 8,500 individuals/m^2 occurred at reference Stations 1 and 2 and below the effluent at Station 3 while 3,000 to 5,000 individuals/m^2 colonized downstream Stations 4, 5, 6,

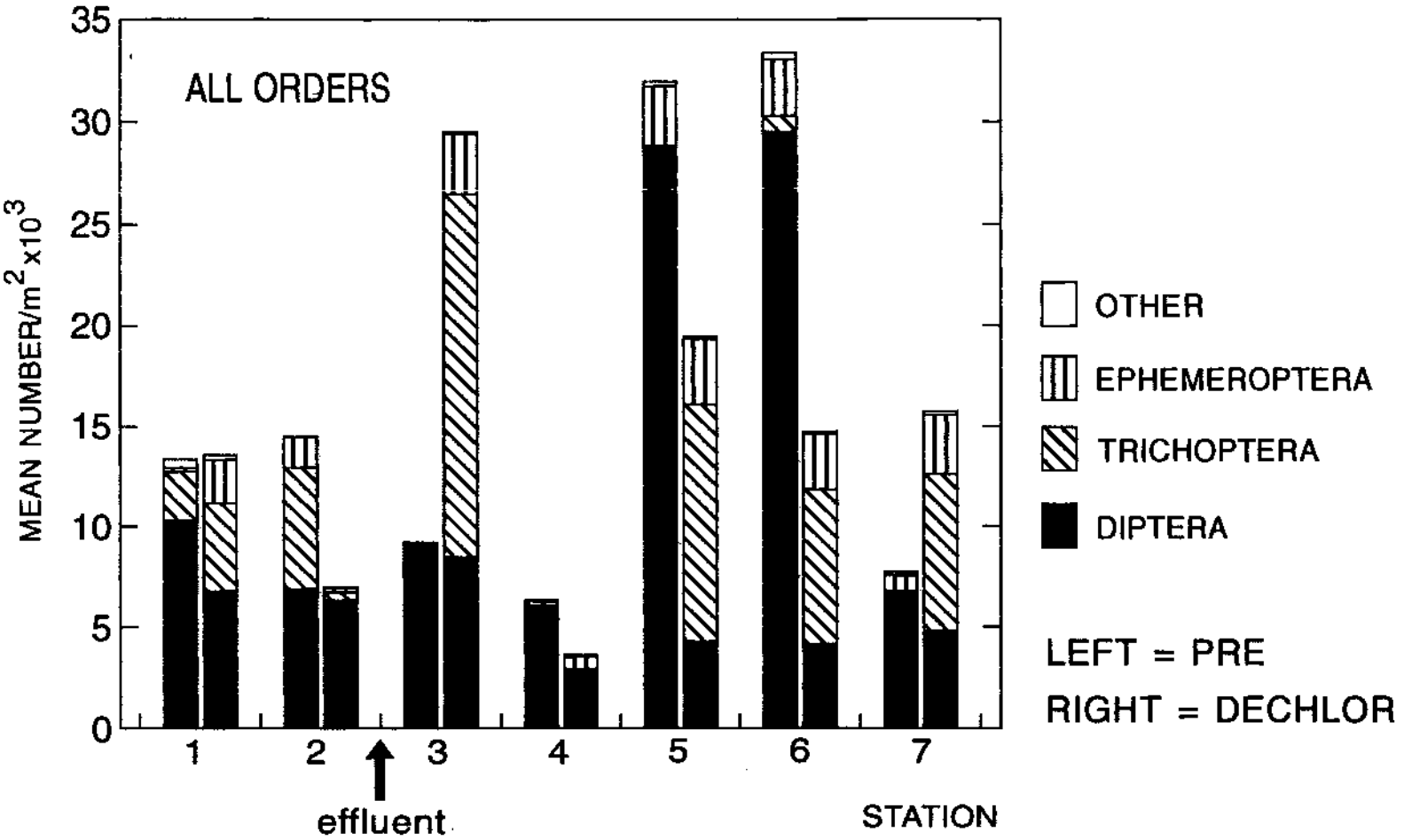

Figure 27. Community composition and total mean density of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before dechlorination (left bar) and almost two years after dechlorination (right bar).

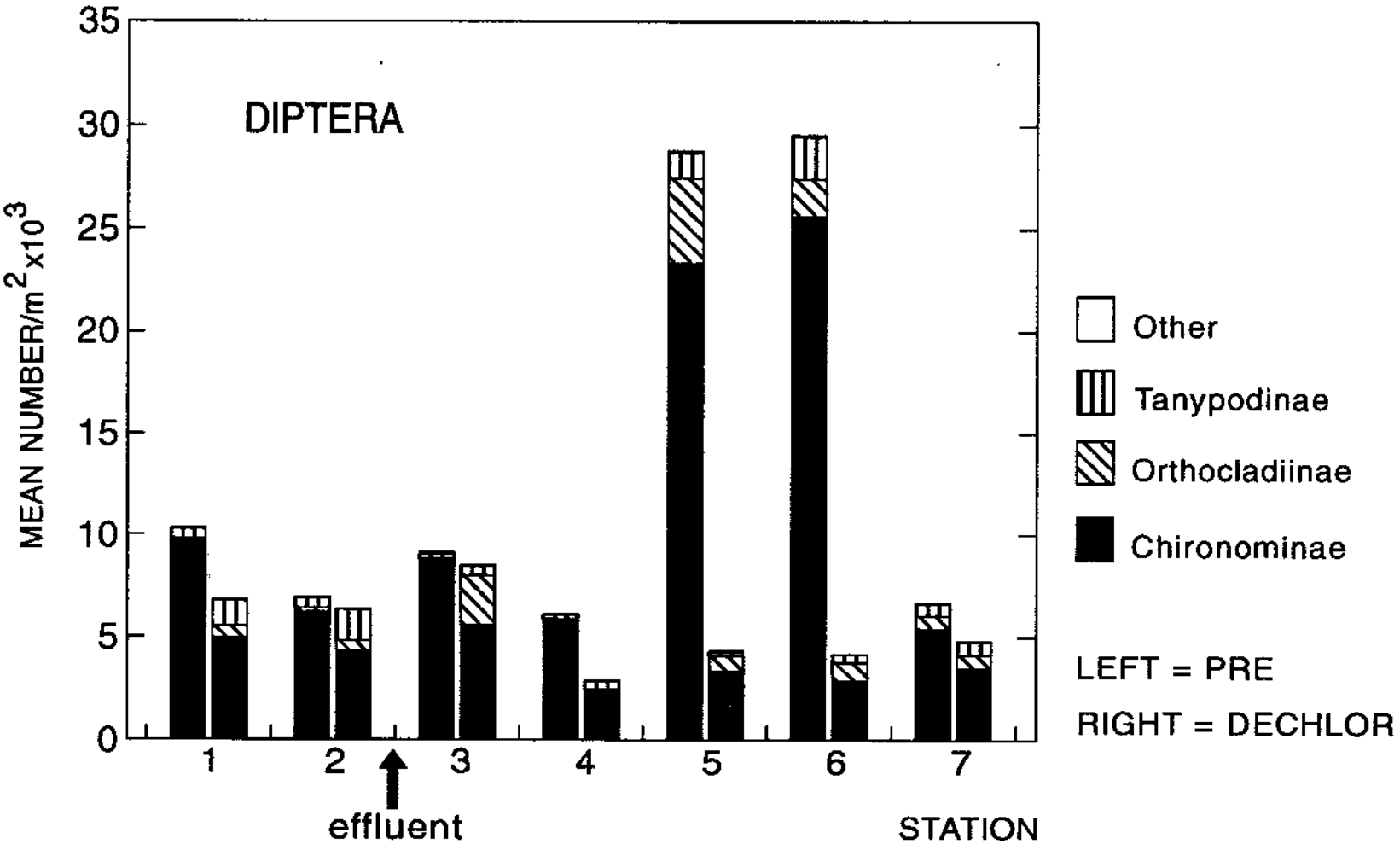

Figure 28. Mean density and taxa composition of Diptera collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before dechlorination (left bar) and almost two years after dechlorination (right bar).

and 7.

Chironominae was the dominant subfamily and accounted for at least 65% of the midge community at all stations in the August 1990 and August 1992 samples. During pre-dechlorination, Stations 5 and 6 supported the largest population of all three chironomid subfamilies. At Stations 1, 2, 3, and 4, Tanypodinae were subdominant and Orthocladiinae were third in abundance while both subfamilies were equally represented at Stations 6 and 7. Orthoclads were only subdominant at Station 5. In the dechlorination sample, the second most common taxa was Tanypodinae at Stations 1, 2, and 4 and Orthocladiinae at Stations 3, 5, and 6; both subfamilies were equally represented at Station 7.

In the pre-dechlorination sample, tanypod populations at reference Stations 1 and 2 were twice as large (500 individuals/m^2 each station) as those at Stations 3 and 4. During the dechlorination sample, three times as many tanypods colonized each reference station (1,400 individuals/m^2) compared to Stations 3 and 4. Stations 3 and 4 each supported twice as many tanypods in August 1992 compared to August 1990. Station 3 had virtually no orthoclads (<20 individuals/m^2) before dechlorination but supported the largest population (2,500 individuals/m^2) among stations during the dechlorination sample.

Trichoptera

During pre-dechlorination, the largest caddisfly populations occurred at reference Stations 1 (2,500 individuals/m^2) and 2 (6,000 individuals/m^2) while small numbers (<150 individuals/m^2) were present at all stations below the effluent except for Station 6 (800 individuals/m^2) (Figure 29). In the dechlorination sample, reference Station 1 supported ten times more caddisflies (4,500 individuals/m^2) compared to reference Station 2. Caddisflies were most abundant below the effluent at Stations 3 (18,000 individuals/m^2) and 5 (12,000 individuals/m^2); Stations 6 and 7 each had almost 8,000 individuals/m^2. Trichopterans were virtually absent (<70 individuals/m^2) from Station 4.

Before dechlorination occurred, Hydropsychidae was the dominant family at reference Stations 1 and 2, Polycentropodidae occupied a majority at Stations 3 and 4, and Hydroptilidae was the most common taxa at Stations 6 and 7. In the dechlorination sample, hydropsychids dominated (>90%) all stations except Station 2 where polycentropids were more prevalent.

In the dechlorination sample, hydropsychid early instars accounted for 38% to 55% of the caddis population at all stations except Station 2. *Hydropsyche* spp. was 25% more abundant than *Potamyia flava* at reference Station 1 and downstream Stations 3 and 5 while Stations 6 and 7 supported twice as many *P. flava* compared to *Hydropsyche* spp..

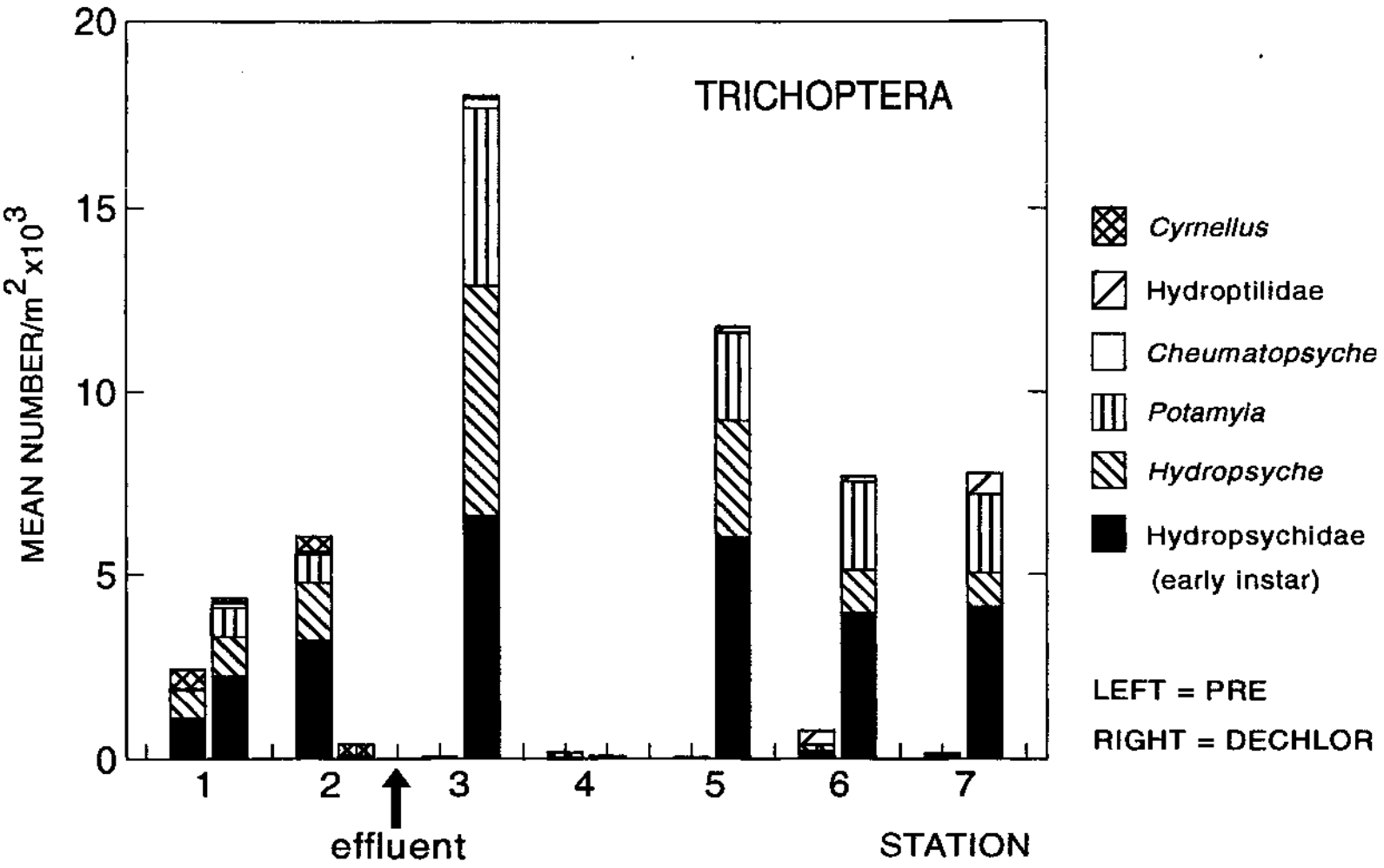

Figure 29. Mean density and taxa composition of Trichoptera collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before dechlorination (left bar) and almost two years after dechlorination (right bar).

Cheumatopsyche spp. was most common at upstream Station 1 (100 individuals/m^2) and downstream Station 3 (250 individuals/m^2) and was virtually absent from all other stations (<20 individuals/m^2).

Ephemeroptera

In the August 1990 pre-dechlorination sample, downstream Stations 5 and 6 supported the largest mayfly populations (3,000 individuals/m^2) followed by reference Station 2 (1,500 individuals/m^2) and Station 7 (700 individuals/m^2) (Figure 30). Mayflies were virtually absent (<20 individuals/m^2) from Stations 3 and 4 and were reduced at reference Station 1 (250 individuals/m^2). In the August 1992 dechlorination sample, Stations 1, 3, 5, 6, and 7 each supported between 2,200 to 3,000 individuals/m^2 while reduced numbers of mayflies (<500 individuals/m^2) occurred at Stations 2 and 4.

Before and after dechlorination was implemented, Tricorythidae was the dominant family at all stations, except for Station 2 where Baetidae was dominant in August 1990. Between pre-dechlorination and dechlorination samples, Heptageniidae, Baetidae, and Isonychiidae each increased in abundance at Stations 3, 5, 6, and 7 below the outfall. In the dechlorination sample, the largest populations of Baetidae (400 to 1,000 individuals/m^2) and *Isonychia* spp. (150 to 300 individuals/m^2) occurred at downstream Stations

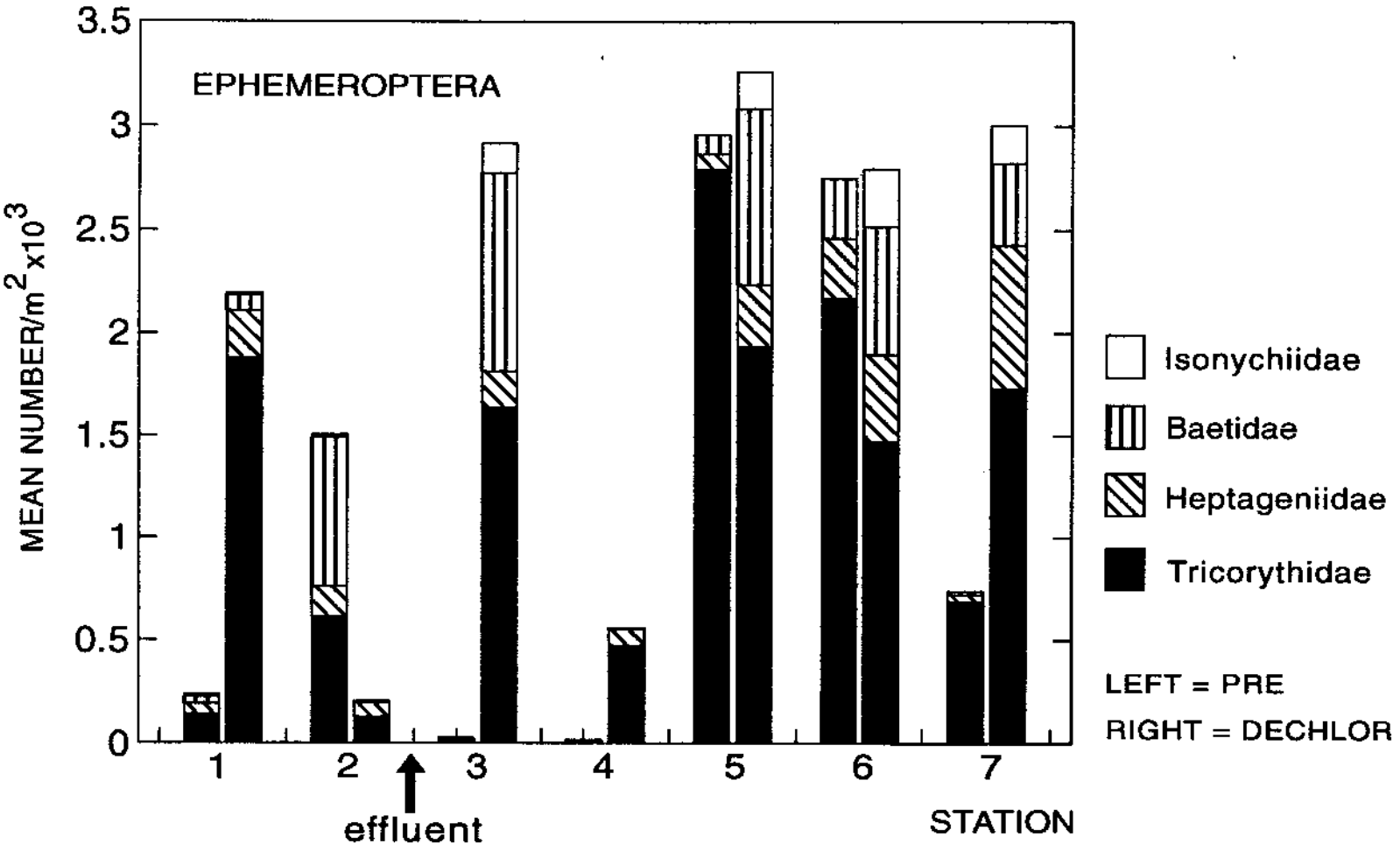

Figure 30. Mean density and taxa composition of Ephemeroptera collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before dechlorination (left bar) and almost two years after dechlorination (right bar).

3, 5, 6, and 7 while both taxa were less abundant at reference Station 1 (80 individuals/m^2 for Baetidae and 10 individuals/m^2 for *Isonychia* spp.). Tricorythidae and Heptageniidae densities were comparable between reference Station 1 (1,900 and 230 individuals/m^2, respectively) and downstream Station 3 (1,650 and 175 individuals/m^2, respectively).

Community Indices

In the pre-dechlorination sample, Brillouin diversity values were 1.3 and 2.4 at reference Stations 1 and 2, respectively; less than 1.0 at Stations 3 and 4 below the outfall; and between 1.4 and 1.6 at Stations 5, 6, and 7 (Figure 31 and Table 14). In the dechlorination sample, diversity values ranged from 2.8 to 3.1 at all stations except for Stations 2 and 4 where diversity decreased to 2.0.

In the August 1990 sample, evenness values were highest at Station 2 (0.55), lowest at Stations 3 and 4 (<0.25), and between 0.33 and 0.42 at Stations 1, 5, 6, and 7. In the dechlorination sample, evenness was between 0.66 and 0.71 at all stations except for Stations 2 and 4 where evenness dropped to 0.50. In the pre-dechlorination sample, taxa richness was highest at Stations 2 and 6 (21 and 22 taxa, respectively) and lowest at Station 4 (8.7 taxa). Between 11 and 16 taxa were found at Stations 1, 3, 5, and 7. In the

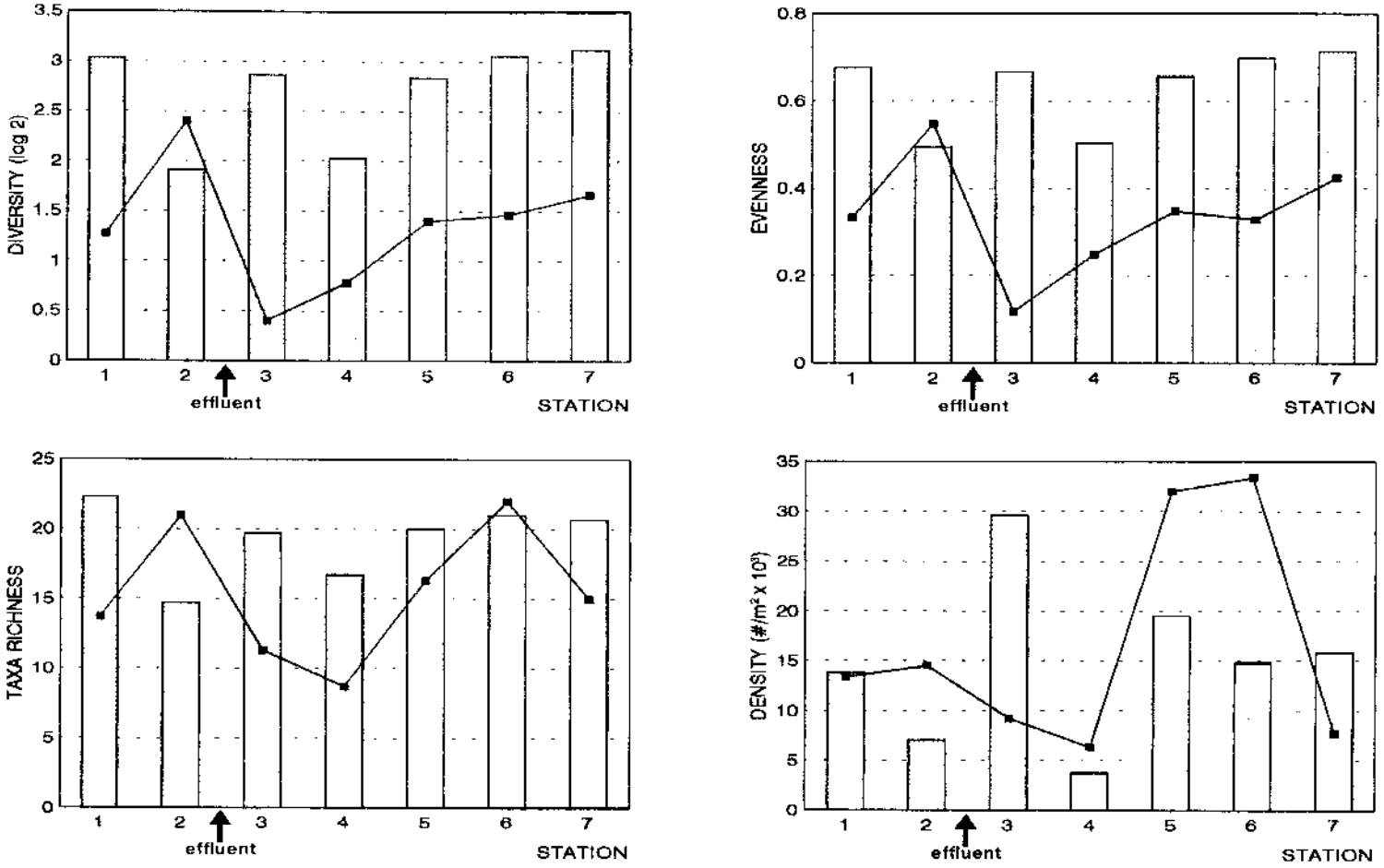

Figure 31. Community indices (mean +/- 1 standard deviation) of macroinvertebrates collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before dechlorination (line) and almost two years after dechlorination (bar).

STATION	1	2	3	4	5	6	7
DIVERSITY							
PRE	1.274	2.405	0.403	0.783	1.396	1.460	1.661
DECHLOR	3.029	1.912	2.863	2.028	2.831	3.054	3.118
EVENNESS							
PRE	0.333	0.548	0.118	0.249	0.347	0.328	0.424
DECHLOR	0.676	0.494	0.667	0.503	0.655	0.698	0.714
RICHNESS							
PRE	13.7	21.0	11.3	8.7	16.3	22.0	15.0
DECHLOR	22.3	14.7	19.7	16.7	20.0	21.0	20.7
DENSITY [1]							
PRE	13370.2	14501.8	9225.1	6344.8	32011.1	33390.7	7718.3
DECHLOR	13790.5	7039.8	29583.8	3710.5	19503.9	14780.6	15795.4

PRE = pre-dechlorination sample in August/September 1990
DECHLOR = dechlorination sample in August 1992
[1] individuals/meter^2

Table 14. Mean values for parameters used to describe macroinvertebrate communities before (PRE) and two years after dechlorination began (DECHLOR). Insects were collected from artificial substrates exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

August 1992 sample, between 20 and 22 taxa were collected at all stations except for Stations 2 and 4 where less than 17 taxa were found.

DISCUSSION

Pre-Dechlorination - Late Summer 1990

Exposure to chlorinated effluent had a negative impact on immature macroinvertebrates within one mile downstream from the outfall. Stations 3 and 4 contained the highest ambient chlorine concentrations (1.04 and 0.45 mg TRC/l, respectively) and exhibited the greatest community impact among stations. Chlorine levels at both stations exceeded the following acute values reported for immature macroinvertebrates: 0.102 mg TRC/l for the mayfly *Stenonema ithaca* (Gregg, 1975, as cited in EPA, 1984); and 0.400 mg TRC/l for the stonefly *Pteronarcys* spp. (Arthur, et al., 1975). Osborne and Davies (1987) found that mean ambient chlorine levels of 0.16, 0.42, and 2.04 mg TRC/l caused significant reductions in caddisfly, mayfly, and stonefly populations and increased the community dominance of Oligochaeta.

Results similar to those observed in the Osborne and Davies (1987) study occurred at Stations 3 and 4 where trichopterans and ephemeropterans were virtually absent and the Chironominae midges (Diptera:Chironomidae) occupied an

overwhelming majority in the community (96% at each station). Damselflies (*Argia* spp.) were barely present at Station 3 while Station 4 was able to support larger numbers. In contrast, reference Stations 1 and 2 supported a more diverse macroinvertebrate fauna where caddisflies occupied 20% to 40% and mayflies 2% to 10% of the community at each station, respectively. When compared to Stations 3 and 4, the chironomid population included twice as many Tanypodinae at both reference stations and ten times as many Orthocladiinae at Station 2. Damselfly populations were present at Station 1 (where the largest population occurred) but decreased substantially at Station 2.

Community indices reflected impact at Stations 3 and 4 where the lowest mean values for diversity, evenness, and taxa richness occurred. The following Shannon diversity values (similar to Brillouin values used in this study) have been suggested as criteria for assessing aquatic life: exceptional >3.50; high 2.50 - 3.49; intermediate 1.50 - 2.49; limited <1.50 (Dickson, et al., 1989). Diversity at Stations 3 and 4 (0.40 and 0.78, respectively) fell into the lower end of the limited use category. At the reference stations, diversity values were at the high end of the limited use range for Station 1 (H = 1.27) and the high end of the intermediate range for Station 2 (H = 2.41).

Some macroinvertebrate community recovery was apparent at Stations 5 (5.2 miles below outfall) and 6 (8.0 miles

below outfall) where ambient chlorine levels fell to 0.14 and 0.08 mg TRC/l, respectively. Diversity improved at Station 5 (H=1.40) and Station 6 (1.46) compared to the stations closest to the outfall. Orthocladiinae and Tanypodinae chironomids occurred in larger numbers at Stations 5 and 6 (1,200 - 4,000 individuals/m^2) compared to Stations 3 and 4 (6.0 - 250 individuals/m^2). The larger population of tanypods at Stations 5 and 6 suggests an improvement in water quality at these locations. Free-living tanypods are more exposed to water column toxicants and are generally more sensitive then the Chironominae and Orthocladiinae.

Mayflies first reappeared in the community at Station 5 where the dominant taxa included *Tricorythodes* spp. and early instar nymphs from the Tricorythidae/Caenidae families. Stations 5 and 6 both supported the largest mayfly communities among all stations. Despite this improvement in the mayfly population below the effluent, reference Station 2 still supported a more diverse community where Baetidae abundance was higher and tricorythids were less dominant. Mayflies recovered quicker from a spatial standpoint compared to caddisflies. Caddisflies were not present in significant numbers below the effluent until Station 6 when Hydroptilidae scrapers colonized the substrates.

Stations 5 and 6 supported the most abundant immature macroinvertebrate population with over 30,000 individuals/m^2

at each station. Densities at reference stations were only half the size of populations found at Stations 5 and 6. One possible explanation for the large densities at these stations was a supply of nutrients, algae, zooplankton, and detritus originating from the effluent that may have provided food for the macroinvertebrate community downstream. Another reason for the high density at Stations 5 and 6 may be due to the absence of competition and predation on the dominant taxa group (Chironomidae).

At Station 7, 18 miles downstream from the outfall, macroinvertebrate densities declined to levels comparable to impacted Stations 3 and 4. However, diversity was highest at Station 7 (H = 1.67) compared to the other downstream stations, and mayflies and tanypod and orthoclad chironomids were better represented compared to Stations 3 and 4. The caddisfly population was still virtually absent at Station 7. Because of its location, Station 7 probably experiences little influence from the effluent (chlorine levels were 0.02 mg TRC/l at Station 7 in August 1990). A factor that may impact this area is nonpoint source pollution originating from a variety of land use practices (i.e., golf course; amusement park; residential neighborhoods) located within the 10 mile distance between Stations 6 and 7. It is unknown whether the lack of caddisflies at Station 7 can be attributed to this potential source of pollution. Ambient toxicity had been observed in lab assays with *Ceriodaphnia*

dubia when exposed to water samples collected at Station 7 in August and September. However, ambient toxicity was also present at Stations 3, 4, 5, and 6 during the same time period (R. Guinn, pers. communication, 1993).

In regard to overall community composition, Chironomidae occupied a majority of the macroinvertebrate communities present on the artificial substrates at all stations. Chironominae was the dominant midge while Tanypodinae and Orthocladiinae were represented in smaller numbers. The Hydropsychidae filterers comprised a majority of the caddisfly community at reference Stations 1 and 2. *C. fraternus* and two hydroptilids were also present. Trichopterans were virtually absent at all downstream stations except for Station 6 where a modest population of hydroptilids occurred. The Ephemeropteran community at downstream Stations 5, 6, and 7 was dominated by *Tricorythodes* spp. and early instars of Tricorythidae-Caenidae families. Mayflies were most diverse at upstream Station 2 where *Tricorythodes* spp. and Baetidae were found in about equal numbers. Heptageniidae and Baetidae were second or third in abundance (depending on the station) after the Tricorythidae complex. At reference Station 1, the mayfly community showed signs of impact where mayfly density and diversity was substantially smaller compared to Station 2 and downstream Stations 5, 6, and 7. The reason for the decline in mayfly populations at Station 1 is unknown.

Damselflies (*Argia* spp.) were present in comparable numbers at Stations 1, 5, 6, and 7 and were virtually absent from Stations 2 and 3.

The Bray-Curtis similarity analysis indicated that Stations 5 and 6 were not significantly different communities ($p=0.253$) and Stations 1, 2, 3, 4, and 7 were similar communities ($p > 0.372$). The high variability between replicates may have influenced the inability of the bootstrap to statistically separate the communities of impacted Stations 3 and 4 from upstream Stations 1 and 2. Other community indices were somewhat difficult to interpret mainly because of the high variability that occurred within some stations. Stations 3 and 4 had the lowest mean values for diversity, evenness, and taxa richness.

Dechlorination - Early Summer 1991

The immature macroinvertebrate community showed signs of recovery directly below the outfall at Station 3 approximately six months after effluent dechlorination began at the Village Creek municipal facility. Prior to dechlorination, mayflies and caddisflies were virtually nonexistent at Station 3. During the spring 1991 sample, a significant increase in density and diversity was observed for chironomids, caddisflies, and mayflies at Station 3. Ephemeroptera density (3,100 individuals/m^2) was twice as high and trichopterans (36,000 individuals/m^2) were six

times more abundant at Station 3 compared to upstream reference Station 1. In the aquatic life use category (Dickson et al., 1989), diversity at Station 3 (H = 2.45) was at the high end of intermediate (1.50 - 2.49) compared to pre-dechlorination when it was considered limited (H = 0.40). Chironomid and mayfly communities also improved further downstream at Stations 4, 5, 6, and 7. The removal of chlorine from the river ecosystem, combined with nutrient loading from the effluent, provided an environment that favored insect recolonization efforts at this previously impacted station.

Recolonization of caddisflies had not yet occurred at stations further downstream from Station 3. Station 4 was the only station below Station 3 to support a caddisfly population, though in much smaller numbers (1,100 individuals/m^2) compared to Station 3 and reference Station 1. It is puzzling that caddisflies recovered at Station 3 (the most chlorine impacted station) while stations located greater distances from the outfall (Stations 5, 6, and 7) remained virtually unpopulated by caddisflies (<50 individuals/m^2). Data from ambient toxicity tests provided little insight into why caddisflies were impacted at some stations. In lab toxicity assays using river water taken on May 22 and again on June 24, *Ceriodaphnia dubia* experienced acute and chronic toxicity at all stations (R. Guinn, pers communication 1993).

Caddisflies at upstream reference Station 2 also had small numbers while Station 1 supported a healthy caddis population. The lack of trichopterans at reference Station 2 suggests that some type of impact occurred at this station. Nonpoint source pollution could have influenced the decline in caddisflies. Rain events occurred on several occasions before and during the exposure period in the river. Declines in the macroinvertebrate community have been observed at this station in other samples. Lab toxicity assays with *C. dubia* have revealed ambient toxicity at Station 2 on an irregular basis (R. Guinn, pers communication, 1993).

In overall community composition, the Chironomidae population was dominated by the Chironominae subfamily at all stations. Station 3 had the largest population of all chironomids but the smallest number of Tanypodinae. The decrease of tanypods at Station 3 below the outfall may be an indication of ambient toxicity still present in the effluent. Tanypods are free-living predaceous midges and tend to be more vulnerable to water column toxicants than the burrowing and tube-building Chironominae and Orthocladiinae.

The trichopteran community at Stations 1 and 3 consisted primarily of Hydropsychidae filter-feeders. Hydropsychid early instars dominated the population and three genera (*Hydropsyche* spp., *Cheumatopsyche* spp., and *P. flava*) were represented. Hydropsychid community composition

was similar at Station 1 above and Station 3 below the effluent. However, densities dramatically increased below the outfall, possibly in response to increases in food supply (primarily zooplankton) that originated from nutrients discharged with the effluent. Zooplankton densities were almost twice as high at Station 3 (980 individuals/liter) compared to reference stations (approximately 500 individuals/liter). Periphyton density was not available at Station 3 for this sample, but density at Station 4 (600 individuals/m^2) was two to three times lower than reference stations (B.Bryan, pers communication, 1993).

Tricorythidae and Heptageniidae comprised a majority of the mayfly community at all stations except Station 3 where Baetidae was the dominant family. Station 3 was the only station where Baetidae and *Isonychia* spp. appeared in substantial numbers (1,300 and 400 individuals/m^2, respectively).

The Bray-Curtis similarity analysis generated a fairly realistic interpretation of community dynamics. Stations 2, 4, 5, 6, and 7 were considered similar communities while Station 1 and Station 3 were each significantly different from all other stations. One driving force behind the analysis was probably the lack of caddisflies at all stations except Stations 1 and 3. The chironomid and mayfly communities were otherwise fairly comparable in density and

diversity between stations.

In contrast to the similarity results, it was difficult to determine relationships among stations based on results from the other community indices. Diversity and evenness was highest at Stations 1 and 4 while mean taxa richness was greatest at Stations 3 and 4 (20.3 and 22.0, respectively) and lowest at Station 6 (13.3). Diversity (range of 2.1 - 3.0) and evenness (range of 0.7 - 0.5) each had a fairly narrow range that made it difficult to detect differences between stations. Mean density was the only parameter that clearly showed the difference between Station 3 and all other stations. Station 3 supported a macroinvertebrate community that was four times larger than Station 1 and over ten times larger than each remaining station.

Dechlorination - Spring 1992

Station 3 (0.1 mile below the outfall) continued to support a diverse and abundant immature macroinvertebrate community a year and a half after effluent dechlorination was implemented. The largest macroinvertebrate population (30,000 individuals/m^2) occurred at Station 3 and diversity (H=2.12) was rated as intermediate (1.50 - 2.49) within the aquatic life use criteria (Dickson et al., 1989). Chironomid populations were four times higher at Stations 3 and 5 (5.2 miles below outfall) compared to all other stations. Caddisfly communities (primarily Hydropsychidae)

were twice as high at Station 3 below the effluent compared to upstream reference Stations 1 and 2 and Station 5 further downstream. It was speculated that the larger populations of chironomids and caddisflies at Station 3 were supported by an artificially larger amount of food originating from the effluent. However, this speculation was not supported by some measurements of the invertebrate food base. At station 3, periphyton density (16,000 individuals/m^2) was similar to Station 1 (13,000 individuals/m^2) and half as much as Station 2; zooplankton density (325 individuals/liter) was slightly lower than both reference stations (475 individuals/liter) (B. Bryan, pers communication, 1993).

Ephemeroptera was the only aquatic insect that showed signs of impact at Station 3, where abundance was 60% to 80% less than reference Stations 1 and 2. The low mayfly density may have resulted from exposure of the substrates above the water surface for approximately 24-48 hours at the end of the third week of colonization. Despite fewer numbers, all mayfly taxa found at other stations were represented at Station 3, except for *Caenis* spp. and *Stenacron* spp..

Community composition tended to be comparable between Station 3 and either reference Station 1 or 2. The Chironomidae midge community was dominated by the Chironominae at all stations except Stations 1 and 7 where equal numbers of Chironominae and Tanypodinae occurred. Orthocladiinae was substantially higher at Stations 3 and 5

compared to all other stations. Tanypodinae, a relatively sensitive chironomid, was slightly lower at Station 3 (1,300 individuals/m^2) compared to reference Stations 1 and 2 (1,700 and 1,500 individuals/m^2, respectively). In the caddisfly community, Station 3 supported the largest population of hydropsychid early instars (three times more than either reference station). Later instars (*Hydropsyche* spp. and *P. flava*) were twice as dense at reference Station 2 compared to Station 3 and reference Station 1. Caddisflies finally returned to Station 5 where they had been virtually absent since the beginning of this study. Station 5 supported proportionately more early instar hydropsychids (90%) than later instars; total trichopteran density was comparable to reference Station 1. Mayfly communities at all stations were comprised primarily of *Tricorythodes* spp. and the Heptageniidae family (early instars and *S. integrum*). Most taxa were represented at both upstream and downstream stations. Mayflies had comparable densities at Stations 1, 2, 5, and 7. Smaller populations were supported at Station 3 and 4 due to impacts unrelated to the effluent. The small numbers of baetids and isonychids at all stations may be attributed to season since larger numbers of these taxa were observed during the May/June 1991 sample.

Macroinvertebrate colonization at Stations 4 and 6 were affected by events that were unrelated to the effluent. After one week of colonization at Station 4, substrates were

found dried out on the riverbank. The substrates were reset for the remaining three weeks of the sample period. The shorter colonization period may have affected the results at Station 4, where the lowest densities for each insect order were found. At Station 6, all substrates were stolen and no data exists for this sample date.

Caddisflies appeared to be impacted at Station 7 where populations were substantially reduced compared to other stations. A likely source of aquatic toxicants at Station 7 is nonpoint pollution from several areas nearby that have a high potential for runoff (i.e., golf course; amusement park; residential neighborhoods). A rain event occurred midway through the sample period and could have triggered the decline in the caddisfly community. In lab toxicity assays with water collected on May 1st, samples from Station 7 were acutely toxic to *C. dubia*. Chronic toxicity affected both survival and reproduction of *C. dubia* at all stations (R. Guinn, pers communication, 1993). The high variability in macroinvertebrate communities between stations was reflected in the Bray-Curtis similarity analysis where all stations were significantly different from each other except for reference Stations 1 and 2. Some of the structural differences that made it difficult to link stations include the following: caddisflies almost lacking at Stations 4 and 7; high density of early instar hydropsychids at Station 3; mayflies poorly represented at Stations 3 and 4; and

chironomid densities lower at Stations 1, 2, 4, and 7 compared to Stations 3 and 5.

Diversity, evenness, and taxa richness were difficult to interpret between stations. Stations 1, 2, and 7 had the highest values for diversity and evenness followed by Stations 3 and 5. Based on these values, one might conclude that Station 7 was comparable to Stations 1 and 2, when in reality the caddisfly and mayfly populations were quite different at Station 7 compared to the reference stations. Results from taxa richness indicated that all stations were comparable (17-21 taxa/station) except for Station 4 where only 11 taxa occurred. The indices did reflect the impact of a shorter colonization period at Station 4 where the lowest values for all parameters were calculated.

Dechlorination - Late Summer 1992

Approximately two years after effluent dechlorination was implemented, the immature macroinvertebrate communities downstream from the outfall appeared to stabilize in abundance and composition. The insect community continued to improve at Station 3 closest to the effluent. For the first time since dechlorination began, diversity at Station 3 (H=2.86) was rated as high (2.50 - 3.49) within the aquatic life use criteria (Dickson et al., 1989). Station 3 also supported the largest macroinvertebrate population (30,000 individuals/m^2) among all stations. Overall community

composition was similar between upstream reference Station 1 and downstream Stations 3, 5, 6, and 7.

The Chironomidae midge community was dominated (65% - 85%) by Chironominae at all stations. At stations above the outfall, Tanypodinae were more abundant than Orthocladiinae while the opposite occurred at Stations 3, 4, 5, and 6 below the outfall. Orthoclads and tanypods were equally represented at Station 7 furthest from the outfall (18 miles downstream). The higher abundance of Tanypodinae at the reference stations and at Station 7 suggests that the effluent may have had a subtle effect on some taxa in the tanypod population at Stations 3, 4, 5, and 6.

In the trichopteran community, all stations were dominated by the filter-feeding Hydropsychidae. The hydropsychid group included early instars and three genera (*Hydropsyche* spp., *P. flava*, and *Cheumatopsyche* spp.). Early instars occupied the largest segment of the population at all stations except at Station 3 where early instars and *Hydropsyche* spp. were equally common. The presence of both early and late instars at Station 3 indicates that water quality at this station can support various life stages of hydropsychids. Trichoptera density was highest at Station 3 and gradually decreased further from the effluent at Stations 5 and 6. Caddisflies were two to four times more common below the effluent than at reference Station 1. The large collector-filterer population of hydropsychids below

the outfall may have been encouraged by an increase in detritus associated with the effluent. However, an increase in food supply downstream was not reflected in zooplankton or periphyton densities. Zooplankton density was similar at Stations 1 and 3 (160 and 190 individuals/l, respectively) while periphyton density was substantially lower at Station 3 (220 individuals/m^2) compared to Station 1 (1200 individuals/m^2) (B. Bryan, pers communication, 1993).

In the mayfly community, Tricorythidae taxa occupied a majority at all stations. A difference between upstream and downstream stations was observed in subdominant mayfly taxa. Heptageniidae taxa were subdominant above the outfall while Baetidae were second in abundance below the outfall. The filtering mayfly, *Isonychia* spp., was over ten times more common below the effluent. *Isonychia* spp. may have had a similar response as the hydropsychid caddisflies to the effluent-induced increase in food supply in the water column. In contrast to the high caddisfly densities found below the effluent, mayfly abundance was only 20% - 30% higher at Stations 3, 5, 6, and 7 compared to reference Station 1.

Macroinvertebrates at Stations 2 and 4 suffered impacts that were not related to the effluent. At Station 2, there was no conclusive explanation for the significant decline in caddisfly and mayfly populations that occurred during the August 1992 sample. In lab toxicity assays, survival and

reproduction was not adversely affected when *C. dubia* was exposed to Trinity River grab samples taken at Station 2 on July 11th and again on August 29th. These results indicate that ambient toxicity was not present in the water column on these dates, although periodic toxicity was observed at Station 2 during the study (R. Guinn, pers communication, 1993). Other possible sources of toxicity at Station 2 are the sediments and/or nonpoint source pollution. During the sample period, river flow increased from 89 cfs to 608 cfs due to a rain event that occurred on August 19th. This event may have helped move pollution into Station 2 in two ways: 1) resuspension of toxicants possibly present in the sediments; 2) runoff from nearby residential areas. Future analysis of benthic communities collected from sediment samples may shed some light on the macroinvertebrate community dynamics occurring at Station 2. Insect colonization at Station 4 may have been affected by sedimentation and changes in river flow caused by a dam and associated construction activities. This physical disturbance in the river occurred approximately 20 meters upstream from the artificial substrates at Station 4. Chironomid, caddisfly, and mayfly populations were all noticeably smaller at Station 4 compared to other stations.

The Bray-Curtis similarity analysis produced a fairly realistic interpretation of community composition among the sampling stations. Reference Station 1 and downstream

Stations 3, 5, 6, and 7 were linked together while the two impacted stations (Stations 2 and 4) were designated as similar communities. Other community indices complimented the Bray-Curtis analysis. Mean values for diversity, evenness, and taxa richness were comparable between Stations 1, 3, 5, 6, and 7 while the lowest values were measured at Stations 2 and 4.

Pre-dechlorination and Dechlorination Comparison

Community composition of immature macroinvertebrates at the order level gave an indication of changes that occurred between pre-dechlorination and dechlorination samples. During pre-dechlorination in August 1990, dipterans almost completely dominated Stations 3 and 4 (within one mile below the effluent) where ambient chlorine levels were highest. Station 5 (5.2 miles downstream from outfall), represented a zone of recovery where mayflies colonized the substrates and ambient chlorine concentration was 0.14 mg TRC/l (seven times less than Station 3). Caddisflies reappeared further downstream at Station 6 (8 miles below the outfall) but populations remained small compared to upstream reference stations. The presence of mayflies and lack of caddisflies at Stations 5 and 6 indicates that caddisflies were more sensitive to chlorine related toxicity than mayflies. At reference Stations 1 and 2, Trichoptera was significantly more abundant than all stations below the effluent while

Ephemeroptera was higher than Stations 3, 4, and 7. Diptera was the only group that was comparable between reference and downstream stations.

By August 1992, almost two years after dechlorination was implemented, caddisflies had repopulated downstream Stations 3, 5, 6, and 7 in densities greater than reference Station 1. Diptera was replaced by Trichoptera as the dominant taxa at Stations 3, 5, 6, and 7. Mayflies successfully returned to Station 3 in numbers comparable to reference Station 1 and downstream Stations 5, 6, and 7. At reference Station 2, a severe reduction in caddisfly and mayfly abundance suggested the presence of nonpoint source pollution in the vicinity during the August 1992 sample. At Station 4, insect colonization was hampered by non-effluent related impacts (dam construction) during the August 1992 sample but showed improvements during the 1991 dechlorination sample when caddisflies and mayflies began to repopulate the substrates.

A more detailed study of community composition at the family, subfamily, and genus levels confirmed that after two years of dechlorination, the Diptera, Trichoptera, and Ephemeroptera populations were generally comparable between reference Station 1 and stations below the effluent. Immature insects were not identified to species and subtle differences may exist at this level between stations.

Diptera were dominated by Chironominae midges at all stations in the August 1990 and August 1992 samples. Dipteran composition became more diverse following dechlorination when larger proportions of Orthocladiinae and Tanypodinae colonized the substrates. Station 3 supported almost no orthoclads in August 1990 and produced the largest population during the August 1992 sample. Between pre-dechlorination and dechlorination samples, Tanypodinae densities improved at Stations 3 and 4 but were always less abundant compared to reference stations and Station 7 furthest from the outfall. The free-living tanypods tend to be more sensitive to water column toxicants and their reduced presence at stations near the effluent may indicate toxicity that was ignored by more tolerant burrowing chironomids.

The caddisfly community showed the most dramatic improvement between pre-dechlorination and dechlorination conditions. At all stations below the outfall, the filter-feeding Hydropsychidae were virtually absent before dechlorination but became the most abundant caddis taxa after dechlorination. In the August 1992 sample, hydropsychids included early instars and three genera (*Hydropsyche* spp., *Potamyia flava*, and *Cheumatopsyche* spp.) that were all present in similar proportions between reference Station 1 and downstream Stations 3, 5, 6, and 7. Higher densities of hydropsychids prevailed at stations

below the effluent compared to upstream stations. Speculation that the larger hydropsychid populations below the outfall were related to benefits from organic pollution originating from the effluent were difficult to quantify. In August 1992, zooplankton density at Station 3 was similar to other stations while periphyton density was six times lower than Station 1 (B. Bryan, pers communication, 1993).

Prior to dechlorination, mayfly communities were present at stations above the effluent and almost nonexistent below at Stations 3 and 4. Mayflies reappeared at downstream Stations 5, 6, and 7 but communities were heavily dominated by one family (Tricorythidae). Two years after dechlorination, mayfly communities were more diverse and abundant at stations below the effluent compared to reference stations. Community composition differed between stations least influenced by the effluent (Stations 1, 2, and 7) and Stations 3, 4, 5, and 6 within 8 miles below the effluent. Tricorythidae was the dominant taxa at all stations but Heptageniidae was subdominant at Stations 1, 2, and 7 while Baetidae was subdominant at remaining stations. By August 1992, the filter-feeding mayfly, Isonychia spp., had established itself at all stations below the effluent where it was absent before dechlorination. A similar functional feeding group, the hydropsychid caddisflies, also recolonized areas below the effluent where it was previously absent. The abundance of Isonychia spp. below the effluent

suggests that the effluent had a positive effect on this genus because it was barely present at reference stations above the effluent.

Community indices generally reflected the improvements observed in macroinvertebrate communities in the river between pre-dechlorination and dechlorination phases. Based on diversity values, aquatic life use (Dickson et al., 1989) at Station 3 was rated as limited before dechlorination and high in the August 1992 sample, a sevenfold increase. At Station 3, evenness was five times higher and taxa richness doubled since dechlorination was implemented. Diversity and evenness approximately doubled at Stations 5, 6, and 7 further downstream from the effluent. Aquatic life use at these stations was rated as high after dechlorination compared to limited (Stations 5 and 6) or intermediate (Station 7) in August 1990.

Low values for diversity, evenness, and taxa richness at Stations 2 and 4 reflected impacts at these two sites in August 1992. At reference Station 2, aquatic life use dropped from intermediate in August 1990 to limited in August 1992.

SUMMARY

The macroinvertebrate community improved after chlorine was removed from the Village Creek municipal effluent prior

to discharge into the Trinity River. After dechlorination was implemented, the following positive changes were observed in communities at stations below the outfall:

1) Trichoptera and Ephemeroptera repopulated Stations 3 and 4 within one mile below the outfall.

2) Chironominae midges and Tricorythidae mayflies decreased in community dominance at Stations 5 and 6 (within 8 miles below the outfall).

3) Collector-filterer functional groups (i.e., Hydropsychidae caddisflies and *Isonychia* spp. mayfly) recolonized all stations below the outfall.

4) Macroinvertebrate taxa present at reference Stations 1 and 2 were also supported at stations below the outfall in similar proportions.

5) The largest macroinvertebrate densities occurred directly below the dechlorinated effluent at Station 3.

6) Almost two years after dechlorination began, community composition was not significantly different between upstream reference Station 1 and downstream Stations 3, 5, 6, and 7 (similarity>0.648; p>0.136; Bray-Curtis similarity index).

7) Based on diversity values, aquatic life use ratings at Station 3 increased from 'limited' to 'high' between pre-dechlorination and dechlorination phases.

The combined densities of Diptera, Trichoptera, and Ephemeroptera comprised approximately 98% of the macroinvertebrate community that colonized the artificial

substrates upstream and downstream of the Village Creek outfall. Representatives from Odonata, Megaloptera, and Coleoptera made up the remainder of each community. In terms of overall density at all stations, Diptera (Chironomidae) or Trichoptera (Hydropsychidae) dominated the macro-invertebrate community while ephemeropteran density was third (10% to 20%). Differences between and pre-dechlorination and dechlorination phases were exhibited at the order level of identification. Impacts from the chlorinated effluent were observed in Trichoptera and Ephemeroptera populations within one mile below the outfall where both groups were virtually absent at Stations 3 and 4. At Stations 5 and 6, the presence of mayflies in higher densities than reference stations indicated that a zone of recovery from chlorine toxicity occurred approximately 5 miles downstream from the outfall. The presence of mayfly populations and lack of caddisfly populations below the chlorinated effluent indicated that some caddisfly taxa were more sensitive to chlorine exposure than some mayfly taxa. During dechlorination in 1991 and 1992, the large densities of caddisflies and mayflies found at Station 3 indicated that these taxa were able to quickly repopulate and become established after chlorine toxicity was removed.

Additional information on community composition was gained from identification to sub-family for Chironomidae, genus for Hydropsychidae, and family for Ephemeroptera.

Diptera were represented primarily by three subfamilies of the Chironomidae midges and incidentally by members of Ceratopogonidae and Empididae. The majority of chironomids were Chironominae while Orthocladiinae or Tanypodinae were subdominant. After dechlorination was implemented, larger densities of orthoclads and tanypods occurred at stations below the effluent. The main difference in chironomid composition between stations upstream and downstream of the outfall was that Tanypodinae were more abundant above while the opposite was true for Orthocladiinae below the outfall. These subtle differences at the sub-family level suggest that further identification to the genus or species level could provide more information on chironomid composition upstream and downstream from the effluent.

The trichopteran community was dominated by the filter-feeding Hydropsychidae while Hydroptilidae and Polycentropodidae were usually represented in small numbers. Since a majority of the caddis community were hydropsychids, a better description of community composition between stations was accomplished by identification to the genus level (when possible). After dechlorination, caddisfly composition was comparable between the reference stations and stations below the effluent. Early instars and three genera of Hydropsychidae were supported in similar proportions at all stations by August 1992. Significantly higher densities of hydropsychids consistently occurred at

Station 3. The large numbers of caddis at Station 3 were thought to be related to organic pollution from the effluent that could stimulate food supplies for invertebrates. However, periphyton and zooplankton densities were not significantly greater below the effluent compared to stations upstream, except during the May/June 1991 sample when zooplankton density was twice as high at Station 3 compared to reference stations (B. Bryan, pers communication, 1993). Greater flow rates exist below the outfall and may have moved larger amounts of material downstream compared to upstream sites.

Mayfly communities included four main families (Tricorythidae, Heptageniidae, Baetidae, and Isonychiidae) and the majority usually consisted of tricorythids or heptagenids. Since only one or two genera were identified in each family, identification to the family level provided an adequate description of the mayfly community among stations. In dechlorination samples, Tricorythidae and Heptageniidae occurred in similar abundance above and below the effluent. Stations below the effluent supported larger populations of Baetidae and *Isonychia* spp. than reference stations.

CHAPTER 4

RESPONSE OF *Corbicula fluminea*

INTRODUCTION

The following chapter will discuss the response of juvenile *Corbicula fluminea* to chlorinated and dechlorinated effluent. Juvenile *C. fluminea* were exposed *in situ* in the West Fork of the Trinity River for a one month duration on five different test dates. The response endpoints were survival and shell length growth. Prior to dechlorination, a baseline *in situ* exposure was completed during August/September 1990. After dechlorination was implemented, four *in situ* exposures were completed within the following dates: 1) July/August 1991; 2) September/October 1991; 3) August/September 1992; and 4) September/October 1992. *In situ* exposures of *C. fluminea* were conducted only during the summer and early fall when the highest number of juvenile clams of suitable size were available from a field source.

RESULTS

Pre-dechlorination

Juvenile clams were exposed *in situ* during August/September 1990 while chlorinated effluent was being discharged into the river. The average ambient total residual chlorine (TRC) concentrations were as follows: Station 3 = 1.1 mg TRC/l; Station 4 = 0.45 mg TRC/l; Station 5 = 0.14 mg TRC/l; Station 6 = 0.08 mg TRC/l; and Station 7 = 0.02 mg TRC/l (R. Guinn, pers communication, 1992). *C. fluminea* experienced 100% mortality at Station 3 directly below the outfall and Station 4 approximately one mile further downstream (Figure 32). All clams survived at the remaining stations.

Mean length gain was highest at Station 2 (7.4 mm) and lowest at Station 5 (2.8 mm) (Figure 32 and Table 15). No shell growth occurred at Stations 3 and 4 due to the acute mortality described above. Chronic toxicity was observed at Station 5 (5.2 miles below the outfall) where juvenile growth was significantly lower compared to Stations 1 and 2 upstream and Stations 6 and 7 further downstream. Reference Stations 1 and 2 were significantly different from each other and all other stations (2 > 1 > 6 = 7 > 5 > 4 = 3; Bonferroni (Dunn) T-test). The minimum significant difference was between 0.15 and 0.69 mm. Coefficients of variation for length gain ranged from 7% to 13% (Table 15).

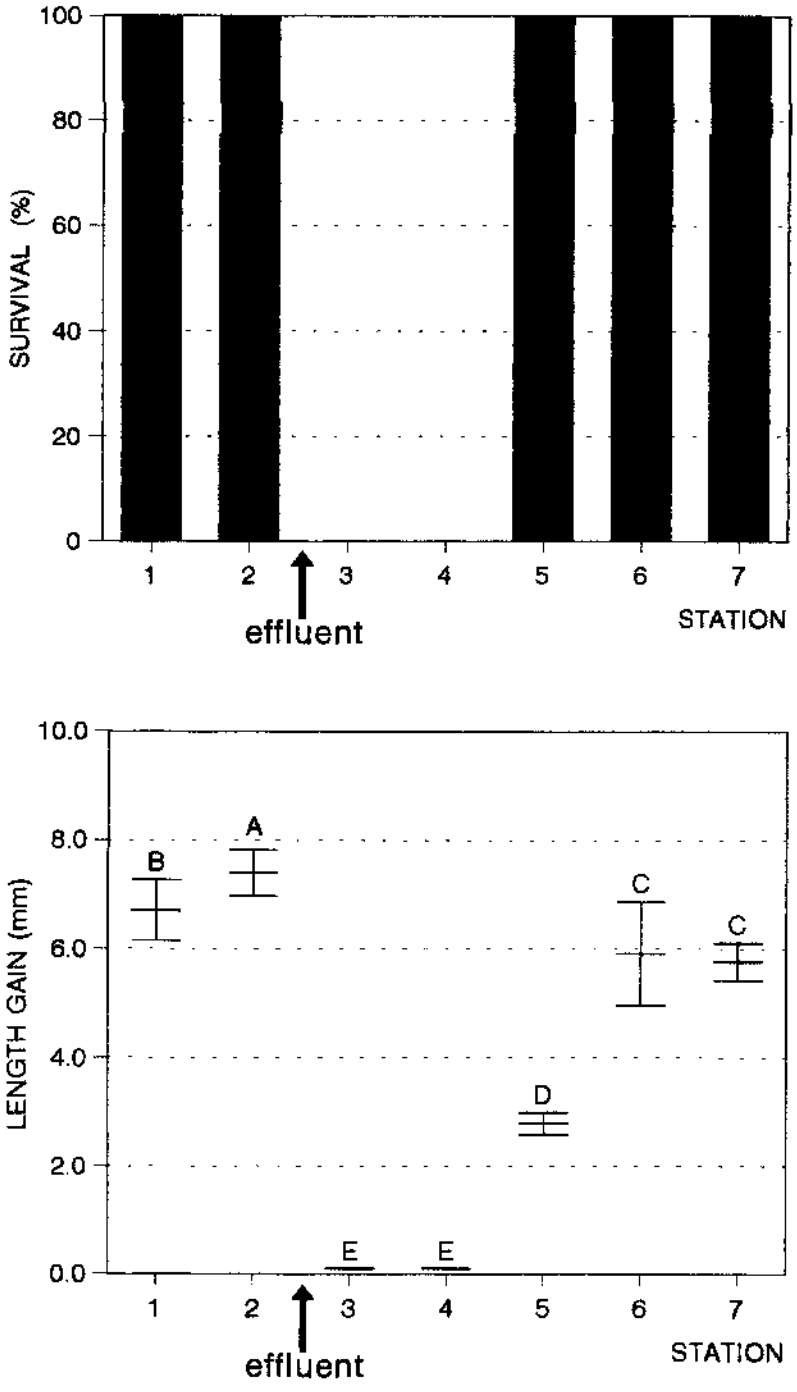

Figure 32. Survival (%) and shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990, before dechlorination began.

STATION	1	2	3	4	5	6	7
Aug 1990							
N	8	9	10	10	10	5	10
MIN	5.50	6.30	0.00	0.00	2.40	5.20	4.80
MAX	7.30	8.10	0.00	0.00	3.30	6.80	6.30
MEAN	6.71	7.40	0.00	0.00	2.79	5.92	5.77
SD	0.666	0.552	0.000	0.000	0.281	0.766	0.481
CV	9.9	7.4	0.0	0.0	10.1	12.9	8.3

SD = Standard Deviation
CV = Coefficient of Variation

Table 15. Descriptive statistics on shell length gains (mm) of caged *in situ* juvenile *Corbicula fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas County, Texas during August/September 1990, before dechlorination began.

The sample size was between 8 and 10 clams for each station except Station 6 where only five clams were recovered. At Station 6, sediment buried the cages containing the other five clams and resulted in total mortality.

Dechlorination

No mortality occurred at any river station during the July/August 1991 test conducted approximately 9 months after dechlorination began (Figure 33). The mean length gain of juvenile *C. fluminea* ranged from 1.1 mm at Station 3 to 6.4 mm at Station 5 (Figure 33 and Table 16). Stations 3 and 4 were significantly different from each other and from all other stations. Clam growth was statistically similar at upstream Stations 1 and 2. Downstream Stations 5, 6, and 7 were not significantly different from each other. The statistical link between upstream and downstream stations occurred between Station 1 and 7 ($5 = 6 = 7 \geq 1 = 2 > 4 > 3$; Tukey's Multiple Comparison). The minimum significant difference was between 0.53 and 0.77 mm. Coefficients of variation ranged from 7% to 14% at all stations except Station 3 where it increased to 24% (Table 16). Sample size was between 8 and 10 clams for each station.

During the September/October 1991 *in situ* exposure, *C. fluminea* survival was not significantly different between stations (Fishers Exact Test) (Figure 34). Survival was 100% at all stations except Station 3 where two clams died. There

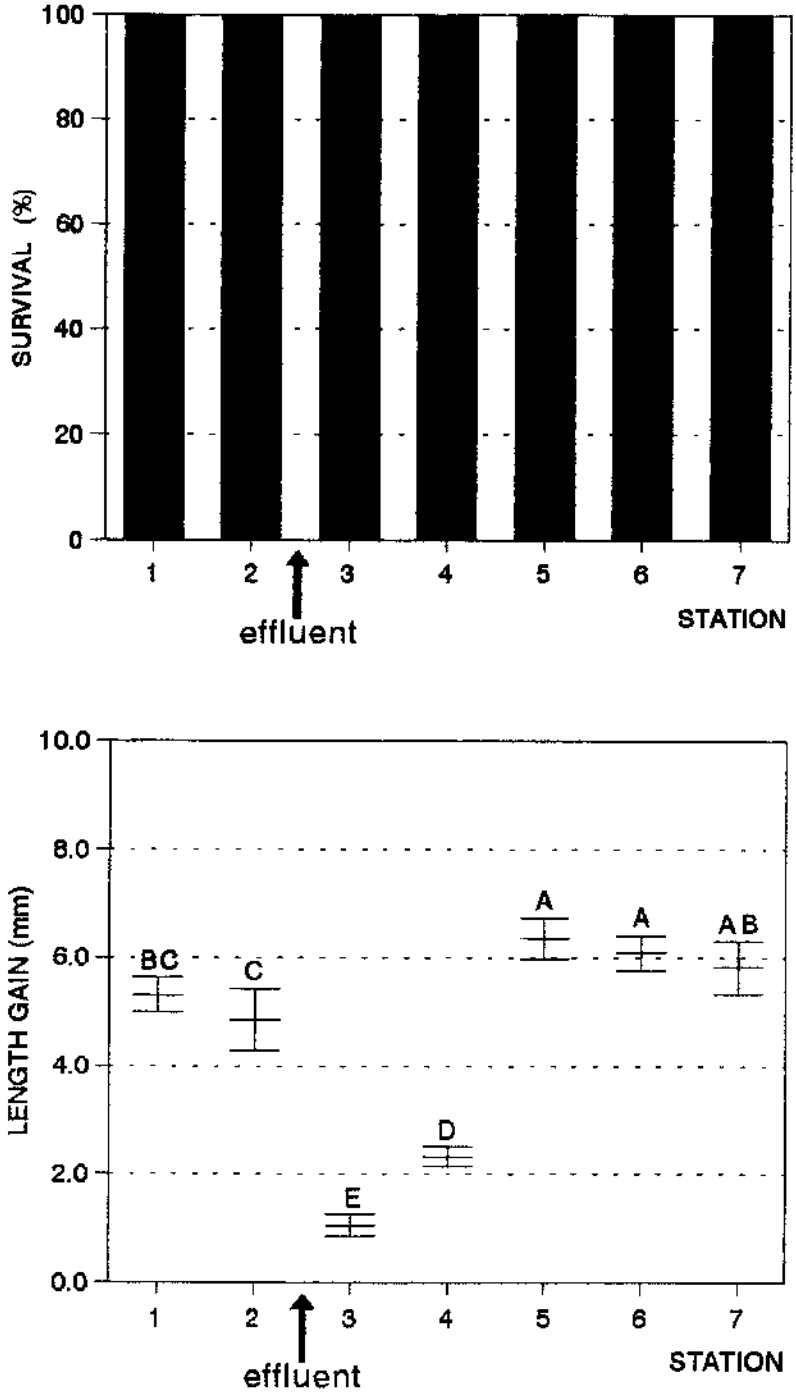

Figure 33. Survival (%) and shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during July/August 1991, approximately seven months after dechlorination began.

STATION	1	2	3	4	5	6	7
July 1991							
N	9	8	9	9	10	10	10
MIN	4.70	3.80	0.80	2.00	5.50	5.30	4.50
MAX	5.90	6.10	1.50	2.60	6.90	6.70	6.40
MEAN	5.31	4.85	1.07	2.33	6.35	6.08	5.82
SD	0.404	0.682	0.255	0.229	0.534	0.454	0.681
CV	7.6%	14.1%	23.9%	9.8%	8.4%	7.5%	11.7%

SD = Standard Deviation
CV = Coefficient of Variation

Table 16. Descriptive statistics on shell length gains (mm) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas County, Texas during July/August 1991, approximately seven months after dechlorination began.

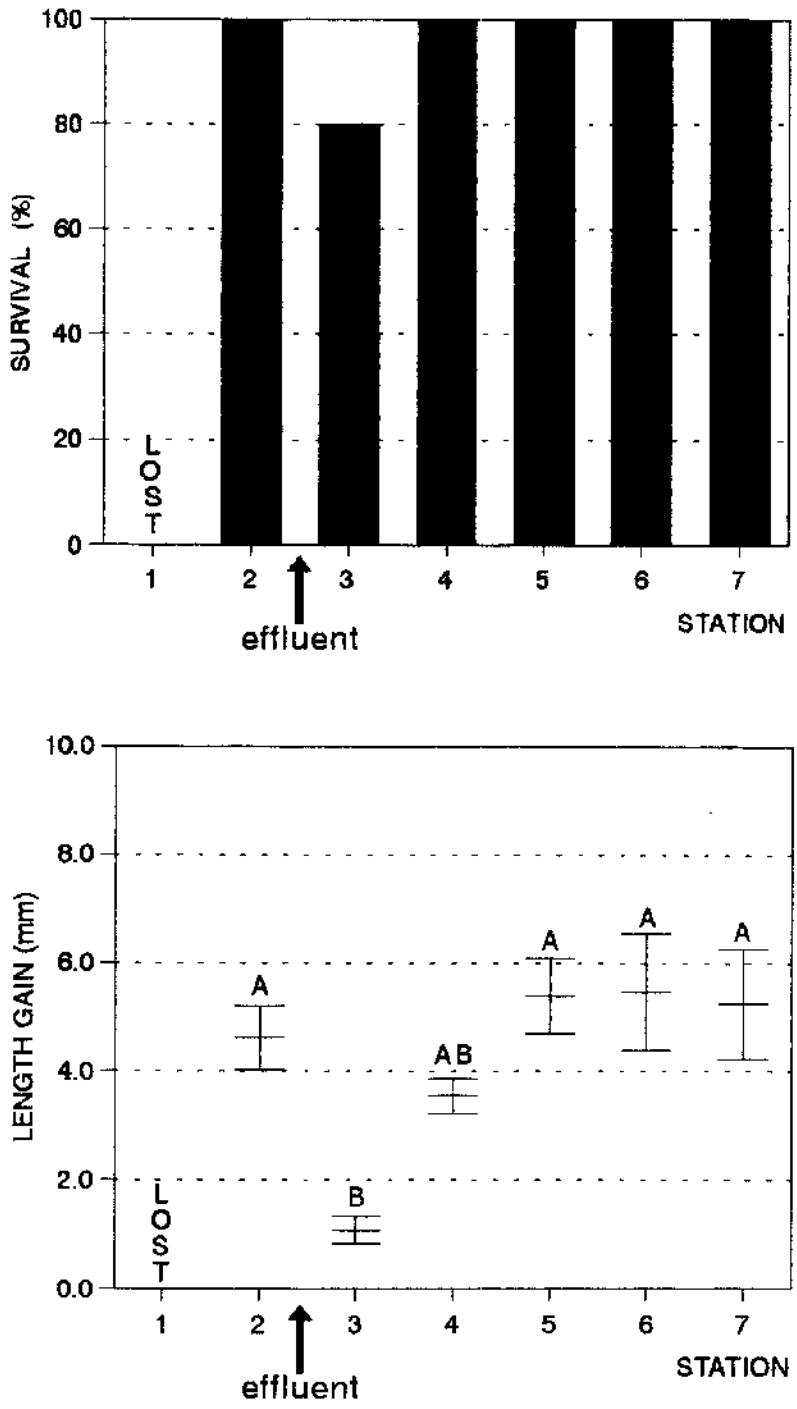

Figure 34. Survival (%) and shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during September/October 1991, approximately nine months after dechlorination began.

STATION	1	2	3	4	5	6	7
Sept 1991							
N	lost	10	10	9	10	8	10
MIN	"	3.18	0.71	2.68	2.98	3.05	2.54
MAX	"	5.65	1.95	3.94	6.45	6.42	6.66
MEAN	"	4.62	1.09	3.55	5.4	5.46	5.24
SD	"	0.820	0.348	0.423	0.967	1.277	1.423
CV	"	17.8	32.0	11.9	17.9	23.4	27.1

SD = Standard Deviation
CV = Coefficient of Variation

Table 17. Descriptive statistics on shell length gains (mm) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas County, Texas during September/October 1991, approximately nine months after dechlorination began.

is no clam data available for Station 1 because the cages were lost in the river. Mean length gain was lowest at Station 3 (1.1 mm) and highest at Station 6 (5.4 mm) (Figure 34 and Table 17). Clam growth was significantly smaller at Station 3 directly below the outfall compared to all other stations except Station 4. Upstream Station 2 and downstream Stations 4, 5, 6, and 7 were not significantly different from each other ($6 = 5 = 7 = 2 = 4 \geq 3$; Dunn's Multiple Comparison). The minimum significant difference was between 2.46 and 3.53 mm. Coefficients of variation ranged from 12% to 32% for all Stations (Table 17). Sample sizes were between 8 and 10 clams for each station.

Approximately 22 months after effluent dechlorination began, a third *C. fluminea* experiment was completed during August 1992 (Figure 35). Survival was 90% to 100% at all stations except Station 4 where survival was significantly reduced when half the clams experienced acute mortality (Fishers Exact Test). During the last ten days of exposure, a physical disturbance occurred at Station 4 and may have impacted clam survival. The condition of the clams was not inspected at the time of the disturbance (see Discussion section). Mean length gains ranged from 1.6 mm at Station 4 to 8.5 mm at Station 1 (Figure 35 and Table 18). Stations 1 and 2 above the outfall and Station 5 below were significantly different from Stations 3 and 4. Stations 6 and 7 were not significantly different from any station

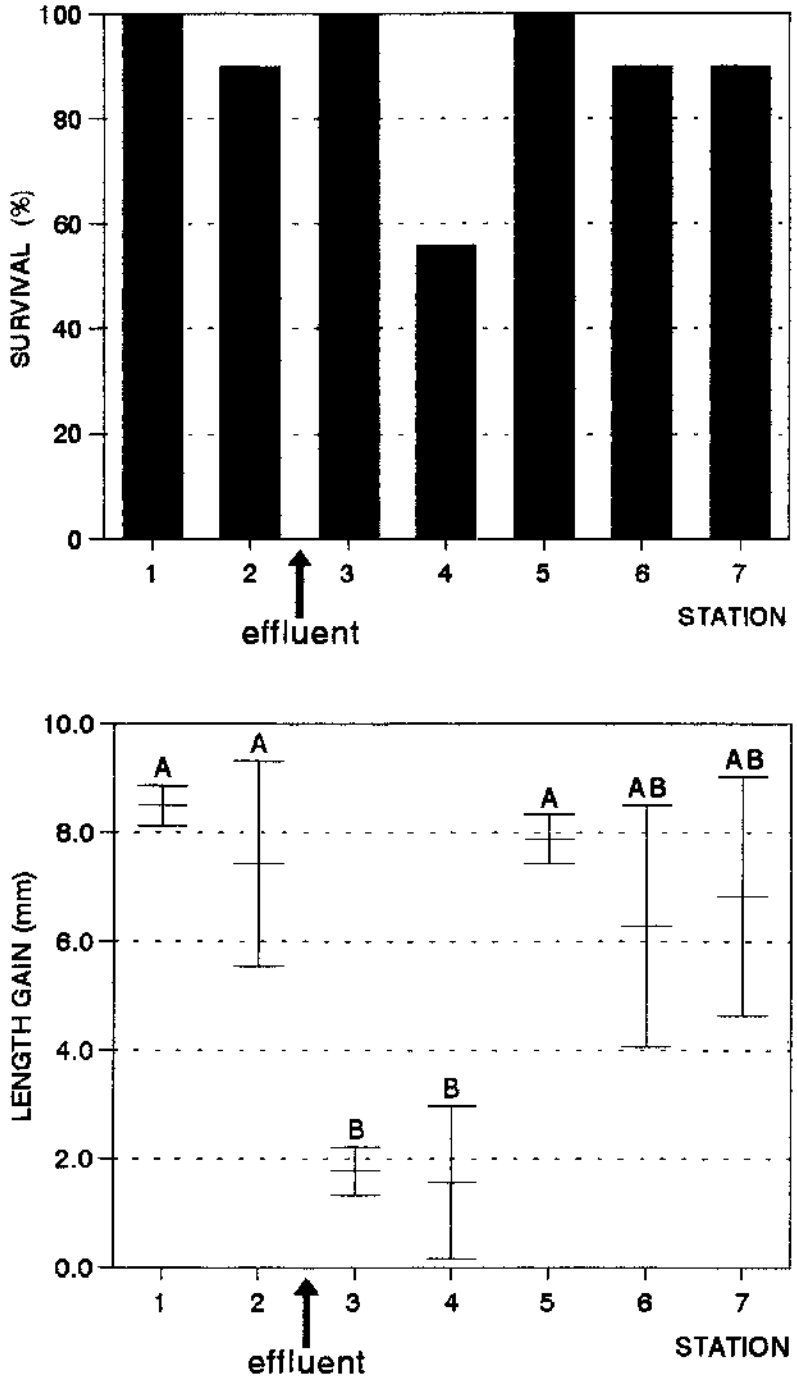

Figure 35. Survival (%) and shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately twenty months after dechlorination began.

STATION	1	2	3	4	5	6	7
Aug 1992							
N	10	10	10	9	10	10	9
MIN	7.55	0.09	0.44	0.00	6.43	0.09	0.17
MAX	9.12	9.03	2.51	3.77	8.74	8.22	9.68
MEAN	8.50	7.44	1.79	1.57	7.89	6.29	6.84
SD	0.521	2.629	0.613	1.829	0.620	3.108	2.842
CV	6.1	35.3	34.3	116.4	7.8	49.4	41.6

SD = Standard Deviation
CV = Coefficient of Variation

Table 18. Descriptive statistics on shell length gains (mm) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August 1992, approximately twenty months after dechlorination began.

(1 = 5 = 2 = 7 ≥ 6 ≥ 3 = 4; Dunn's Multiple Comparison). The minimum significant difference was between 5.27 and 5.66 mm. Coefficients of variation were less than 10% at Stations 1 and 5 while Stations 2, 3, 6, and 7 were between 30% and 50% (Table 18). Station 4 had the highest coefficient with 116%. Sample size was between 9 and 10 clams for each station.

During the final dechlorination *in situ* exposure conducted in September/October 1992, survival was statistically equal between all stations (Fishers Exact Test) (Figure 36). *C. fluminea* survival was 100% at Stations 1, 2, 3, and 5 and 90% at Stations 4, 6, and 7. Mean shell growth ranged from 0.9 mm at Station 3 to 6.4 mm at Station 5 (Figure 36 and Table 19). Juvenile clams experienced significantly reduced growth directly below the outfall at Station 3 compared to all other stations except Station 4. Upstream Stations 1 and 2 and downstream Station 7 were not significantly different from Stations 4, 5, and 6. However, Stations 5 and 6 were different from Station 4 (5 = 6 = 2 ≥ 1 ≥ 7 ≥ 4 = 3; Dunn's Multiple Comparison). The minimum significant difference was between 2.71 and 3.04 mm. Coefficients of variation ranged from 10% to 20% at Stations 1, 2, and 5 and increased to 35% to 70% at Stations 3, 4, 6, and 7 (Table 19). Between nine and ten clams were retrieved at each station.

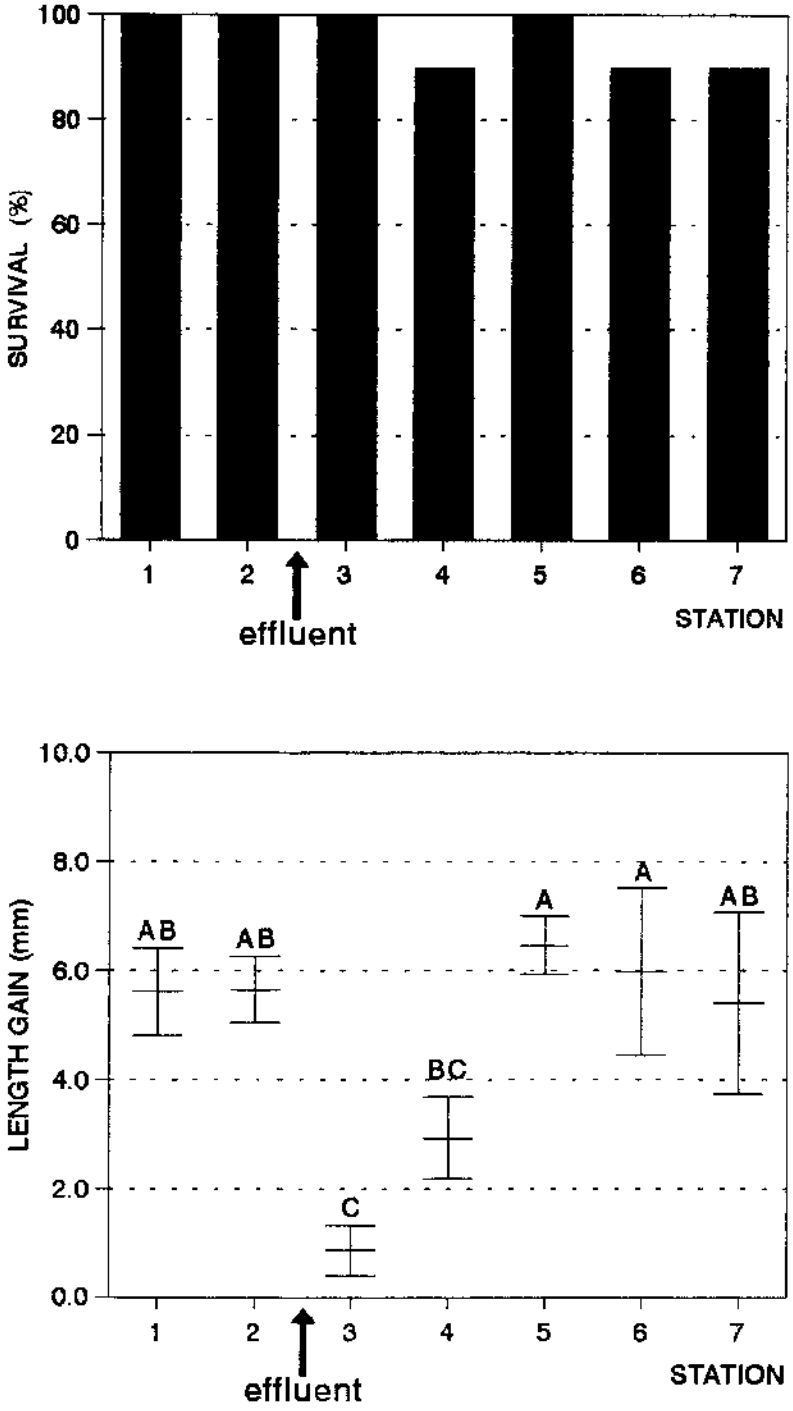

Figure 36. Survival (%) and shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during September/October 1992, almost two years after dechlorination began.

STATION	1	2	3	4	5	6	7
Sept 1992							
N	10	10	9	10	10	10	9
MIN	3.27	3.85	0.13	0.00	4.53	0.00	0.00
MAX	7.21	6.67	1.85	3.56	7.28	7.31	7.74
MEAN	5.61	5.64	0.86	2.93	6.45	5.97	5.40
SD	1.120	0.853	0.600	1.051	0.754	2.147	2.156
CV	20.0	15.1	69.7	35.9	11.7	35.9	39.9

SD = Standard Deviation
CV = Coefficient of Variation

Table 19. Descriptive statistics on shell length gains (mm) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during September/October 1992, almost two years after dechlorination began.

Pre-dechlorination and Dechlorination Comparison

The overall response of juvenile *C. fluminea* to effluent dechlorination is illustrated in Figures 37 and 38 and Tables 20 and 21. Data from all four dechlorination sample dates were combined to form a grand mean for survival and length gain for each station. Prior to dechlorination, no clams survived at Stations 3 and 4 compared to 100% survival at all other stations. After dechlorination was implemented, juvenile *C. fluminea* survival was between 80% and 100% at all stations. Survival was not significantly different between stations during dechlorination (Fishers Exact Test).

Juvenile *C. fluminea* growth was higher at Stations 3, 4, and 5 for each *in situ* test during dechlorination compared to pre-dechlorination results (Figure 38). Growth increased at Stations 3 and 4 from zero at both stations to mean length gains of 1.2 mm and 2.6 mm, respectively. Mean growth at Station 5 doubled from 2.8 mm before dechlorination to 6.5 mm during dechlorination.

During dechlorination, juvenile *C. fluminea* growth was not significantly different between upstream Stations 1 and 2 and downstream Stations 5, 6, and 7. Clams at Stations 3 and 4, within one-mile below the outfall, were significantly smaller than all other stations ($5 = 1 = 6 = 7 = 2 > 4 = 3$; Dunn's Multiple Comparison). Grand mean growth ranged from 5.7 mm to 6.5 mm for Stations 1, 2, 5, 6, and 7. The

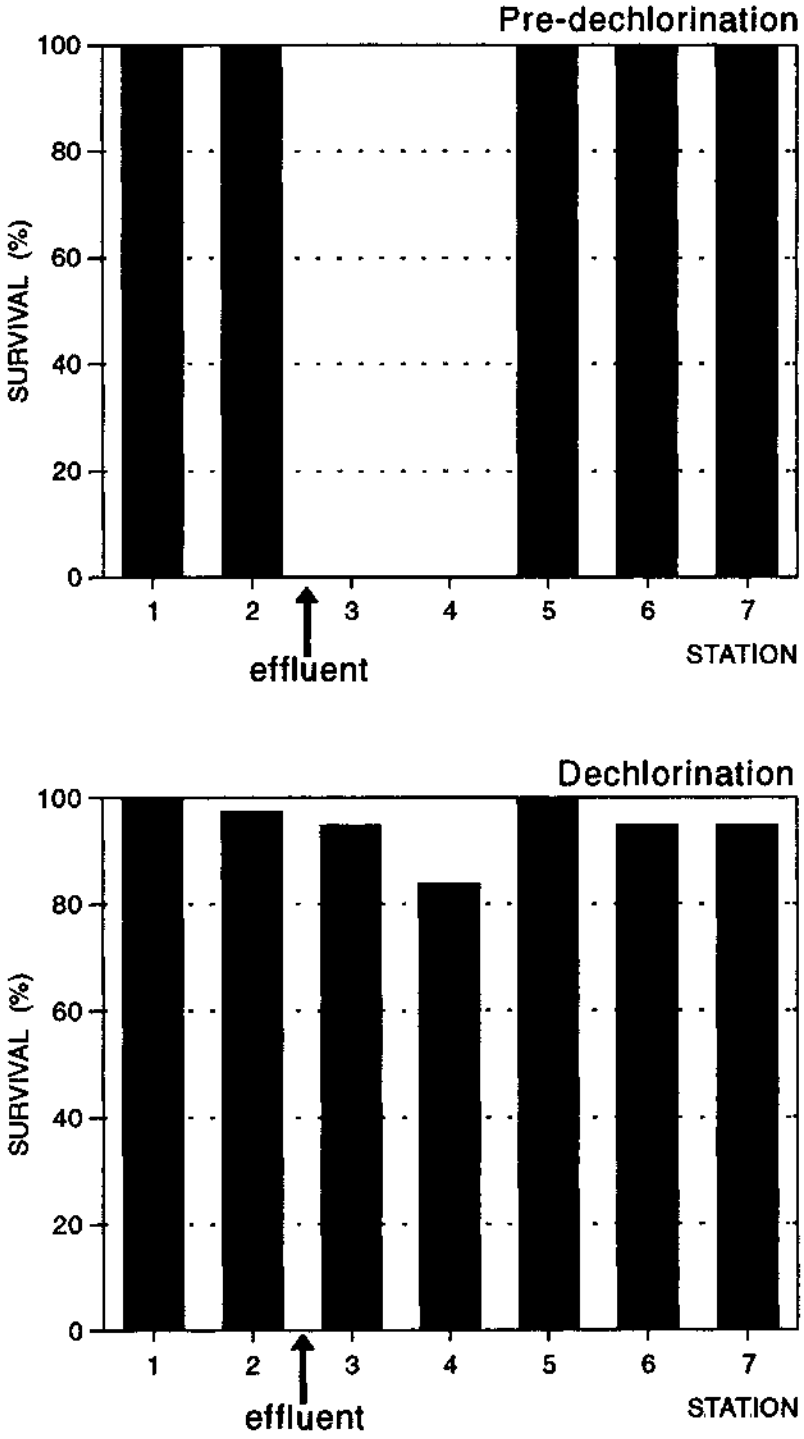

Figure 37. Survival (%) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before (top) and after (bottom) dechlorination began. Pre-dechlorination data was from August/September 1990 test and dechlorination data combined all four *in situ* tests from 1991-92.

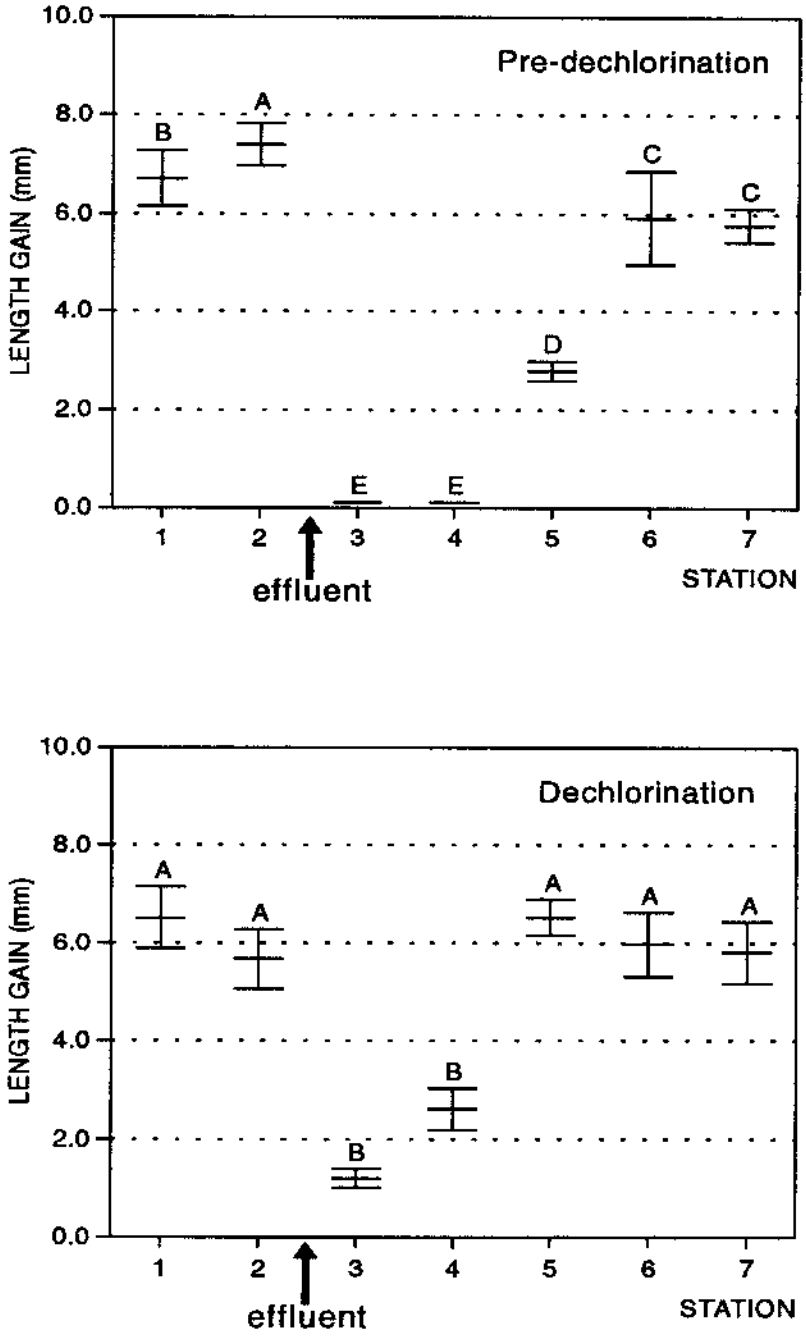

Figure 38. Shell growth (mean and 95% confidence interval) of caged *in situ* juvenile *C. fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before (top) and after (bottom) dechlorination began. Pre-dechlorination data was from August/September 1990 test and dechlorination data combined all four *in situ* tests from 1991-92.

STATION	1	2	3	4	5	6	7
Pre-decl							
N	8	9	10	10	10	5	10
MIN	5.50	6.30	0.00	0.00	2.40	5.20	4.80
MAX	7.30	8.10	0.00	0.00	3.30	6.80	6.30
MEAN	6.71	7.40	0.00	0.00	2.79	5.92	5.77
SD	0.666	0.552	0.000	0.000	0.281	0.766	0.481
CV	9.9	7.4	0.0	0.0	10.1	12.9	8.3

SD = Standard Deviation
CV = Coefficient of Variation

Table 20. Descriptive statistics on pre-dechlorination shell length gains (mm) of caged *in situ* juvenile *Corbicula fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas during August/September 1990.

STATION	1	2	3	4	5	6	7
Dechlor[1]							
N	29	38	38	37	40	38	38
MIN	3.27	0.09	0.13	0.00	2.98	0.00	0.00
MAX	9.12	9.03	2.51	3.94	8.74	8.22	9.68
MEAN	6.51	5.68	1.21	2.61	6.52	5.98	5.81
SD	1.644	1.845	0.584	1.269	1.145	1.979	1.935
CV	25.2	32.5	48.1	48.7	17.5	33.1	33.3

[1] = data combined from all four dechlorination *in situ* tests
SD = Standard Deviation
CV = Coefficient of Variation

Table 21. Descriptive statistics on dechlorination shell length gains (mm) of caged *in situ* juvenile *Corbicula fluminea* exposed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

smallest grand mean length gains occurred at Station 3 with 1.2 mm. The minimum significant difference was between 1.4 mm and 3.1 mm. Coefficients of variation ranged from 18% to 50% for all stations during dechlorination (Table 21). Total dechlorination sample size was between 37 and 40 clams at each station except Station 1, where only 29 clams were retrieved because cages were lost in the October 1991 experiment.

Comparison with *Ceriodaphnia dubia*

The response to chlorinated and dechlorinated effluent by *in situ C. fluminea* and *C. dubia* (R. Guinn, pers communication, 1993) in chronic toxicity lab tests is illustrated in Figure 39. During the pre-dechlorination tests, both *C. fluminea* growth (6.7 and 7.4 mm/individual) and *C. dubia* neonate production (29 and 27 neonates/female) were highest and proportionately equal at upstream reference Stations 1 and 2, respectively. Within 1 mile below the outfall at Stations 3 and 4, both organisms experienced acute mortality and no neonate production or clam growth occurred. At Station 5, *C. fluminea* growth (2.8 mm) and *C. dubia* neonate production (10.1 neonates) were each approximately 60% less than the reference stations. At Stations 6 and 7, respectively, clam growth (approximately 6.0 mm for each station) was slightly lower than reference stations, while *C. dubia* neonate production (7 and 11

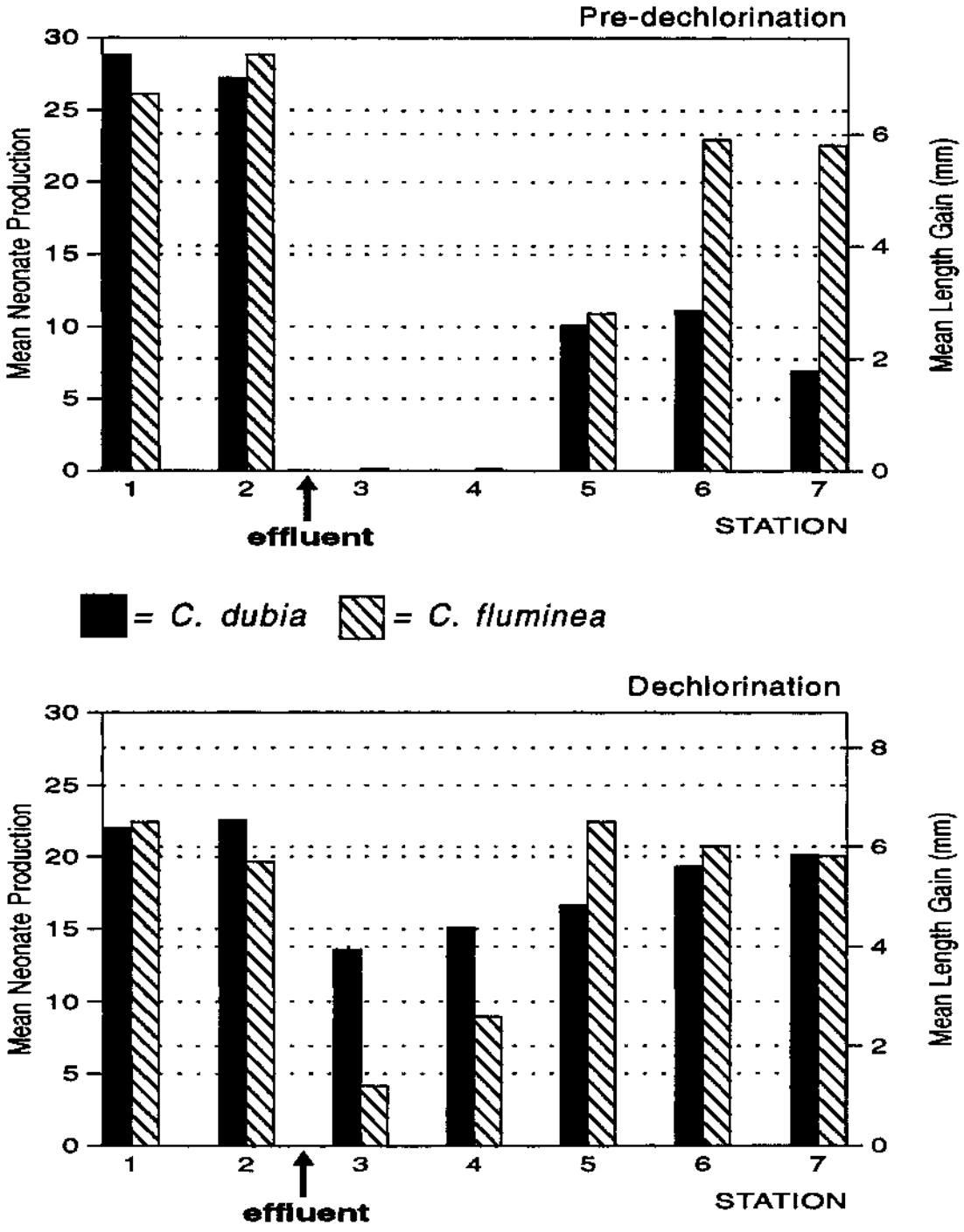

Figure 39. Comparison of responses from *Ceriodaphnia dubia* in 7-day ambient toxicity laboratory tests & caged juvenile *C. fluminea* in 30-day *in situ* tests in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas before (top) & after (bottom) dechlorination. *C. dubia* data was based on exposure to monthly grab samples from 3 samples during predechlorination & 17 samples during dechlorination.

neonates) was comparable to Station 5 and almost a third less than reference stations.

During dechlorination tests, *C. fluminea* growth (1.0 and 2.5 mm) was lowest at Stations 3 and 4, respectively, while *C. dubia* reproduction (13.5 to 16.5 neonates) was lowest at Stations 3, 4, and 5. *C. fluminea* growth was proportionately two to three times less than *C. dubia* neonate production at Stations 3 and 4. Clam growth (5.7 to 6.5 mm) and *C. dubia* production (19 to 22 neonates) were both comparable at Stations 1, 2, 6, and 7.

Growth Trends

During late summer *in situ* tests (August 1990 and 1992), *C. fluminea* tended to exhibit higher minimum, maximum, and mean shell growth compared to clams exposed during the early fall (September/October 1991 and 1992) (Table 22). Growth data was compiled from unimpacted Stations 1, 2, 5, 6, and 7. During the August tests, growth ranged from 4.8 - 8.1 mm (mean = 6.5 mm) in 1990 and 5.0 - 9.7 mm (mean = 8.0 mm) in 1992. During the September/October tests, growth ranged from 2.5 - 6.7 mm (mean = 5.2 mm) in 1991 and 3.3 - 7.7 mm (mean = 6.1 mm) in 1992. Mean ambient water temperature ranged from 28.3 °C to 28.8 °C during the summer tests and decreased to 25.0 °C to 25.6 °C during the September/October tests (Table 22). Water temperatures during the July 1991 test (28.6 °C) were comparable to the

Test Date	July/Aug 1991	August 1992	Aug/Sep 1990	Sept/Oct 1991	Sept/Oct 1992
Temperture[1] (° C)					
MIN	19.6	21.5	21.6	18.9	20.6
MAX	36.5	35.1	35.2	32.6	34.5
MEAN	28.6	28.3	28.8	25.0	25.6
SD	2.33	2.04	2.37	2.56	2.89
Clam Growth[2]					
MIN	3.8	5.0	4.8	2.5	3.3
MAX	6.9	9.7	8.1	6.7	7.7
MEAN	5.7	8.0	6.5	5.2	6.1

[1] =water temperature data was compiled from USGS gaging stations near Stations 1 & 7

[2] = clam growth data was compiled from Stations 1, 2, 5, 6, and 7

Table 22. Descriptive statistics on ambient water temperatures and *C. fluminea* growth (shell length in mm) for each *in situ* test period in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

August tests but clam growth was lower (3.8 to 6.9 mm; mean = 5.7 mm). Minimum and maximum temperatures were lower during the fall (18.9°C minimum; 34.5°C maximum) compared to the late summer (21.5°C minimum; 36.5°C maximum).

Test Date	Initial Size[1]	Growth [1]	p	R^2
July 1991	3.1 - 6.3	3.8 - 6.9	p=0.001;	R^2=0.22
Sept 1991	3.0 - 4.0	2.5 - 6.7	p=0.134;	R^2=0.06
Aug 1992	3.4 - 4.9	5.0 - 9.7	p=0.005;	R^2=0.17
Sept 1992	4.0 - 5.5	3.3 - 7.7	p=0.004;	R^2=0.17

[1] = minimum and maximum range of shell lengths (mm) from Stations 1, 2, 5, 6, and 7.

Table 23. Initial size and resulting growth of *in situ* caged juvenile *C. fluminea* placed for 30 days in the West Fork of the Trinity River, Tarrant and Dallas Counties, Texas.

Table 23 above shows the relationship between initial shell sizes used for the dechlorination *in situ* tests and associated length gains. Some *C. fluminea* sample populations at the unimpacted stations showed a trend between initial shell size and growth, where smaller initial sizes tended to grow more than larger initial sizes. Statistically significant relationships were found between initial size and growth for sample dates July 1991, August 1992, and September 1992 where initial sizes were within 1.5 to 3.0 mm of each other (p=0.001, p=0.005, p=0.004, respectively). Coefficients of determination ranged from 0.17 to 0.22. No significant growth trend was exhibited during the September 1991 test where initial clam sizes were all within 1.0 mm of each other (p=0.134 and R^2=0.06).

DISCUSSION

Pre-dechlorination

Prior to dechlorination, all caged juvenile *C. fluminea* were killed within one mile downstream from the outfall during the August 1990 *in situ* exposure. Clam shell growth was impacted 5.2 miles downstream from the outfall at Station 5 where juveniles were significantly smaller than all other stations, exclusive of Station 3 and 4 where there was 100% mortality. Chlorine appeared to be the toxicant responsible for the mortality at Stations 3 and 4 and perhaps for the reduced growth at Station 5. During August and September 1990, the average ambient total residual chlorine (TRC) concentrations were highest at Station 3 (1.1 mg TRC/l) and Station 4 (0.45 mg TRC/l) and decreased with increasing distance from the outfall (Station 5 = 0.14 mg TRC/l; Station 6 = 0.08 mg TRC/l; and Station 7 = 0.02 mg TRC/l) (R. Guinn, pers communication, 1992). Juvenile *C. fluminea* at upstream reference Stations 1 and 2 and downstream Stations 6 and 7 (7.9 and 17.3 miles below outfall, respectively) all exhibited significantly higher mean growth compared to impacted Stations 3, 4, and 5.

Chlorine exposure has been shown to cause acute and chronic toxicity in several aquatic organisms at ambient levels less than 1.0 mg TRC/l (Brungs, 1973; Brooks and Seegert, 1977; EPA, 1984). In *C. fluminea*, sub-lethal

effects to chlorine exposure were documented by Sappington (1987, as cited by Doherty, 1990) who found that significant reductions occurred in glycogen levels and siphoning behavior in clams exposed to 0.30 mg TRC/l for 30 days (twice as high as TRC measured at Station 5 where reduced growth occurred). In acute four day tests, exposures of 0.73 mg monochloramine/l resulted in significant reductions in glycogen.

Dechlorination

Results of the dechlorination *in situ* tests indicated that caged juvenile *C. fluminea* were able to survive in the Trinity River within one-mile below the dechlorinated outfall. Ambient chlorine concentrations were consistently below detection (< 0.01 mg TRC/l) at all stations downstream from the outfall after dechlorination was implemented (R. Guinn, pers communication, 1992). Periodic mortality of only 10% - 20% was observed during the 1991 season and the September/October 1992 test at upstream Station 2 and downstream Stations 3, 4, 6, and 7. There was no significant difference in survival between all seven stations during each of these sample dates.

In August/September 1992, survival was significantly reduced at Station 4 where half the clams experienced acute mortality. During the last ten days of exposure, a temporary dam structure was unexpectedly constructed 20 meters

upstream from the clams at Station 4 and may have created a physical disturbance (i.e., sedimentation, changes in river flow). However, the mortality appeared to occur soon after placement in the river because the dead clams showed virtually no growth while the live clams doubled in size. The fact that no clams died at Station 3 during the same exposure time suggests that an additional perturbation had occurred at Station 4. Station 4 was located within 30 meters downstream of a heavily traveled bridge and dumping could have potentially occurred at this location.

During dechlorination, juvenile *C. fluminea* growth improved at stations that were impacted prior to dechlorination. Before dechlorination, clams at Station 5 were significantly smaller than clams at Stations 1, 2, 6, and 7. Dechlorination recovery was apparent at Station 5 where juvenile mean length gains were typically among the two highest for each exposure period. At Stations 3 and 4, *C. fluminea* were able to survive and grow after dechlorination but the smallest clams were consistently found at both stations compared to all other stations. Mean length gains were typically four to five times higher at Stations 1, 2, 5, 6, and 7 compared to Station 3 for each test date. Clams at Station 4 usually grew twice as large as clams at Station 3. Station 4 clams experienced the highest growth during the September/October 1991 sample when mean length gains were approximately one millimeter less than

gains at upstream Station 2. Juvenile *C. fluminea* at Station 4 consistently had the second lowest growth rates for all test dates.

Minimum significant differences for shell length gains changed considerably between experiments. The minimum significant difference was less than 1.0 mm for August 1990 and July 1991 analyses, increased to approximately 2.5 to 3.5 mm for July 1991 and September 1992 analyses, and was largest for the August 1992 analysis with approximately 5.5 mm. As a consequence of this variation in minimum significant difference, some analyses appeared more conservative than others (i.e., difference required was so large that most stations were not significantly different from each other) and vice versa. For example, in the July 1991 test, *C. fluminea* mean growth was >4 mm and >2.5 mm less at Stations 3 and 4, respectively, compared to all other stations. Results from Tukey's multiple comparison test showed that Stations 3 and 4 were significantly different from each other and all other stations for the July 1991 experiment. In contrast, in the August 1992 test, clams at Stations 6 and 7 showed a mean length gain >4 mm greater than Station 3, yet all three stations were not significantly different from each other (Dunn's Multiple Comparison). These somewhat contradictory conclusions may be partially explained by the large variability that occurred at a station where an individual clam died soon after

placement in the river and consequently showed little or no growth. (These dead clams were consistently included in all statistical analyses in order to eliminate any bias among stations). Though dead clams (no growth) influenced the degree of variation at a station, "normal" growth patterns also contributed to high variability within a station. For instance, when dead clams were removed from the August 1992 data analysis, heteroscedasticity persisted because clams had a wide range of shell growth at Stations 6 and 7 and a narrow range at Station 4. Regardless of what the causes were for variability at a station, larger variances among stations translated into larger minimum significant differences required between stations.

Pre-dechlorination and Dechlorination Comparison

Improvements in caged juvenile *C. fluminea* survival and shell growth demonstrated that portions of the Trinity River study area have recovered from chlorine pollution. Juvenile clam growth at Station 5 (5.2 miles downstream from the outfall) was significantly impacted prior to dechlorination. After dechlorination, Station 5 was comparable to reference Stations 1 and 2 and downstream Stations 6 and 7.

Within one mile below the outfall, at Stations 3 and 4, *C. fluminea* survival increased from zero (pre-dechlorination) to almost 100% (dechlorination). Juvenile clams at Stations 3 and 4 exhibited shell growth during

dechlorination that was non-existent before dechlorination. During dechlorination, however, grand mean growth of clams at Station 3 (mean = 1.2 mm) and Station 4 (mean = 2.6 mm) was significantly lower compared to all other stations (mean range of 5.7 - 6.5 mm). Overall juvenile clam growth at Station 4 was twice as high as Station 3 but less than half the growth recorded at all other stations. Based on growth results, caged *C. fluminea* at Station 4 (0.7 miles below outfall) appeared to suffer less impact compared to Station 3 directly below the discharge. Clams at Station 3 may have been exposed to the effluent mixing zone while Station 4 clams were downstream of this zone.

The chlorinated effluent did not appear to impact *C. fluminea* survival or growth at Stations 6 and 7 located downstream from the outfall approximately 8 and 17 miles, respectively. During dechlorination, grand mean growth was similar (5.7 mm to 6.5 mm) among reference Stations 1 and 2 and downstream Stations 5, 6, and 7.

Dechlorination results indicate that reduced growth in caged juvenile *C. fluminea* continues to be impacted at Stations 3 and 4 by chronic toxicity still present in the dechlorinated effluent. Organophosphorus compounds (including the pesticide diazinon) are the suspected toxicants remaining in the effluent (R. Guinn, pers. communication, 1993). Diazinon has been documented as a toxicant to some aquatic organisms. In acute toxicity tests

with zooplankton, the 48-hour LC50 of diazinon was lowest for *C. dubia* with 0.35 ug/l (Norberg-King et al., 1989), followed by *Daphnia magna* (0.7 ug/l) (Dortland, 1980) and *Daphnia pulex* (0.8 ug/l) (Saunders and Cope, 1966). In 96-hour tests with *Pimephales promelas*, the Diazinon LC50 was 4500 ug/l (Jarvinen and Tanner, 1982).

The chronic toxicity present in the dechlorinated effluent could have created at least three potential scenarios that ultimately resulted in suppressed growth in juvenile *C. fluminea* at Stations 3 and 4: 1) a decrease in available food sources such as algae, protozoans, and bacteria; 2) a physiological stress that impaired a clams ability to filter food from the water or to properly ingest food; 3) a combination of 1 and 2.

During the August 1991 and 1992 dechlorination *in situ* tests, Station 3 had lower densities of periphyton (210 to 225 individuals/m^2) compared to reference Station 1 (1200 to 1420 individuals/m^2) and Stations 4 through 7 further downstream (800 to 9,000 individuals/m^2); however, periphyton at Station 3 was only slightly lower than upstream Station 2 (320 to 400 individuals/m^2). Despite similar periphyton densities at Stations 2 and 3, *C. fluminea* growth was five times higher at Station 2. At Station 4, periphyton density was five to ten times higher and clam growth was doubled compared to Station 3; however, clam growth at Station 4 remained significantly lower than

reference stations and downstream stations that had similar periphyton densities. At reference Station 2, periphyton density was lower than Station 4 but clam growth was significantly higher. The periphyton results indicate that physiological effects from toxicants in the dechlorinated effluent, rather than lack of food, played a larger part in reduced clam growth at Stations 3 and 4.

Comparison with *Ceriodaphnia dubia*

Prior to dechlorination, *C. dubia* in laboratory toxicity tests and *in situ* caged *C. fluminea* had similar responses at Stations 1 and 2 above and Stations 3, 4, and 5 below the effluent. The highest clam growth and *C. dubia* neonate production were observed at reference Stations 1 and 2. Both species experienced acute mortality at Stations 3 and 4 within one mile below the chlorinated outfall. At Station 5 (5 miles downstream), *C. dubia* production and *C. fluminea* growth were both reduced compared to reference Stations 1 and 2. Response of each test species differed at Stations 6 and 7, where impact on *C. dubia* production continued but *C. fluminea* growth improved to levels comparable to reference stations.

During dechlorination tests at Stations 3 and 4, *C. dubia* and *C. fluminea* both experienced chronic effects instead of the acute mortality observed before dechlorination. Chronic toxicity appeared to be present at

Stations 3 and 4 where productivity in *C. dubia* and shell growth in *C. fluminea* were both significantly reduced compared to all other stations. At Stations 3 and 4, *C. fluminea* may have been more sensitive to toxicity still present in the dechlorinated effluent as evidenced by the proportionately lower shell growth compared to *C. dubia* neonate production. Between pre-dechlorination and dechlorination at Station 5, *C. fluminea* growth more than doubled while *C. dubia* reproduction improved but continued to be reduced and was comparable to Stations 3 and 4. For *C. dubia*, dechlorination improvements finally occurred at Stations 6 and 7 (8 and 18 miles downstream, respectively) where neonate production doubled since pre-dechlorination tests and was comparable to reference stations.

The 30 day *in situ* exposure tests with caged juvenile *C. fluminea* and seven day lab toxicity tests with *C. dubia* were conducted during the same time periods. Despite different response endpoints, exposure periods, and test designs, both organisms exhibited similar patterns in impact and recovery at stations downstream from the effluent during pre-dechlorination and dechlorination. Both species responded to ambient toxicity present in the dechlorinated effluent as expressed through reduced growth (*C. fluminea*) and neonate production (*C. dubia*) at Stations 3 and 4.

Growth Trends

Seasonal trends in juvenile *C. fluminea* growth were noticeable between experiments conducted during late summer (August) and early fall (September/October). Mean clam growth was between 6.5 - 8.0 mm during the August tests and fell to between 5.2 - 6.1 mm during the fall tests. The slower growth rates in the fall may have been affected by a decrease of approximately three degrees in mean water temperatures between the summer (28.3°C to 28.8°C) and fall (25.0°C to 25.6°C) tests. Absolute minimum and maximum values for water temperature and *C. fluminea* growth both tended to be lower in the fall compared to the summer. McMahon and Williams, (1986) suggested that in North Texas waters, maximum growth in *C. fluminea* may occur between 25°C and 30°C.

Initial shell size of *C. fluminea* appeared to influence growth rates in some sample populations. The wider the size range between clams within a particular test, the more obvious the trend. When a sample population had initial sizes within 1.5 to 3.0 mm of each other, smaller sizes tended to exhibit more growth than larger initial sizes. Sample populations of initial sizes all within 1.0 mm of one another did not show the same trend, probably due to the narrower size range. Despite the significant trend indicated by linear regression analysis for initial size and growth, the amount of variation attributed to initial size was less

than 25% (coefficients of determination ranged from 0.17 to 0.22). Based on these results, initial size was a poor predictor of growth for juvenile clams in this study. In contrast, several researchers have documented the inverse relationship that can occur between *C. fluminea* growth rate and an individual's shell size (Aldridge and McMahon, 1978; Britton et al., 1979; Buttner and Heidinger, 1980; Doherty, et al., 1990).

SUMMARY

Effluent dechlorination by the Village Creek municipal wastewater treatment plant resulted in some improvements in survival and growth of caged juvenile *C. fluminea* at stations downstream from the outfall in the West Fork of the Trinity River. When exposed to chlorinated effluent in 1990, no clams survived within one mile below the outfall (Stations 3 and 4) and clam growth was impacted five miles downstream (Station 5). After dechlorination was implemented in 1991, *C. fluminea* survival was no longer impacted at Stations 3 and 4 and growth was not significantly reduced at Station 5. However, at Stations 3 and 4, *in situ* juvenile clams continued to exhibit significantly reduced shell growth compared to upstream reference Stations 1 and 2 and downstream Stations 5, 6, and 7. Chronic toxicity, that continued to affect juvenile *C. fluminea* growth after dechlorination, may have been caused by organophosphorus

compounds recently detected in the dechlorinated effluent (R. Guinn, pers communication, 1993).

As an *in situ* biomonitor, juvenile *C. fluminea* responded to the presence of toxicants in both the chlorinated and dechlorinated municipal wastewater effluent through the endpoints of mortality and reduced shell growth. Previous studies have successfully used *C. fluminea* as an *in situ* biomonitor of water quality, including the exposure to wastewater effluent (Dickson, et al., 1989), copper (Belanger, et al., 1990), and thermal discharges (Foe and Knight, 1987).

When exposed to ambient water from stations above and below the chlorinated and dechlorinated effluent, the responses between *C. dubia* exposed in laboratory toxicity tests and *in situ* caged juvenile *C. fluminea* were comparable. At stations directly below the outfall, both species experienced acute mortality before dechlorination and chronic effects after dechlorination was implemented. Chronic effects were more severe in *C. fluminea* than *C. dubia* when exposed to toxicants still present in the dechlorinated effluent. Some have alleged that *C. dubia* is too sensitive for use as a biomonitor for water quality. However, results from this study suggest that *in situ* juvenile *C. fluminea* and the lab test organism *C. dubia* each exhibited a similar pattern of response to acute and chronic toxicity in the effluent.

CHAPTER 5

SUMMARY

After the Village Creek municipal effluent was dechlorinated prior to discharge, significant improvements were observed in the diversity and density of immature macroinvertebrates collected on artificial substrates and the survival of *in situ* caged *Corbicula fluminea*, at stations below the outfall in the West Fork of the Trinity River. Positive responses in aquatic biota were documented directly below the effluent and within six months after dechlorination was implemented. Despite improvements after chlorine removal, other toxicants were found to occur in the dechlorinated effluent. *C. fluminea* responded to the additional toxicants through reduced shell growth but it was not possible to determine whether some taxa in the macroinvertebrate community were also impacted.

In the dechlorination macroinvertebrate samples, caddisflies and mayflies recolonized stations where they were absent before dechlorination and chironomid sub-family diversity increased. At all stations above and below the outfall, the caddis community was dominated by Hydropsychidae (three genera and four species) or

Hydroptilidae (2 genera). Polycentropodidae (1 species) occurred in minor numbers. Stations within five miles downstream of the effluent supported larger densities of hydropsychids compared to reference stations. The mayfly community consisted of five main families present upstream and downstream of the effluent. At all stations, Tricorythidae (1 genus) tended to occupy a majority of the community, Heptageniidae (2 genus and 3 species) was usually sub-dominant and Caenidae (1 genus) was the least common family. Baetidae (1 genus) and Isonychiidae (1 genus) were more prevalent below the outfall. Chironomids were identified only to sub-family but dechlorination improvements were detected at this level. Orthocladiinae and Tanypodinae increased their abundance and Chironominae decreased in dominance at all stations below the dechlorinated effluent.

The presence of significantly larger densities of macroinvertebrates below the dechlorinated effluent (especially of hydropsychid filterers) would suggest a relationship between organic loading and the municipal effluent. Total dissolved solids and nitrates (phosphorus data was not available) were two parameters that tended to have slightly higher concentrations directly below the effluent compared to reference stations. However, it was difficult to discern a pattern or make a quantitative connection between potential food supply (total dissolved

solids, periphyton, zooplankton) and invertebrate abundance. The only sample that showed a noticeable trend was during the May/June 1991 sample when Station 3 (directly below the outfall) supported the largest hydropsychid population of any station and zooplankton density was twice as high at Station 3 compared to reference stations (B. Bryan, pers communication, 1993).

During the dechlorination study, caged juvenile *C. fluminea* were able to survive within one mile and successfully grow within five miles below the effluent. Clams did consistently experience chronic toxicity within one mile below the dechlorinated outfall, possibly due to organophosphorus compounds (i.e., diazinon) detected in the effluent. At stations more than five miles below the dechlorinated effluent, *C. fluminea* tended to have slightly higher growth rates compared to stations upstream. There was no apparent relationship between clam growth and periphyton density.

When exposed to the Village Creek effluent, a similar pattern of response was observed between caged *in situ C. fluminea* and *C. dubia* in lab toxicity tests. Survival in clams and *C. dubia* were both impacted before dechlorination while clam growth and *C. dubia* production were both reduced during the dechlorination study.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Effluent dechlorination was successful in eliminating chlorine toxicity from the West Fork of the Trinity River below the Village Creek facility. Within six months after dechlorination began, significant improvements were observed in both immature macroinvertebrate community composition on artificial substrates and survival and growth of *in situ* juvenile *C. fluminea*. However, the presence of other toxicants (i.e., organophosphorus compounds) in the dechlorinated effluent were revealed through chronic toxicity experienced by *C. fluminea* (reduced growth) and *C. dubia* (reduced production). Based on the taxonomic levels used in this study, it appeared that the aquatic insect communities were not impacted by toxicants in the dechlorinated effluent. However, identification to the species level (especially for Chironomidae) could provide additional information and potentially reveal differences between stations above and below the dechlorinated outfall.

Juvenile *C. fluminea* worked well as a biomonitor of pollution in the Trinity River. *C. fluminea* responded to both acute (mortality) and chronic (reduced shell growth)

levels of toxicity originating from the effluent. As a test organism, juvenile *C. fluminea* were easy to collect and process and data interpretation was relatively straightforward. One drawback with *C. fluminea* was that the juvenile size class was not abundant in the field until mid-summer after the first major spate of young. *In situ* tests were not conducted in the spring or early summer because of this limitation. The likelihood of finding enough juvenile clams was also influenced by flooding and the resulting burial and potential mortality of young.

The upstream reference stations were not entirely unimpacted during the study. Macroinvertebrates (caddis and/or mayfly) at Station 2 appeared to be periodically influenced by unidentified toxicants. Nonpoint source pollution may have been the source of these toxicants. Station 7, 18 miles downstream from the outfall, also exhibited impacts that were not related to the effluent.

Several additions to this study could have provided a better understanding of the dynamics of the aquatic community following removal of one toxicant and the persistence of others. The following activities could potentially improve a study of this nature:

1) Conduct a survey of the freshwater mussel community in the West Fork of the Trinity River and compare with the response of caged *in situ C. fluminea*.

2) Culture *C. fluminea* in the lab so that a supply of

juveniles would be available year round for biomonitoring tests.

3) Choose macroinvertebrate indicator species that are tolerant and intolerant to chlorine; monitor presence and absence of these species before and after dechlorination.

4) Sub-sample macroinvertebrates and debris collected from artificial substrates; this would enable more time to be spent on identification and less time on sorting.

5) Identify macroinvertebrates to lower taxonomic levels (i.e., species or genus) especially for chironomids; some changes can be detected in midge communities at the sub-family level (as evidenced in this study) but valuable information on various tolerances within each sub-family are only revealed at the genus or species level.

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