ABSTRACT

How Does Material Adsorbed onto Flood-Borne Sediment Affect Lake Zooplankton?

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The effect of materials adsorbed onto flood-borne sediments on *Brachionus calyciflorus* was investigated in this study. Sediment samples were collected from the North Bosque River and South Bosque River which drain into Lake Waco for five months during flood events and low water flow. Measurements for population parameter changes were made after the rotifer cysts were subjected the materials leached off the flood-borne sediment (elutriates).

B. calyciflorus population growth and birth rates were inhibited and their death rates were stimulated when subjected to ambient elutriate concentrations containing contaminants from the North Bosque River. Population parameters remained statistically unaltered for the South Bosque River samples.

How does the Material Adsorbed onto Flood-Borne Sediment Affect Lake Zooplankton Populations?

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DEDICATION

This thesis is dedicated to my family and Michael for all their love and support.

CHAPTER ONE

Introduction

Recent studies have examined the effects of released chemical substances from flood-borne sediments on organisms in the aquatic environment (Calow 1994; Phillips and Walling 1995). Kirk and Gilbert (1990) studied the effect of fine suspended clay particles containing contaminants on rotifers. They found that rotifers were sensitive to the clays (Table 1). In general, nutrients stimulate and toxic pollutants inhibit planktonic communities. Marzolf (1989) found that zooplankton life expectancy declined after eating dissolved organic protein adsorbed onto clay particles. This thesis examines the effect of sediment-borne nutrients and/or pollutants on *Brachionus calyciflorus*. The objective of this thesis is to estimate the suppression of (1) population growth, (2) reproductive rates, and (3) death rates, and (4) monitor any induced cyclomorphotic spine length alterations through continuous exposure to chemicals extracted from flood-borne sediments.

Sediment transport and deposition is a process in reservoirs that significantly influences the population dynamics of the aquatic system. The physical accumulation of sediment in reservoirs alone would indicate

Table 1. Effects of suspended clay on the population growth rate (r_m) of four species of rotifers.

		Fine clay		Concentration	
		Control		50-100 mg/L	
Rotifer species	Food concentration (cells/ml)	X ± SE	n	X ± SE	n
B.calyciflorus	2000	.32 ± .2	5	.03 ± .1 s	5
K. cochlearis	5000	.22 ± .01	5	.24 ± .01 ns	5
K. crassa	2500	.27 ± .01	5	.04 ± .1 s	5
S. pectinata	2500	1.08 ± .1	4	1.01 ± .05 ns	4

s= $p<.05$ (t tests of control vs. each clay treatment)

Kirk and Gilbert 1990

this potential importance in ecosystem structure and function.
Sediment is not only the major water pollutant by weight and volume but is also a major carrier and catalyst for nutrients, pesticides, organic residues, pathogenic organisms, and other pollutants (Thornton et al. 1990). The nutrient/pollution interaction is complex and not very well understood.

Watershed runoff influences the quantity and quality of material delivered to an aquatic system. The larger drainage basins associated with reservoirs may result in greater annual flows entering reservoirs than lakes. Larger drainage basins and greater flows also indicate the potential for greater sediment and nutrient loads to reservoirs. As watershed size increases, the potential for interception and/or deposition of transported particulate matter increases, so the sediment delivery ratio is inversely proportional to the watershed area (Bishop 1977, Thornton et al. 1990). However, since the relationship between drainage area and sediment delivery ratio is logarithmic and not linear, the absolute quantity of sediment and its adsorbed constituents delivered continues to increase with increased drainage area.

The two main sites in this study, North and South Bosque Rivers, drain directly into Lake Waco, the main water supply for the city of Waco and a flood control reservoir built by the U.S. Army Corps of Engineers. The Bosque River Basin is located in central Texas and drains an area of approximately 4297.28 km^2. The watershed is primarily rangeland (55%), with cropland and pastureland making up roughly 38% of the remaining land use (Table 2). The basin also has dairy farms near the headwaters of the basin, which produce elevated nitrate concentrations in the rivers. Soils are primarily clay and silty clay in the uplands of the watershed, with gravely clays and clay-loam in the lowlands near the stream channels (Bishop 1977). Hydrologically, the North and South Bosque basins exhibit a rapid time-to-peak during storm events due to the high runoff potential of the clay soils and the extensive drainage network.

Knowledge about suspended sediments from contributing watershed soils plays an important role in studying contaminants and/or nutrients in river systems for multiple reasons (Thornton et al. 1990). Fine silt and clay particles have a high adsorptive capacity for phosphorus, dissolved organic acids and other nutrients or contaminants. Because stream processing increases the concentration of fine particulate organic matter, reservoirs receive large proportions of fine particulate matter and dissolved organic matter from the surrounding basin (Bishop 1977, Brady 1990, Thornton et al. 1990).

Rainfall initiates sediment transport from the watersheds into the rivers. A high concentration of suspended clay and silt particles appear as an effect of floods or elevated flows carrying a large quantity of clay

Table 2. Components of the Bosque Basin watershed.

Description	% Coverage
Urban	1.64
Other Populated Land	1.40
Highways	0.57
Cropland	20.16
Cropland, Irrigated	0.13
Pastureland and Hayland	17.51
Pastureland and Hayland, Irrigated	0.19
Horticultural Land	0.06
Horticultural Land, Irrigated	0.03
Confined Feeding Operations	0.12
Open Rangeland	30.89
Brushy Rangeland	25.49
Water	0.95
Farm Ponds	0.20
Swamp	0.04
Strip Mines	0.18
Recreation Land	0.45

Referenced from the internet: http\\www.txwww.cr.usgs.

into the lake. Sediment, particulate organic matter, and adsorbed constituents are readily washed into the stream during storm events and transported downstream to the reservoir.

There is a finite amount of energy in rainfall that determines the rate of erosion and transport of particulate matter from the watershed to the stream (Thornton et al. 1990). For rivers this transport may occur through a series of storm events with intermittent periods of deposition and processing in the stream between storm events. Thunderstorms are the most common type of flood-producing storm in the Bosque basin (Bishop 1977, U.S. Army Corps of Engineers 1970, U.S. Department of Commerce 1995).

There is a strong seasonal component to suspended solids transport. This seasonality is a function of watershed land-use. A variety of tillage, planting, and other agricultural practices during the year result in varied nutrient/toxin contribution into the bed-load (Thornton et al. 1990).

The suspended inorganic particles have direct and indirect effects on the ecology of turbid water. Inorganic suspended sediments suppress the survivorship and fecundity of filter-feeding zooplankton and especially B.*caylciflorus* (McCabe and O'Brien 1983, Zurek 1982). Figure 1 is a diagram of probable direct and indirect effects of suspended sediments on some major groups of planktonic organisms. Suspended

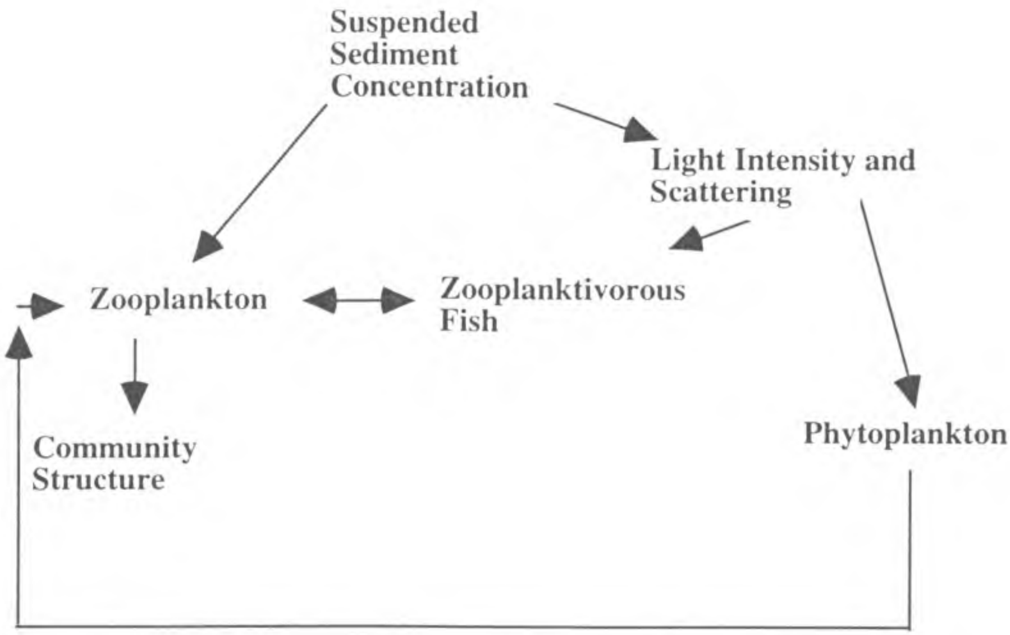


Figure 1. Diagram of probable direct and indirect effects of suspended sediments on some of the major groups of planktonic organisms.

sediments affect zooplankton populations directly primarily by inhibiting the ingestion of phytoplankton (Arruda et al. 1983, McCabe and O'Brien 1983, Zurek 1982). Gliwicz (1986) and Kirk and Gilbert (1990) found that contaminants adsorbed onto clays affect the population dynamics of filter-feeders. The organic compounds bound to the clay particles are a main food source for filter-feeding zooplankton in the lake. Filter-feeding zooplankton reduce the abundance of clay suspended in the water.

In nature, it is difficult to distinguish the effect of sediment-borne materials on zooplankton because of other concomitant direct and indirect effects of clay suspensoids. For example, suspended sediments may indirectly affect zooplankton populations by affecting other components of the planktonic ecosystem. These effects are mediated by an increase in light attenuation and scattering (Kirk 1985). Increased inorganic turbidity decreases light penetration, which in turn decreases phytoplankton primary production and biomass (Grobellar 1985, Hoyer and Jones 1983). Phytoplankton comprise one of the main food sources for rotifers and other zooplankton, and are often present at growth-limiting concentrations (Edmondson 1965, Gliwicz 1986, Hbrackova-Esslova 1963, Pact et al. 1983, Tessier 1986, Tessier and Goulden 1982, Weglenska 1971). Thus, any decrease in phytoplankton abundance may affect zooplankton populations.

Another possible indirect effect of suspended sediments on zooplankton populations occurs via the effect of suspended sediments on visually-searching zooplanktivorous fish. *B. calyciflorus* account for approximately 50% or more of the zooplankton production in many freshwater systems which makes them an important food source for fish as well as other rotifers, copepods, malacostracans, and insect larvae (Covich and Thorp 1991, Hoff and Snell 1993). *B. calyciflorus* are suitable food for larval fish because they inhabit littoral zones where the larval fish tend to hide from larger predatory fish (Hoff and Snell 1993). Increased inorganic turbidity decreases the ability of the zooplanktivorous fish to locate their prey and thus decreases their predation rate on these prey (Gardner 1981, Vinyard and O'Brien 1976). The turbidity in suspended sediment concentrations makes field observations of predator and prey interactions difficult (Gardner 1981).

The existence of strong indirect effects of suspended sediments on zooplankton population is the primary justification for the use of a controlled laboratory approach. In the laboratory, the direct effect of suspended sediment can be observed in isolation from its indirect effects. If laboratory experiments are designed using conditions similar to those in the field, then their results can explain field observations and experiments. For example, if the minimum suspended sediment concentration at which various direct and indirect effects become important is known from laboratory experiments, then the results of a given set of field observations could be interpreted in terms of the relative importance of various direct and indirect effects on the zooplankton community.

The disadvantages of a laboratory approach stem from the same source as its advantages. The laboratory approach is unlikely to yield predictive data appropriate to the field situation where the concentrations and effects of toxins may fluctuate either by changes in the pollution input or by natural variations in the properties of the site itself such as flow, pH, or temperature. Thus, the precise application of laboratory experiments to any given set of field observations depends on knowledge of all the important factors operating in the field and of how they interact. The applicability of laboratory experiments to field situations can be increased by studying the effect of more than one factor on zooplankton populations, and by studying how those factors interact in the laboratory. The way forward is to improve our knowledge of the relative effects of magnitude, duration, and frequency of exposure by controlled laboratory experimentation. (Seager and Maltby 1989).

CHAPTER TWO

Literature Review

Sediment Toxicity Research

A developing concern over the quality of materials adsorbed onto sediments in water and the effect they have on the ecosystem has resulted in a rapid increase in sediment toxicity research (Kortelained 1994). Very little is known of the mechanistic basis of responses and recovery of aquatic organisms and communities exposed to pulses of common pollutants after heavy rainfall. Little is known about how aquatic organisms and communities respond to the physical and chemical changes in receiving waters brought about by transient pollution events. The stimulatory and/or inhibitory effects related to the relationship of nutrients and pollution adsorbed onto flood-borne sediments have not been studied thoroughly (Seager and Maltby 1989). To study flood-borne sediment it is important to study these factors: the type and concentration of contaminants, the location of the contaminant in the sediment, the chemistry of the sediment, and the original source, terrestrial or aquatic, of the sediment (Moll and Mansfield 1991).

A. Source of Sediment Pollution Transport

Pollution may enter a water system from many sources. Natural pollution may occur as vegetation and organisms in and along the edge of the water die and decompose. Contaminants may also enter the water as urban or rural runoff or municipal waste discharge. Agricultural and industrial plant discharge introduces many exotic, persistent compounds into the water. Contaminants also enter water by direct application or inadvertent drifting of droplets and vapors via storm runoff and seepage. The amounts and frequency of rainfall or irrigation water affect the volume of runoff, leaching and transport of these toxic chemicals. In cases of rainfall, they are carried into streams where they become a factor in the aquatic environments (Calow 1994, Phillips and Walling 1995). With time, most pollution entering a waterway can be assimilated into the system and organisms will either acclimate, disappear, or evolve to the extent to which they are capable of degrading even the most resistant compounds.

When the pollutant load entering a water system exceeds the assimilative capacity of the system, contaminant concentrations build up. Pollutants are transported throughout the ecosystem through the tissues of animals that have come in direct contact with the chemicals or feed on contaminated plants or animals. Contaminants are lost through volatilization, degradation by chemical and biological processes, removal in runoff water when in solution, or attached to soil particles or organic materials. The surface sediments are then a very critical source of potentially harmful pollution (Jordan 1979).

B. Source of Flood Events

Thunderstorms, tropical cyclones, and tropical waves are the three major types of flood-producing storms which may reasonably be expected within the Bosque basin. Each such storm is capable of producing extensive flooding within the basin (Bishop 1977, U.S. Corp of Engineers 1970).

Tropical cyclones develop mainly over open warm water and decrease in intensity as they move inland. They sometimes deliver floods of greater magnitude than any other atmospheric disturbance. The Bosque basin is not immune from tropical cyclone-derived flood waters because of its distance inland from the Texas Gulf Coast (Bishop 1977, U.S. Corp of Engineers 1970).

Tropical waves represent the greatest potential threat of dangerous flood-producing rains in central Texas. They produce tropical cyclone-like rainfall amounts and are more common than tropical cyclones. However, with the utilization of upper air charts and satellite photographs they can be easily tracked. Tropical waves are essentially weak troughs of low pressure. Their movement is east to west and the place or origin of waves affecting central Texas is the southern periphery of the Azores-Bermuda high (Bishop 1977, U.S. Corp of Engineers 1970).

Tropical waves are common in Texas from June through September with highest incidence in August and September (Report for the Brazos River Authority 1976). Their effects are normally confined to the Coastal Plain because on moving inland they are altered by orographic and diurnal factors. Stronger waves, however, do affect inland areas and "waves that reach the Balcones Escarpment are profoundly influenced by this orographic barrier". The result is heavy rainfalls in areas immediately adjacent to the scarp (Bishop 1977).

Thunderstorms are the most common type of flood-producing storm in the Bosque basin. They produce floods of short duration and initial high intensity. While all areas have experienced rainfalls and floods which are record setting for the specific region, central Texas has been the locus of rainfalls unequaled in magnitude or intensity in the coterminous United States (Bishop 1977). However, because of short duration and initial high intensity, they commonly produce flash floods of moderate volume and short duration. They do not normally produce floods of such magnitude as to fill the flood storage capacity of Lake Waco and therefore do not represent major flood threats to the City of Waco (U.S. Army Corp of Engineers 1970).

A. Development of Toxicity Test Techniques

In the broadest sense, a toxicity test is a determination of the biological effects of some substance or environmental condition and includes the use of organisms to detect or to measure the concentration of substances or to indicate the nature of the physical conditions in the environment (Jordan 1979). Toxicity tests determine the concentration of a chemical which causes either death, or some altered physiological process reflecting interference with the normal life cycle of the test organism. Toxicity data provide information about the mechanisms of toxicity, synergistic or antagonistic interactions with various environmental parameters and the overall impact of stresses with respect to mortality, growth, reproduction, respiration, and behavior (U.S. EPA 1981.) Originally, toxicity tests were short-term tests used to determine the possible harmful effects new chemical compounds or drugs could have on humans as evidenced by their effects on laboratory animals. In the early 1900's the first water quality related bioassays were done (Jordan 1979). In 1937, Ellis (1937) described the effects of various concentrations of a great number of substances on aquatic life. He provided a rationale for the use of standard test animals in aquatic bioassay procedures. Currently, the 19th edition of *Standard Methods* *for the Examination of Water and Wastewater* contains detailed procedures used for conducting bioassays on a wide variety of aquatic organisms including: algae, phytoplankton, zooplankton, coral, annelids, crustaceans, aquatic insects, mollusks, and fishes.

Toxicity tests have been performed for well over a hundred years. Unfortunately, most data collected before 1951 are virtually useless due to the lack of a standardized technique that allows comparison. In 1951, however, Doudorooff evolved such a protocol that details the procedure to be followed and emphasizes knowledge of test water quality .

Toxicity tests are useful for a variety of purposes that include determining: (a) suitability of environmental conditions for aquatic life, (b) favorable and unfavorable environmental factors, such as DO, pH, temperature, salinity or turbidity, (c) effect of environmental factors on waste toxicity, (d) toxicity of wastes to a test species or organism, (e) relative sensitivity of aquatic organisms to an effluent or toxicant, (f) amount and type of waste treatment plans needed to meet water pollution control requirements, (g) permissible effluent discharge rates, (h) compliance with water quality standards and regulations, effluent requirements, and discharge permits. In such regulatory assessments, toxicity data are used in conjunction with receiving water and site specific discharge data on volumes, dilution rates, and exposure times and concentrations .

B. Chronic Toxicity Tests

A chronic toxicity test involves a stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. "Chronic" should be considered a relative term depending on the life span of an organism. A chronic toxic effect can be measured in terms of reduced population growth, reduced reproduction, etc., in addition to lethality .

C. Elutriate Technique for Bioassessment of Sediment Contaminants

In order to find out the impact of resuspended sediment-borne substances, nutrients and/or toxicants on *B. calyciflorus*, I used the Elutriate Test. The Elutriate Test was developed as a broad-spectrum leaching procedure primarily to determine the solubility of contaminants subject to release when dredged sediments were deposited in open water. The method was subsequently formalized and promulgated as the Standard Elutriate Test by the EPA in 1977 to study the potential liberation and toxic effects of contaminants during resuspension (Daniels et al. 1989, Davalos-Lind 1996).

D. Test Organisms

In studying the biological response of an aquatic system to a particular stress, several members of various trophic levels within the ecosystem should be studied, if possible, to accurately define the effects of the stress (Patrick 1950). The prime considerations in selecting test organisms are: their sensitivity to the factors under consideration; their geographical distribution, abundance, and availability within a practical size range throughout the year; their recreational, economic, and ecological importance and relevance to the purpose of the study; their abiotic requirements and whether these requirements approach the conditions normally found at the study site; the availability of culture methods for rearing them in the laboratory and a knowledge of their physiological and nutritional requirements; and their general physical condition and freedom from parasites and disease. Test species should be endemic, ecologically significant, sensitive to pollutants and easily cultured in the laboratory (Jordan 1979).

Brachionus calyciflorus as Test Organisms

A. Life History and Reproduction

In the past five years, rotifers have become vastly used bioassay organisms. *B. calyciflorus*, who are one of the smallest metazoans at 350 um, are valuable bioassay organisms because they are easily cultured and maintained in the laboratory. Rotifer cysts can be purchased inexpensively through Florida Aqua Farms, Inc. When placed in the

proper environment, *B. calyciflorus* cysts hatch synchronously in 72
hours at 25°C with continuous fluorescent lighting. The hatchlings come out swimming and begin rapid asexual reproduction. In absence of a stimulus, *B. calyciflorus* can produce thousands of identical offspring through parthenogenesis (Figure 2). Female life spans at 25°C are 6-8 days. They have remarkable reproductive capacity. They produce their first offspring when they are about 18 hours old and continue producing throughout their lives. Lifetime fecundity for a single asexual female is 20-25 daughters if food supply is adequate and water quality is good. During peak production, days 1-4 of a female's life, eggs are extruded every 4-6 hours and hatch after another 12 hours. These high fecundities and short developmental times give rotifers some of the highest population growth rates recorded for animals (r= 0.7 to 1.4 offspring/ female/ day) (Hoff and Snell 1993). Given the ability to reproduce rapidly, rotifers may account for 50% or more of the zooplankton production, depending on the prevailing conditions (Covich and Thorp 1991). This production, in turn, can be an important food source for other rotifers, cyclopoid and calanoid copepods, malacostracans, insect larvae, and fish.

B. Functional Role in the Ecosystem

Rotifers play an important role in aquatic ecosystem nutrient

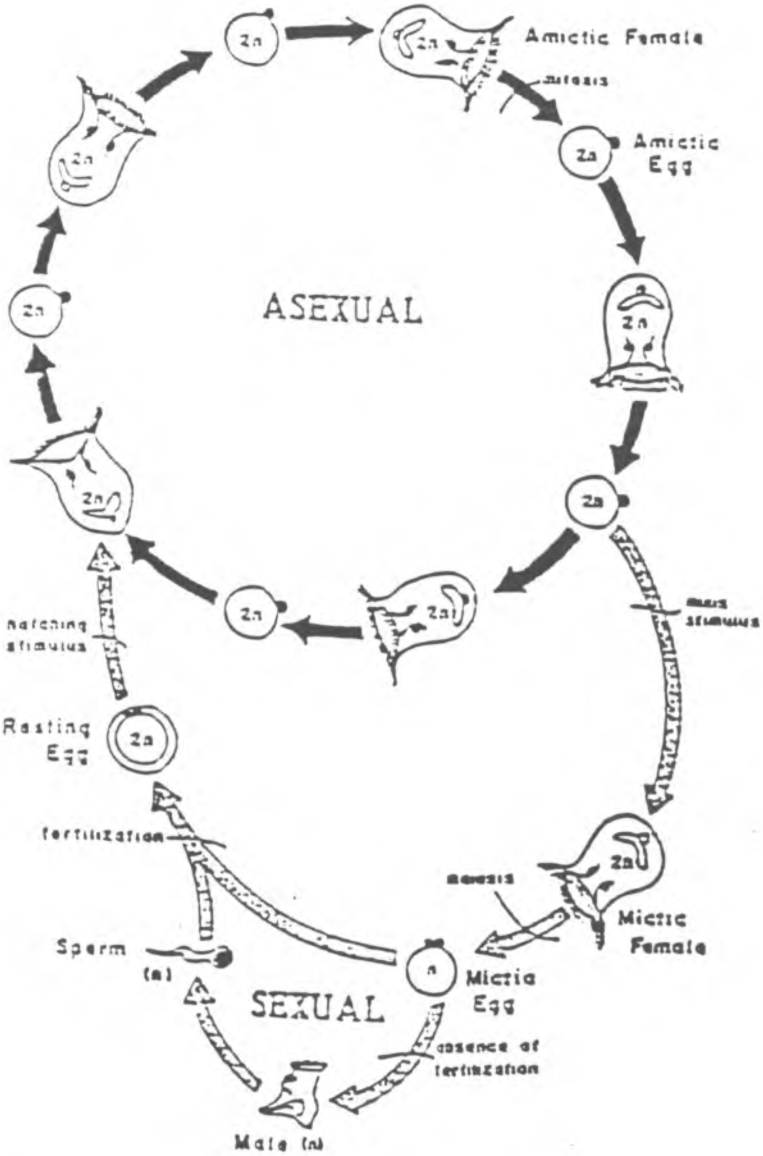


Figure 2. Rotifer life cycles illustrating the combination of asexual and sexual reproduction.

(Hoff and Snell 1987)

cycling. They are cosmopolitan and common in eutrophic ponds and lakes like Lake Waco. They live as suspension feeders (Halbach 1979). Their grazing rates can be seasonally intense, sometimes affecting algal species composition and water quality. They convert a large portion of food into biomass, making it available for higher trophic levels. All carnivorous fishes pass through an early growth stage in which zooplankton are the major source of food. Rotifers can exceed 5000 individuals per liter in some habitats (Covich And Thorp 1991). Upon reaching larger size and becoming piscivorous, large fishes are more or less dependent upon the plankters, for the small fishes taken as food usually feed regularly upon the microscopic forms (Reid 1976). Feeding rates on zooplankton generally are referred to as filtration or clearance rates and are measured as microliters of water cleared of a certain food type per animal per unit time (i.e., ul/animal/hr). The clearance rates are commonly between 1 and 10ul/ animal/hr, whether determined in the laboratory or in the field. However, a few species can achieve levels exceeding 50 ul/animal/hr (Bogdan and Gilbert 1987, Bogdan et al. 1980, Wallace and Starkweather 1985). Using estimates of clearance rates, Starkweather (1987) noted that even moderately sized rotifers with body volumes of about 10^{-3} ul process enormous amounts of water with respect to their size: >1000 times their own body volume each hour. Ingestion rates (biomass consumed per animal per unit time) are also very high for rotifers. An adult rotifer may consume food resources equal to ten times its own dry weight per day. Therefore, by having assimilation efficiencies (i.e., assimilation divided by ingestion) of between 20% and 80%, rotifers convert a good deal of their food to animal biomass, which may be passed on to the next trophic level (Starkweather 1980, 1987).

The abundance and species composition of rotifers often reflect the trophic status of lakes. For example, Hillbricht-Ilkowsha (1983) Walz (1987) and others have reported changes in the maximal total population density of several orders of magnitude when lakes were subject to intense eutrophication. Individual species sometimes undergo dramatic population changes during those periods. Walz et al. (1987) showed that the density of rotifers in Lake Constance increased its maximum population levels 280-fold over a period of 28 years. Population declines also have been seen. Edmondson and Litt (1982) indicated that rotifers were abundant during the years when Lake Washington had elevated concentrations of dissolved phosphorus, low water transparency, and high algal densities. However , as these water quality parameters improved, the population of rotifers declined dramatically. Overall, there was at least a 20-fold increase and then decline during a period of 15 15 years (Covich and Thorp 1991).

C. Environmental Toxicology

Because rotifers fill an important ecological role and are relatively easy to raise in the laboratory, interest is growing in their use for aquatic toxicity testing. The response of rotifers to a variety of toxicants has been characterized in both natural and laboratory populations. For example, effects of various insecticides and herbicides on natural rotifer population have been investigated (Hurlber et al. 1972, Kaushik et al. 1985). In general, rotifers seem to serve as good indicators of environmental water quality and the use of rotifer population dynamics as sensitive indicators of toxicity has been promoted (Halbach 1984, Halbach et al. 1981, 1983). Many short-term, acute toxicity tests with a variety of substances have been conducted using rotifers, e.g., insecticides, heavy metal, free ammonia, sodium dodecyl sulfate (Snell and Persoone 1991).

B. calyciflorus are sensitive to many chemicals and toxicants. Snell et al. (1991) found that *B. calyciflorus* sensitivities to toxicants can span about five orders of magnitude. With respect to the metals, *B. calyciflorus* was most sensitive to silver and least sensitive to selenium. Silver, tributyl tin, copper, and mercury were very toxic, whereas zinc, cadmium, nickel and selenium were only moderately toxic. Aluminum and lead were not toxic at their solubility limits in standard freshwater. *B. calyciflorus* was sensitive to the pesticide PCP and the herbicide 2,4-

D. *B. calyciflorus* sensitivity to 1-chloro-2,4-dinitrobenzene (CDNB)
and sodium dodecyl sulfate (SDS) was similar to that to PCP. Sodium hypochlorite (NaOCl) was highly toxic, whereas free ammonia, chloroform, acetone, hexane, dichloroaniline, and diesel fuel were only moderately toxic. The pesticides fenitrothion and chloropyrifos (Dursban) were quite toxic to *B. calyciflorus*, but the organophosphate trichlorofon was moderately toxic. The low coefficients of variation for each compound indicate a high degree of reproducibility for *B. calyciflorus* acute toxicity tests.

B. calyciflorus are excellent test organisms for ecotoxicology studies because of their role in the trophic level of the food chain. They are filter-feeders who feed on microalgae such as *Chlorella vulgaris* and *Selenastrum capricornutum.* Kasai and Hatakeyama found that these two green algae are susceptible to common commercial herbicides used on pest plants and, pecan orchids, and crop plants. Since *B. calyciflorus* can not detoxify such chemicals, they must store them. This can cause a biomagnification effect when predatory fish prey on the "toxic" rotifers and larger fish feed on the then "toxic" smaller fish (Kasai and Hatakeyama 1993, Cambell 1995, Kirk and Gilbert 1990, Janssen et al. 1994, Snell et al. 1991).

Toxicants in the Aquatic Environment

In a natural water system, pollutants are discharged from a variety of sources including: municipal and industrial wastewater outfalls, nonpoint sources, accidental spills and dredged material disposal (Qasim 1979). The pollutants may settle out near their discharge points or may travel downstream for a considerable distance adsorbed to fine particles.

Engler studied the chemical pollutants which may be associated with various sediment phases and reported that:

A particular element or molecule can be present (be partitioned) in a sediment in one or more of several locations. The possible locations include: (a) the lattice of crystalline minerals, (b) the interlayer positions of phyllosilicate (clay) minerals, (c) adsorbed on mineral surfaces, (d) associated with hydrous iron and manganese oxides are hydroxides which can exist as surface coatings or discrete particles, (e) absorbed or adsorbed with organic matter which can exist as surface coatings or discrete particles, and (f) dissolved in the sediment interstitial water. These locations also represent a range in the receiving water. This range extends from stable components in the mineral lattices, which are essentially insoluble, to soluble compounds dissolved in the sediment interstitial water, which are readily mobile (Engler 1976).

Several investigators suggest that the release or resuspension of pollutants into the water when the sediment is disturbed is a complex function of the sediment soil characteristics and the temperature, pH, redox potential and concentrations of other ions and compounds in the water and sediments (Engler 1976; Gambrell et al 1996; Patrick et al 1996; Chen et al 1986). Traditional physical-chemical analyses may yield considerable quantitative data, but investigators can not use these data directly to predict the bioavailability or toxicity of the resuspended contaminants to aquatic organisms.

CHAPTER THREE

Study Area and Background

The Bosque River Basin is located in central Texas and drains an area of approximately 4297 km^2. It's four main rivers, North Bosque River, South Bosque River, Middle Bosque River, and Hog Creek drain directly into Lake Waco, a flood control reservoir used as the main water supply for the city of Waco and built by the U.S. Army Corps of Engineers (Report prepared for the Brazos River Authority 1976). The watershed is primarily rangeland (55%), with cropland and pastureland making up roughly 38% of the remaining land use (Report prepared for the Brazos River Authority 1976). The basin also has dairy farms near the headwaters of the basin, and these areas produce elevated nitrate levels in the rivers due to the agricultural runoff. Soils are primarily clay and silty clays in the uplands of the watershed, with gravely clays and clay-loam in the lowlands near the stream channels (Bishop 1977). Hydrologically, the basin exhibits a rapid time-to-peak during storm events due to the high runoff potential of the clay soils and the extensive drainage network.

A. Sample Sites

Because of suspected water quality problems, one sampling site on the South Bosque River and two sites along the North Bosque River were selected. The South Bosque and North Bosque rivers were selected since they receive influences from two types of watershed soils, Blackland Prairie and the Grand Prairie, respectively. The South Bosque River samples were taken from Old Lorena Road bridge in McLennan County, Texas at approximately Latitude 31° 31' 00", Longitude 097°15'00". The North Bosque Delmar Ranch samples were taken one mile downstream from a U.S.G.S. gage station in Bosque County, Texas at approximately Latitude 31° 40' 10", Longitude 97° 28' 09". The North Bosque Hico samples were taken near a U.S.G.S. gage station in Hamilton County, Texas at Latitude 31°58'41", Longitude 98°02'04".

The South Bosque River has a drainage area of 1000 km^2. Industrial and municipal utilities from McGregor contribute to the runoff into South Bosque River. Much of the sediment runoff into the South Bosque waters comes from the Blackland Prairies (Bishop 1977, Report prepared for the Brazos River Authority 1976). Much of the soil is undulating alkaline to slightly acid from the Austin-Stephen-Eddy Association. Soils are deep to shallow darker, calcareous, clayey soils over chalk or limestone. Most of the land adjacent to the river is used for row feed crops. A portland cement factory is located in the immediate vicinity of the South Bosque site. The rest of the land is used for a landfill, residential and municipal purposes (Bishop 1977).

The North Bosque River is the principal contributor to Lake Waco. The two sites sampled were in Hico in Hamilton County, Texas and Delmar Ranch which is in Bosque County, Texas. Hico has a drainage area of approximately 930 km^2. Delmar Ranch has a drainage area of approximately 2968 km^2. The North Bosque River has its headwaters in the open, gently rolling terrain. The North Bosque River flows southeasterly thorough low hills and exposed chalk and limestone escarpments and terraces in the Grand Prairie area. The narrow stream bed and banks are characterized by outcroppings of limestone and clay strata (Bishop 1977).

Approximately 70% of the North Bosque basin is devoted to livestock grazing. Many pastures and meadows in the bottomlands have been planted in coastal bermudagrass for beef and dairy herds. And approximately 30% of the area is cultivated for row feed crops. Most of the industry in this area is agriculture and supportive businesses. (Report prepared for the Brazos River Authority 1976)

B. Soil Composition of Sample Sites

Figure 3 details the soil outlay within the entire Bosque Basin. The South Bosque consists of mainly slowly permeable shale and some

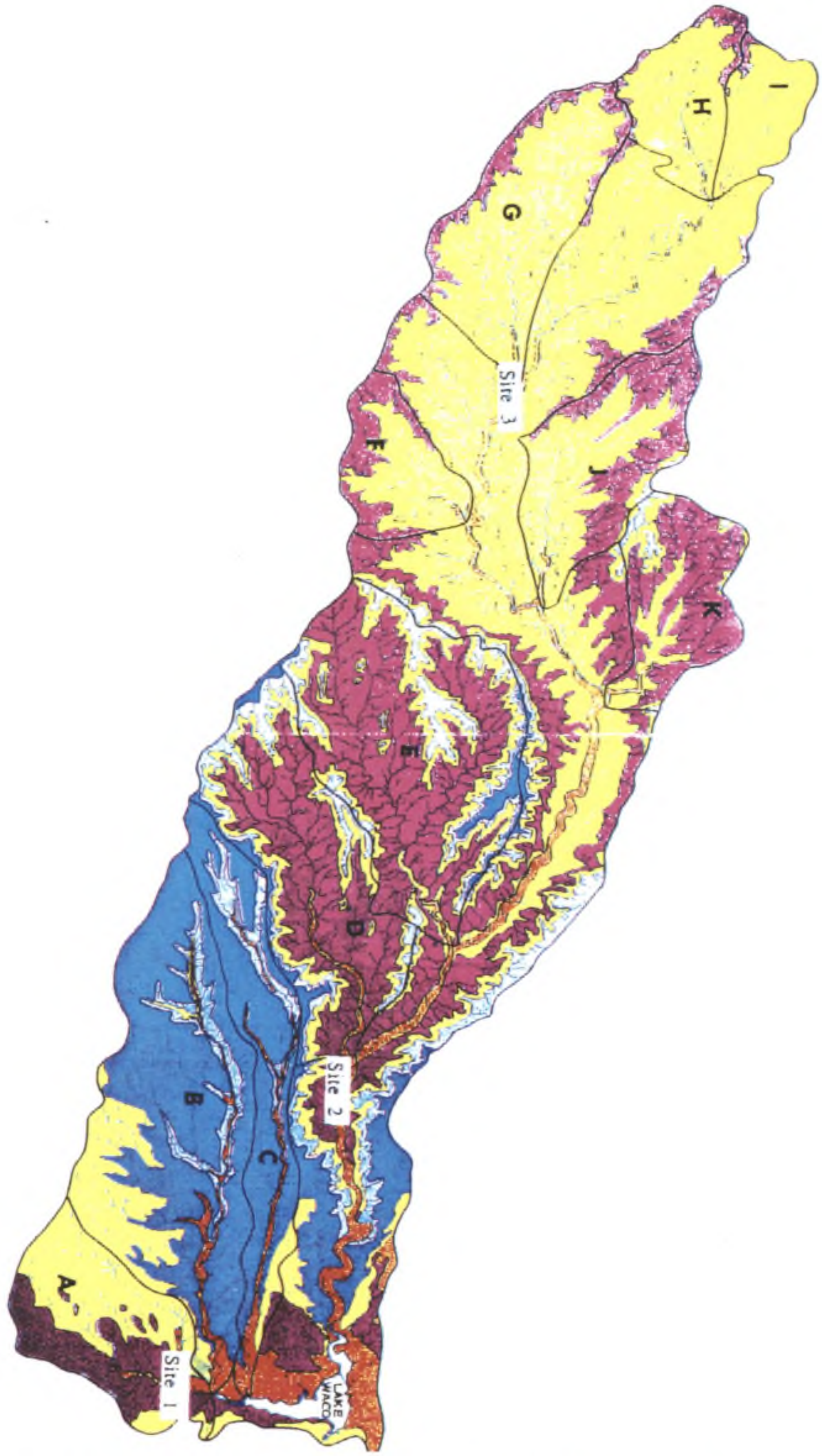


Figure 3. Sample sites and soil composition along the Bosque basin.

(Bishop 1977)

more permeable sand and gravel. Shale has high runoff potential.
It's soils having very slow infiltration rates when thoroughly wetted. The more permeable sand and gravel have a more moderate infiltration rate when thoroughly wetted. The soils are moderately fine to moderately coarse in texture.

The North Bosque at Delmar Ranch consists of permeable sands and gravel, slowly permeable limestone, and some slowly permeable shale. The sands and gravel have moderate infiltration rates. The slowly permeable limestone and shale have a high runoff potential but slow infiltration rate when thoroughly wetted. The soils are more coarse than the South Bosque.

The North Bosque at Hico contains mostly limestone. Limestone is slowly permeable and has high runoff potential. The soils at Hico are very coarse.

CHAPTER FOUR

Methods

It was my purpose to assess the influence of contaminants and/or nutrients adsorbed onto flood-borne suspended sediment on *Brachionus calyciflorus* populations. I accomplished this by measuring the population growth rate, birth rate, death rate, and cyclomorphosis of spine lengths. The population dynamics and spine lengths were measured for rotifers exposed to ambient 1:1 (water volume: sediment weight) conditions, and 2:1 and 4:1 dilutions.

A. Field Procedures

A number of physical characteristics of floods are important in considering the impact of flooding on aquatic organisms and therefore determine the sampling times: the frequency of flooding; peak flow (magnitude); total flood runoff volume (the discharge above an arbitrary baseflow); the rate of discharge increase or decrease; the lag time (the time between the center of mass of a rainstorm and the center of mass of associated runoff (Cooke and Doornkamp 1974).

In addition, the sediment load is an important physical characteristic of floods. Direct runoff is the primary method of distributing sediment to the river during rainfall. Direct runoff is the volume of water and sediment mixture production produced from rainfall. Direct runoff includes surface runoff and the interflow which moves through the soil within the watershed. Baseflow is the volume of water and sediment mixture representing the groundwater contribution (Arnold et al. 1993, Gburek et al. 1991, Gordon et al. 1992). I took suspended sediment samples during both floodflow and regular flow events for comparison.

I used a hydrograph to determine when to sample during floodflow for the two stations at the North Bosque River (Table 3). The hydrograph is a graph showing level, velocity or discharge of water in a river channel plotted against time (Figure 4) (Bowen 1982). Figures 5 and 6 are hydrograph separation graphs that were generated with the U.S.G.S gage discharge data and filtered through the FILTER software program. This software "filters" the data three times and gives a baseflow assessment. For example, in Figure 5, the baseflow during the month of September in 1996 was approximately 2000 cfs.

I collected samples three times for the South Bosque site, 4/8, 8/29, 9/19, four times for the Delmar Ranch site, 4/8, 5/31, 8/19, 9/19, and five times for the Hico site, 4/8, 5/31, 8/17 8/29, 9/19 during the 1996 season. Table 3 indicate the discharges

Table 3. Sampling dates and their corresponding water discharges.
*The South Bosque site lacked a U.S.G.S discharge gage. Low water flow and high flow were estimated for the South Bosque River site by water depth. The two North Bosque River sites flow patterns was estimated by hydrographs (figures 6 and 7).

Site	Sampling Date	Discharge	Description of Flow
South Bosque	4/8/96	1 ft *	low flow
	8/29/96	2.36 ft *	
	9/19/96	4.20 ft *	high flow
Delmar Ranch	4/8/96	136 cfs	low flow
	5/31/96	304 cfs	
	8/29/96	14,243 cfs	high flow
	9/19/96	1203 cfs	high flow
Hico	4/8/96	45 cfs	low flow
	5/31/96	269 cfs	
	8/17/96	290 cfs	
	8/29/96	1870 cfs	high flow
	9/19/96	421 cfs	high flow

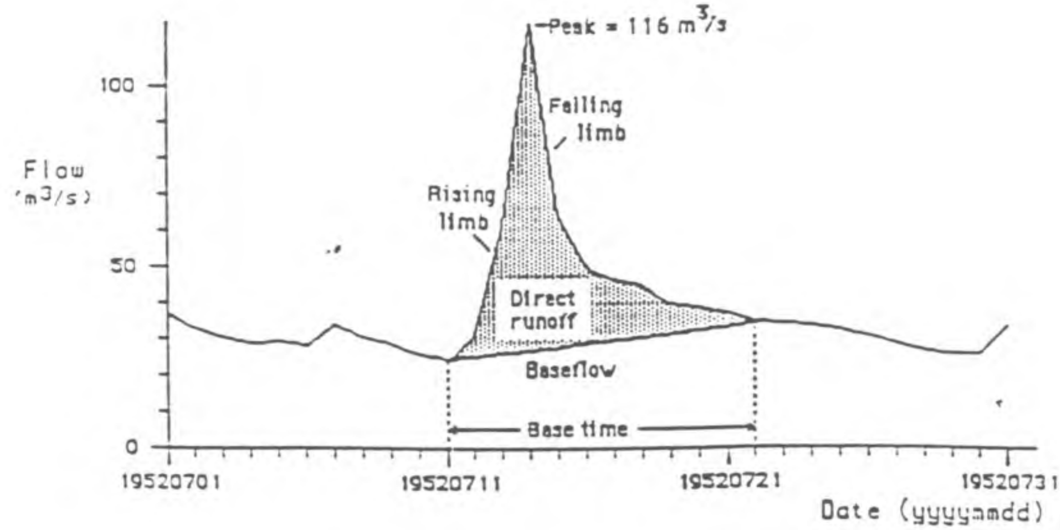


Figure 4. Typical flood hydrograph developed using daily data. (Gordon et al. 1992)

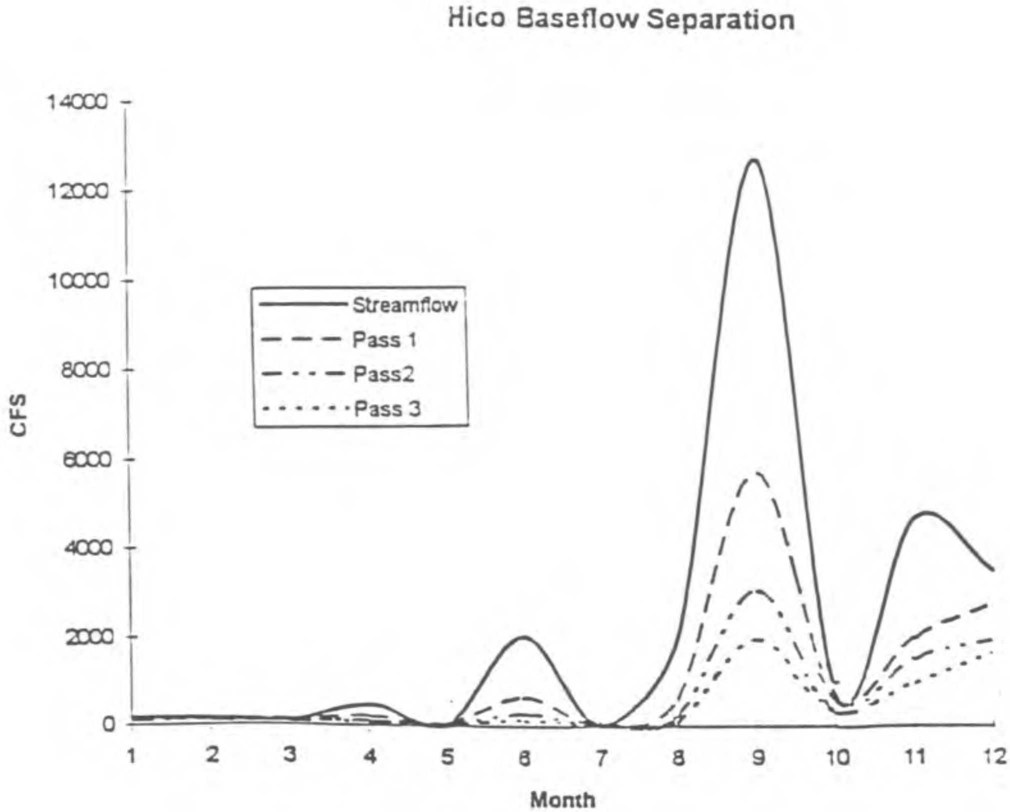


Figure 5. Hico baseflow separation graph for 1996.

Delmar Ranch Baseflow - 1996

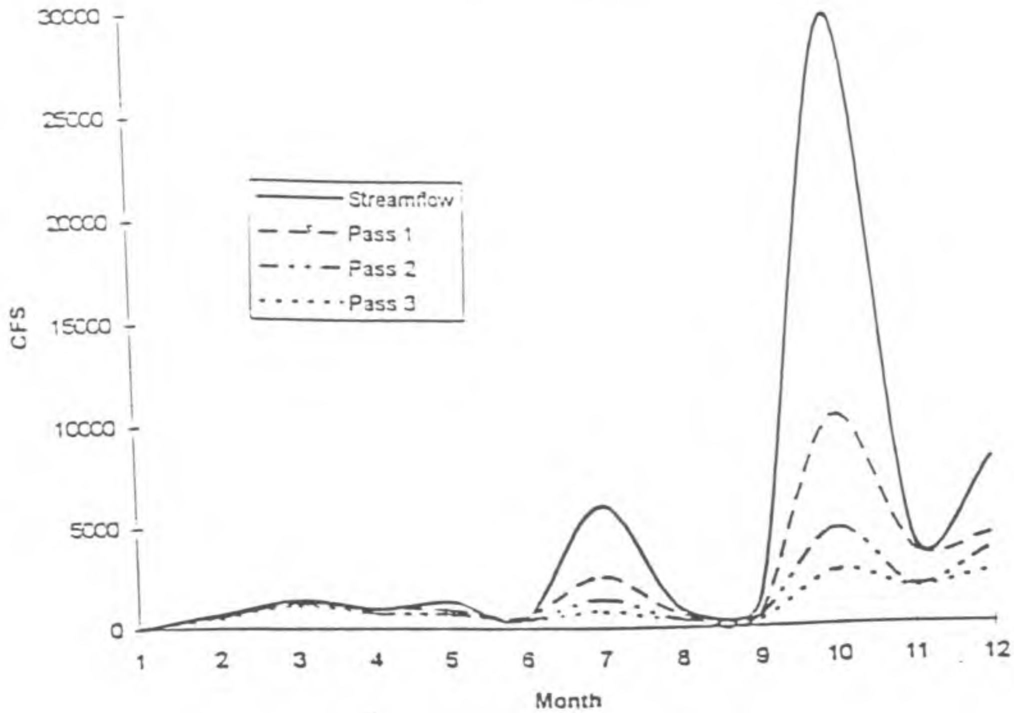


Figure 6. Delmar Ranch separation graph for 1996.

corresponding with the sample dates listed previously. Figures 5 and
6 show that samples taken on 4/8 and 5/31 were near baseflow and samples were taken during floodflow on 8/17, 8/29, and 9/19. The stream discharge at the Hico site was taken from a U.S.G.S. gage which is monitored on the U.S.G.S. internet site every fifteen minutes. The Valley Mills U.S.G.S. discharge gage was used to monitor flow for Delmar Ranch. A hydrograph showed that there was only a 1% difference of the flow at Valley Mills and Delmar Ranch since they are less than one mile from each other. The South Bosque does not contain a working U.S.G.S. gage. I estimated flood stage by taking depth measurements from the same location.

To simulate what happens when river water with suspensoids reaches Lake Waco, surface sediment water was collected with a polyethylene bucket and pored through a polyethylene funnel and a no. 25 mesh net plankton filter into one gallon polyethylene bottles. In addition, Lake Waco water by the dam was collected in one gallon polyethylene bottles and filtered through a no. 25 mesh net plankton filter. This water was the source used for the resuspended sediment in the bioassays. All equipment was washed five times with 5% muriatic acid, neutralized with sodium bicarbonate, and rinsed five times with glass-distilled water.

B. Test Organism and Bioassays

I placed fifty *B. calyciflorus* cysts obtained from Florida Aqua Supplies, Inc. into 100 ml aliquots of elutriates in 250 ml glass Erlenmeyer flasks. I shook the flask for 30 seconds before placing them in the incubator. The flasks were incubated at 25 C and provided with 500 lux of continuous light. Hatching was synchronous 72 h after exposure to the elutriate. The rotifers began asexual reproduction approximately 24 h after hatching. The cultures were continued for 10 d after initial emergence.

Two identical flasks were prepared for each sample site- 10 ml aliquots were taken from one flask daily for counts and replaced by 10 ml from the second flask to maintain the population density. I measured the population growths, reproductive potential, mortality, and cyclomorphosis of spine lengths every 24 h. I retrieved rotifers with a 10 ml Hensen-Stempel pipette and counted using a high resolution Nikon dissecting microscope at 180x magnification and a Ward counting wheel. Adult, dead organisms and eggs were counted. Each sample took approximately ten minutes to count.

C. Bioassays

To find the impact of resuspended sediment-borne substances (nutrients and/or toxicants), I used the Elutriate Test developed by the

USEPA/Corps of Engineers in 1974 (Keeley & Engler 1974). In the
laboratory, I filtered water samples from the North Bosque River, South Bosque River, and Lake Waco. 0.5L water increments were first filtered through 1.5 µm Fisher glass fiber filters and then through 0.7 µm Whatman GF/F. All water samples were filtered within 24 h of collection. Because all of the samples could not be dried and weighed due to the chance of chemical loss through heating, two Fisher and two Whatman filters from each sample site were dried for 24 h at 75 C in a drying oven and weighed. I calculated the average of the sediment concentrate on the two sets of filters which represented the weights for the rest of the samples that were used in the toxicity tests (Table 4). The filters containing adsorbed materials on sediment were wrapped in plastic film, stored in petri dishes and frozen at -4 ±1 C until needed for toxicity tests. The filtered Lake Waco water was refrigerated at 4 C until needed for the toxicity tests.

Sediment to Lake Waco water ratios of ambient 1:1 conditions, and 1:2, 1:4 dilutions (i.e. sediment: water) were prepared by vigorously shaking the previously filtered and frozen sediment and Lake Waco water for 24 h a shaker at 250 excursions per minute. I poured the elutriate into 250 ml polyethylene cone centrifuge tubes and centrifuged in a Beckman automatic refrigerated centrifuge at 7500 rpm for 30 m at 4 C. I then filtered the supernatant through pre-washed 0.45 µm

Table 4. Percent sediment on filters. 0.5 L water samples from the South Bosque River and North Bosque River at Delmar and Hico were filtered through 1.2μm and then through 0.7μm glass fiber filters to obtain materials adsorbed onto suspended sediment particles. * denotes only 1.2μm Fisher G8 used on 4/8/97.

Site	Sampling Date	Sediment Concentration mg L^{-1}	% Sediment on 0.7um filter	% Sediment on 1.2 um filter
S.B.	4/8/96*	62.11	0	100
D.R.	4/8/96*	28.28	0	100
Hico	4/8/96*	12.92	0	100
S.B.	5/31/96	NA	NA	NA
D.R.	5/31/96	1436.75	3.70	96.30
Hico	5/31/96	1139.67	13.79	86.21
Hico	8/17/96	233.37	20.50	79.50
S.B.	8/29/96	782.58	67.45	35.55
D.R.	8/29/96	1988.31	6.38	93.32
Hico	8/29/96	534.89	17.61	82.39
S.B.	9/19/96	526.03	51.67	48.33
D.R.	9/19/96	569.10	9.76	90.24
Hico	9/19/96	158.46	5.10	94.90

Millipore HA 47 mm membrane filters. Afterwards, I took pH and conductivity. measurements for the elutriates.

I fed the rotifers daily. In the beginning of the experiment 1 ml of Roti-Rich, plus 1 ml of *Chlorella vulgaris* at three million cells ml^{-1} supplied the daily nutrients required for active organisms (Hoff and Snell 1993). However, in cases of low population growth in which a scum build-up developed, I decreased the amount of food until the cloudy appearance ceased.

Culturing and contamination became a problem with *Chlorella vulgaris*.. So, I conducted a preliminary experiment to test the viability of the *B. calyciflorus* on three different feeding regimens: *Chlorella vulgaris* + Roti-Rich, Roti-Rich alone, and no food. Figure 7 shows the results from the experiment. Equivalent rotifer population growths resulted when fed *C. vulgaris* + Roti-Rich and Roti-Rich alone. Thus, all cultures subsequently were fed Roti-Rich alone for their daily nutritional intake.

D. Calculation of Growth Rates, Birth Rates and Death Rates

Few dynamic properties of most zooplankton populations can be determined by simple, periodic sampling and counting of populations, even when the samples are separated by very short intervals. Changes in population size can be measured, but the usually continuous nature of reproduction and death normally precludes determination of birth

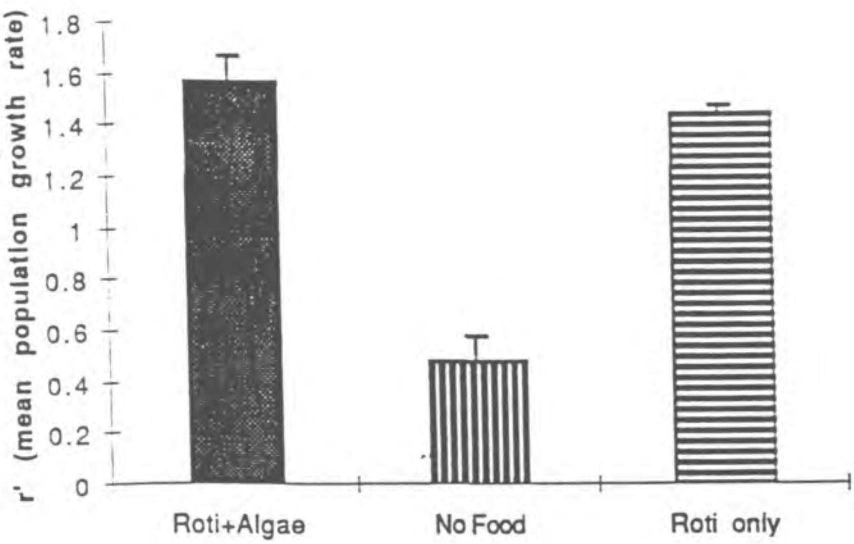


Figure 7. Food experiment with Roti-Rich alone, Roti-Rich plus *Chlorella vulgaris*, and no food.

and death rates from counting alone.

The coefficient of population growth rate (r') can be calculated from the following equation:

$$r' = (\ln Nt_2 - \ln Nt_1)/(t_2 - t_1)$$

N_1 and N_2= Population size at times t_1 and t_2.

The prime on r' is used to emphasize that it is calculated from measurements of population size rather than being derived from age specific mortality and natality. Thus r' is used here as a measure of the actual or net rate of population growth. One of the properties that has shown distinct promise for the analysis of birth and death rates on a population basis is the abundance of eggs. The ratio of eggs/female (E) observable in a sample at any moment can be converted to a finite measure of population birth rate, eggs per female per day (B), by the relation:

$$B = E/ND$$

E= Eggs in the population

N= Adults in population

D= Egg development time in days

The basis of this relation is that the eggs present at a given moment will be the increment that will be added to the population during the next period of time D. If the age distribution of the eggs is uniform, the fraction of eggs hatching during a day is 1/D. Thus, for each female present initially, the number added during the next day is E/D.

The birth rate can be described as a finite rate of reproduction since it pertains to the events of a full day. This can be converted to an instantaneous coefficient of birth:

$$b' = \ln (B+1)$$

B= Population birth rate

b'= Intrinsic birth rate

This birth rate term, as it is being used in this connection, means the rate at which free swimming immature individuals enter the population, not the rate at which eggs are being laid. Thus, it is actually the hatching rate, not the laying rate of the population of females as seen in the samples on which the egg ratio is based.

It is common to regard the instantaneous coefficient of population growth (r) to be composed of a birth rate (b) and a death rate (d). A coefficient of mortality can be calculated as the difference between the natural logarithms of the population sizes in a manner analogous to that for r'. It is as if hatching stopped at a moment and the population decreased for one day. The equation used is:

$$d = \ln N_t - \ln (N_t - C)$$

N= Population size at time t

C= Number of animals dying during the day

E. Cyclomorphosis Calculations

Phenotypic variation such as cyclomorphosis is an important adaptive mechanism in rotifers. Cyclomorphosis is the phenotypic change in body size, spine length, pigmentation, or ornamentation found in successive generations in zooplankton. These changes are phenotypic alterations in a single population that are related to physical, chemical, biologic features of the environment (Covich & Thorp 1991).

Cyclomorphosis in rotifers is a complex process that is not well understood. Physical, chemical, and predatory environmental stresses can induce phenotypic variation in zooplankton. Halbach has shown that under overwhelming stress, *B. calyciflorus* undergoes spine length variation (Halbach 1979). The most studied cyclomorphosis stress inducer has been predation. However, excessive turbidity and chemical changes in the water can also attribute to spine length alterations (Covich and Thorp 1993, Halbach 1979, Hoff and Snell 1993).

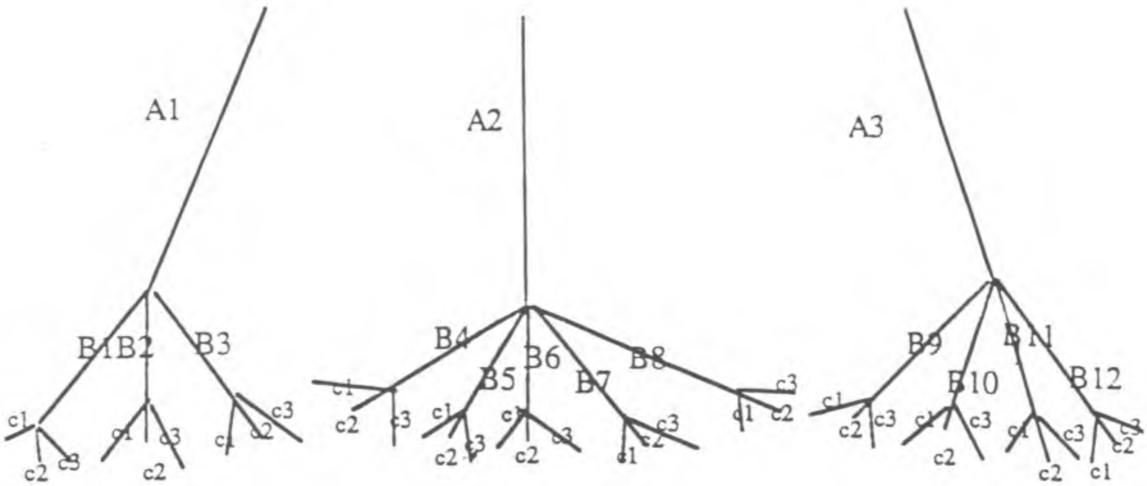
One hundred rotifers of reproductive age per site were measured for any cyclomorphosic changes in anterolateral spine lengths. Since the peak reproductive ages of B. calyciflorus were between days 1 through 4, I chose to make spine measurements on day 3. All measurements were made on a Zeiss compound microscope at 10x magnification. The sizes were measured by a Whipple Grid that was calibrated to 1 µm by an ocular micrometre. The mean relative length of the anterolateral spines were measured using the equation (Halbach 1979):

$$\frac{\text{Spine length(D)}}{\text{Lorica length (L)}}$$

F. Data Analyses

A factorial design was constructed from basic building block designs. Figure 8 depicts the completely randomized partial hierarchical experimental design used to set up statistical tests that were used (Kirk 1968).

I used Analysis of Variance (ANOVA) to determine the relation of the elutriates (the independent variable) measured to the *B. calyciflorus* population growth, death rate, birth rate, and spine length changes (the dependent variable). The ANOVA tested for significant differences



Legend

A1= South Bosque

A2= Hico

A3= Delmar Ranch

B= Samples from 4/8-9/19-1996

c1= 1:1 concentration

c2= 2:1 concentration

c3= 4:1 concentration

Figure 8. Completely randomized partial hierarchical experimental design.

between the two rivers, among the samples taken at different discharges within the river, and among the three different concentrations, 1:1, 2:1, and 4:1.

If significant differences were detected using the ANOVA test, the Dunnett's stepwise multiple comparison test procedure was used to test the differences between the controls and the individual samples taken on different days at the three sites, South Bosque, North Bosque at Delmar Ranch, and North Bosque at Hico. Dunnett's multiple comparison statistic tests for comparisons involving a control mean. The object of Dunnett's is to compare a number of treatment levels with a control condition. If the observed tD value is greater than the tabled value of tD, the comparison is declared to be significant (Kirk 1968). The statistical program, SAS, was used to analyze all the data. The probability value was set at 0.05 for all the tests used. The control and all toxicity tests were replicated three times (n=3).

A simple linear regression analysis was used to determine the relation of the water flow discharges to the population parameters, the mean population growth, r_m, the mean birth rate, b_m, and the mean death rate, d_m. Regression analysis measures the cause and effect relationship between a dependent variable (population dynamics) and one or more independent variables (water flow discharges). These statistical data analyses were done with the Statview program.

CHAPTER FIVE

Experimental Results

In general, *B. calyciflorus* are susceptible to the inhibitory effects of suspended sediments when exposed to the North Bosque elutriates but not the South Bosque elutriates. There were significant differences between the controls and the *B. calyciflorus* mean population growth rate, mean birth rate, and the mean death rate, when exposed to resuspended sediment from regular flow and high flood events at the North Bosque Delmar Ranch and Hico stations. None of the elutriates caused cyclomorphotic spine length changes in any of the toxicity tests.

ANOVA Results

None of the cyclomorphosis results showed significance ($p>0.05$). For mean population growth rate (r_m), mean birth rate (b_m), and the mean death rate (d_m), the ANOVA showed significant differences ($p<0.05$) among the sample dates within the river (B in Figure 8) and among the three concentrations, 1:1, 2:1, and 4:1 (C in Figure 8).

A. <u>Population Growth Rates</u>

Tables 5-7 lists the results from the Dunnett's test for the *B. calyciflorus* mean population growth rate. There were significant differences (t>tD) between the control and the *B. calyciflorus* mean population growth rate (n=3) for 1:1 concentration samples taken during baseflow and floodflow at the North Bosque River at Delmar Ranch and Hico. However, the South Bosque River 1:1 ambient concentration samples showed no significant differences from the controls. None of the 2:1 and 4:1 dilution samples from any of the sample sites were different from the controls.

B. <u>Birth Rates</u>

Tables 8-10 lists the results from the Dunnett's multiple comparison tests for the *B. calyciflorus* mean birth rates. There was no consistent pattern of response. Similar to the population growth rate tests, no significant differences were found between the South Bosque River samples at all three concentrations and the controls. The Delmar Ranch samples were significantly different (t>tD) from the controls for samples taken at base flow (136 and340 cfs) at a 1:1 and at samples taken at base flow (136 and340 cfs) at a 1:1 and at baseflow

Table 5. Dunnett's Multiple Comparison Test results for *B. calyciflorus* population growth rates subjected to elutriates at a 1:1 (water: sediment) concentration. P value set at 0.05. *Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD=3.51).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	-0.43	0.13	3.20 ns
	3	2.36 ft *	-0.40	0.15	2.02 ns
	3	14.2 ft *	-0.10	0.20	.92 ns
Delmar Ranch	3	136 cfs	-1.08	0.18	6.76 s
	3	304 cfs	-0.92	0.24	4.12 s
	3	14,243 cfs	-01.10	0.22	3.70 s
	3	1203 cfs	-0.82	0.16	4.20 s
Hico	3	45 cfs	-1.11	0.33	4.52 s
	3	269 cfs	-0.64	0.24	2.89 ns
	3	290 cfs	-0.71	0.19	3.50 ns
	3	1870 cfs	-1.49	0.12	3.62 s
	3	421 cfs	-0.89	0.23	3.93 s

Table 6. Dunnett's Multiple Comparison Test results for *B. calyciflorus* population growth rates subjected to elutriates at a 2:1 (water: sediment) concentration. P value set at 0.05. *Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 3.51).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	-0.27	0.10	3.31 ns
	3	2.36 ft *	-0.18	0.10	1.28 ns
	3	14.2 ft *	-0.28	0.11	2.45 ns
Delmar Ranch	3	136 cfs	-0.58	0.21	2.15 ns
	3	304 cfs	-0.41	0.13	2.22 ns
	3	14,243 cfs	-0.37	0.21	2.01 ns
	3	1203 cfs	-0.50	0.14	1.96 ns
Hico	3	45 cfs	-0.50	0.21	.82 ns
	3	269 cfs	-0.36	0.09	.32 ns
	3	290 cfs	-0.44	0.17	2.18 ns
	3	1870 cfs	-0.34	0.21	1.94 ns
	3	421 cfs	-0.39	0.18	2.21 ns

Table 7. Dunnett's Multiple Comparison Test results for *B. calyciflorus* population growth rates subjected to elutriates at a 4:1 (water: sediment) concentration. P value set at 0.05. *Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 3.51).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	-0.12	0.11	2.41 ns
	3	2.36 ft *	-0.23	0.10	1.47 ns
	3	14.2 ft *	-0.01	0.10	.076 ns
Delmar Ranch	3	136 cfs	-0.56	0.13	1.23 ns
	3	304 cfs	-0.33	0.24	1.81 ns
	3	14,243 cfs	-0.10	0.10	.098 ns
	3	1203 cfs	-0.38	0.12	.99 ns
Hico	3	45 cfs	-0.21	0.10	.54 ns
	3	269 cfs	-0.06	0.10	.12 ns
	3	290 cfs	-0.26	0.21	1.22 ns
	3	1870 cfs	-0.17	0.15	.88 ns
	3	421 cfs	-0.30	0.18	1.31 ns

Table 8. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean birth rates subjected to elutriates at a 1:1 (water: sediment) concentration. P value set at 0.05. * Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean	SD	t test
South Bosque	3	1 ft *	-0.22	0.06	1.80 ns
	3	2.36 ft *	-0.44	0.05	2.96 ns
	3	14.2 ft *	-0.29	0.01	2.42 ns
Delmar Ranch	3	136 cfs	-0.68	0.06	5.04 s
	3	304 cfs	-0.66	0.04	4.01 s
	3	14,243 cfs	-0.14	0.06	1.07 ns
	3	1203 cfs	-0.21	0.08	1.04 ns
Hico	3	45 cfs	-0.60	0.15	4.84 s
	3	269 cfs	-0.42	0.08	2.95 ns
	3	290 cfs	-0.30	0.15	2.32 ns
	3	1870 cfs	-0.55	0.14	3.06 s
	3	421 cfs	-0.24	0.25	2.21 ns

Table 9. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean birth rates subjected to elutriates at a 2:1 (water: sediment) concentration. P value set at 0.05. * Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	-0.26	0.04	1.02 ns
	3	2.36 ft *	-0.18	0.08	1.12 ns
	3	14.2 ft *	-0.12	0.02	1.87 ns
Delmar Ranch	3	136 cfs	-0.48	0.09	2.98 s
	3	304 cfs	-0.25	0.05	1.17 ns
	3	14,243 cfs	-0.16	0.08	1.99 ns
	3	1203 cfs	-0.09	0.09	.64 ns
Hico	3	45 cfs	-0.38	0.11	1.22 ns
	3	269 cfs	-0.23	0.09	2.14 ns
	3	290 cfs	-0.14	0.10	.97 ns
	3	1870 cfs	-0.31	0.18	2.11 ns
	3	421 cfs	-0.31	0.14	1.62 ns

Table 10. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean birth rates subjected to elutriates at a 4:1 (water: sediment) concentration. P value set at 0.05. * Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	-0.02	0.02	.94 ns
	3	2.36 ft *	-0.19	0.03	.75 ns
	3	14.2 ft *	-0.26	0.06	1.23 ns
	3	136 cfs	-0.23	0.07	.95 ns
Delmar Ranch	3	304 cfs	-0.20	0.09	.44 ns
	3	14,243 cfs	-0.01	0.04	.82 ns
	3	1203 cfs	-0.02	0.08	.33 ns
Hico	3	45 cfs	-0.33	0.12	.85 ns
	3	269 cfs	-0.16	0.06	1.23 ns
	3	290 cfs	-0.15	0.11	1.12 ns
	3	1870 cfs	-0.14	0.01	1.42 ns
	3	421 cfs	-0.14	0.09	.74 ns

(136 cfs only) at a 2:1 concentration. Hico samples were significantly different from the controls at baseflow (45 cfs) and at floodflow (1870 cfs) at a 1:1 concentration.

C. Death Rate

Dunnett's multiple comparison test showed a significant increase (t>tD) in the death rate of *B. calyciflorus* exposed to North Bosque Delmar Ranch samples taken at baseflow (136 cfs) and at floodflow (1203 cfs and 14,243 cfs) and Hico samples taken at baseflow (45 cfs) and floodflow (421 and 1870 cfs) at a 1:1 concentration (Table 11). There were no significant differences between the rotifers exposed to South Bosque River samples at any of the concentrations and the controls (Tables 12-13).

Simple Linear Regression Test

A cause and effect relationship exists between the water flow discharge and the population parameters, r_m, b_m, and d_m, for 1:1 concentration samples (Table 14). Regression analysis showed that approximately 70% of the charges in the mean population growth for *B. calyciflorus* exposed to South Bosque and North Bosque Delmar Ranch and Hico samples were explained by the water discharge. Table 15

Table 11. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean death rates subjected to elutriates at a 1:1 (water: sediment) concentration. P value set at 0.05. * Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	0.021	0.022	.819 ns
	3	2.36 ft *	0.049	0.047	1.95 ns
	3	14.2 ft *	0.039	0.052	1.54 ns
Delmar Ranch	3	136 cfs	0.45	0.17	3.00 s
	3	304 cfs	0.2	0.083	1.75 ns
	3	14,243 cfs	0.51	0.10	3.30 s
	3	1203 cfs	0.37	0.070	5.644 s
Hico	3	45 cfs	0.42	0.18	7.23 s
	3	269 cfs	0.096	0.033	2.74 ns
	3	290 cfs	0.091	0.049	2.65 ns
	3	1870 cfs	0.31	0.075	4.65 s
	3	421 cfs	0.24	0.090	4.32 s

Table 12. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean death rates subjected to elutriates at a 2:1 (water: sediment) concentration. P value set at 0.05. *Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	0.049	0.038	.26 ns
	3	2.36 ft *	0.1	0.1	.94 ns
	3	14.2 ft *	0.0071	0.0054	.34 ns
Delmar Ranch	3	136 cfs	0.24	0.088	1.24 ns
	3	304 cfs	0.093	0.012	.77 ns
	3	14,243 cfs	0.221	0.085	1.27 ns
	3	1203 cfs	0.142	0.09	2.054 ns
Hico	3	45 cfs	0.1	0.014	2.1 ns
	3	269 cfs	0.093	0.067	1.21 ns
	3	290 cfs	0.15	0.075	2.87 ns
	3	1870 cfs	0.12	0.089	2.89 ns
	3	421 cfs	0.012	0.009	.087 ns

Table 13. Dunnett's Multiple Comparison Test results for *B. calyciflorus* mean death rates subjected to elutriates at a 4:1 (water: sediment) concentration. P value set at 0.05. * Represents water levels. Mean ** represents the average means of all replicates (n) minus the average mean of the control (significance of t when t> tD; tD= 2.97).

Site	n	Discharge	Mean **	SD	t test
South Bosque	3	1 ft *	0.039	0.052	.24 ns
	3	2.36 ft *	0.009	0.006	.65 ns
	3	14.2 ft *	0.003	0.002	.42 ns
Delmar Ranch	3	136 cfs	0.068	0.087	.90 ns
	3	304 cfs	0.008	0.010	.53 ns
	3	14,243 cfs	0.200	0.054	.85 ns
	3	1203 cfs	0.090	0.015	.95 ns
Hico	3	45 cfs	0.027	0.017	2.00 ns
	3	269 cfs	0.009	0.005	.19 ns
	3	290 cfs	0.013	0.010	.23 ns
	3	1870 cfs	0.030	0.009	.089 ns
	3	421 cfs	0.014	0.007	.099 ns

Table 14. Percent variation (r^2) of population parameters (intrinsic rate of natural increase (r_m), the birth rate (b_m), and the death rate (d_m)) explained by discharge.

		Concentration (vol: wt)			
Sample Site	Parameter	1 to 1	2 to 1	4 to 1	n
South Bosque	r_m	0.7	0.0023	0.15	3
	b_m	0.87	1	0.62	
	d_m	0.54	0.11	0.09	
Delmar Ranch	r_m	0.71	0.25	0.36	4
	b_m	0.77	0.15	0.5	
	d_m	0.41	0.19	0.003	
Hico	r_m	0.67	0.46	0.33	5
	b_m	0.003	0.02	0.21	
	d_m	0.64	0.05	0.03	

Table 15. A relationship between the discharge and sediment concentration was determined through regression, r^2, analysis. *Represents flood levels.

Sample Site	Discharge (cfs)	Sediment Concentration (mg L^{-1})	r^2
South Bosque	1 f*	62.11	0.83
	2.36 f*	782.58	
	14.20 f*	1526.03	
Delmar Ranch	136	28.28	0.57
	304	1436.75	
	14243	1988.31	
	1203	569.1	
Hico	45	12.92	0.59
	269	1139.67	
	290	233.37	
	1870	1534.89	
	421	158.46	

showed a high cause and effect relationship between the water discharges and the death rates for *B. calyciflorus* exposed to Delmar Ranch and Hico elutriates at a 1:1 concentration. High birth rates were also related to water discharges for rotifers exposed to 1:1 concentration, South Bosque River and North Bosque River Delmar Ranch elutriates.

A regression analysis showed a high cause and effect relationship between the sediment concentration and flood levels at (136 cfs only) at a 2:1 concentration. Hico samples were significantly different from the controls at baseflow (45 cfs) and at floodflow (1870 cfs) at a 1:1 concentration and the flood levels are particularly strong, 83%. The North Bosque River at Delmar Ranch and Hico were not as strong, 57% and 59%, respectively.

CHAPTER SIX

Discussion

An aquatic environment is a complex ecosystem consisting of many physical, chemical, and biological systems that are inseparably interrelated. Abiotic components of the aquatic environment that affect the life and survival of *B. calyciflorus* have been studied for only the past ten years. Tolerance ranges for a number of pollutants for *B. calyciflorus* have been presented in the literature review. When different toxic and nontoxic materials are present in an aquatic environment, they may interact to either increase or decrease the overall toxic effect upon the organism. The interaction of pollutants and the subsequent effect this interaction may have on a given organism within an aquatic system is variable and difficult to predict.

Contaminants as well as nutrients will affect *B. calyciflorus* population dynamics. This relationship will determine zooplankton community structure in freshwater systems. The main point of my study was to compare the effects of contaminants/nutrients obtained from the South Bosque and North Bosque Rivers during flood events and regular flow on the population growth, fertility, and death rates on

B. calyciflorus. I also attempted to detect spine length alterations caused by environmental stress induced by materials adsorbed onto suspended sediments.

A. Sediments and Populations

Previous experimental studies have shown that suspended sediments directly decrease the survivorship and reproduction of zooplankton, but few previous lab studies have examined the effect of suspended sediments on rotifers. However, my results show that 12 to 1988 mg L^{-1} suspended sediment caused inhibition in *B. calyciflorus* population growth and reproduction, and induced rapid mortality. Zurek (1982) found that 100 to 2000 mg L^{-1} suspended sediment caused rapid mortality in cladoceran populations. McCabe and O'Brien (1983) found that suspended sediments decreased the survivorship and fecundity of cladocera when present at turbidities of 10 to 30 NTU.

Two in situ enclosure experiments concerning the effect of suspended sediments on zooplankton populations have been conducted. Cuker (1987) found that adding 100 g m^{-2} d^{-1} suspended clay to 10 m^3 enclosures decreased the abundance of both copepods and cladocerans relative to control enclosures after four weeks. Neither reduction was statistically significant, however, and rotifer population numbers were not determined (Cuker 1987). Threkeld and Soballe (1988) added 10 to

100 mg L^{-1} suspended sediments to 7 m^3 tanks at the start of three separate experiments. In general, there were no clear effects of suspended sediments on zooplankton populations. These experiments were conducted using unreplicated factorial designs, so many effects of suspended sediments may have been obscured by interactions with other manipulated parameters (Threlkeld and Soballe 1988).

Several observational studies provided indirect evidence that high suspended sediment concentrations suppress zooplankton populations. Hart (1986, 1987), in his studies of a turbid reservoir in South Africa, found a negative correlation between turbidity and the abundance of a variety of cladocerans, rotifers, and copepods over a seven year period. Threlkeld (1986) showed that the abundance of cladocerans decreased during a flood of turbid water into a turbid reservoir in Oklahoma. However, he also found that two other species of cladocerans increased during the period of high turbidity. In the Neusiedlersee, Herzig (1975) found a positive correlation between the death rate of filter-feeders and turbidity. The abundance of zooplankton declined in an Amazonian floodplain lake during periods of high inorganic turbidity (Carvalho 1984).

There are few field data concerning the effect of suspended sediments specifically on rotifer population dynamics. Holland et al. (1983) studied rotifer populations in the lakes and swamps of the

Atchafalaya River basin. Rotifer abundance was higher in less turbid
water upper basin environments than in the more turbid lower basin water, but this pattern was confounded by the fact that phytoplankton abundance was also higher in the upper basin. Within the turbid lower basin, the seasonal abundance of most rotifer species was negatively correlated with turbidity. However, phytoplankton abundance was also negatively correlated with high turbidity (Holland et al. 1983). Sheil and Walker (1984) compared the zooplankton assemblages in two river-reservoir systems in Australia. They found that the less turbid Murray River and its associated reservoirs were dominated by crustacean zooplankton, whereas the more turbid Darling River was dominated by rotifers. Clay suspended in lake water may be an important factor in limiting primary production by decreasing availability of light and of nutrients (Gliwicz 1986). Gliwicz (1986) found that the concentration of suspended clay or silt particles maximize as an effect of floods carrying a large quantity of clay particles from the sediments during high turbid events. He also found the filter-feeding zooplankton greatly reduce the abundance of clay suspended in the water. All the intestines in each filter-feeder he examined contained high amounts of clay. The organic compounds bound to the clay particles seem to be the major food source for filter-feeders in the lake (Gliwicz 1986).

Adalsteinsson (1979) provided a very complete data set concerning rotifer population dynamics in turbid water. Lake Myvatn, Iceland, contains two basins, a deeper (mean depth 3.3 m), less turbid South basin, and a shallower (mean depth 1 m), more turbid North basin. In the South basin, where the suspended sediment concentration is usually below 5 mg L^{-1}, zooplankton are present, often at densities greater than 20 L^{-1}. However, zooplankton populations are very rare in the turbid North basin, possibly as a result of the higher suspended sediment concentrations, which often reach 20 mg L^{-1} and may exceed 40 mg L^{-1}. In addition, there were no obviously confounding indirect effects of suspended sediments on the zooplankton. In general, there were no large differences in nutritious phytoplankton abundance between the two basins. Both basins experienced a bloom of the blue-green algae, *Anabaina flos-aquae*, although the bloom lasted about 2 weeks longer in the North basin than in the South.

B. The North Bosque River Vs. The South Bosque River

The studies by Gliwicz (1986) and Cuker (1987) previously mentioned insinuate that suspended sediment soil texture could be an important component of understanding nutrient and pollutant adsorption and distribution in water systems. In the U.S. Department of Agriculture classification system, sandy soils are considered coarse in texture and clayey soils are fine in texture. The finer the texture of a soil, the greater the effective surface exposed by its particles. The surface area per unit mass of clay is very high because of the small size of the individual particles. Fine colloidal clay has about 10,000 times as much surface area as the same weight of medium sized sand. Since the adsorption of water, nutrients, contaminants, and gas and the attraction of particles for each other are all surface phenomena, clay particles have greater nutrients and pollutants adsorption capabilities than sand particles (Brady 1990).

Since most of the South Bosque River and North Bosque River contain clay and silt sediment particles except at Hico, similar inhibition results would be expected. However, the results from this study showed contrasting results in the North Bosque River and the South Bosque River. The South Bosque River showed no inhibition in the population dynamics measured and the North Bosque River at Hico and Delmar Ranch showed inhibition in all the population parameters measured.

The difference in results at the two rivers is explained by the different land use at the two basins. The South Bosque River basin is utilized for residential, municipal, and some row crop purposes. In contrast, 70% of the North Bosque River basin is used for livestock grazing and 30% for row crops (Report prepared for the Brazos River Authority 1976). These different types of land uses contribute different types of nutrients and contaminants such as pesticides and herbicides the rivers.

C. Suspended Sediment Concentration

Laboratory experiments are most meaningful if they are conducted under conditions that are relevant to conditions encountered in the field. In this study, suspended sediment particle concentrations and sizes that were collected from natural environments were used. Arruda (1980) and Arruda et al. (1983) found that increasing the sediment concentration decreases rates of ingestion, clearance and incorporation of *C. vulgaris* by cladocera. My results showed that the ambient concentrations were effective in inhibiting *B. calyciflorus* population growth and reproduction and increased their mortality rate. However, diluting the concentrations to a two parts water and one part sediment and four parts water and one part sediment caused no effect in any of the population dynamics measured. Thus, a threshold existed between the 1:1 ambient concentration and the 2:1 dilute concentration. This is most obvious in the North Bosque River samples.

D. Regular Flow Vs Floodflow

River sediment could be contributed through several means during flood events and in regular water flow conditions. In this experiment, *B. calyciflorus* exposed to North Bosque Delmar Ranch and Hico resuspended elutriates showed an increased mortality rate and reproduction and population inhibition at both regular flow and floodflow. Sorensen et al. (1977) found that eroded soils produce the most quantity of suspended solids. "Sand, silt, and clay are dislodged by rainfall, wind, and overland flow and carried into streams and lakes from rural and agricultural areas, forest, and urban areas. Sediment resuspended in the course of the stream (from groundwater) is also an important type of suspended solids." Groundwater contains stored nutrients and toxins that accumulate over time (Chapman 1981, Bowen 1982, Cooke and Doornkamp 1974). When induced by flood events or other wet conditions, groundwater discharge is stimulated and releases the stored nutrients and toxins (Gburek et al. 1991). Also, during high rainfall events, the nutrients and contaminants accumulate on soil and plants in the surrounding watershed are released into the river.

There are several explanations to clarify why inhibition and increased mortality rates from the North Bosque River sites during regular water flow occurred. The stability of the river banks is an important factor to consider. Highly erodable river banks can contribute to suspended sediment into the water after light rain or heavy wind activity (Brady 1990). Chepil's studies (1945, 1951, 1955) indicated the important contribution of wind on soil erosion. "...The relatively dry natural environment can easily be upset by inappropriate agricultural and grazing practices and development." Benthic communities can also contribute to resuspension of sediment in fluvial water.

The size of a sediment particle contributes to the distribution of sediment during low water flow events. There are few data concerning the

size-distribution of suspended particles in nature, but most show that
fine particles tend to dominate (Kennedy et al. 1981, Smith and Syvitske 1982, G.-Toth 1984, Shiel 1985, Gliwicz 1986, Threlkeld 1986, Hart 1988). Large suspended silt particles may be present in extremely well-mixed environments such as the upper basins of reservoirs (Nakamura and Adachi 1981), but simple consideration of Stoke's law shows that in most lakes and reservoir's fine particles will tend to stay in suspension much longer (Hutchinson 1967). Since clay has a low settling velocity because of its small size, sediment is continuously being distributed in fluvial systems through groundwater return flow during low velocity water flow.

E. Biological Effect

Parametric statistical analyses were used to identify all statistically significant differences between the experimental and the control samples. The two basic assumptions for using a parametric test were met. These assumptions are: (1) the populations are normally distributed and (2) the variances are equal (Kirk 1968).

The materials adsorbed onto the flood-borne sediment did not statistically alter the *B. calyciflorus* population parameters in all cases but they did not stimulate them either. None of the experimental samples showed stimulation for any of the population parameters in comparison with the control even though the South Bosque River showed no statistically significant differences in any of the population parameters at all three concentrations and the North Bosque River showed no significant differences at 2:1 and 4:1 dilutions. It is evident from these observations that a biological factor exists inhibiting the mean population growth rate, mean birth rate, and increasing the mean death rate at the North Bosque River at Hico and Delmar Ranch and at the South Bosque River at 1:1 ambient conditions, and 2:1 and 4:1 dilutions.

F. Effects of Suspended Sediment on Zooplankton From the River to Reservoir

Integration of information about zooplankton existence in reservoirs yields a pattern that suggests that reservoirs behave as continuous flow processors (Thornton et al. 1990). The processes involved are both physical and biological. Materials exported from the catchment basin into the reservoir undergo change, and the water that leaves the reservoir through the outflow structure at the dam is of distinctly different quality.

The distribution of zooplankton populations in freshwater is not well understood, especially rotifers (Covich and Thorp 1991). The observed horizontal distribution of zooplankton in a reservoir is presented in Figure 9a (Vannote et al. 1980). This distribution pattern is general and is most likely driven by resources entering the reservoir from the river.

Vannote et al. (1980) found that exports, silts and clays, nutrients, DOC, algae, and bacteria from the river supply the reservoirs. Figure 9b illustrates the hypothetical pattern of exports from the river into the reservoir and their fate as they move toward the dam. The decreases downstream result from deposition of particulate materials as current velocity declines and the load-carrying capacity decreases. Nutrients and contaminants adsorbed to particles are transported to the sediments by this mechanism. Small particles are maintained in suspension by wind-generated currents or by their colloidal properties. The algae exported from the river into the reservoir suffer losses resulting from limited light availability.

As a response to the export from the river that is induced by wind and flood events, zooplankton population density decrease from the upstream river end to the reservoir dam. Figure 9c illustrates the zooplankton response to river exports (Thornton et al. 1990). Filter feeding organisms respond to changing quantity or quality of food resources by altering their reproductive performance.

The main purpose of my study was to determine the impact of materials transported on flood-borne sediments on zooplankton. In particular, I studied the impact on *B. calyciflorus.* Suspended solids exported from the river into the reservoir can have biochemical, chemical, and biological effects on freshwater systems.

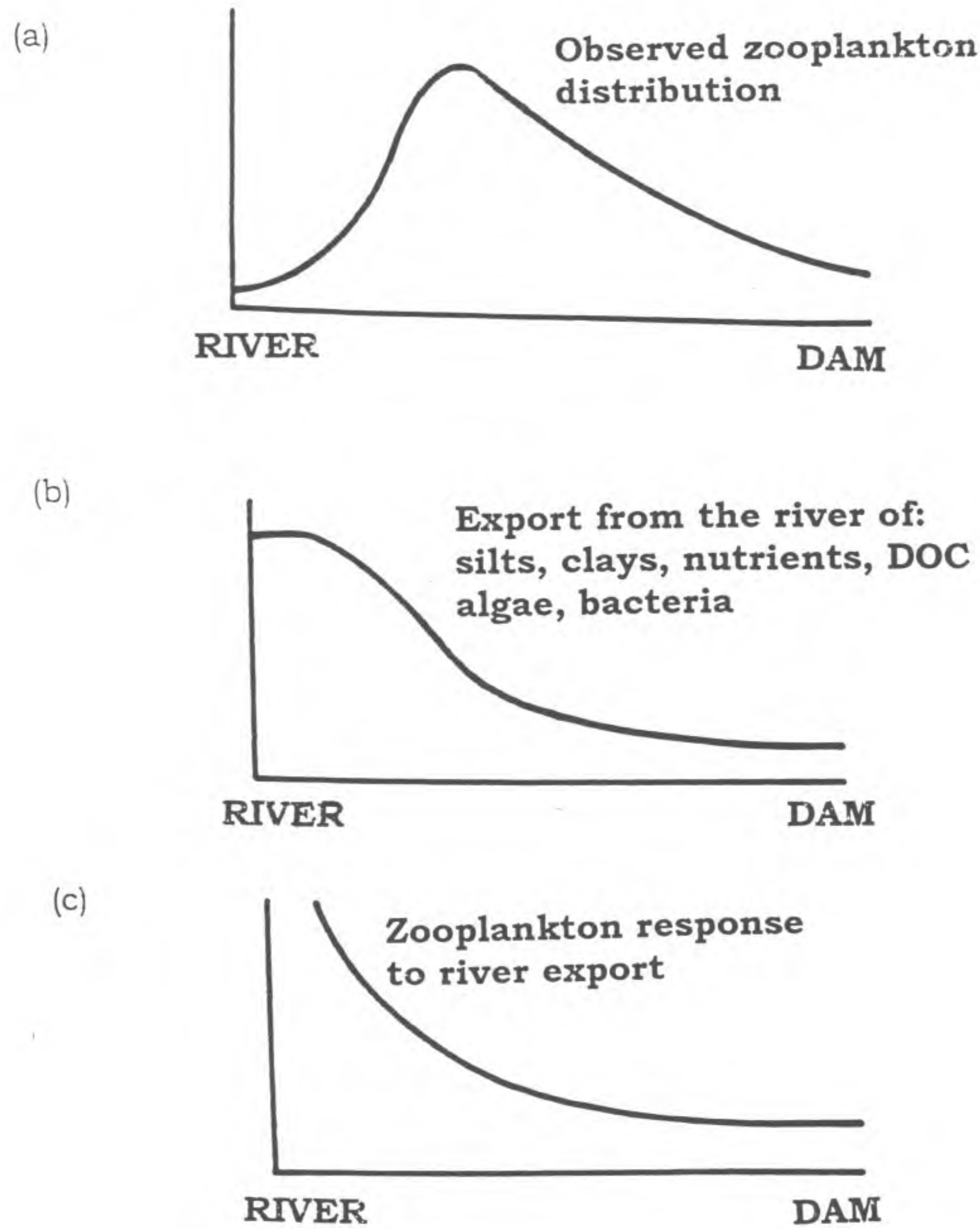


Figure 9. An illustration of the several trends that develop for zooplankton from the river to the dam.

Table 16 lists some of these effects (Wilber 1983). The clays, silts, and sands were the vehicles of the toxicants in this experiment. However, natural organic matter and wastewater organic particles are also capable of adsorbing toxicants in fluvial water systems.

Table 16. Classification of suspended solids and their probable major impacts on freshwater ecosystems.

Suspended Solids	Biochemical, Chemical, and Physical Effects	Biological Effects
Clays, silts, sand	Sedimentation, erosion & abrasion, turbidity (light reduction), habitat change	Respiratory interference, habitat restriction, light limitation
Natural organic matter	Sedimentation, DO utilization	Food sources, DO effects
Wastewater organic particles	Sedimentation, DO utilization, nutrient source	DO effects
Toxicants sorbed to particles	All of the above	Toxicity

(Wilber 1983)

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