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# The Use of *Lemna minor* L. (Lemnaceae) as Assessor of Water Quality in the Big South Fork River, Cumberland Plateau, Tennessee-Kentucky

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

Margaret Dianne Parks

December, 1993

DEDICATION

This thesis is dedicated to my sister

Sharyn Parks Magee

without whose assistance I could not have persevered

and

to my major professor

Clifford C. Amundsen

a steadfast source of encouragement and enlightenment

ACNOWLEDGMENTS

I would like to thank my committee members, Dr. James D. Caponetti, Dr. J. Frank McCormick and Dr. B. Eugene Wofford, for their comments and assistance over the past three years.

## ABSTRACT

This research investigated the applicability of the response of Lemna minor L. as an indicative, site specific, bioassay of water quality within a riverine system. This standardized bioassay test (ASTM, 1992) does not quantify or specify pollutants, but provides a qualitative, seasonal assessment of phytotoxicity at the primary producer level of the food chain for sample sites. Our intention was to interpret the growth constraints of accessible site water collections in terms of the integrated capability of the BSF as a biotic substrate.

It was concluded that Lemna minor can detect river contaminination by quantifying growth responses. The method can be expanded to encompass food chain impacts of contaminants at various sites at various times, and to identify places needing more intensive analyses and quantifications.

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# I. Introduction

The Big South Fork of the Cumberland River is the premier feature of a National River and Recreation area now operated by the National Park Service. It flows north across the Kentucky-Tennessee border on the Cumberland Plateau, and is formed by conjuction of the Clear Fork and the New River. The entire watershed has been used extensively in the past for timber logging, strip mining, agriculture, and industry; and each usage has left a legacy of water pollution.

This project investigates the possibility of using Lemna minor L. as a quick bioassay for water quality throughout a riverine system. Procedures have been adapted from Wunchen Wang (1990) and Section 8211 of the Standard Methods 18th Edition (ATSM, 1992). The test does not quantify or specify pollutants, but provides a qualitative, seasonal assessment of phytotoxicity at the primary production level of the food chain. Testing is nonelaborate, inexpensive, and replicable. The National Park Service has previously commissioned chemical analyses of water quality, detailing oil and gas exploitation, tourist development, and acid coal mine waste point sources (Rickard, Kunkle, and Wilson, 1986).

Bioassay project results provide a useful indication of water

quality from the major tributaries down to Lake Cumberland, and include two site-specific tributaries of interest. The most contaminated site in the study is Upper Bear Creek tributary, focus of a strip mine stream quality amelioration effort conducted by government agencies (Burr, 1993). In contrast to the lack of improvement stated in the amelioration report, responses in bioassays indicate improvement at the level of resolution provided by Lemna minor.

## II. Literature Review

### The Big South Fork

The Big South Fork of the Cumberland River is formed by the confluence of two main tributaries, the Clear Fork and the New River. It flows north 77 miles before joining the Cumberland River at Burnside, Kentucky (Safley, 1970). It is the third largest Cumberland River tributary, with a total drainage area of 1,382 square miles (Safley, 1970). Much of the area is operated as a National River and Recreation Area by the National Park Service. The section of the river between the Tennessee-Kentucky border and the Blue Heron mine is designated as a Kentucky wild river (U. S. Park Service, 1985).

Surface water quality within the system has been severely impacted by past and present land use. The Park Service is presently investigating the mitigation and abatement of chronic and incidental toxic contamination to the system, and has reported contamination of point sources by main tributaries (Rickard, Kunkle, and Wilson, 1986). The waterways have been polluted by a combination of coal mining, oil and gas exploration, agricultural runoff, erosion from forestry practices, and sewage discharge from private and

urban sources. Recreational development of the area by the U. S. Army Corps of Engineers and the National Park Service began in 1978 (Rickard, Kunkle, and Wilson, 1986).

There are only a few light industries in the watershed, none of which are major water users (Rickard, Kunkle, and Wilson, 1986). Their Water Quality Report also states that waters in the western half of the recreation area are relatively undisturbed, while the eastern and southern regions have degraded water quality of varying intensity. The report refers to over 150 active and abandoned oil and gas well sites, with a network of access roads; contamination from households and the sewage plant in Oneida, Tennessee; roadside garbage dumps which are impacted during flood events; and erosion from mining, agricultural and forestry practices. The low flows in the late summer tend to concentrate pollutants in the system. A summary of the main water quality parameters used by the Water Quality Report, and main tributary streams documented by severity of pollution is listed in Appendix D.

The Big South Fork is located in a region of mild winters and humid, warm to hot summers. Prevailing winds from the south and southwest bring moisture into the area, with heavy rainstorms and flooding most likely from December through

March (U. S. Park Service, 1985). The largest average river flows are recorded in March, and the smallest in October (Rikard, Kunkle, and Wilson, 1986). The National Climatic Data Center at Asheville, North Carolina, has compiled precipitation data for Oneida, Tennessee. Their data (see Appendix B) define the number of days during which one inch or more of rain was recorded. During the period of our water sample collections, there were six months between April 1991 and April 1992 during which at least one inch fell at one time. There were two months during which one inch fell at least twice. In one case, the greatest monthly rainfall event was recorded four days prior to our field collection. One inch rainfalls following a relatively dry period of 0.2 or less for seven days is considered a storm event for monitoring by the Tennessee Department of Health and the Environment. There was one storm event during our period of collection (Appendix B).

The geology of the Big South Fork is of Pennsylvanian and Mississippean strata which have been faulted and eroded into an intensely dissected topography. Valley bottoms contain colluvial material, with little flood plain present in the gorges (Safley, 1970). Soils are derived chiefly from the sandstones and shales of the underlying bedrock, as well as limestone and alluvium of the bottom of the main gorge

(Safley, 1970). There is a resistant cap of Pennsylvanian rocks, and soils tend to be of low fertility (Rickard, Kunkle, and Wilson, 1986). A summary of the main geologic formations cut through by the primary tributaries and the main river are listed in Appendix G.

The vegetation of the area is still in a successional state after extensive and repeated harvests. It is dominated by a mixed pine-oak-hickory forest, with white pine and hemlock present in the coves (Rickard, Kunkle, and Wilson, 1986). Vegetation of the gorges is poorly documented, but consists mainly of mixed mesophytic forest with many Cumberland Plateau endemics. Habitat varies from xeric to mesophytic to hydric, but the microclimates of the gorge are not well detailed. A list of the primary association types documented by Safley in 1970 is listed in Appendix C. The area is inhabited by typical southeastern United States wildlife, including white-tail deer, red and grey fox, bobcat, feral hogs, gray squirrel, wild turkey, and ruffed grouse (Rickard, Kunkle, and Wilson, 1986). A project to reintroduce the black bear to the region is currently underway. During the course of the Park Service Water Quality Study, 44 fish species and 215 benthic macroinvertebrates were documented in the primary river system (Rickard, Kunkle, and Wilson, 1986).

## Lemna minor

Lemna minor is a recommended macrophyte for toxicity testing (Wang, 1990). Known commonly as duckweed or duckmeat, the plant is an extremely fast-growing aquatic monocot. The flattened, leaflike thallus ("frond") (Hillman, 1961) floats on the surface of still or slightly moving water. Reproduction of the "fronds" is usually vegetative (Hillman, 1961), and during the course of the experiments, no flowering was observed. New "daughter" fronds are produced from two pockets on each side of the narrower end of the older "mother" frond, near the point at which the root arises; and are alternately produced from side to side (Hillman, 1961). The upper surface of the frond is highly cutinized and unwettable, with stomates located on the upper surface. Vascular bundles are greatly reduced, and roots are adventitious, with no root hairs. The lower surface of the frond absorbs nutrients, with the roots serving primarily as anchors and tangles to keep the fronds together in colonies (Hillman, 1961). The fronds are generally lens-shaped, with the lower surface of the frond flat or slightly convex (Godfrey and Wooten, 1979). Fronds range from green to yellowish green, and margins are entire. The Lemna minor grown during the course of the experiment were usually light yellowish green, with dark green being typical of the stock

solution specimens grown in complete nutrient solution. Fronds are broadly obovate to almost obovate, between two to four millimeters long, symmetric to slightly asymmetric (Godfrey and Wooten, 1979). Colonies of three or more thalli are common, with colony breakup common after production of six to seven thalli. Each thallus is a few cells thick, with large intercellular air spaces. Calcium oxalate crystals accumulate, and are deposited in large parencymous cells in the thallus or in the root cortex and epidermis (Sculthorpe, 1967).

Light intensity and temperature influence the rate of multiplication and growth of the thallus (Sculthorpe, 1967). Varying effects of nitrogen and potassium in relation to light intensities, with poor light leading to an excess of these nutrients, has also been cited by Sculthorpe. Development of the root has been correlated to the carbohydrate/protein ratio of the thallus, with a longer root produced when the ratio is greater than unity; and a decrease in root length linked to a fall in potassium levels or light intensity (Sculthorpe, 1967).

Colonies of Lemna minor are commonly found in still or slightly moving water, where they may break up at the slightest touch. During the course of the experiment, frond

number counts were made before any breakup. Duckweed development is reported at all light intensities from full sun to below 50 foot-candles in dense shade. Temperatures between 20°C and 30°C are most favorable to growth (Sculthorpe, 1967).

A large number of early investigators have documented the mineral nutrition of Lemna minor (Wang, 1990). Duckweed responds as a typical higher plant, growing in light on a complete inorganic medium containing the major elements plus iron, manganese, molybdenum, boron, zinc, copper, gallium, and a trace of chlorine (Sculthorpe, 1967). Low nitrate levels reduce multiplication rates, frond areas, clorophyll contents, protein contents, and respiration; and increase starch content and root lengths in Lemna minor, with the longest roots occurring under conditions of high carbon assimilation and low nitrogen (Sculthorpe, 1967). It is reported that low potassium levels decrease root lengths, multiplications rates, net assimilation, and frond areas, with similar effects reported for calcium (Sculthorpe, 1967).

The optimum temperature for frond multiplication rates is reported by Sculthorpe to be approximately 29°C, with thermoperiodic effects not noted. Senescence and rejuvenation cycles result in different frond sizes in the

population, and care was taken during the course of the experiment to select test fronds of approximately equal size.

Lemna minor can use organic molecules directly (Hillman and Cully, 1978), and is often found in polluted waters. It forms a large matted surface over the sewage containment lagoons at Bandy Creek (Headquarters, NPS, Big South Fork). Lemna minor may accumulate toxic metal. Boron is accumulated at a rate proportional to its external concentration, possibly as a deterrent to predators (Hillman and Cully, 1978). Exposure to Triton molecules produced in the manufacture of pesticides and herbicides cause a decrease in frond development (Caux, et al., 1988). Excess copper sulfate causes chlorosis, reduced frond size, the continued attachment of daughter fronds to mothers, and the reduction of the photosynthetic uptake of $CO_2$ (Filbin and Hough, 1979). Pentachlorophenol (PCP) is reported as inhibiting the photosynthetic oxygen production of Lemna minor (Huber, et al., 1982).

## III. Sampling Methods

Nine water collection sites were chosen in Kentucky and Tennessee: New River Bridge (New River), Burnt Mill Bridge (Clear Fork), Leatherwood Ford, Station Camp, TN; Blue Heron Mine, Yamacraw Bridge, Alum Ford, KY; Bandy Creek, TN; Upper Bear Creek, KY. These sites include both major tributaries, and the Leatherwood Ford through Alum Ford sites represent the conditions of the river from confluence to the Lake Cumberland pool head. Bandy Creek and Upper Bear Creek are low-order stream tributaries. Upper Bear Creek was selected because it drains an open strip mine site currently undergoing outflow reclamation efforts, where pollutants have been well documented. In contrast, Bandy Creek, which drains a modern waste-treatment system, is now among the most pristine streams in the park (NPS, extant data). Sites were chosen to be accessible by vehicle.

Six daylight water collection trips were made: 5 June 1991, 16 July 1991, 11 October 1991, 18 December 1991, 25 March 1992, and 20 August 1992. Dates were chosen to anticipate seasonal variations in expected precipitation and river flow. When logistics allowed, all sites were sampled for each trip. Buckets of water were dipped from the river and funnelled into sterile water collection containers. Site pH, water

temperature, and flow readings were taken (Appendix A). Redox was recorded when possible. At the request of the NPS, selected samples from the nearby, but discrete, Obed River were also tested in adjunct runs (Appendix F).

## IV. Laboratory Methods

*Lemna minor* plants with only two fronds, no buds, no chlorosis, and no holes in the fronds from tanks in the University of Tennessee Greenhouse 1 of Hesler Biology Building were used. Fifteen plants, or a total of 30 fronds, were selected and weighed, then grown under controlled conditions in petri dishes containing either stock solution or river water collected at one of the nine sites. After five days, plants were removed from the growth chamber, and frond counts and weights were determined. Three replicates of each sample were made, and each sample site collection run was repeated three times.

Ten milliliters of each of three stock solutions adapted from Wang (1990) plus 0.3 ml of Murashige FeEDTA solution were diluted into a one-liter flask to make 10-fold strength algal nutrient solution. The Wang solution is as follows:

| | | |
|---|---|---|
| Stock A | $NaNO_3$ | 22,500 mg/liter |
| | $NaHCO_3$ | 15,000 mg/liter |
| | $K_2HPO_4$ | 1,040 mg/liter |
| Stock B | $MgCl_2 \cdot 6H_2O$ | 12,164 mg/liter |
| | $CaCl_2 \cdot 2H_2O$ | 4,410 mg/liter |

| | | |
|---|---|---|
| | $FeCl_3$ | 96 mg/liter |
| | $Na_2EDTA \cdot 2H_2O$ | 300 mg/liter |
| | $MnCl_2$ | 264 mg/liter |
| Stock C | $MgSO_4 \cdot 7H_2O$ | 14,700 mg/liter |
| | $H_3BO_3$ | 185.5 mg/liter |
| | $ZnCl_2$ | 3.270 mg/liter |
| | $CoCl_2$ | 0.780 mg/liter |
| | $NaMoO_4 \cdot 2H_2O$ | 7.260 mg/liter |
| | $CuCl_2$ | 0.009 mg/liter |

Toxicity testing conditions as detailed by Wang were:

| | |
|---|---|
| Test type | Static |
| Temperature | 27-28°C |
| Light | 86 uE/$m^2$/s, cool-white fluorescent |
| Photoperiod | Continuous |
| Test vessel | 100 x 15-mm culture dish |
| Test solution | 15 ml |
| Fronds/vessel | 20 (10 colonies) |
| Replicates | 4 |
| Control solution | 10-fold algal nutrient solution |
| Test duration | 120 hours |
| End point | Frond count increase |

Solutions not available through our sources were amended by

James D. Caponetti (1991) as follows:

| | | |
|---|---|---|
| Stock A | $NaNO_3$ | 22,500 mg/liter |
| | $NaHCO_3$ | 15,000 mg/liter |
| | $K_2HPO_4$ | 1,040 mg/liter |
| Stock B | $MgCl_2 \cdot 6H_2O$ | 12,164 mg/liter |
| | $CaCl_2 \cdot 2H_2O$ | 4,410 mg/liter |
| | $MnCl_2 \cdot 4H_2O$ | 415 mg/liter |
| Stock C | $MgSO_4 \cdot 7H_2O$ | 14,700 mg/liter |
| | $H_3BO_3$ | 185.5 mg/liter |
| | $ZnCl_2$ | 3.27 mg/liter |
| | $CoCl_2$ | 0.78 mg/liter |
| | $NaMoO_4 \cdot 2H_2O$ | 7.26 mg/liter |
| | $CuCl_2 \cdot 2H_2O$ | 0.01 mg/liter |

Growth chamber conditions in the University of Tennessee Botany facility were as follows:

| | |
|---|---|
| Test type | Static |
| Temperature | 25°C |
| Light | 2500 foot-candles, cool-white fluorescent |
| Photoperiod | Continuous |
| Test vessel | 100 x 15-mm petri dish |

| | |
|---|---|
| Test solution | 15 ml |
| Fronds/vessel | 30 (15 colonies) |
| Replicates | 3 |
| Control solution | 10-fold algal nutrient medium |
| Test duration | 5 days |
| End points | Frond count and biomass |

## V. Results

Biomass measurements were dropped early in the study. Data was inconsistent and non-quantifiable; the biomass consistency and hydration state would increase, decrease or remain constant (zero) with no regularity or predictability. Frondcount proved much more straight-forward and repeatable.

Endpoint frond counts were made for each replicated sample site, in order, from headwaters to the Cumberland pool, followed by the two streams. Sample abbreviations are as follows: New River Bridge (NRB), Burnt Mill Bridge (BMB), Leatherwood Ford (LF), Station Camp (SC), Blue Heron Mine (BHM), Yamacraw Bridge (YB), Alum Ford (AF), Bandy Creek (BC), and Upper Bear Creek (UBC). Milepoint approximations are: NRB milepoint 81.5, BMB milepoint 75.5, LF milepoint 70, SC milepoint 62.5, BHM milepoint 46, YB milepoint 41, AF milepoint 36, BC milepoint at river conjunction approximately 71, and UBC milepoint at river conjunction approximately 50.5.

Endpoint frond counts indicated several broad generalizations concerning pH. Frond counts were consistently higher in lab media controls of pH 5.0 (C5.0) and 7.5 (C7.5), with the best growth results occurring in the pH 5.0 samples. Measurements of pH values in the controls after the 120-hour

growth periods indicated that pH shifts occurred in the solutions. In the 5.0 control samples, shifts to pH's between 7.0 and 7.6 were recorded, probably as a result of algal growth on the Lemna minor fronds and roots. Algal cultures are known to adjust pH's in nutrient solutions (Caponetti, 1992). Smaller shifts occurred in the 7.5 control samples, usually toward the 8.0 pH range.

Frondcount totals are higher for Leatherwood Ford (LF), Station Camp (SC) and Blue Heron Mine (BHM) sample sites in the mainstream of the river, indicated by a slight bell-shaped curve in most of the figures. Leatherwood Ford (LF) provided the highest total frondcount for the 25 March 1992 and 20 August 1992 trips (378 and 365 total fronds, respectively). Station Camp provided the highest count for the 5 June 1991 trip (302 total fronds). Total frond count for Blue Heron Mine was highest for the 18 December 1991 trip (406 total fronds).

Frondcount totals in the two tributaries, New River (NRB) and Clear Fork (BMB), tend to be equivalent to the central portion of the river, with Clear Fork consistently higher than New River. In the 16 July 1991 trip, the Clear Fork (BMB) samples had the highest frondcounts (257 total fronds). In the 11 October 1991 trip, the New River Bridge (NRB) site

totalled highest (325 total fronds).

Tables 1-18 and their associated Figures 1-18 depict the six collection trips by test run. Tables 19-24 and their associated Figures 19-24 show total endpoint frond counts for the three test runs by collection trip. Figures are set at equivalent frond counts on the y-axis, to facilitate comparison. Table 25 shows the grand total frondcounts for each trip.

Table 1. 5 June 1991 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 77 | Lab media |
| C7.5B | 62 | |
| C7.5C | 64 | |
| Total | 203 | |
| | | |
| C5.0A | 47 | Lab media |
| C5.0B | 71 | |
| C5.0C | 63 | |
| Total | 181 | |
| | | |
| NRB-A | 29 | New River Bridge, TN |
| NRB-B | 25 | |
| NRB-C | 26 | |
| Total | 80 | |
| | | |
| BMB-A | 12 | Burnt Mill Bridge, TN |
| BMB-B | 24 | |
| BMB-C | 26 | |
| Total | 62 | |
| | | |
| LF-A | 28 | Leatherwood Ford, TN |
| LF-B | 30 | |
| LF-C | 26 | |
| Total | 84 | |
| | | |
| SC-A | 34 | Station Camp, TN |
| SC-B | 38 | |
| SC-C | 31 | |
| Total | 103 | |
| | | |
| BHM-A | 28 | Blue Heron Mine, KY |
| BHM-B | 29 | |
| BHM-C | 29 | |
| Total | 86 | |
| | | |
| YB-A | 31 | Yamacraw Bridge, KY |
| YB-B | 27 | |
| YB-C | 28 | |
| Total | 86 | |
| | | |
| AF-A | 22 | Alum Ford, KY |
| AF-B | 22 | |
| AF-C | 19 | |
| Total | 63 | |
| | | |
| UBC-A | 23 | Upper Bear Creek, KY |
| UBC-B | 20 | |
| UBC-C | 24 | |
| Total | 67 | |

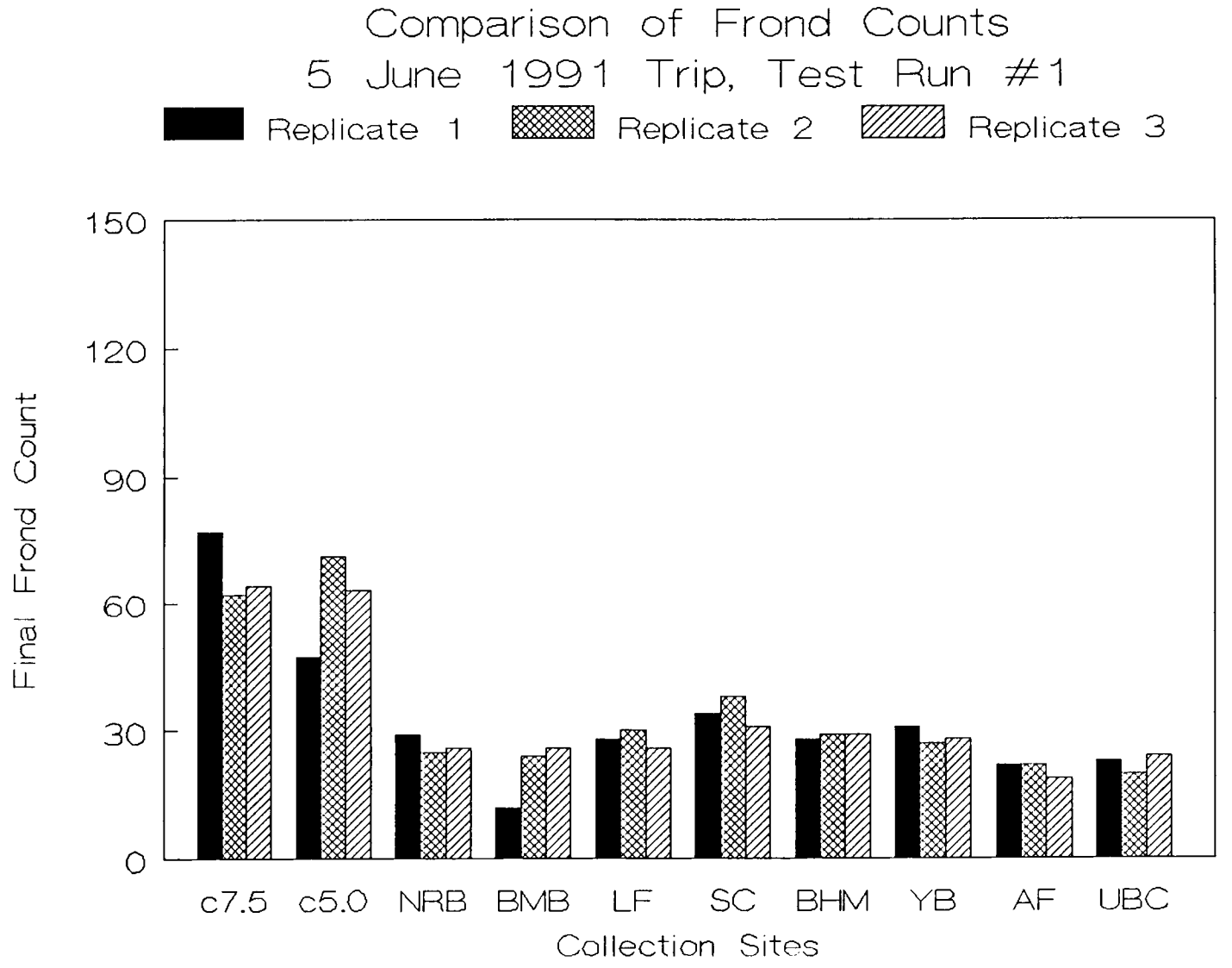


Figure [illegible] 5 June 1991 Trip, Test Run #1

Table 2. 5 June 1991 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 56 | Lab media |
| C7.5B | 41 | |
| C7.5C | 34 | |
| Total | 131 | |
| | | |
| C5.0A | 58 | Lab media |
| C5.0B | 52 | |
| C5.0C | 46 | |
| Total | 156 | |
| | | |
| NRB-A | 24 | New River Bridge, TN |
| NRB-B | 25 | |
| NRB-C | 23 | |
| Total | 72 | |
| | | |
| BMB-A | 22 | Burnt Mill Bridge, TN |
| BMB-B | 24 | |
| BMB-C | 29 | |
| Total | 75 | |
| | | |
| LF-A | 25 | Leatherwood Ford, TN |
| LF-B | 22 | |
| LF-C | 23 | |
| Total | 70 | |
| | | |
| SC-A | 36 | Station Camp, TN |
| SC-B | 34 | |
| SC-C | 29 | |
| Total | 99 | |
| | | |
| BHM-A | 27 | Blue Heron Mine, KY |
| BHM-B | 25 | |
| BHM-C | 24 | |
| Total | 76 | |
| | | |
| YB-A | 25 | Yamacraw Bridge, KY |
| YB-B | 26 | |
| YB-C | 30 | |
| Total | 81 | |
| | | |
| AF-A | 23 | Alum Ford, KY |
| AF-B | 23 | |
| AF-C | 19 | |
| Total | 81 | |
| | | |
| UBC-A | 17 | Upper Bear Creek, KY |
| UBC-B | 18 | |
| UBC-C | 16 | |
| Total | 51 | |

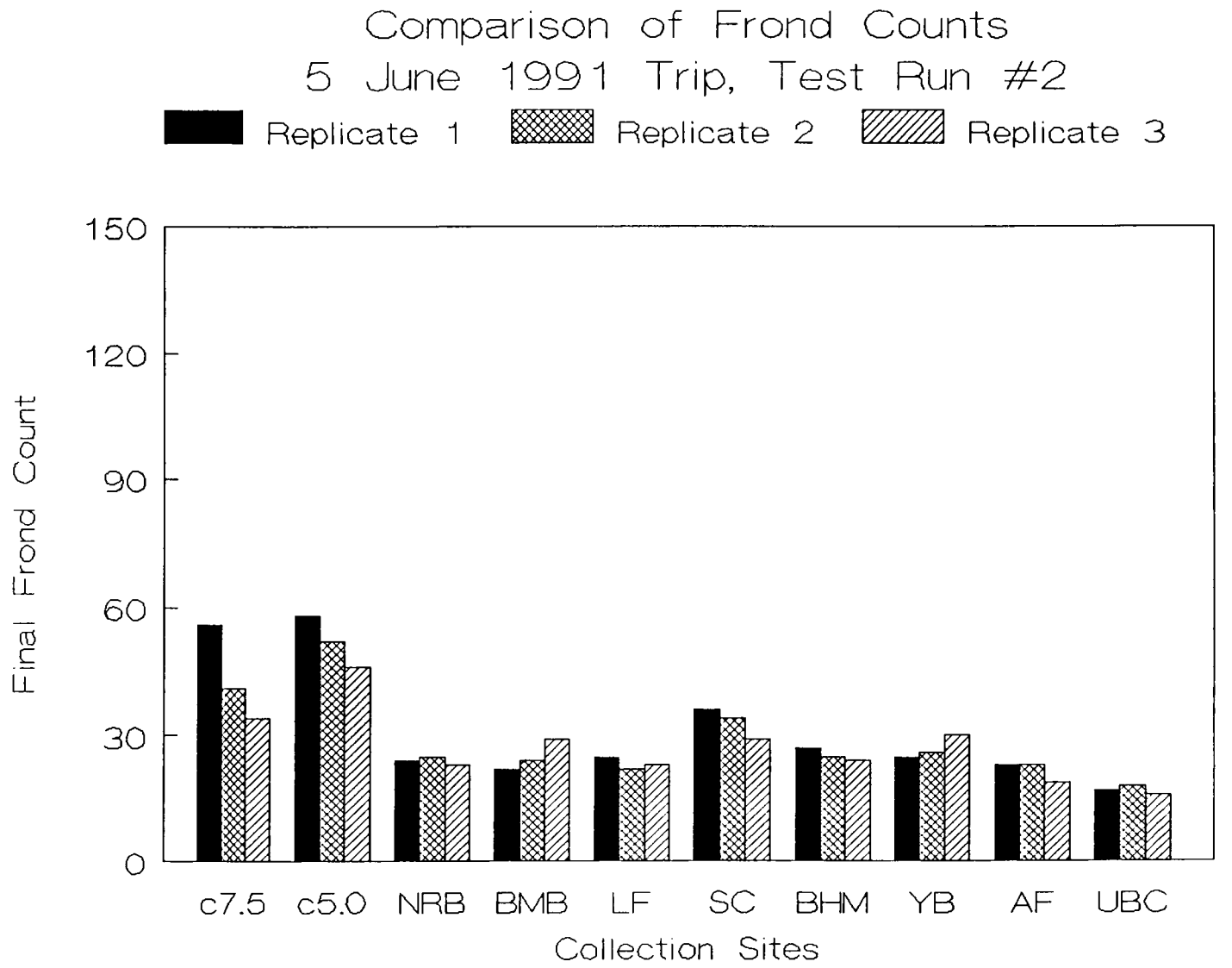


Figure 2. 5 June 1991 Trip Totals, Second Run

Table 3. 5 June 1991 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 97 | |
| C5.0C | 150 | |
| Total | 338 | |
| | | |
| NRB-A | 31 | New River Bridge, TN |
| NRB-B | 28 | |
| NRB-C | 30 | |
| Total | 89 | |
| | | |
| BMB-A | 36 | Burnt Mill Bridge, TN |
| BMB-B | 31 | |
| BMB-C | 36 | |
| Total | 103 | |
| | | |
| LF-A | 33 | Leatherwood Ford, TN |
| LF-B | 31 | |
| LF-C | 33 | |
| Total | 97 | |
| | | |
| SC-A | 16 | Station Camp, TN |
| SC-B | 36 | |
| SC-C | 48 | |
| Total | 100 | |
| | | |
| BHM-A | 33 | Blue Heron Mine, KY |
| BHM-B | 29 | |
| BHM-C | 30 | |
| Total | 92 | |
| | | |
| YB-A | 35 | Yamacraw Bridge, KY |
| YB-B | 32 | |
| YB-C | 31 | |
| Total | 98 | |
| | | |
| AF-A | 30 | Alum Ford, KY |
| AF-B | 29 | |
| AF-C | 31 | |
| Total | 90 | |
| | | |
| UBC-A | 20 | Upper Bear Creek, KY |
| UBC-B | 28 | |
| UBC-C | 16 | |
| Total | 64 | |

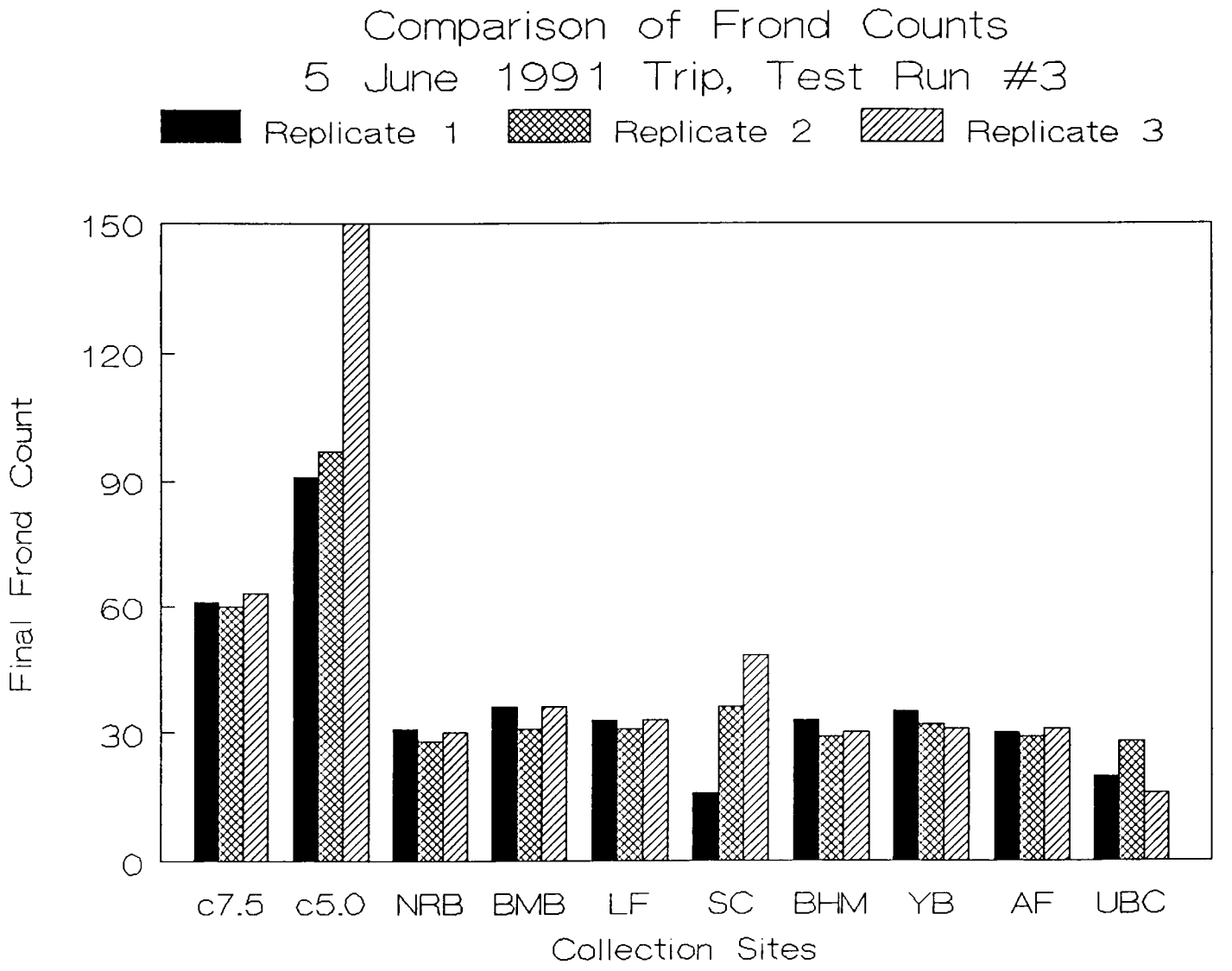


Figure [illegible] 5 June 1991 Trip Totals, Third Run.

Table 4. 16 July 1991 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 62 | Lab media |
| C7.5B | 63 | |
| C7.5C | 89 | |
| Total | 214 | |
| | | |
| C5.0A | 84 | Lab media |
| C5.0B | 90 | |
| C5.0C | 98 | |
| 3Total | 272 | |
| | | |
| NRB-A | 26 | New River Bridge, TN |
| NRB-B | 29 | |
| NRB-C | 28 | |
| Total | 83 | |
| | | |
| BMB-A | 27 | Burnt Mill Bridge, TN |
| BMB-B | 26 | |
| BMB-C | 22 | |
| Total | 75 | |
| | | |
| LF-A | 25 | Leatherwood Ford, TN |
| LF-B | 23 | |
| LF-C | 23 | |
| Total | 71 | |
| | | |
| BHM-A | 24 | Blue Heron Mine, KY |
| BHM-B | 28 | |
| BHM-C | 25 | |
| Total | 77 | |
| | | |
| YB-A | 25 | Yamacraw Bridge, KY |
| YB-B | 25 | |
| YB-C | 27 | |
| Total | 77 | |
| | | |
| AF-A | 18 | Alum Ford, KY |
| AF-B | 22 | |
| AF-C | 24 | |
| Total | 64 | |
| | | |
| BC-A | 27 | Bandy Creek, TN |
| BC-B | 24 | |
| BC-C | 26 | |
| Total | 77 | |
| | | |
| UBC-A | 26 | Upper Bear Creek, KY |
| UBC-B | 24 | |
| UBC-C | 35 | |
| Total | 85 | |

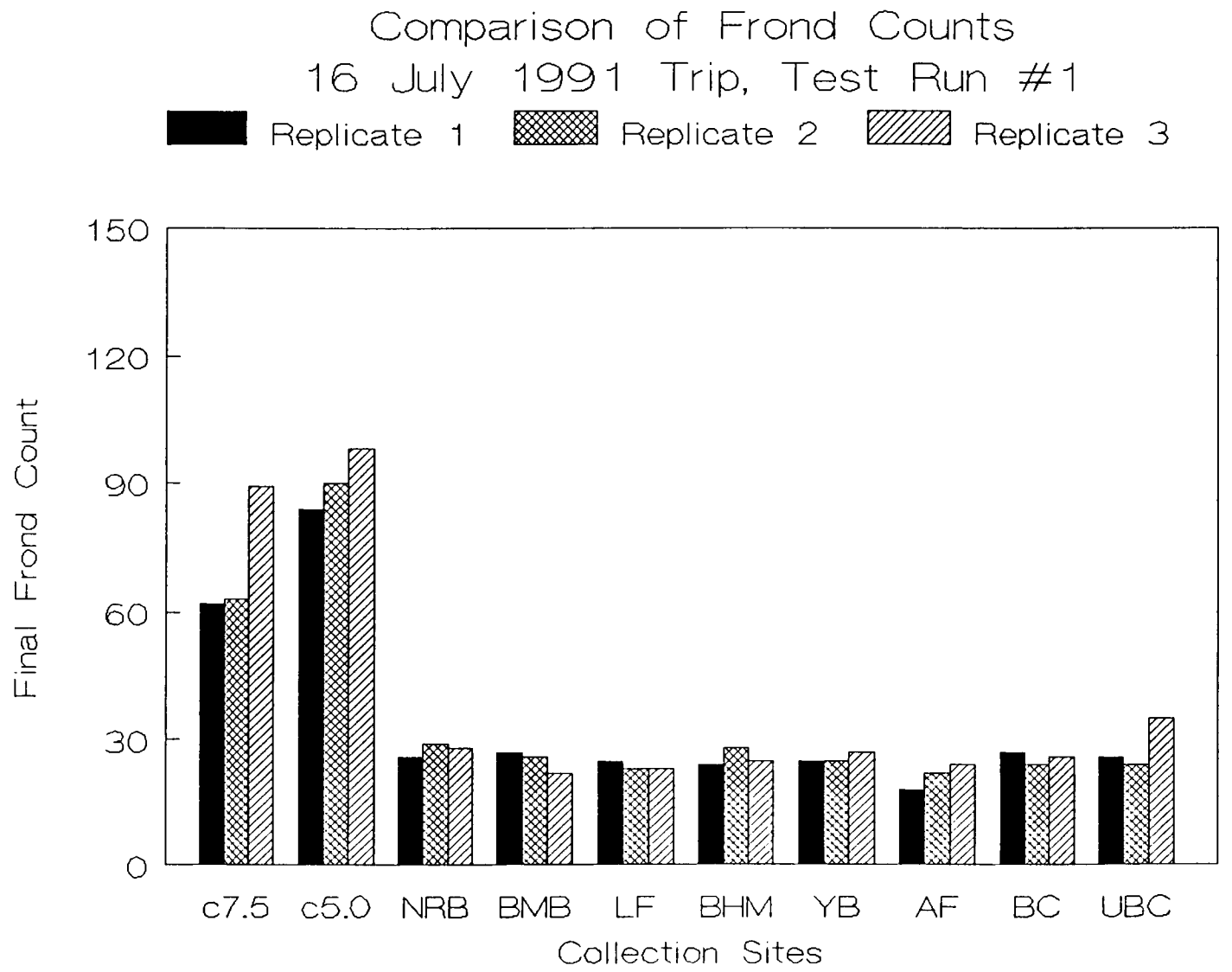


Figure 1. 16 July 1991 Trip Totals, First Run

Table 5. 16 July 1991 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 54 | Lab media |
| C7.5B | 60 | |
| C7.5C | 82 | |
| Total | 196 | |
| | | |
| C5.0A | 80 | Lab media |
| C5.0B | 66 | |
| C5.0C | 58 | |
| Total | 204 | |
| | | |
| NRB-A | 16 | New River Bridge, TN |
| NRB-B | 15 | |
| NRB-C | 15 | |
| Total | 46 | |
| | | |
| BMB-A | 15 | Burnt Mill Bridge, TN |
| BMB-B | 16 | |
| BMB-C | 16 | |
| Total | 47 | |
| | | |
| LF-A | 13 | Leatherwood Ford, TN |
| LF-B | 15 | |
| LF-C | 15 | |
| Total | 43 | |
| | | |
| BHM-A | 18 | Blue Heron Mine, KY |
| BHM-B | 15 | |
| BHM-C | 12 | |
| Total | 45 | |
| | | |
| YB-A | 15 | Yamacraw Bridge, KY |
| YB-B | 16 | |
| YB-C | 13 | |
| Total | 44 | |
| | | |
| AF-A | 18 | Alum Ford, KY |
| AF-B | 13 | |
| AF-C | 18 | |
| Total | 49 | |
| | | |
| BC-A | 15 | Bandy Creek, TN |
| BC-B | 17 | |
| BC-C | 18 | |
| Total | 50 | |
| | | |
| UBC-A | 13 | Upper Bear Creek, KY |
| UBC-B | 18 | |
| UBC-C | 15 | |
| Total | 46 | |

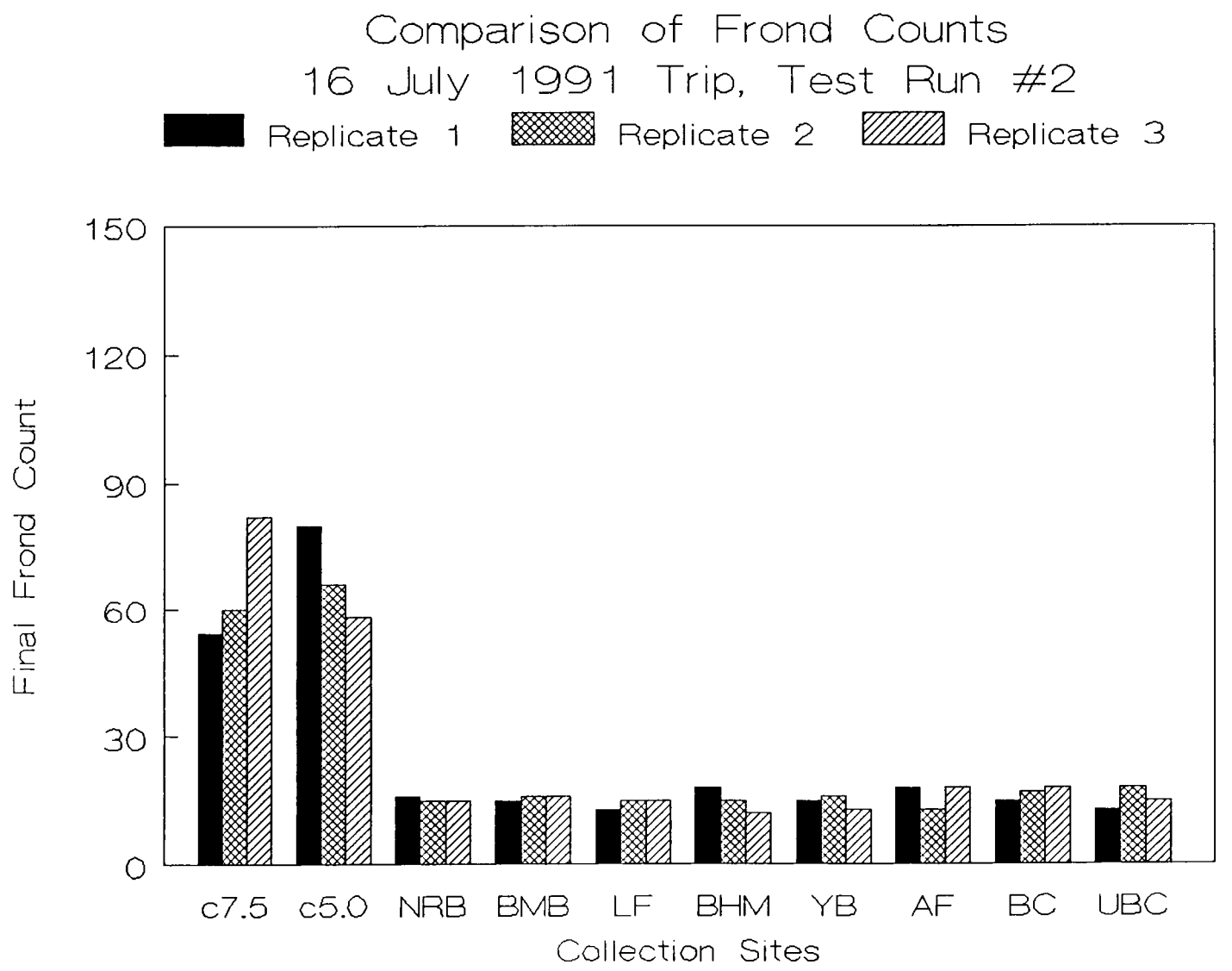


Figure 5. 16 July 1991 Trip Totals, 5 Trial Run

Table 6. 16 July 1991 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 97 | |
| C5.0C | 150 | |
| Total | 338 | |
| | | |
| NRB-A | 30 | New River Bridge, TN |
| NRB-B | 32 | |
| NRB-C | 30 | |
| Total | 92 | |
| | | |
| BMB-A | 30 | Burnt Mill Bridge, TN |
| BMB-B | 32 | |
| BMB-C | 33 | |
| Total | 95 | |
| | | |
| LF-A | 32 | Leatherwood Ford, TN |
| LF-B | 33 | |
| LF-C | 28 | |
| Total | 93 | |
| | | |
| BHM-A | 32 | Blue Heron Mine, KY |
| BHM-B | 29 | |
| BHM-C | 31 | |
| Total | 92 | |
| | | |
| YB-A | 28 | Yamacraw Bridge, KY |
| YB-B | 32 | |
| YB-C | 39 | |
| Total | 99 | |
| | | |
| AF-A | 35 | Alum Ford, KY |
| AF-B | 35 | |
| AF-C | 28 | |
| Total | 93 | |
| | | |
| BC-A | 31 | Bandy Creek, TN |
| BC-B | 30 | |
| BC-C | 29 | |
| Total | 90 | |
| | | |
| UBC-A | 29 | Upper Bear Creek, KY |
| UBC-B | 28 | |
| UBC-C | 29 | |
| Total | 86 | |

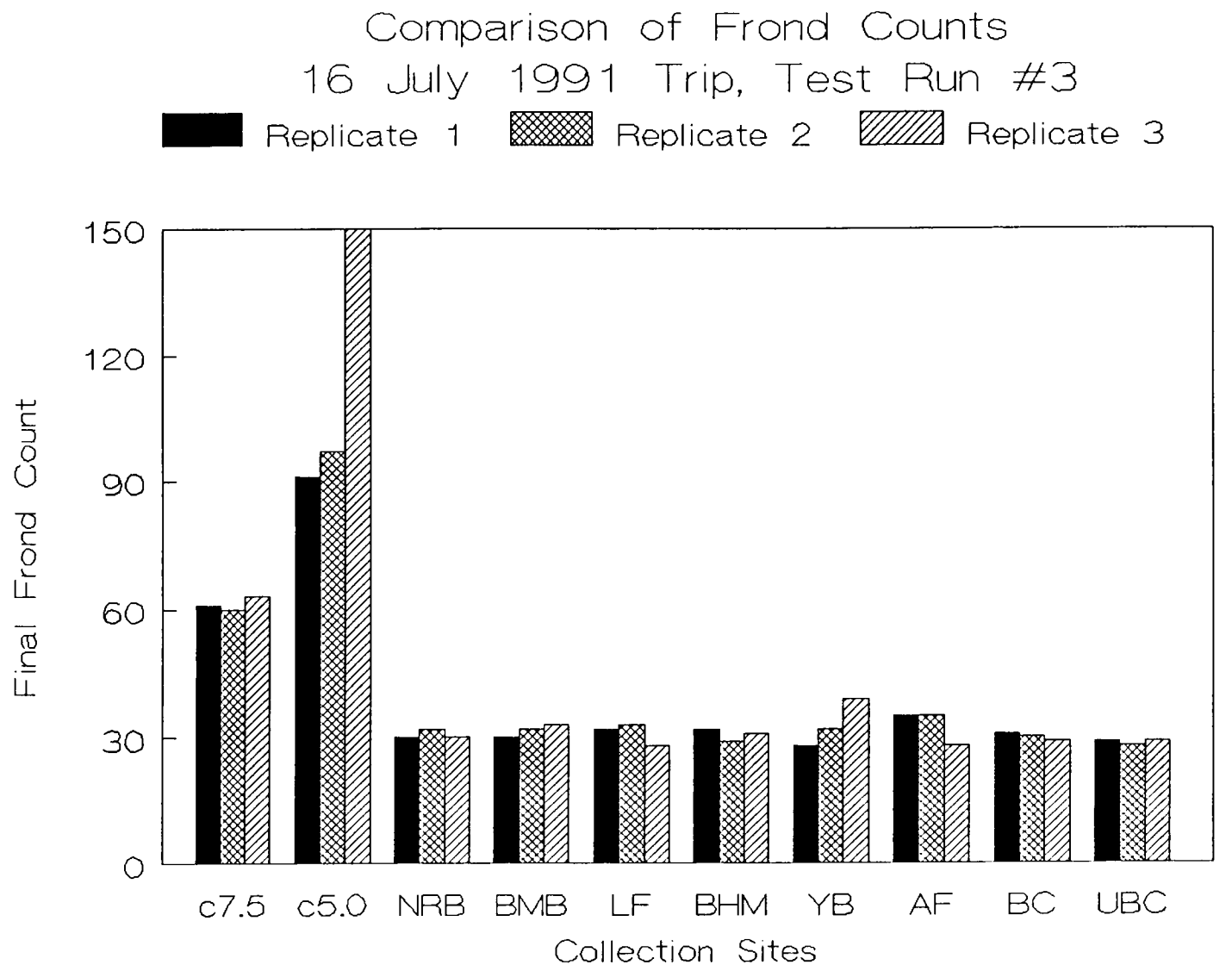


Figure 6. 16 July 1991 Trip Test Run Third Run

Table 7. 11 Oct 1991 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 46 | Lab media |
| C7.5B | 48 | |
| C7.5C | 47 | |
| Total | 141 | |
| | | |
| C5.0A | 62 | Lab media |
| C5.0B | 65 | |
| C5.0C | 162 | |
| Total | 189 | |
| | | |
| NRB-A | 25 | New River Bridge, TN |
| NRB-B | 29 | |
| NRB-C | 27 | |
| Total | 81 | |
| | | |
| BMB-A | 17 | Burnt Mill Bridge, TN |
| BMB-B | 14 | |
| BMB-C | 19 | |
| Total | 50 | |
| | | |
| LF-A | 16 | Leatherwood Ford, TN |
| LF-B | 20 | |
| LF-C | 12 | |
| Total | 48 | |
| | | |
| BHM-A | 16 | Blue Heron Mine, KY |
| BHM-B | 14 | |
| BHM-C | 18 | |
| Total | 48 | |
| | | |
| AF-A | 18 | Alum Ford, KY |
| AF-B | 24 | |
| AF-C | 24 | |
| Total | 66 | |
| | | |
| BC-A | 22 | Bandy Creek, TN |
| BC-B | 13 | |
| BC-C | 15 | |
| Total | 50 | |

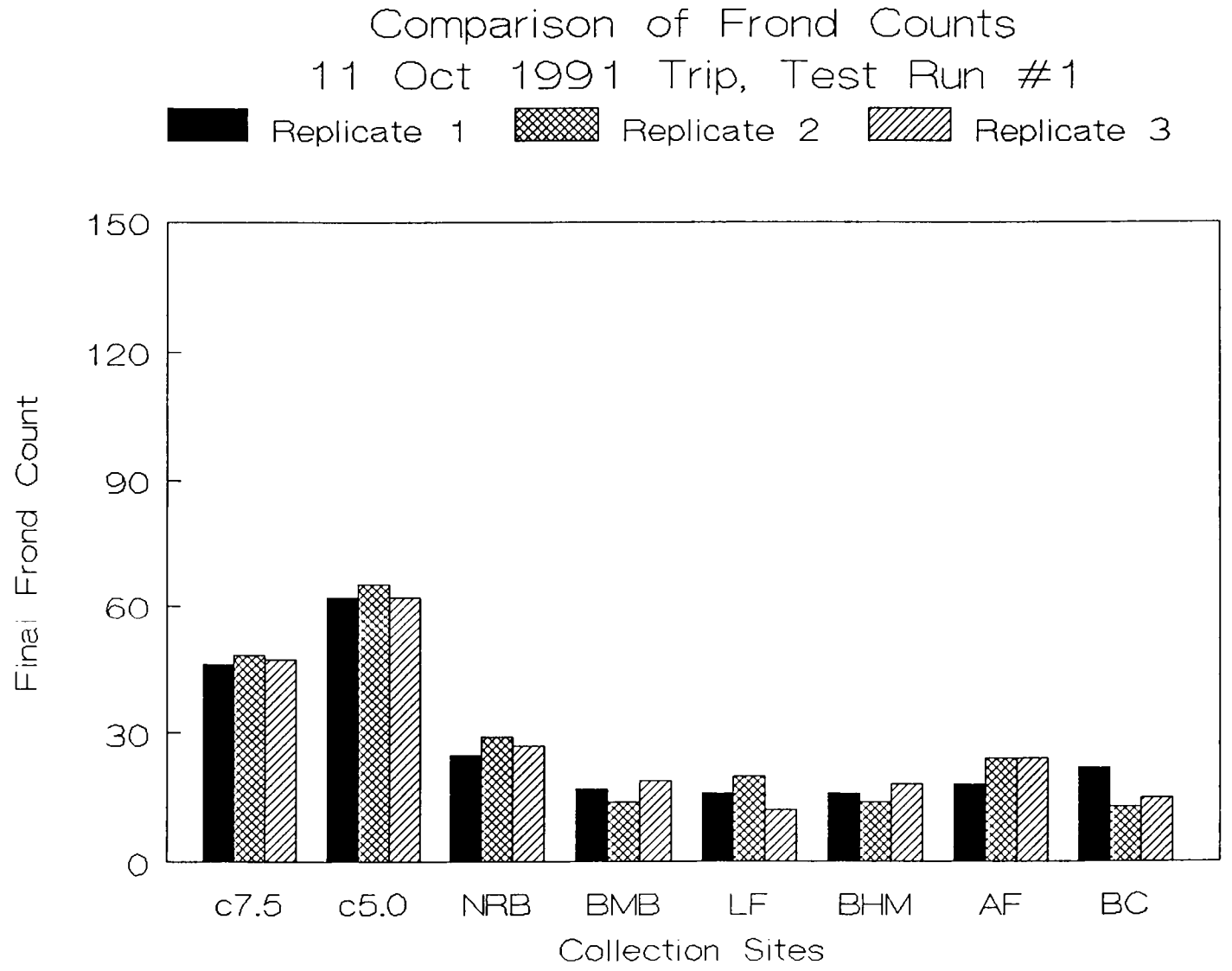


Figure 7. [illegible] 1991 Trip Test Run, First Run

Table 8. 11 Oct 1991 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 70 | Lab media |
| C7.5B | 60 | |
| C7.5C | 64 | |
| Total | 194 | |
| | | |
| C5.0A | 127 | Lab media |
| C5.0B | 135 | |
| C5.0C | 132 | |
| Total | 394 | |
| | | |
| NRB-A | 34 | New River Bridge, TN |
| NRB-B | 34 | |
| NRB-C | 38 | |
| Total | 106 | |
| | | |
| BMB-A | 29 | Burnt Mill Bridge, TN |
| BMB-B | 34 | |
| BMB-C | 30 | |
| Total | 93 | |
| | | |
| LF-A | 32 | Leatherwood Ford, TN |
| LF-B | 33 | |
| LF-C | 37 | |
| Total | 102 | |
| | | |
| BHM-A | 29 | Blue Heron Mine, KY |
| BHM-B | 35 | |
| BHM-C | 29 | |
| Total | 93 | |
| | | |
| AF-A | 34 | Alum Ford, KY |
| AF-B | 37 | |
| AF-C | 35 | |
| Total | 106 | |
| | | |
| BC-A | 30 | Bandy Creek, TN |
| BC-B | 30 | |
| BC-C | 30 | |
| Total | 90 | |

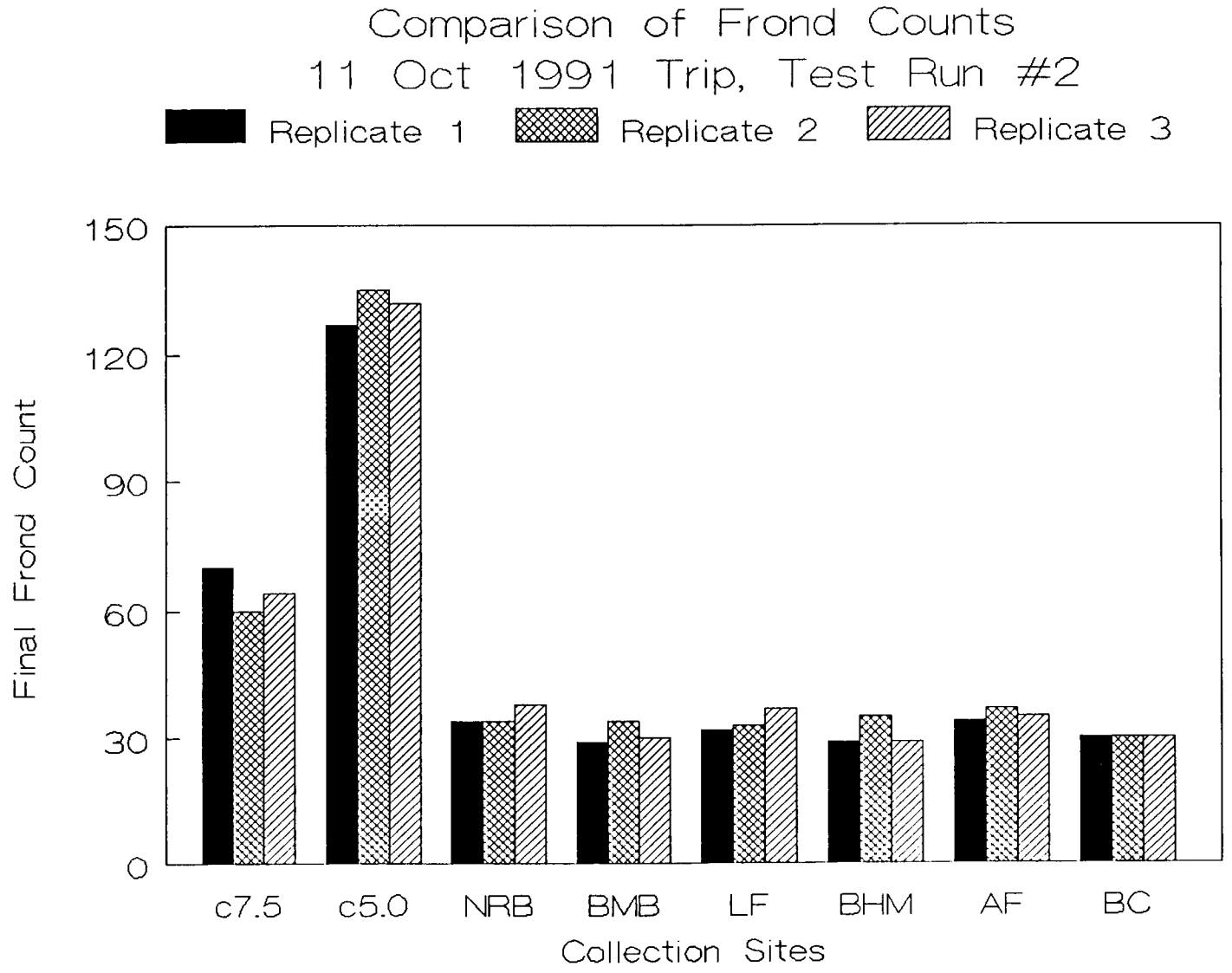


Figure 8. 11 October 1991 Trip Totals, Second Run

Table 9. 11 Oct 1991 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 52 | Lab media |
| C7.5B | 35 | |
| C7.5C | 49 | |
| Total | 136 | |
| | | |
| C5.0A | 55 | Lab media |
| C5.0B | 49 | |
| C5.0C | 66 | |
| Total | 170 | |
| | | |
| NRB-A | 44 | New River Bridge, TN |
| NRB-B | 48 | |
| NRB-C | 46 | |
| Total | 138 | |
| | | |
| BMB-A | 30 | Burnt Mill Bridge, TN |
| BMB-B | 37 | |
| BMB-C | 32 | |
| Total | 99 | |
| | | |
| LF-A | 36 | Leatherwood Ford, TN |
| LF-B | 29 | |
| LF-C | 34 | |
| Total | 99 | |
| | | |
| BHM-A | 32 | Blue Heron Mine, KY |
| BHM-B | 31 | |
| BHM-C | 30 | |
| Total | 93 | |
| | | |
| AF-A | 40 | Alum Ford, KY |
| AF-B | 40 | |
| AF-C | 34 | |
| Total | 114 | |
| | | |
| BC-A | 30 | Bandy Creek, TN |
| BC-B | 31 | |
| BC-C | 32 | |
| Total | 93 | |

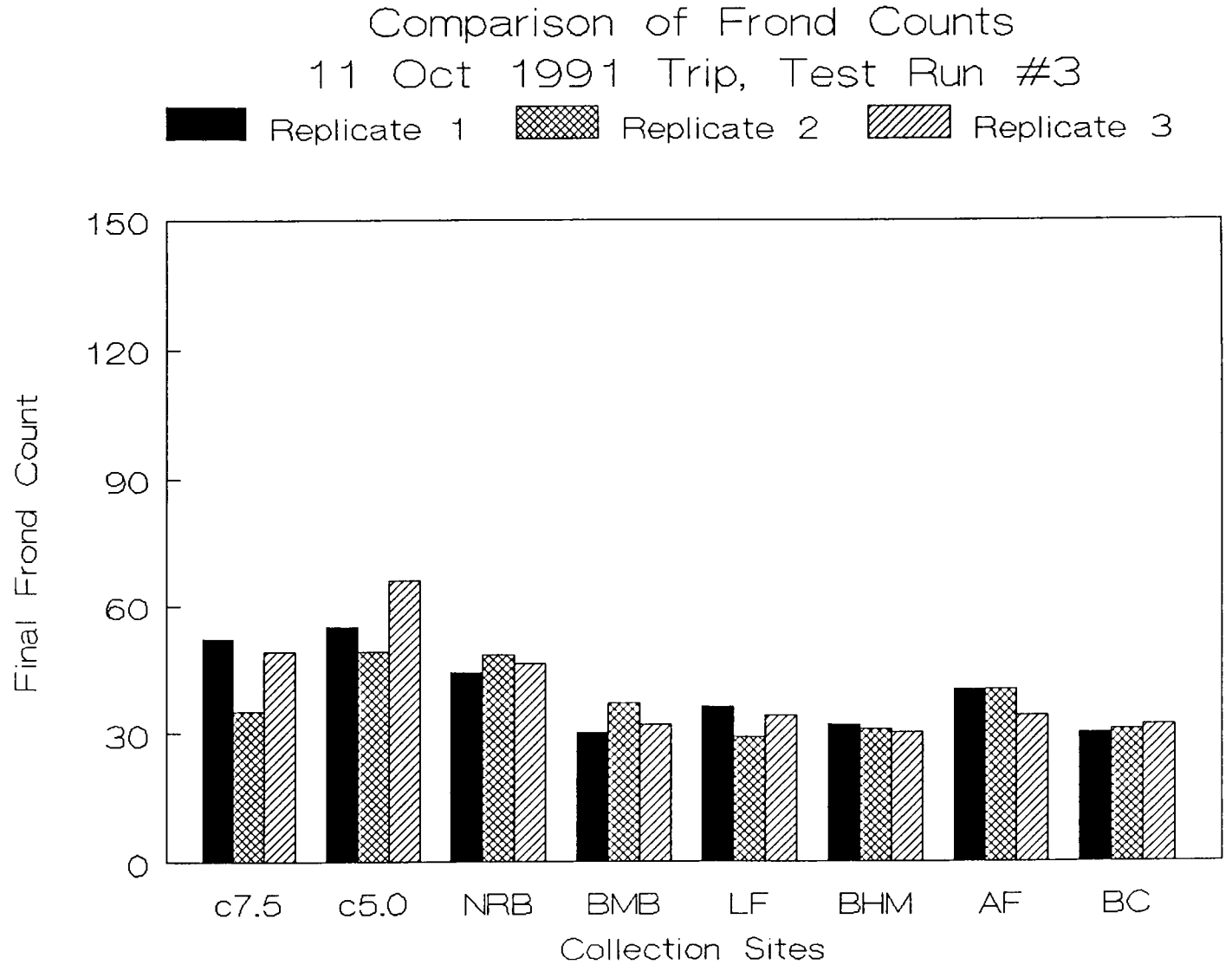


Figure 9. 11 October 1991 Trip Test Run, Third Run.

Table 10. 18 Dec 1991 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 88 | Lab media |
| C7.5B | 99 | |
| C7.5C | 92 | |
| Total | 279 | |
| | | |
| C5.0A | 100 | Lab media |
| C5.0B | 124 | |
| C5.0C | 119 | |
| Total | 343 | |
| | | |
| NRB-A | 29 | New River Bridge, TN |
| NRB-B | 36 | |
| NRB-C | 30 | |
| Total | 95 | |
| | | |
| BMB-A | 31 | Burnt Mill Bridge, TN |
| BMB-B | 27 | |
| BMB-C | 28 | |
| Total | 86 | |
| | | |
| LF-A | 28 | Leatherwood Ford, TN |
| LF-B | 28 | |
| LF-C | 32 | |
| Total | 88 | |
| | | |
| SC-A | 28 | Station Camp, TN |
| SC-B | 31 | |
| SC-C | 30 | |
| Total | 89 | |
| | | |
| BHM-A | 31 | Blue Heron Mine, KY |
| BHM-B | 33 | |
| BHM-C | 28 | |
| Total | 92 | |
| | | |
| YB-A | 24 | Yamacraw Bridge, KY |
| YB-B | 27 | |
| YB-C | 28 | |
| Total | 79 | |
| | | |
| AF-A | 30 | Alum Ford, KY |
| AF-B | 27 | |
| AF-C | 29 | |
| Total | 86 | |
| | | |
| BC-A | 15 | Bandy Creek, TN |
| BC-B | 18 | |
| BC-C | 17 | |
| Total | 50 | |
| | | |
| UBC-A | 21 | Upper Bear Creek, KY |
| UBC-B | 24 | |
| UBC-C | 18 | |
| Total | 63 | |

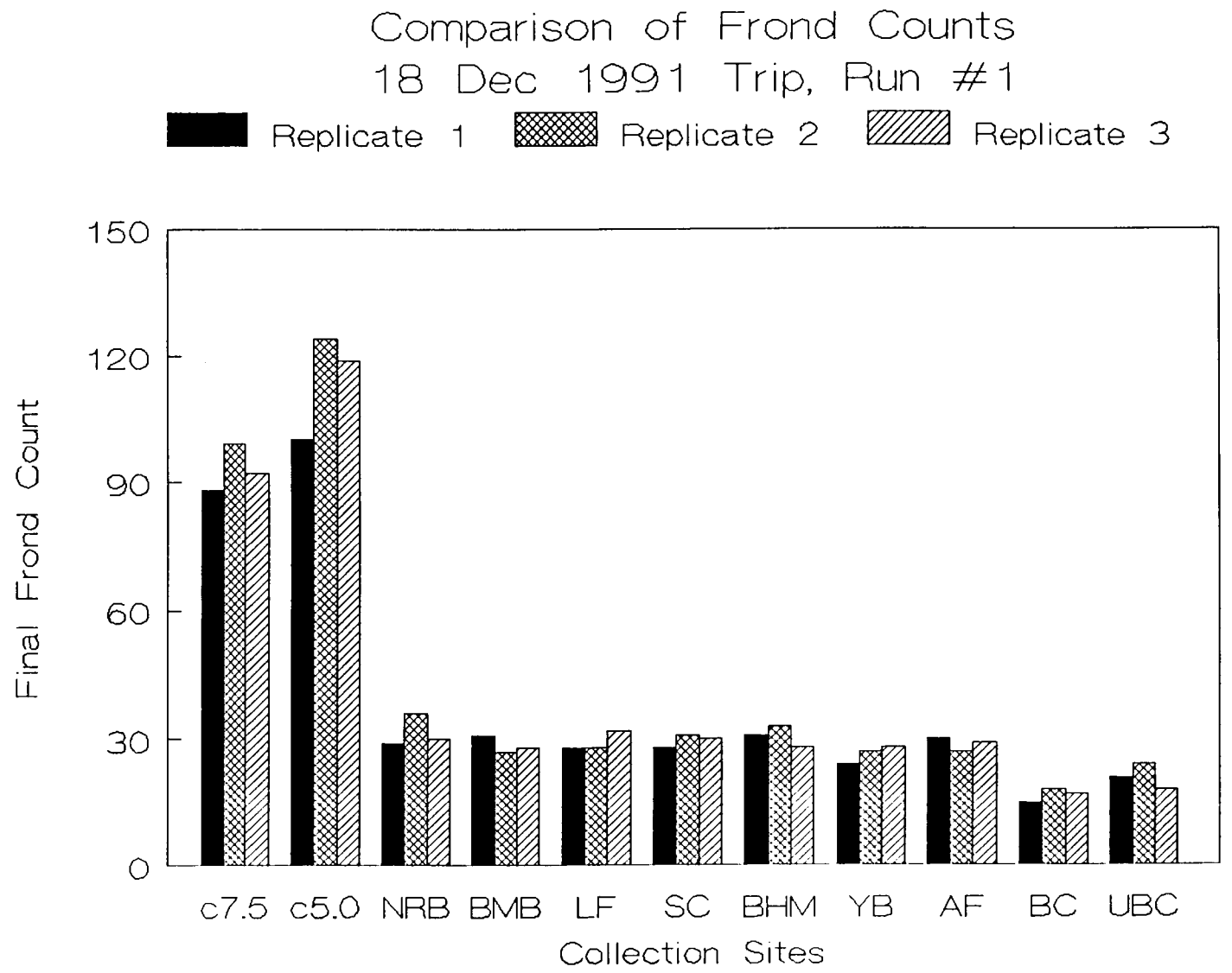


Figure 11. 18 December 1991 Trip Totals, First Run

Table 11. 18 Dec 1991 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 70 | Lab media |
| C7.5B | 60 | |
| C7.5C | 64 | |
| Total | 194 | |
| | | |
| C5.0A | 127 | Lab media |
| C5.0B | 135 | |
| C5.0C | 132 | |
| Total | 394 | |
| | | |
| NRB-A | 41 | New River Bridge, TN |
| NRB-B | 63 | |
| NRB-C | 58 | |
| Total | 162 | |
| | | |
| BMB-A | 64 | Burnt Mill Bridge, TN |
| BMB-B | 65 | |
| BMB-C | 57 | |
| Total | 186 | |
| | | |
| LF-A | 61 | Leatherwood Ford, TN |
| LF-B | 50 | |
| LF-C | 63 | |
| Total | 174 | |
| | | |
| SC-A | 47 | Station Camp, TN |
| SC-B | 54 | |
| SC-C | 51 | |
| Total | 152 | |
| | | |
| BHM-A | 58 | Blue Heron Mine, KY |
| BHM-B | 57 | |
| BHM-C | 60 | |
| Total | 175 | |
| | | |
| YB-A | 56 | Yamacraw Bridge, KY |
| YB-B | 55 | |
| YB-C | 47 | |
| Total | 158 | |
| | | |
| AF-A | 48 | Alum Ford, KY |
| AF-B | 51 | |
| AF-C | 57 | |
| Total | 156 | |
| | | |
| BC-A | 32 | Bandy Creek, TN |
| BC-B | 36 | |
| BC-C | 37 | |
| Total | 105 | |
| | | |
| UBC-A | 36 | Upper Bear Creek, KY |
| UBC-B | 24 | |
| UBC-C | 37 | |
| Total | 97 | |

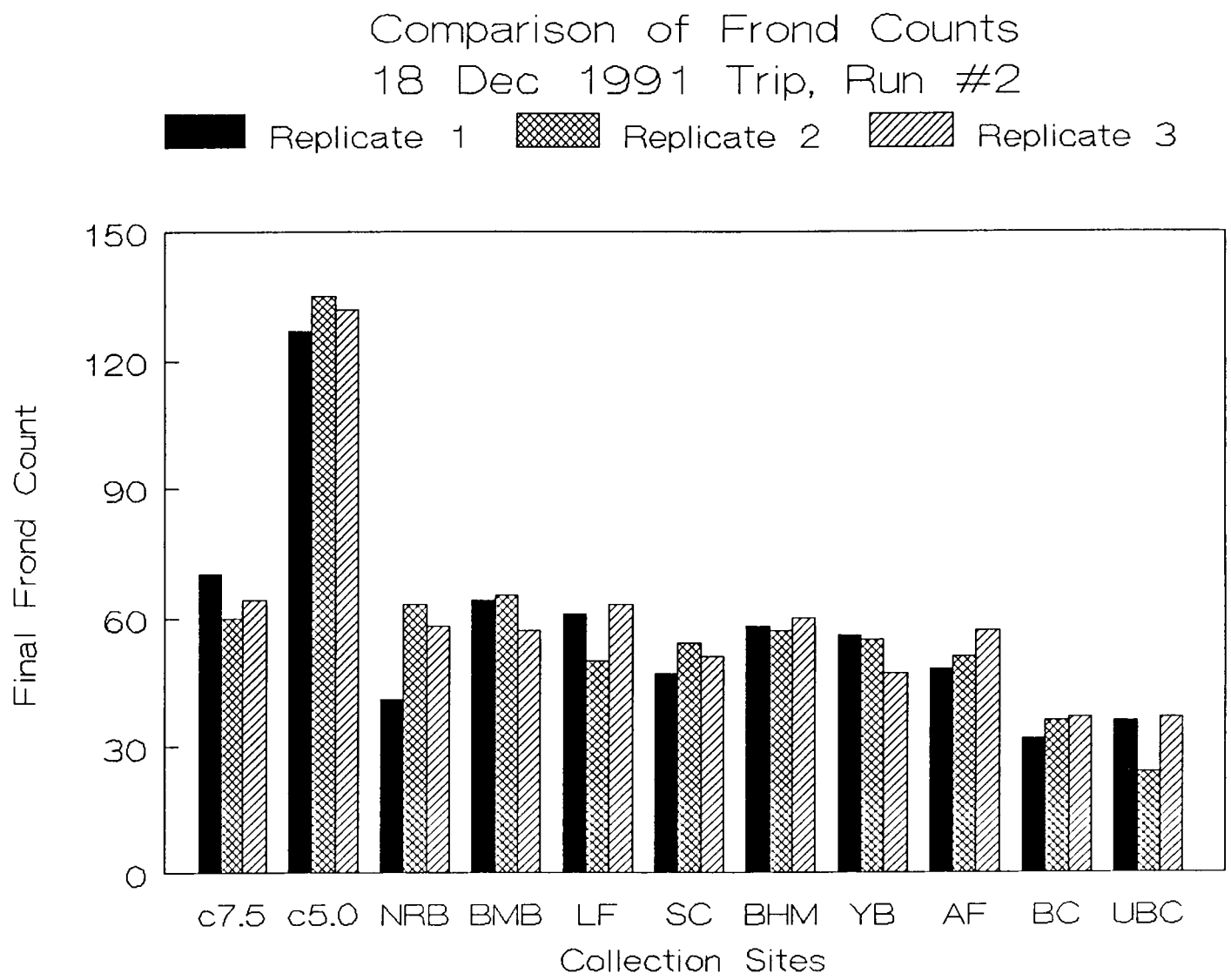


Figure 11. 18 December 1991 Trip Totals, Second Run

Table 12. 18 Dec 1991 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 52 | Lab media |
| C7.5B | 35 | |
| C7.5C | 49 | |
| Total | 136 | |
| | | |
| C5.0A | 55 | Lab media |
| C5.0B | 79 | |
| C5.0C | 66 | |
| Total | 200 | |
| | | |
| NRB-A | 37 | New River Bridge, TN |
| NRB-B | 28 | |
| NRB-C | 45 | |
| Total | 110 | |
| | | |
| BMB-A | 49 | Burnt Mill Bridge, TN |
| BMB-B | 38 | |
| BMB-C | 47 | |
| Total | 134 | |
| | | |
| LF-A | 29 | Leatherwood Ford, TN |
| LF-B | 33 | |
| LF-C | 45 | |
| Total | 107 | |
| | | |
| SC-A | 37 | Station Camp, TN |
| SC-B | 45 | |
| SC-C | 42 | |
| Total | 124 | |
| | | |
| BHM-A | 49 | Blue Heron Mine, KY |
| BHM-B | 38 | |
| BHM-C | 47 | |
| Total | 134 | |
| | | |
| YB-A | 33 | Yamacraw Bridge, KY |
| YB-B | 34 | |
| YB-C | 29 | |
| Total | 96 | |
| | | |
| AF-A | 32 | Alum Ford, KY |
| AF-B | 34 | |
| AF-C | 31 | |
| Total | 97 | |
| | | |
| BC-A | 28 | Bandy Creek, TN |
| BC-B | 30 | |
| BC-C | 27 | |
| Total | 85 | |
| | | |
| UBC-A | 31 | Upper Bear Creek, KY |
| UBC-B | 31 | |
| UBC-C | 27 | |
| Total | 89 | |

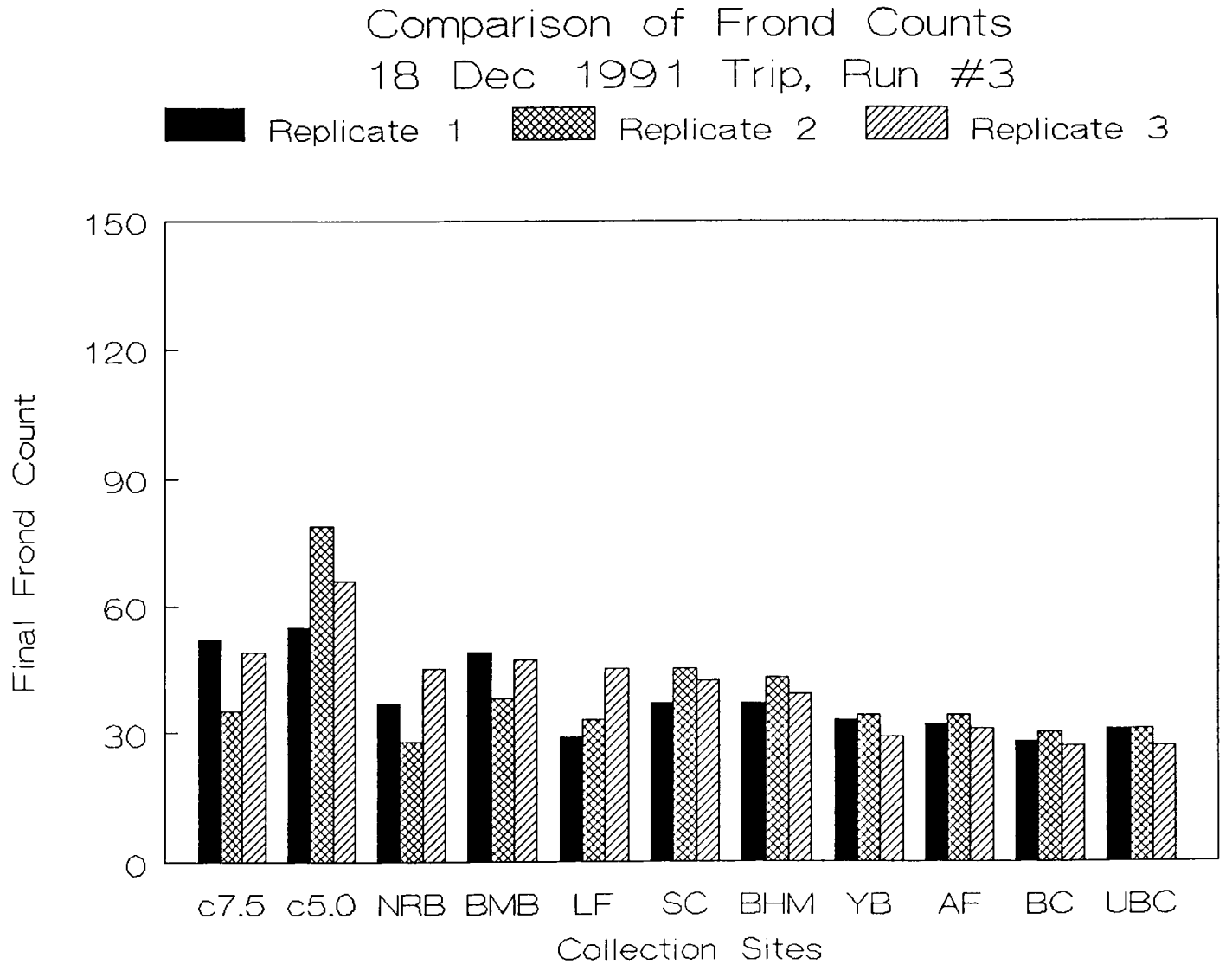


Figure 12. 18 December 1991 Trip, Test Jar, Third Run.

Table 13. 25 March 1992 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 85 | Lab media |
| C7.5B | 68 | |
| C7.5C | 103 | |
| Total | 256 | |
| | | |
| C5.0A | 132 | Lab media |
| C5.0B | 128 | |
| C5.0C | 122 | |
| Total | 382 | |
| | | |
| NRB-A | 35 | New River Bridge, TN |
| NRB-B | 38 | |
| NRB-C | 47 | |
| Total | 120 | |
| | | |
| BMB-A | 35 | Burnt Mill Bridge, TN |
| BMB-B | 37 | |
| BMB-C | 41 | |
| Total | 113 | |
| | | |
| LF-A | 41 | Leatherwood Ford, TN |
| LF-B | 42 | |
| LF-C | 40 | |
| Total | 123 | |
| | | |
| SC-A | 46 | Station Camp, TN |
| SC-B | 47 | |
| SC-C | 50 | |
| Total | 143 | |
| | | |
| BHM-A | 55 | Blue Heron Mine, KY |
| BHM-B | 41 | |
| BHM-C | 53 | |
| Total | 149 | |
| | | |
| YB-A | 39 | Yamacraw Bridge, KY |
| YB-B | 40 | |
| YB-C | 39 | |
| Total | 118 | |
| | | |
| AF-A | 35 | Alum Ford, KY |
| AF-B | 42 | |
| AF-C | 34 | |
| Total | 111 | |
| | | |
| BC-A | 21 | Bandy Creek, TN |
| BC-B | 25 | |
| BC-C | 28 | |
| Total | 74 | |
| | | |
| UBC-A | 29 | Upper Bear Creek, KY |
| UBC-B | 28 | |
| UBC-C | 31 | |
| Total | 88 | |

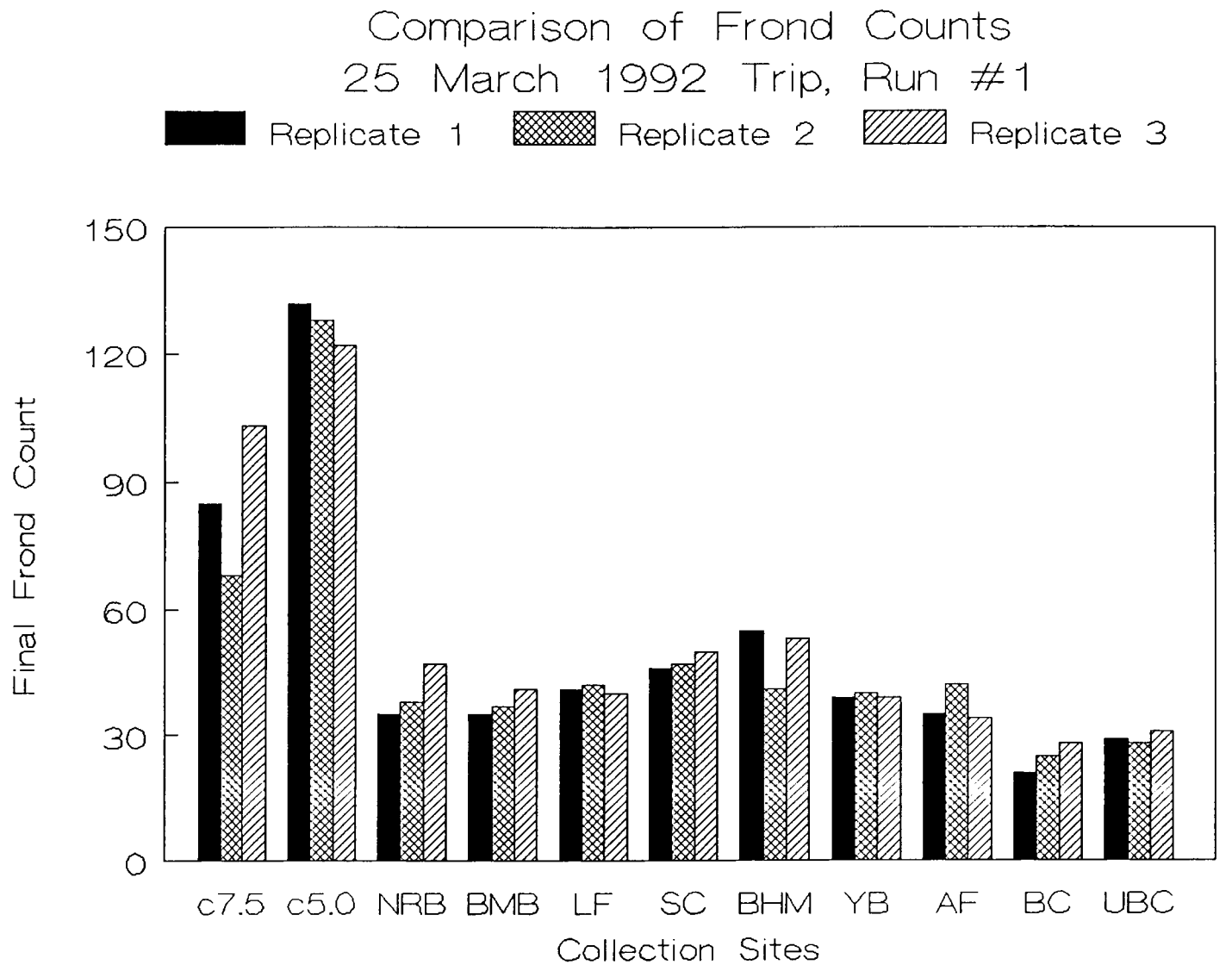


Figure 10. 25 March 1992 Trip Totals, First Run

Table 14. 25 March 1992 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 97 | |
| C5.0C | 150 | |
| Total | 338 | |
| | | |
| NRB-A | 50 | New River Bridge, TN |
| NRB-B | 38 | |
| NRB-C | 38 | |
| Total | 126 | |
| | | |
| BMB-A | 45 | Burnt Mill Bridge, TN |
| BMB-B | 37 | |
| BMB-C | 39 | |
| Total | 121 | |
| | | |
| LF-A | 40 | Leatherwood Ford, TN |
| LF-B | 50 | |
| LF-C | 43 | |
| Total | 133 | |
| | | |
| SC-A | 41 | Station Camp, TN |
| SC-B | 55 | |
| SC-C | 41 | |
| Total | 137 | |
| | | |
| BHM-A | 41 | Blue Heron Mine, KY |
| BHM-B | 41 | |
| BHM-C | 42 | |
| Total | 124 | |
| | | |
| YB-A | 50 | Yamacraw Bridge, KY |
| YB-B | 52 | |
| YB-C | 49 | |
| Total | 151 | |
| | | |
| AF-A | 40 | Alum Ford, KY |
| AF-B | 51 | |
| AF-C | 44 | |
| Total | 135 | |
| | | |
| BC-A | 31 | Bandy Creek, TN |
| BC-B | 33 | |
| BC-C | 36 | |
| Total | 100 | |
| | | |
| UBC-A | 30 | Upper Bear Creek, KY |
| UBC-B | 29 | |
| UBC-C | 27 | |
| Total | 86 | |

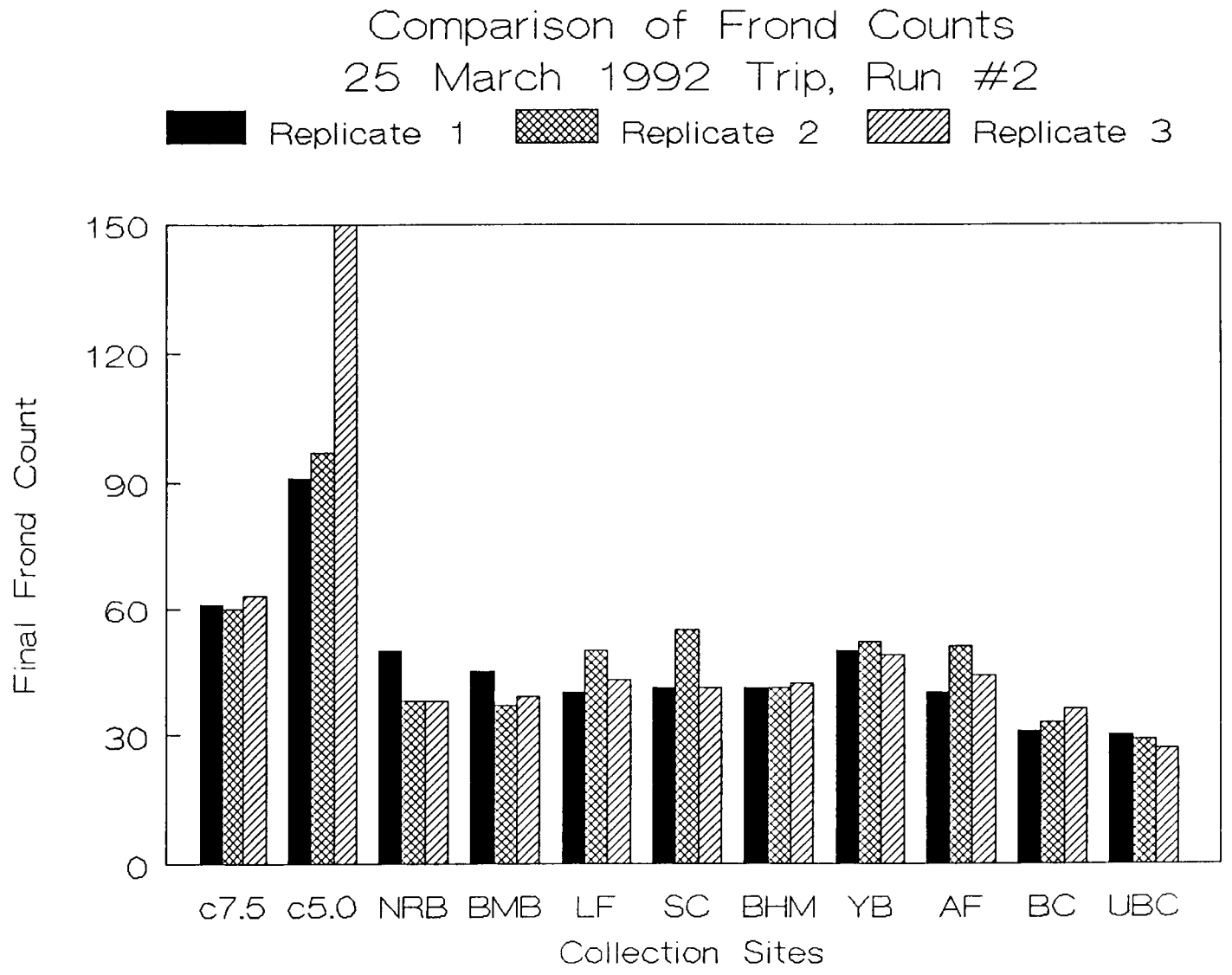


Figure 11. 25 March 1992 Trip Totals, Second Run

Table 15. 25 March 1992 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 52 | Lab media |
| C7.5B | 35 | |
| C7.5C | 49 | |
| Total | 136 | |
| | | |
| C5.0A | 55 | Lab media |
| C5.0B | 49 | |
| C5.0C | 66 | |
| Total | 170 | |
| | | |
| NRB-A | 38 | New River Bridge, TN |
| NRB-B | 34 | |
| NRB-C | 27 | |
| Total | 99 | |
| | | |
| BMB-A | 40 | Burnt Mill Bridge, TN |
| BMB-B | 37 | |
| BMB-C | 44 | |
| Total | 121 | |
| | | |
| LF-A | 46 | Leatherwood Ford, TN |
| LF-B | 40 | |
| LF-C | 36 | |
| Total | 122 | |
| | | |
| SC-A | 27 | Station Camp, TN |
| SC-B | 33 | |
| SC-C | 34 | |
| Total | 94 | |
| | | |
| BHM-A | 38 | Blue Heron Mine, KY |
| BHM-B | 34 | |
| BHM-C | 29 | |
| Total | 101 | |
| | | |
| YB-A | 32 | Yamacraw Bridge, KY |
| YB-B | 32 | |
| YB-C | 29 | |
| Total | 93 | |
| | | |
| AF-A | 45 | Alum Ford, KY |
| AF-B | 44 | |
| AF-C | 32 | |
| Total | 121 | |
| | | |
| BC-A | 30 | Bandy Creek, TN |
| BC-B | 30 | |
| BC-C | 31 | |
| Total | 91 | |
| | | |
| UBC-A | 20 | Upper Bear Creek, KY |
| UBC-B | 25 | |
| UBC-C | 21 | |
| Total | 66 | |

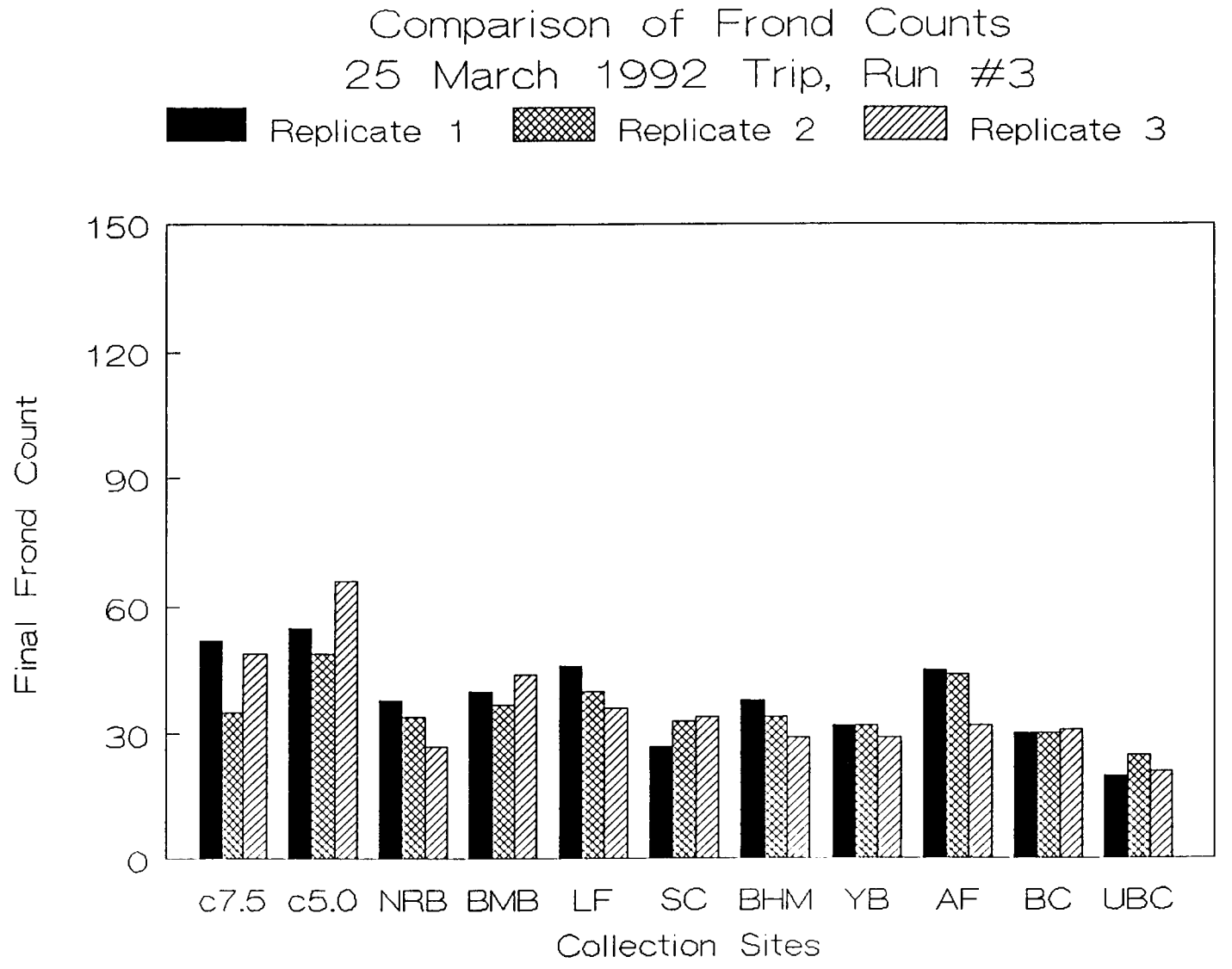


Figure 15. 25 March 1992 Trip, Tubidy, Third Run

Table 16. 20 Aug 1992 Trip Totals, First Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 85 | Lab media |
| C7.5B | 68 | |
| C7.5C | 103 | |
| Total | 256 | |
| | | |
| C5.0A | 132 | Lab media |
| C5.0B | 128 | |
| C5.0C | 122 | |
| Total | 382 | |
| | | |
| NRB-A | 44 | New River Bridge, TN |
| NRB-B | 38 | |
| NRB-C | 44 | |
| Total | 126 | |
| | | |
| BMB-A | 39 | Burnt Mill Bridge, TN |
| BMB-B | 44 | |
| BMB-C | 47 | |
| Total | 130 | |
| | | |
| LF-A | 49 | Leatherwood Ford, TN |
| LF-B | 49 | |
| LF-C | 53 | |
| Total | 151 | |
| | | |
| SC-A | 58 | Station Camp, TN |
| SC-B | 46 | |
| SC-C | 45 | |
| Total | 149 | |
| | | |
| BHM-A | 50 | Blue Heron Mine, KY |
| BHM-B | 38 | |
| BHM-C | 41 | |
| Total | 129 | |
| | | |
| YB-A | 39 | Yamacraw Bridge, KY |
| YB-B | 39 | |
| YB-C | 43 | |
| Total | 121 | |
| | | |
| AF-A | 37 | Alum Ford, KY |
| AF-B | 44 | |
| AF-C | 44 | |
| Total | 125 | |
| | | |
| BC-A | 42 | Bandy Creek, TN |
| BC-B | 40 | |
| BC-C | 35 | |
| Total | 117 | |
| | | |
| UBC-A | 35 | Upper Bear Creek, KY |
| UBC-B | 33 | |
| UBC-C | 42 | |
| Total | 110 | |

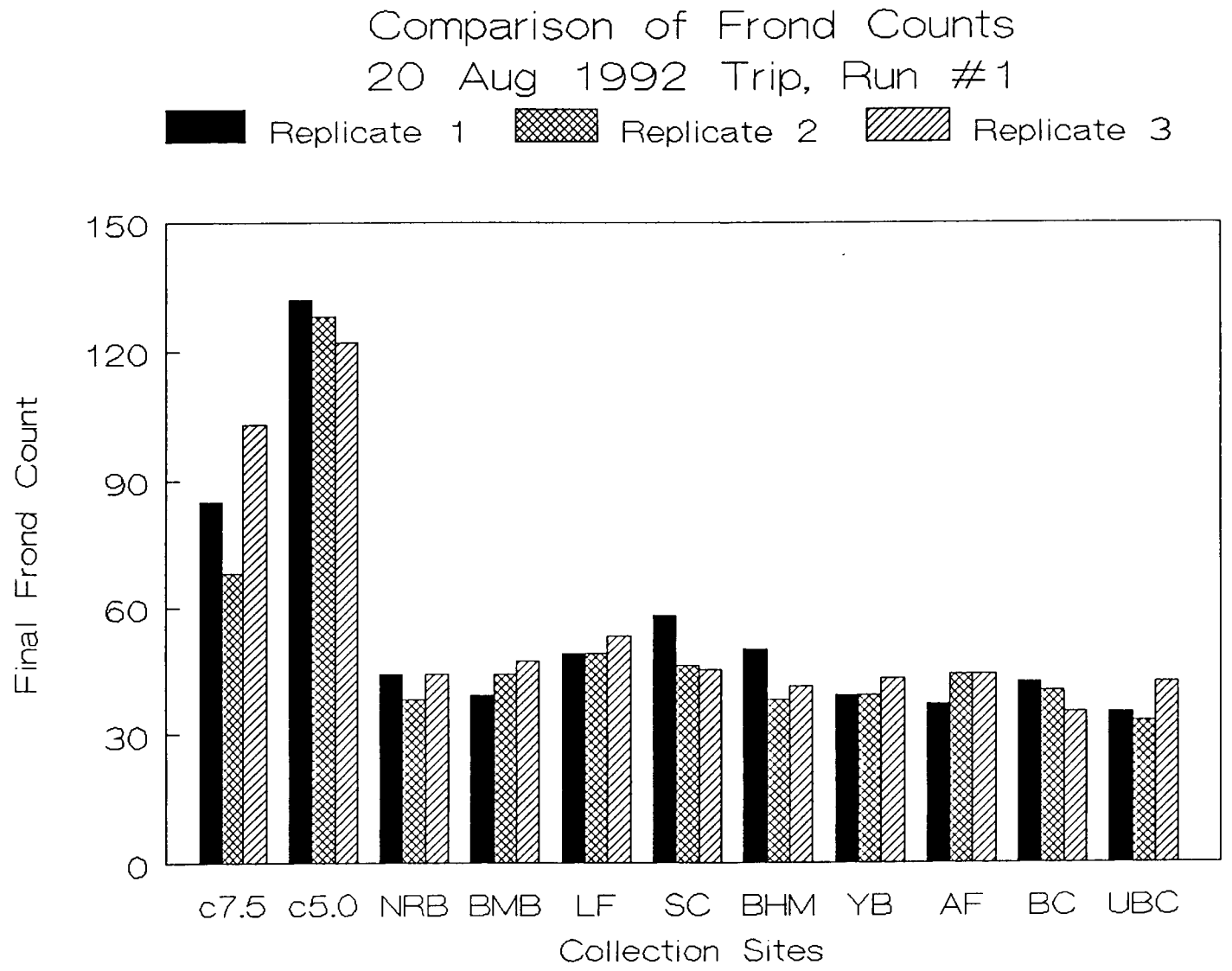


Figure 16. 20 August 1992 Trip Totals, First Run.

Table 17. 20 Aug 1992 Trip Totals, Second Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 197 | |
| C5.0C | 194 | |
| Total | 282 | |
| | | |
| NRB-A | 42 | New River Bridge, TN |
| NRB-B | 32 | |
| NRB-C | 42 | |
| Total | 116 | |
| | | |
| BMB-A | 38 | Burnt Mill Bridge, TN |
| BMB-B | 40 | |
| BMB-C | 34 | |
| Total | 112 | |
| | | |
| LF-A | 39 | Leatherwood Ford, TN |
| LF-B | 39 | |
| LF-C | 44 | |
| Total | 122 | |
| | | |
| SC-A | 40 | Station Camp, TN |
| SC-B | 34 | |
| SC-C | 41 | |
| Total | 115 | |
| | | |
| BHM-A | 37 | Blue Heron Mine, KY |
| BHM-B | 39 | |
| BHM-C | 40 | |
| Total | 116 | |
| | | |
| YB-A | 43 | Yamacraw Bridge, KY |
| YB-B | 42 | |
| YB-C | 44 | |
| Total | 129 | |
| | | |
| AF-A | 37 | Alum Ford, KY |
| AF-B | 51 | |
| AF-C | 43 | |
| Total | 131 | |
| | | |
| BC-A | 35 | Bandy Creek, TN |
| BC-B | 38 | |
| BC-C | 30 | |
| Total | 103 | |
| | | |
| UBC-A | 26 | Upper Bear Creek, KY |
| UBC-B | 39 | |
| UBC-C | 32 | |
| Total | 97 | |

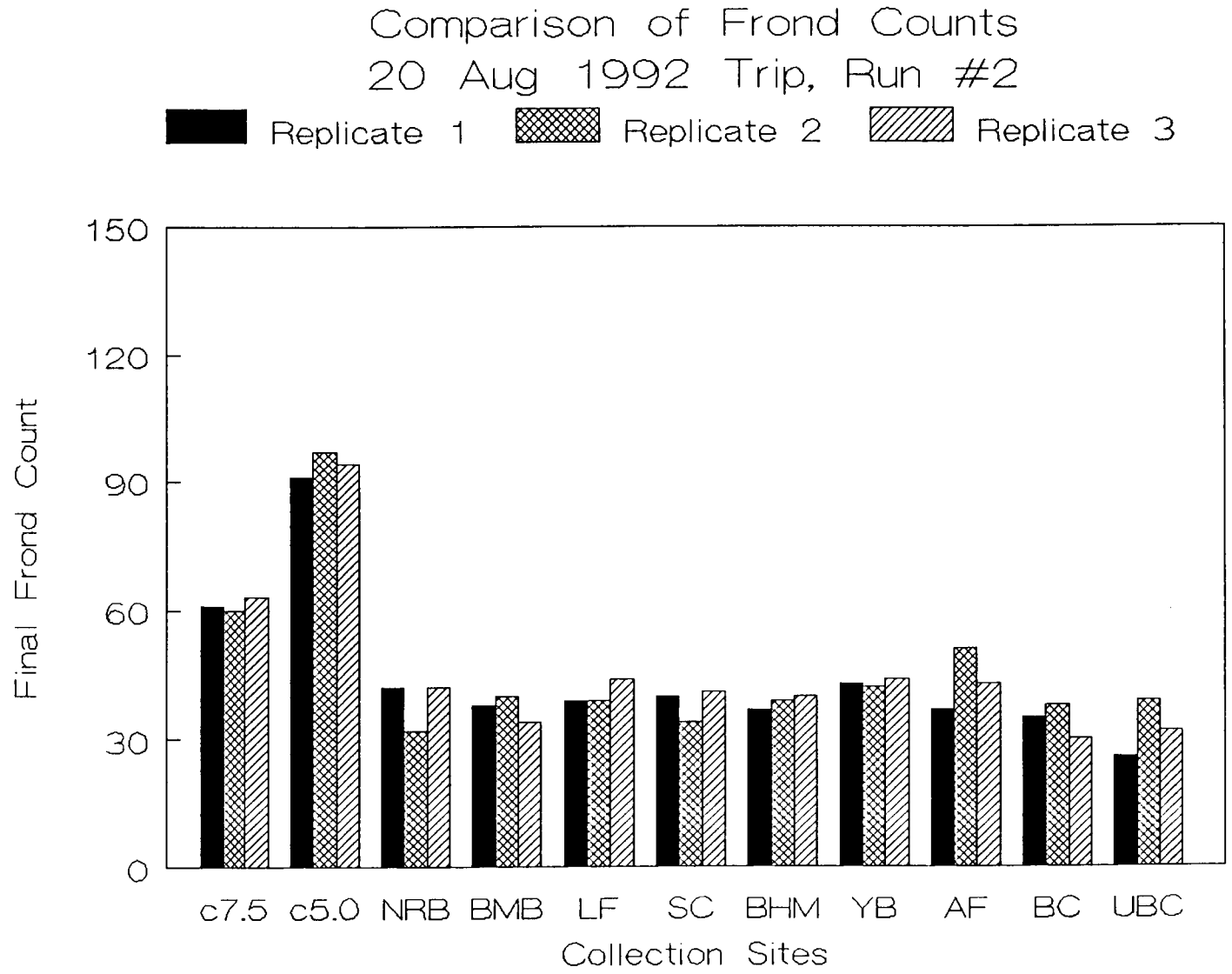


Figure 17. 20 August 1992 Trip Trial, Second Run

Table 18. 20 Aug 1992 Trip Totals, Third Run

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 49 | Lab media |
| C7.5B | 52 | |
| C7.5C | 51 | |
| Total | 152 | |
| | | |
| C5.0A | 61 | Lab media |
| C5.0B | 76 | |
| C5.0C | 63 | |
| Total | 200 | |
| | | |
| NRB-A | 36 | New River Bridge, TN |
| NRB-B | 36 | |
| NRB-C | 41 | |
| Total | 113 | |
| | | |
| BMB-A | 41 | Burnt Mill Bridge, TN |
| BMB-B | 41 | |
| BMB-C | 40 | |
| Total | 122 | |
| | | |
| LF-A | 30 | Leatherwood Ford, TN |
| LF-B | 30 | |
| LF-C | 32 | |
| Total | 92 | |
| | | |
| SC-A | 30 | Station Camp, TN |
| SC-B | 33 | |
| SC-C | 32 | |
| Total | 95 | |
| | | |
| BHM-A | 42 | Blue Heron Mine, KY |
| BHM-B | 38 | |
| BHM-C | 33 | |
| Total | 113 | |
| | | |
| YB-A | 35 | Yamacraw Bridge, KY |
| YB-B | 36 | |
| YB-C | 34 | |
| Total | 105 | |
| | | |
| AF-A | 32 | Alum Ford, KY |
| AF-B | 32 | |
| AF-C | 39 | |
| Total | 103 | |
| | | |
| BC-A | 30 | Bandy Creek, TN |
| BC-B | 33 | |
| BC-C | 30 | |
| Total | 93 | |
| | | |
| UBC-A | 34 | Upper Bear Creek, KY |
| UBC-B | 31 | |
| UBC-C | 39 | |
| Total | 104 | |

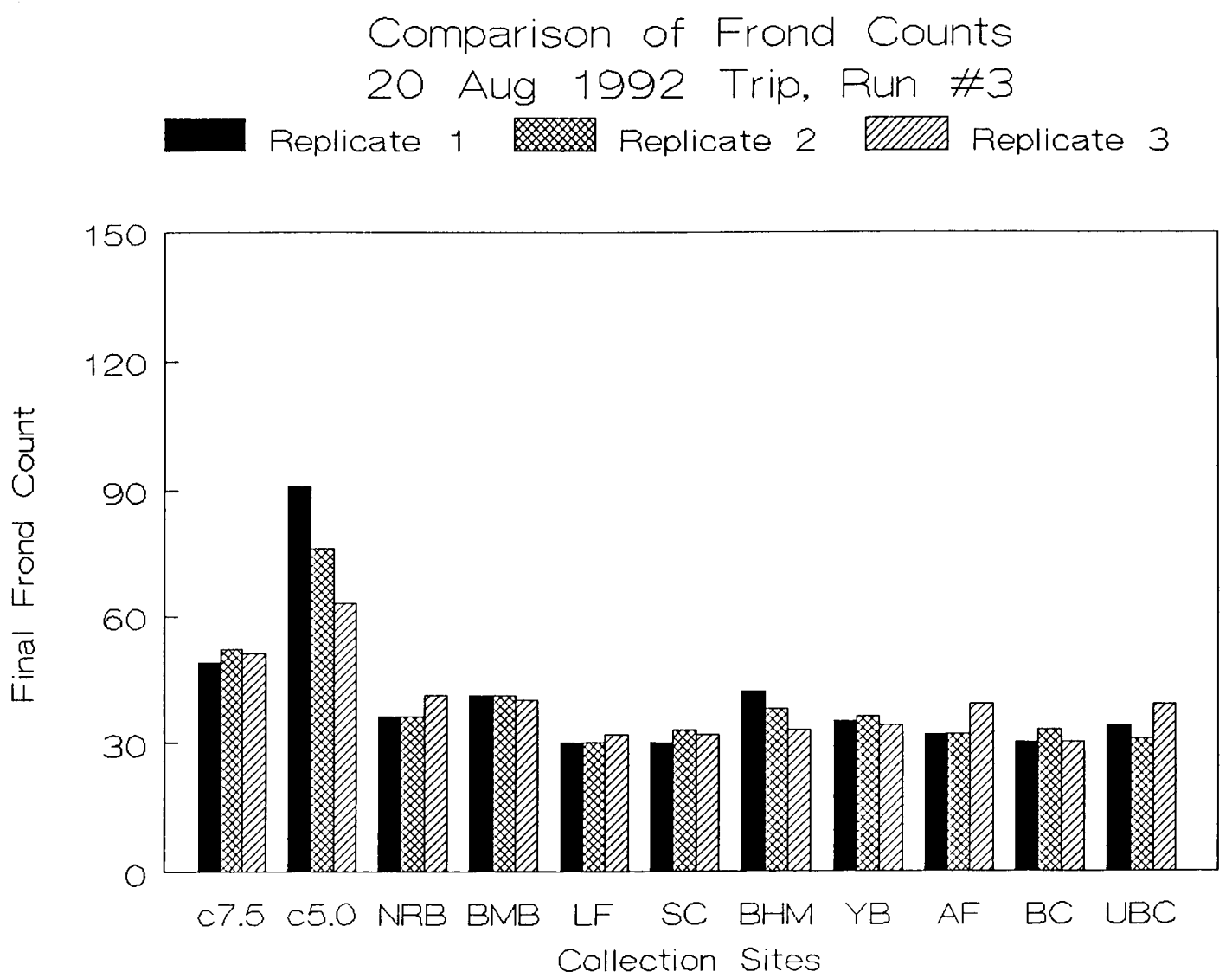


Figure 1[illegible] 20 August 1992 Trip Totals, Third Run

Table 19. Endpoint Frond Count Trip Totals

5 June 1991 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 203 | 131 | 184 |
| C5.0 | 181 | 156 | 338 |
| NRB | 80 | 72 | 89 |
| BMB | 62 | 75 | 103 |
| LF | 84 | 70 | 97 |
| SC | 103 | 99 | 100 |
| BHM | 86 | 76 | 92 |
| YB | 86 | 81 | 98 |
| AF | 63 | 65 | 90 |
| BC | -- | -- | -- |
| UBC | 67 | 51 | 64 |

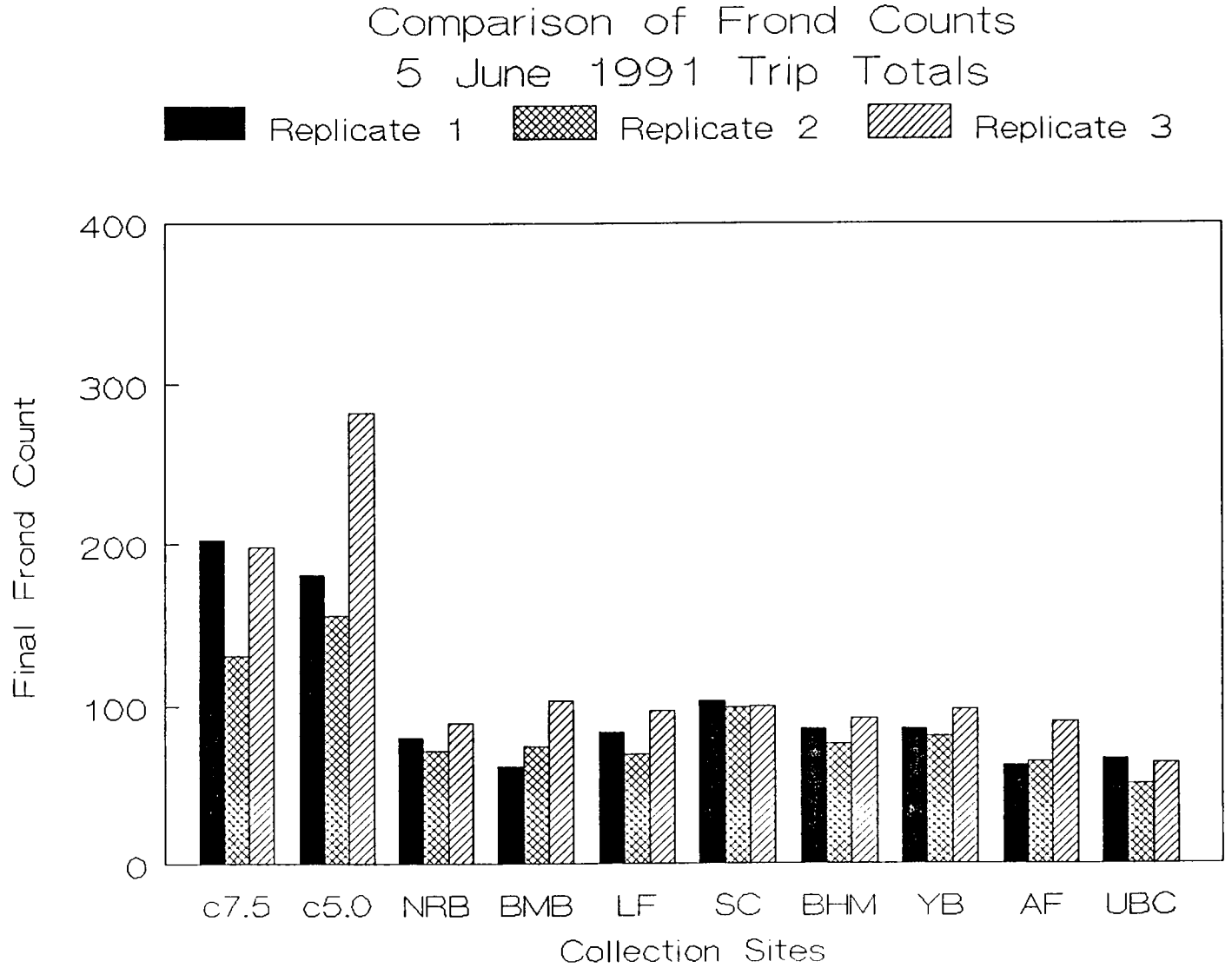


**Figure 19. Endpoint Frond Count Trip Totals, 5 June 1991**

Table 20. Endpoint Frond Count Trip Totals

16 July 1991 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 214 | 196 | 184 |
| C5.0 | 272 | 204 | 338 |
| NRB | 83 | 46 | 92 |
| BMB | 75 | 47 | 135 |
| LF | 71 | 43 | 93 |
| SC | -- | -- | -- |
| BHM | 77 | 45 | 92 |
| YB | 77 | 44 | 99 |
| AF | 64 | 49 | 98 |
| BC | 77 | 50 | 90 |
| UBC | 85 | 46 | 86 |

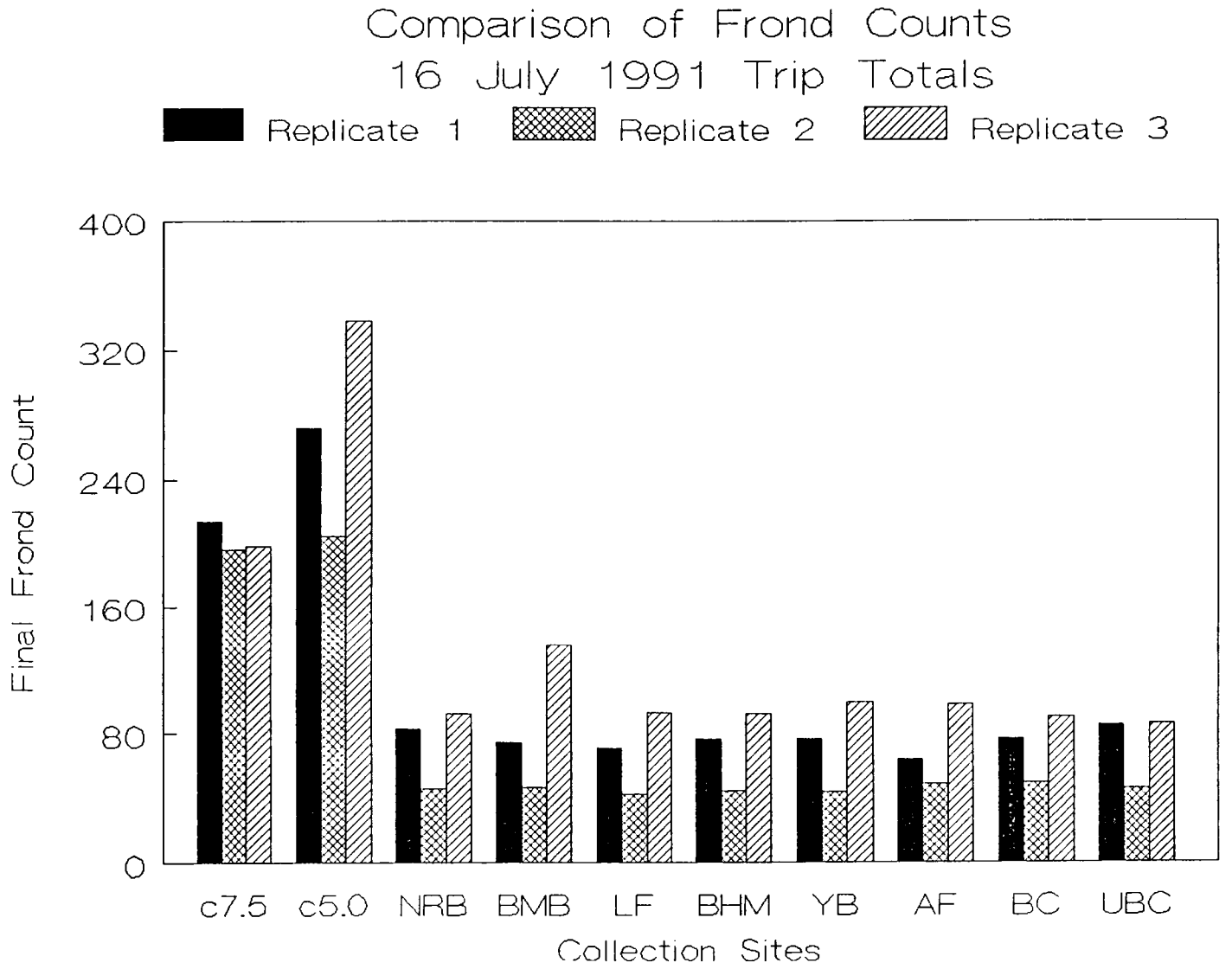


**Figure 20. Endpoint Frond Count Trip Totals, 16 July 1991**

Table 21. Endpoint Frond Count Trip Totals

11 Oct 1991 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 141 | 194 | 136 |
| C5.0 | 189 | 394 | 170 |
| NRB | 81 | 106 | 138 |
| BMB | 50 | 93 | 99 |
| LF | 48 | 102 | 99 |
| SC | -- | -- | -- |
| BHM | 48 | 93 | 93 |
| YB | -- | -- | -- |
| AF | 66 | 106 | 114 |
| BC | 50 | 90 | 93 |
| UBC | -- | -- | -- |

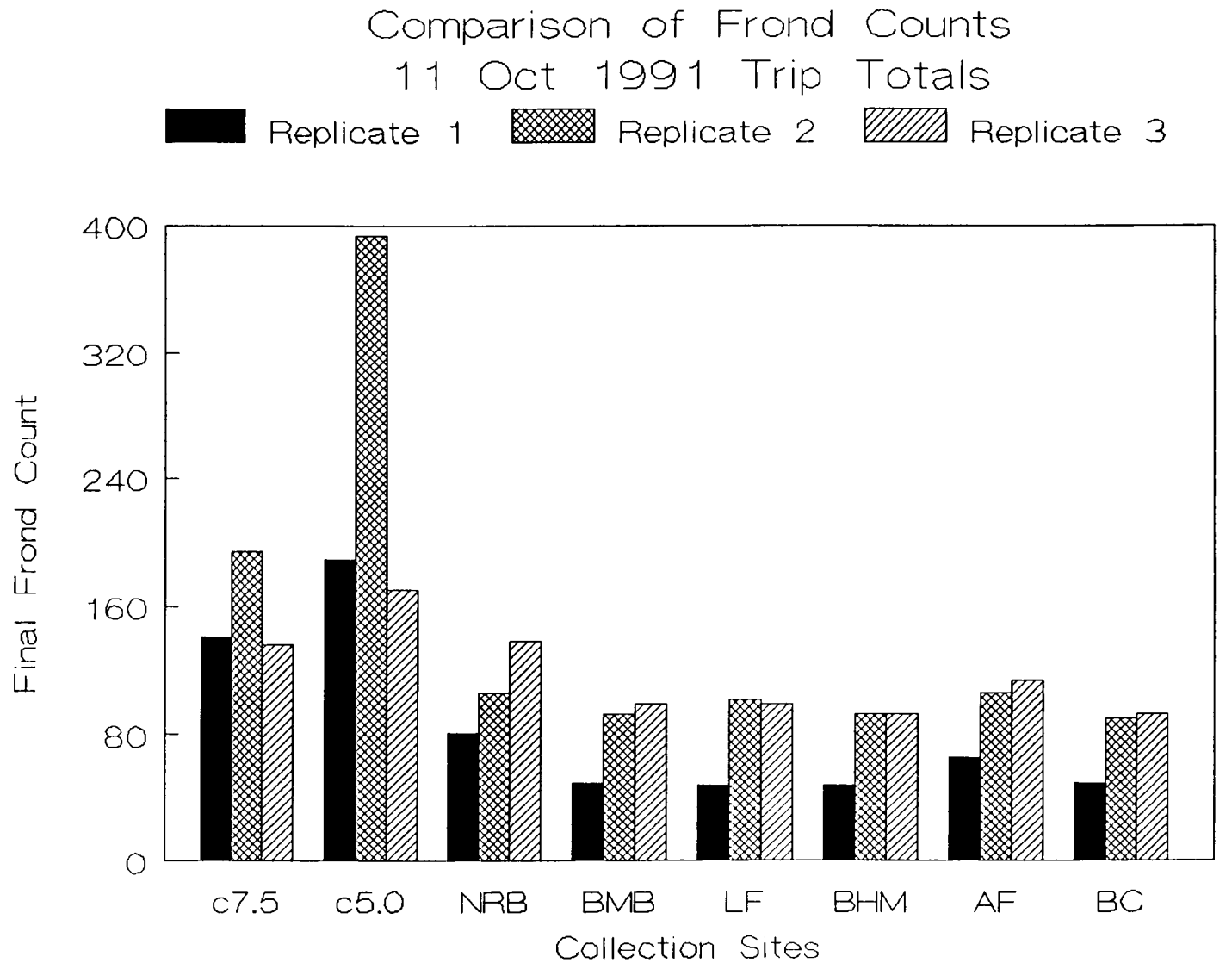


**Figure 21. Endpoint Frond Count Trip Totals, 11 October 1991**

Table 22. Endpoint Frond Count Trip Totals

18 Dec 1991 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 279 | 194 | 136 |
| C5.0 | 343 | 394 | 200 |
| NRB | 95 | 162 | 110 |
| BMB | 86 | 186 | 134 |
| LF | 88 | 174 | 107 |
| SC | 89 | 152 | 124 |
| BHM | 92 | 175 | 119 |
| YB | 79 | 158 | 96 |
| AF | 86 | 156 | 97 |
| BC | 50 | 105 | 85 |
| UBC | 63 | 97 | 89 |

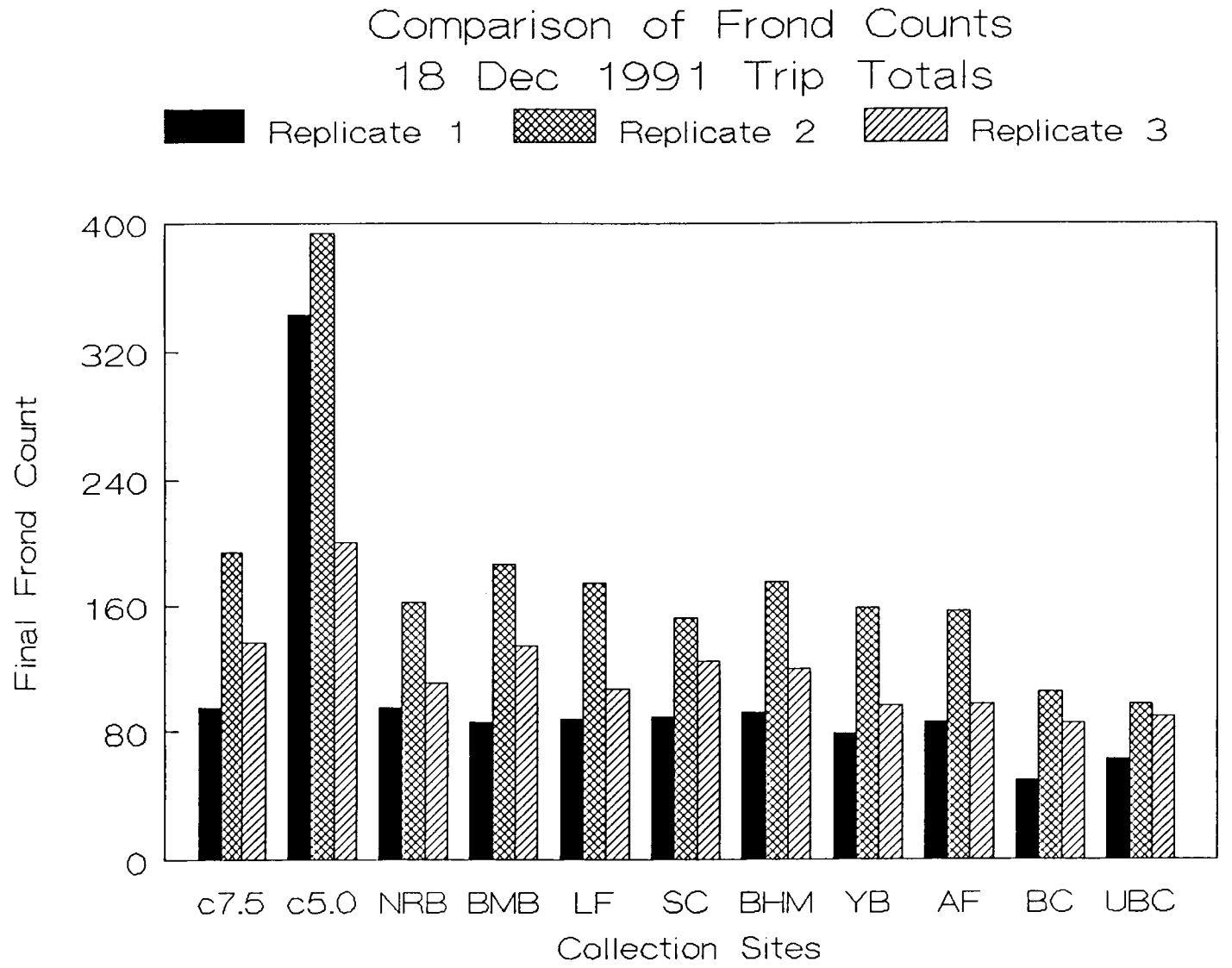


**Figure 22. Endpoint Frond Count Trip Totals, 18 December 1991**

Table 23. Endpoint Frond Count Trip Totals

25 March 1992 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 256 | 184 | 136 |
| C5.0 | 382 | 338 | 170 |
| NRB | 120 | 126 | 99 |
| BMB | 113 | 121 | 121 |
| LF | 123 | 133 | 122 |
| SC | 143 | 137 | 94 |
| BHM | 149 | 124 | 101 |
| YB | 118 | 151 | 93 |
| AF | 111 | 135 | 121 |
| BC | 74 | 100 | 91 |
| UBC | 88 | 86 | 66 |

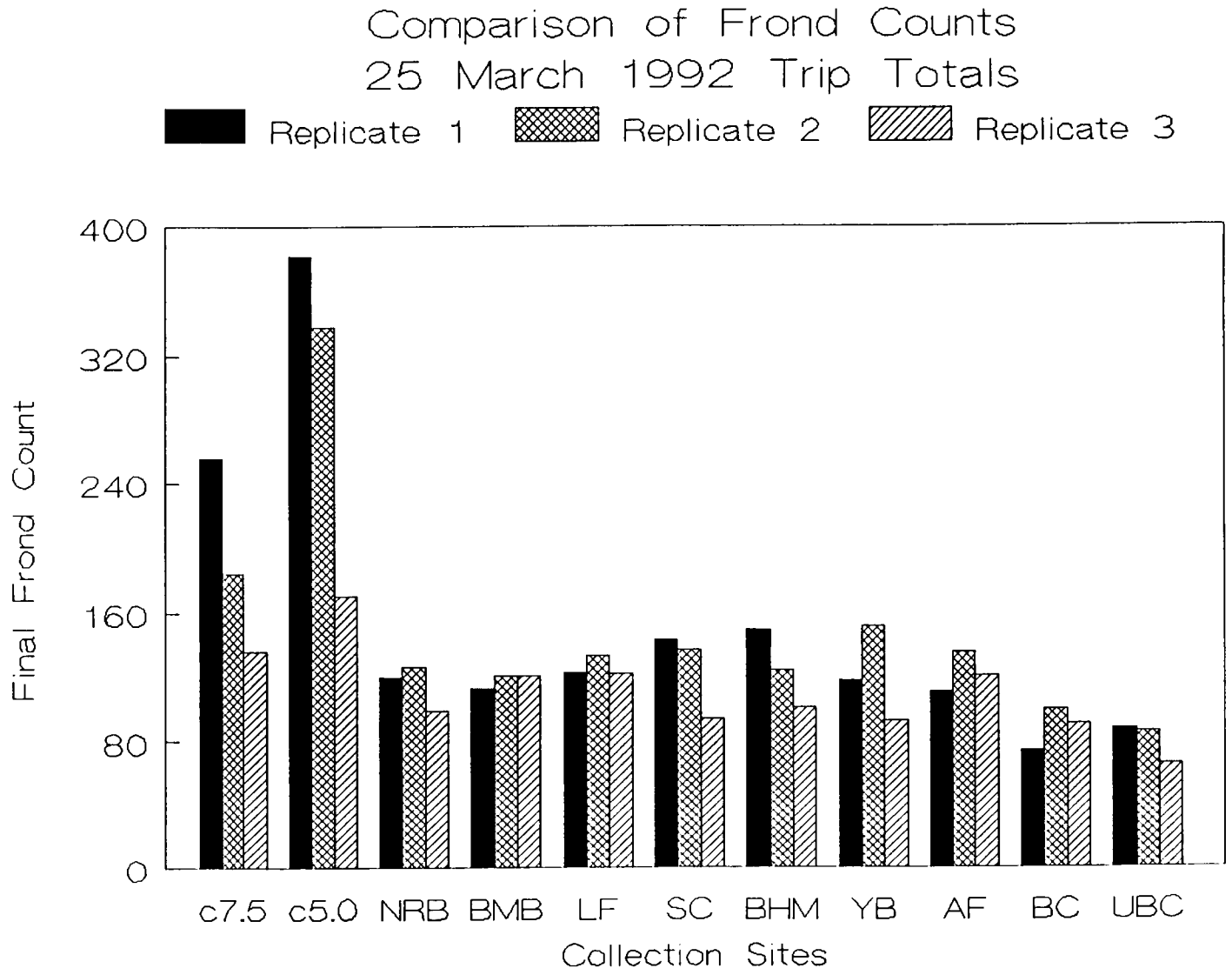


**Figure 23. Endpoint Frond Count Trip Totals, 25 March 1992**

Table 24. Endpoint Frond Count Trip Totals

20 Aug 1992 Trip

| Site | Run 1 Totals | Run 2 Totals | Run 3 Totals |
|---|---|---|---|
| C7.5 | 256 | 184 | 152 |
| C5.0 | 382 | 282 | 200 |
| NRB | 126 | 116 | 113 |
| BMB | 130 | 112 | 122 |
| LF | 151 | 122 | 92 |
| SC | 149 | 115 | 95 |
| BHM | 129 | 116 | 113 |
| YB | 121 | 129 | 105 |
| AF | 125 | 131 | 103 |
| BC | 117 | 103 | 93 |
| UBC | 110 | 97 | 104 |

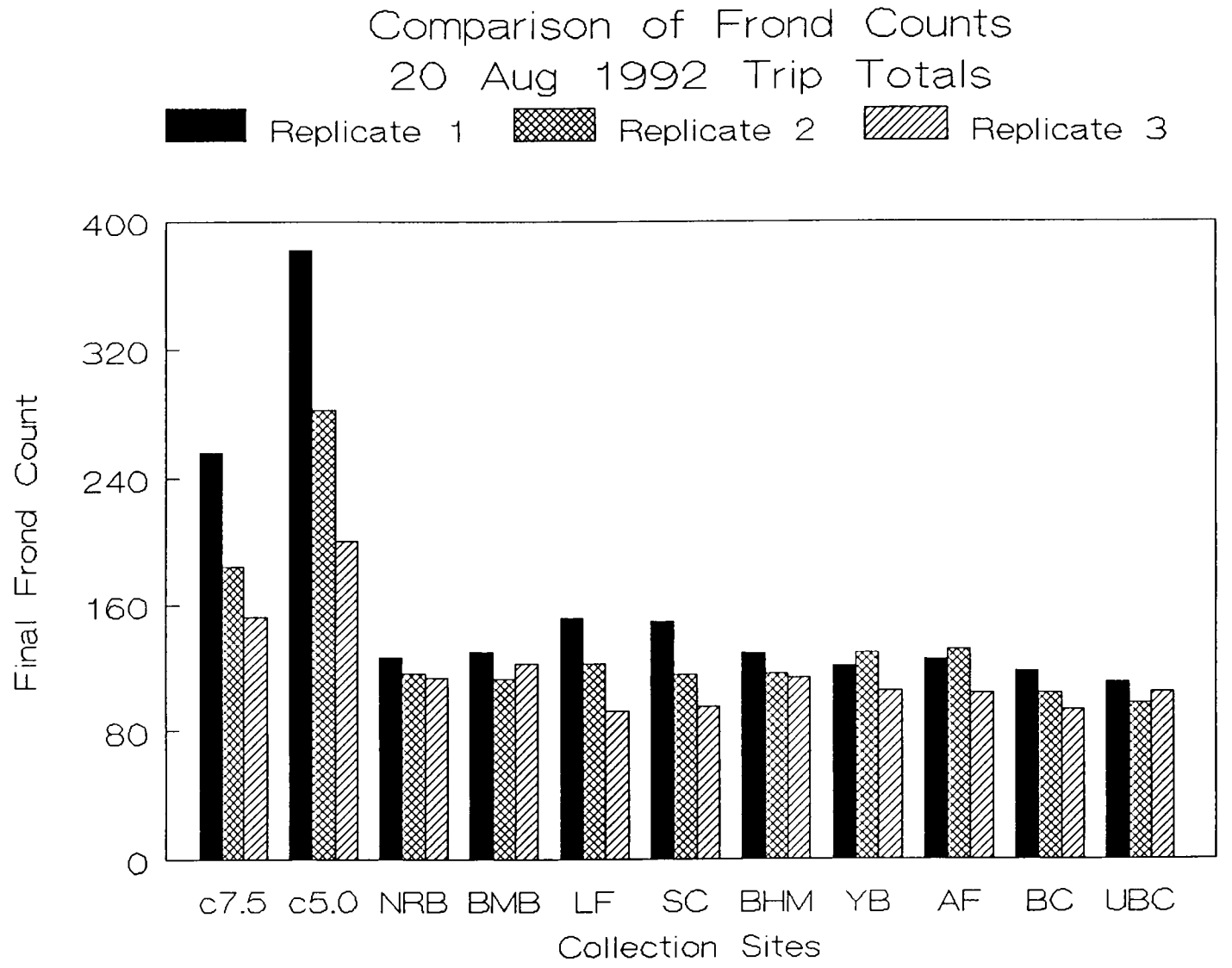


**Figure 24. Endpoint Frond Count Trip Totals, 20 August 1992**

Table 25. Total Frondcount Numbers by Trip

| Site | 5 Jun | 16 Jul | 11 Oct | 18 Dec 91 | 25 Mar | 20 Aug 92 |
|---|---|---|---|---|---|---|
| NRB | 241 | 221 | 325 | 367 | 345 | 355 |
| BMB | 240 | 257 | 242 | 406 | 355 | 364 |
| LF | 251 | 207 | 249 | 369 | 378 | 365 |
| SC | 302 | -- | -- | 365 | 374 | 359 |
| BHM | 254 | 214 | 234 | 386 | 374 | 358 |
| YB | 265 | 220 | -- | 333 | 362 | 355 |
| AF | 218 | 211 | 286 | 339 | 367 | 359 |
| BC | -- | 217 | 233 | 240 | 265 | 313 |
| UBC | 182 | 217 | -- | 249 | 240 | 311 |
| Total | 1953 | 1764 | 1569 | 3054 | 3060 | 3139 |
| A-Tot* | 1204 | 1110 | 1336 | 1867 | 1819 | 1801 |

*The adjusted total uses only the five sites which were sampled at each trip (NRB, BMB, LF, BHM, AF) and disregards the sites where data are missing (SC, YB, BC, UBC).

## VI. Discussion

Statistical tests were run using the Tukey test, at the suggestions of the University of Tennessee Computer Center consultants (Muenchen, 1993). The Tukey test gives the "most realistic" comparisons of appropriate statistical tests, and controls the experimentwise error rate (Meunchen, 1993). The test contrasts each variable against all others, and determines significance by dividing .05/number of contrasts; it is harder to get significance than by other tests, such as the Scheffe, but is more reliable. The statistical data has a problem in that all the sites were not able to be sampled for each trip; so the programs threw out incomplete trips (the first three). This was compensated for by running individual tests using contrast statements for site-by-site analyses.

The two controls, pH 7.5 and pH 5.0, were significantly different from all the other samples, in all cases. The pH 5.0 controls consistently gave the greatest frondcounts. After the first few sets of results, I randomly sampled the pH's of the endpoint frondcounts within the petri dishes, and found that the pH had adjusted upwards toward the pH 7.0 - pH 7.4 range. Dr. James D. Caponetti (1992) suggested that algae growing on the roots was responsible for the change in

pH.

The two stream tributaries, Upper Bear Creek (UBC) and Bandy Creek (BC) tested significantly different from the main river, as well as from the controls. Upper Bear Creek (UBC) was consistently the lowest frondcount, probably resulting from the acid strip mine drainage. Bandy Creek (BC), one of the most pristine streams in the park, was also consistently low in frondcount; possibly due to low, comprehensive, nutrient availability as a result of the sewage treatment impoundment upstream.

Differences between the sites within a trip were not statistically significant, but differences between seasons were. The Tukey test which compared all sites for the complete seasons of 18 December 1991, 25 March 1992 and 20 August 1992 (winter, spring and summer) gave the following:

| Season | Mean Frondcount | Tukey Significance |
|---|---|---|
| 18 Dec 91 | 41.822 | A |
| 25 Mar 92 | 40.422 | A |
| 20 Aug 92 | 40.022 | A |
| 11 Oct 91 | 29.689 | B |
| 5 Jun 91 | 26.756 | B, C |
| 16 Jul 91 | 23.778 | C |

Comparisons were river sites only, with no controls (pH 5.0

and pH 7.5) or streams (BC and UBC). The Tukey test shows significance between seasons, with the first three listed not being significantly different under the relatively harsh comparisons of the Tukey (all A). The 11 Oct 91 and 5 Jun 91 show as not significantly different (both B), as do the 5 Jun 91 and the 16 Jul 91 (both C). The statistical data is only approximate, due to the missing factors, and indicates that under different criteria, more definite differences would occur. The figures showing the actual frond counts are probably a better indication of differences than the statistical data.

The site comparison data shows that unlike seasons, sites are not significantly different from one another along the length of the main river:

| Site | Mean Frondcount | Tukey Significance |
|---|---|---|
| BHM | 41.963 | A |
| BMB | 41.667 | A |
| LF | 41.185 | A |
| SC | 40.667 | A |
| NRB | 39.519 | A |
| AF | 39.444 | A |
| YB | 38.889 | A |
| BC | 30.296 | B |
| UBC | 29.630 | B |

All sites with the same letter (A or B) are not significantly different from one another.

The precipitation and river flow (cfs) data in Appendix B show that the highest river flow within a week before the sampling date was in March 1992; the lowest was October 1991. This data matches the rainfall records. The statistical data indicates the highest mean frondcount in December 1991, followed by March 1992. October 1991 was in the middle range (see Tukey test by season, above). The adjusted total frondcounts (Table 24) agree with the statistical data, with the highest total in December 1991, followed by March 1992. The flood on 14 December 1991 (17361 cfs), four days before the collection date (18 December 1991) may have churned allocthanous nutrients into the river and decreased any pollution due to flow volume as well. The October flow was the lowest on all three of the guage measure sites (New River, Clear Fork and Stearns; see Appendix B); low flows may bring about a concentration of pollutants. The frondcounts in October 1991 are in the middle of the range, indicating that allocthanous input and pollutants may not have seriously impacted the river at that time. March 1992 was the second highest in frondcount, possible reflecting the increased flow at that time (highest on all three guages, excluding the December 1991 flood). The flow rate is not the only factor

affecting nutrient availability, as demonstrated by the frondcount numbers.

## VII. Conclusions

The total frondcount numbers are consistently higher in the pH 5.0 and pH 7.5 controls than in any of the river or stream tributary frondcounts. The Upper Bear Creek strip mine site has the lowest consistent frondcounts. It is unlikely that any contaminants in the samples are beneficial to the growth of Lemna minor.

The Park Service can be confident that except for extraordinary circumstances, Lemna minor will grow in the BSF. Using Lemna minor as a test surrogate, the BSF provides a suitable habitat for a wide range of consumers in the detritus and grazing food chains. Lemna minor provides a more accurate analysis of river contamination than fish studies, because it typifies any habitat limitations at the base of the food chain. The river indicates a continued support of vascular systems at the food base level. The colonies of Justicia americana which proliferate in shallow edges and on bars along the river indicate that abundant resources are available for the producer level. On this basis, as well as the Lemna experiments, over the period sampled, the river could support an adequate food base potential.

It seems likely that under Park Service management, the BSF will continue to improve in quality. However, this study does not quantify or identify pollutants; it quantifies Lemna responses. The implications are that there is a resource restriction on the growth of Lemna in the samples. This would agree with the results that the greatest frond counts correspond to the flood flows in December 1991, and the lowest frond counts correspond to the lowest river flows measured in July 1991. The study targets water that has not been analyzed for control criteria; some resources may be sufficient, some may not.

There has been an improvement in the response of vascular plants (Lemna), especially in the case of the Upper Bear Creek amelioration project. However, this study can make no predictions as to the continuing quality of the water.

This method can be expanded to encompass food chain impacts of contaminants at various sites at various times, and to identify places needing more intensive analyses and quantifications.

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# APPENDICES

## Appendix A: Site Collection Information

Site NRB: New River Bridge (Colonel Joe Cecil Bridge), TN. On Route 27 South from Oneida. Collection site underneath the bridge, on the gravel fill. Site surrounded by riverine forest, including sycamore, alder, sumac and a few pines.
5 June 1991 Collection Trip: Current 1-2 mph, river turbid, approximately 30 meters wide.
16 July 1991 Collection Trip: River very low and murky, down 1-1/2 feet from June. Current is practically nonexistant (1 mph), greenish with algae. Surrounded by lush riverine growth; including phlox, oenothera, Queen Anne's lace, crown vetch, red clover, yellow oxalis, smartweed, plantain, foxtail (Fordiam grass), fleabane, elderberry, sycamore and alder.
11 October 1991 Collection Trip: Very foggy below the bridge; river very murky, with little noticeable flow.
18 December 1991: River green and slow, has been 10-12 feet higher. Is a sunny day, with an air temperature of 20°F.

Site BMB: Burnt Mill Bridge, TN. Collection at campground below the bridge, on sandy put-in. River has sandy bottom, surrounded by riverine forest, with lush growth of Justicia americana on sandbars.
5 June 1991 Collection Trip: River clear brown, flow 4-5 mph.
16 July 1991 Collection Trip: River clear brown, flow 3-4 mph, approximately one foot lower than June. Lush Justicia americana on river bars, and overhanging riverine vegetation.
11 October 1991 Collection Trip: River clear, with 3-4 mph flow.
18 December 1991: River is green and swift, up to the bank tree butts. The day is clear, with an air temperature of 22°F.

Site LF: Leatherwood Ford, TN. Collection site to the left of the foot bridge, below the guage station. Forest cover sycamore, alder, sweet gum, leatherwood, green ash, river birch, pines, and hemlock. Small population of Justicia americana on river bars.
5 June 1991 Collection Trip: River greenish from minerals, mostly clear, with little algae.
16 July 1991 Collection Trip: Pollution floating on water surface, very slow flow (2 mph). River one foot lower than June. Murky greenish-brown water, with banded sculpin feeding on algae. Stage 4.29, cfs 116.7. Lush riverine vegetation.
11 October 1991 Collection Trip: Low guage, below mount at Bridge sill. River clear, with flow of 2-3 mph.
18 December 1991: River is green and swift, has been >30

feet higher (up to the gazebo handrail). River stage 6.60 feet. Air temperature 25°F on a sunny day.

Site SC: Station Camp, TN. Old horse crossing. Collection site on east side of the river. Wooded island in the center of the river, with the near edge current slower than the far side of the island. Surrounded by riverine forest, stand of river birch at river edge, and Justicia americana on river bars.
5 June 1991 Collection Trip: River current beyond island 4-5 mph. (750-1000 cfs.)

Site BHM: Blue Heron Mine, KY. Collection site at end of boat ramp. Site is an old ford, with few trees near the edges, and riverine forest beyond.
5 June 1991 Collection Trip: River is fast-flowing, with small rapids approximately 3 mph.
16 July 1991 Collection Trip: River down two feet from June. Current 5 mph, clear brown, with some algae.
18 December 1991 Collection Trip: Storm event a few days ago sent water over 40 feet deep before falling. River is green in color, and swift. Air temperature 30°F.

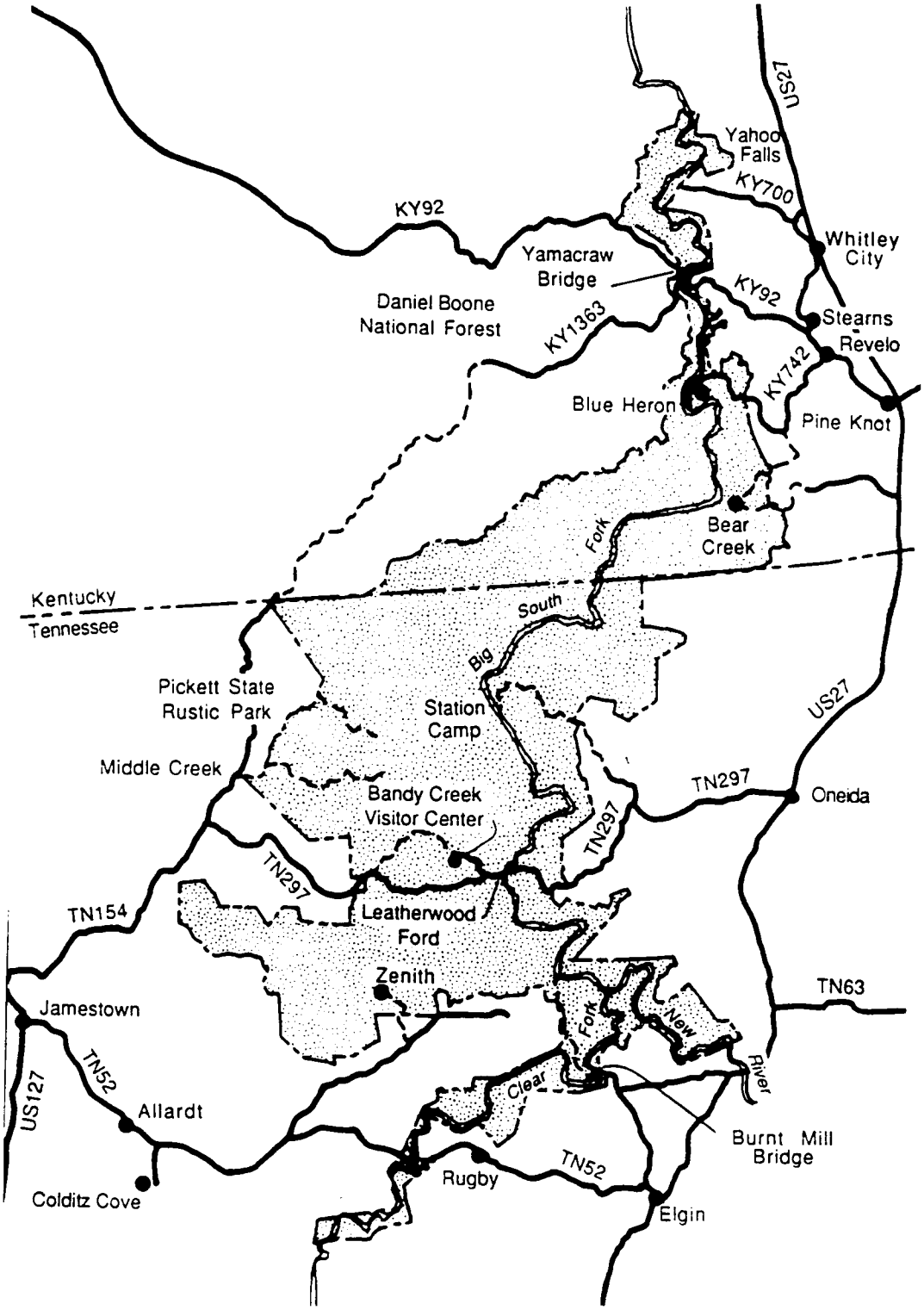
Site YB: Yamacraw Bridge, KY. Collection site on narrow corridor of U. S. Park Service land. River bank very sandy, surrounded by riverine forest with river birch and sycamore.
5 June 1991 Collection Trip: River very murky.
16 July 1991 Collection Trip: Water down three feet from June. Current 2 mph, mucky brown with green algal tinge from pollution. The edge of the river is sandy and muddy.
18 December 1991 Collection Trip: River surface over 50 meters below the bridge, has been 30 feet higher. Current is swift, and green in color.

Site AF: Alum Ford, KY. Collection site is an embayment of the Cumberland reservoir, above the boat ramp (under construction). There is much unconsolidated backfill, with a very unstable substrate (step in and sink fast). Site is the contact point between Mississippean and Pennsylvanian strata, consisting of limestone and siltstone. Riverine forest of sycamore, river birch, alder and black willow; upland forests on the bluff.
5 June 1991: River is muddy and greenish-brown. There was a coal seam fire in the adjacent area in early spring, 2-3 years previously. Site was underwater in May 1991.
16 July 1991: No discernable current, river edge very mucky with fill mud. River greenish-brown, down 3 feet from June.
18 December 1991: Water is medium high, green and turbid. River surface had been approximately 11 feet higher (absorbed by the Cumberland Reservoir). Air temperature 35°F; day is

breezy and sunny.

Site BC: Bandy Creek, TN. Collection site in stream below campground sewage treatment pools. One of the most visually pristine sites in the project, with dark clear water, a rocky bottom, and vegetation overhang of buttonbush, holly, rhododendron, hemlock and ferns. Aquatic inhabitants include crayfish and minnows. Stream has standing pools, with a current of <1 mph.
16 July 1991 Collection Trip:
18 December 1991: Sunny, air temperature 34°F. River down to normal size, with steady, clear flow.

Site UBC: Upper Bear Creek, KY. From Route 27 North of Oneida, take the unmarked road to the right where a solitary line pole stands, across from the BFW and animal clinic; pass the lumber yard on the right, take the left fork of the road and stop at the first culvert on the right side of the road. Collection site overhung with vegetation; sweetgum, alder and hemlock.
5 June 1991: Shallow, silt-bottom stream below tiny waterfall, with boulders and discolored silt from mineral plume; has mineral accumulations (mostly iron) on bottom rocks. Water striders present on surface, and the grey fungus Sphrotolis (an ascomycete) and iron-reducing bacteria present. Sample date is pre-reclamation of limestone trench system for open strip mine.




Map of the Big South Fork National River and Recreation Area (BSFNRRA) (The Best of the BSFNRRA, 1990).

Site pH, Temperature °C, Redox

| Site | Trip 1 | Trip 2 | Trip 3 | Trip 4 | Trip 5 | Trip 6 |
|---|---|---|---|---|---|---|
| New | 7.3 | 6.4 | 6.4 | 6.8 | 6.9 | 7.4 |
| River | 23 | 25 | 14 | 4 | 7 | 22 |
| Bridge | 190 | 200 | -- | 55 | -- | 45 |
| Burnt | 7.2 | 6.0 | 6.3 | 7.0 | 7.3 | 7.3 |
| Mill | 25 | 25 | 13 | 3 | 7 | 20 |
| Bridge | 186 | 60 | -- | 22 | -- | 52 |
| Leather- | 7.7 | 6.7 | 6.3 | 6.8 | 6.9 | 7.4 |
| wood | 24 | 27 | 15 | 4 | 7 | 21 |
| Ford | 190 | 170 | -- | 70 | -- | 52 |
| Station | 7.5 | -- | -- | 7.2 | 6.7 | -- |
| Camp | 26 | -- | -- | 6 | 7 | -- |
| | 180 | -- | -- | -- | 35 | -- |
| Blue | 7.5 | 6.8 | 6.6 | 6.8 | 6.7 | 7.4 |
| Heron | 26 | 30 | 17 | 4 | 7 | -- |
| Mine | 150 | 130 | -- | 40 | 50 | 60 |
| Yamacraw | 7.3 | 6.6 | -- | 6.8 | 6.7 | 7.3 |
| Bridge | 26 | 28 | -- | 5 | 8 | 22 |
| | 80 | 140 | -- | 40 | 30 | 66 |
| Alum | 7.3 | 6.4 | 6.3 | 7.0 | 6.5 | 7.6 |
| Ford | 26 | 30 | 17 | 5 | 8 | 25 |
| | 165 | 115 | -- | 70 | -- | 55 |
| Bandy | -- | 6.6 | 6.7 | 7.3 | 7.8 | -- |
| Creek | -- | 22 | 8 | 5 | 6 | -- |
| | -- | 155 | -- | 50 | 50 | -- |
| Upper | 5.0 | 6.6 | -- | 6.7 | 6.7 | -- |
| Bear | 20 | 22 | -- | 3 | 8 | -- |
| Creek | 240 | 25 | -- | -- | 10 | -- |

Missing data not available.

## Test Run Dates for Seasonal Trips

| Trip | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| 5 Jun 91 | 6-7 Jun 91 | 24-25 Jun 91 | 18 Dec 92 |
| 16 Jul 91 | 18-19 Jul 91 | 2,5 Sept 91 | 18 Dec 92 |
| 11 Oct 91 | 4 Aug 91 | 2 Dec 92 | 19 Dec 92 |
| 18 Dec 91 | 29-30 Jul 91, 2 Aug 91 | 7-8 Dec 92 | 20 Dec 92 |
| 25 Mar 92 | 23, 25 Sept 92 1, 8 Oct 92 | 15 Dec 92 | 20 Dec 92 |
| 20 Aug 92 | 8, 17 Oct 92 | 16-17 Dec 92 | 21 Dec 92 |

## Appendix B: Precipitation and Flow Data

### Precipitation Data, National Weather Service, for Oneida, TN

| Month/Year | | Total (inches) | Day/Amount | | # Days 0.1 | 0.5 | 1.0 |
|---|---|---|---|---|---|---|---|
| April | 1991 | 2.34 | 15 | 0.85 | 6 | 2 | 0 |
| May | 1991 | 3.40 | * | * | 5 | 1 | 0 |
| June | 1991 | 4.33 | 13 | 1.36 | 5 | 3 | 2 |
| July | 1991 | 5.59 | 9 | 2.40 | 4 | 2 | 2 |
| Aug | 1991 | 4.34 | * | * | 5 | 2 | 0 |
| Sept | 1991 | 2.80 | 25 | 2.15 | 2 | 2 | 1 |
| Oct | 1991 | 1.50 | 15 | 1.00 | 2 | 2 | 1 |
| Nov | 1991 | 2.80 | 22 | 2.20 | 3 | 1 | 1 |
| Dec | 1991 | * | | | | | |
| Jan | 1992 | 2.93 | 3 | 1.45 | 4 | 2 | 1 |
| Feb | 1992 | 3.72 | 24 | 1.20 | 6 | 2 | 2 |
| Mar | 1992 | 4.55 | 19 | 0.95 | 11 | 4 | 0 |
| Apr | 1992 | 1.95 | 21 | 0.81 | 3 | 1 | 0 |
| May | 1992 | 4.42 | 30 | 0.96 | 4 | 2 | 0 |
| June | 1992 | 6.14 | 13 | 1.15 | 8 | 6 | 4 |
| July | 1992 | 6.36 | 3 | 1.15 | 13 | 5 | 1 |
| Aug | 1992 | * | | | | | |

* Data not reported (National Climatic Data Center, 1991, 1992). Storm in December exceeded all guages by 30 feet.

Daily Mean CFS, Stearns, KY, U. S. Dept. Interior Data

| Sampling Dates | Max Flow w/in week | | Min Flow w/in week | |
|---|---|---|---|---|
| 5 June 91 | 28 May | 2932 cfs | 5 Jun | 400 cfs |
| 16 July 91 | 9 Jul | 1205 cfs | 16 Jul | 198 cfs |
| 11 Oct 91 | 6 Oct | 75 cfs | 11 Oct | 53 cfs |
| 18 Dec 91 | 14 Dec | 17361 cfs | 18 Dec | 18 cfs |
| 25 Mar 92 | 19 Mar | 5940 cfs | 18 Mar | 1184 cfs |
| 20 Aug 92 | 15 Aug | 2445 cfs | 20 Aug | 427 cfs |

Daily Mean CFS, New River and Clear Fork Bridges, TN, U. S. Dept. of Interior Data

| Sampling Date | CFS New River | CFS Clear Fork |
|---|---|---|
| 5 June 91 | 243 cfs | 59 cfs |
| 16 July 91 | e100 cfs* | 48 cfs |
| 11 Oct 91 | 17 cfs | 8.1 cfs |
| 18 Dec 91 | 833 cfs | 581 cfs |
| 25 Mar 92 | 895 cfs | e700 cfs* |
| 20 Aug 92 | 68 cfs | 134 cfs |

* e = estimated

## Appendix C: Vegetation Types

Vegetation types are reported from Safley (1990).

Gorge vegetation is River Birch Type at low elevation, with the gorge bottom on alluvium.

Tulip Poplar-Sweetgum Type: lies upslope from the River Birch Type, on the first terrace positions.

Red Cedar Type: forms a narrow band on Mississippean strata, between 870 - 1000 feet above the gorge.

White Oak-Chinquapin Type: lower slopes within the gorge, above the Red Cedar Type.

Tulip Poplar-Hemlock Type: mesic sites on north-facing ridges and draws within the gorges.

White Oak-Virginia Pine Type: most common on middle and lower slopes of north-facing gorge draws.

Shortleaf Pine-White Oak Type: upper slopes of north-facing draws.

White Oak-Virginia Pine Ridge Type: wide elevation range alog north-facing ridges.

North Red Oak-Chestnut Oak-White Oak Type: northerly ridges with thinner soils.

White Oak-Hemlock-Chestnut Oak Type: most extensive type, on south-facing draws of gorges and mountain draws.

White Oak-Beech Type: Lower portions of south-facing ridges and leads within gorges, between 900 - 1120 feet elevation.

White Oak-Chestnut Oak Type: steep, south-facing gorge ridges.

Virginia Pine-White Pine Type: upper slopes of south-facing gorge ridges.

Virginia Pine-White Oak Type: on gorge crest.

Northern Aspect White Oak Type: north-facing plateau slopes except at the uppermost levels.

Southern Aspect White Oak Type: on south-facing plateau slopes.

Virginia Pine-White Oak-Blackjack Oak Thpe: midslope on southern aspects after recent disturbance.

Virginia Pine Type: on thin soils, upper slopes and hill crests.

Primarily deciduous forests with a heavy mix of pines or cedar. White oak is dominant. Mixed mesophytic forests on protected sites. Much unstable secondary growth after clearcutting and strip mining.

## Appendix D: Water Quality Parameters
## BSFNRRA Water Quality Report 1982-1984

Temperature directly affects aquatic life, with increasing temperature depressing the oxygen content and increasing the biodegradation of organics and tolerances of species.

The pH is affected by acid mine drainage. Pyrite and air are transformed to ferric sulfate, then to sulfuric acid. Extremely low pH leads to sterile streams, and produces a metallic yellow-orange precipitate. Some buffering of the pH is due to alkaline earths and carbonates of mine drainages.

Increased chloride indicates pollution. Fecal coliform bacteria indicates the presence of sewage. Iron and Manganese are indicative of mining, as are sulfates.

The Clear Fork is rated as slightly polluted. New River has moderate pollution, and Bear Creek is severly polluted.

Bandy Creek is a sandstone stream, with extremely good water quality below Leatherwood Ford. Sediments from past logging have been affected by the large campground complex, with increased iron. The sewage treatment plant discharges into the creek.

Grassy Fork, above Yamacraw Bridge, is of excellent quality. Laurel Fork, at Station Camp, is very good. They are both sandstone streams.

No Business Creek below Station Camp is a limestone stream with no significant problems. Station Camp Creek, also a limestone stream, shows no significant impact.

Slightly polluted streams are the Clear Fork, Laurel Fork, North White Oak Creek and Williams Creek. Clear Fork is a major tributary which has been exposed to agriculture, mining, oil and gas exploration and pollution from White Oak Creek. Laurel Fork is generally clean, with some runoff, erosion, and sewage from the compound. North White Oak Creek is contaminated by agricultural runoff. Williams Creek is one of only two reasonably clear streams in the east central portion of the Big South Fork area; the Grassy Fork of Williams Creek is the other.

Moderately polluted streams include New River, Pine Creek, Pucheoncamp branch of Williams Creek and the White Oak Creek tributary of the Clear Fork. The New River has been impacted by coal mining, oil and gas exploration, agriculture and forestry; with extensive strip mining in the headwaters.

Pine Creek flows from Oneida to the Narrows; channelization upstream, high sewage sediment load and bacteria, surface mining and oil and gas exploration has impacted the stream. The Puncheoncamp of Williams Creek contains acid mine drainage from small abandoned strip mines. The White Oak Creek tributary of the Clear Fork has been exposed to forestry and oil and gas exploration.

Severely polluted streams include Bear Creek, Roaring Paunch Creek and Rock Creek. Bear Creek contains acid mine drainage. Roaring Paunch Creek, near Blue Heron, is impacted by acid mine, agricultural and domestic runoff. The lower reaches of Rock Creek, above Yamacraw Bridge, is contaminated with acid mine drainage.

## Appendix E: Big South Fork Non-point Watershed Project

A non-point source is one without significant rainfall (1/4 inch or less in any one day) of greater than or equal to one inch within 24 hours. The Knoxville Environmental Field Office of the Tennessee Division of Health and Environment is sampling storm events on the three tributaries of Upper Bear Creek to determine how much contaminant from the strip mine amelioration project is flushed downstream. Some reclamation has been done by the Tennessee Department of Conservation, but storm events indicate no improvement in Upper Bear Creek.

Tests are conducted for inorganic arsenic (As), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), nickle (Ni), potassium (K), sodium (Na), and zinc (Zn). Chlorine (Cl), total Kjeldahl nitrogen (N), ammonia N, total organic N, phosphate, and sulfates are also tested for. Metals in high concentrations have been measured in Bear Creek at the mouth and in the Big South Fork below the confluence with Bear Creek. Bear Creek has been declared to be a health hazard (Basher, 1990). The following concentration data are from the Basher report.

### 1. Metals in Bear Creek and BSF Cumberland River October 24, 1988

| Metal | Bear Creek mg/kg | BSF above Bear Creek ppm | BSF below Bear Creek ppm |
|---|---|---|---|
| Al | 4900.0 | 854.0 | 1680.0 |
| As | 3.46 | <0.194 | 1.56 |
| Cd | <0.010 | <0.010 | <0.010 |
| Cr | 5.50 | 2.03 | 4.40 |
| Cu | 6.45 | 2.24 | 3.63 |
| Fe | 11000.0 | 2370.0 | 4620.0 |
| Pb | 7.40 | 2.33 | 5.0 |
| Mn | 503.0 | 475.0 | 1100.0 |
| Hg | 0.019 | 0.016 | 0.019 |
| Ni | 12.7 | 6.32 | 12.4 |
| Zn | 39.5 | 11.5 | 26.0 |

2. Comparison of Metals in Bear Creek with State and Federal Maximum Contaminent Levels

| Metal | Bear Creek mg/kg | State MCL mg/l | Federal MCL mg/l |
|---|---|---|---|
| Al | 4900.0 | 0.2 | 0.05 (s) |
| As | 3.46 | 0.05 | 0.05 (p) |
| Cd | <0.010 | 0.010 | 0.005 (p) |
| Cr | 5.50 | 0.05 | 0.05 (i)<br>0.1 (p) |
| Cu | 6.45 | 1.0 (s) | -- |
| Fe | 11000.0 | 0.3 (s) | -- |
| Pb | 7.4 | 0.05 | 0.05 (i)<br>0.02 (p) |
| Mn | 503.0 | 0.05 (s) | -- |
| Hg | 0.019 | 0.002 | 0.002 (p) |
| Ni | 12.7 | -- | -- |
| Zn | 34.5 | 5.0 (s) | -- |

* s = secondary (taste/odor)
  i = interim
  p = proposed

3. Metals in Bear Creek in Multiples of Maximum Contaminant Levels (MCL)

| Thousands of MCL's | Multiples of MCL's |
|---|---|
| Al approx. 100 | As approx. 60-75 |
| Fe approx. 35 | Cr approx. 50-60 |
| Mn approx. 10 | Cu approx. 10 |
| | Pb approx. 350 |
| | Hg approx. 15 |
| | Zn approx. 10 |

Storm events in the three Bear Creek tributaries show high levels of total metals and solids, and stressed levels of pH, conductivity, and turbidity.

## Appendix F: Obed River Graphs

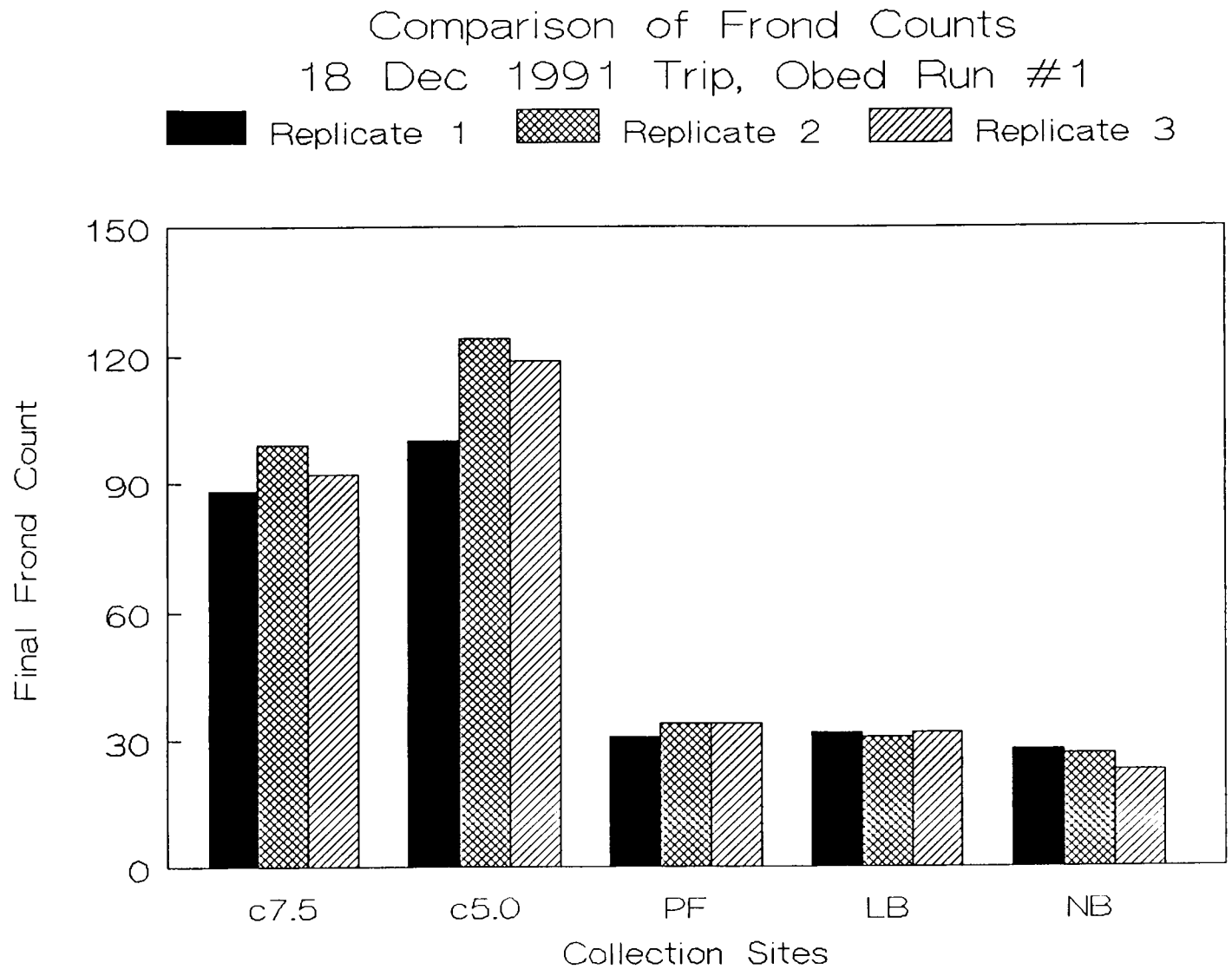
At the request of the U. S. Park Service, frond counts were quantitated for the nearby, discrete Obed River. Graphs were constructed at the same scale as those of the Big South Fork, to facilitate comparison. The total frondcounts showed seasonal variation between the three seasons in which the river was sampled (December 1991, March 1992 and August 1992). Frondcounts were highest for March 1992 (total frondcount 1103), followed by December 1991 (total frondcount 1078) and August 1992 (total frondcount 955). The Potters Ford site gave consistently higher total frondcounts (endpoint frondcount 1125) than either Nemo Bridge or Lilly Bridge. The difference between the Nemo Bridge and Lilly Bridge site was insignificant (endpoint frondcounts 1005 and 1006, respectively). This study did not include precipitation data for the Obed River, so comparisons cannot be made with results from the Big South Fork. Total frondcounts are summarized as follows:

1: Frondcount Totals for Obed River

| Site | Dec 1991 | Mar 1992 | Aug 1992 | Total |
|---|---|---|---|---|
| PF | 382 | 398 | 345 | 1125 |
| NB | 338 | 364 | 303 | 1005 |
| LB | 358 | 341 | 307 | 1006 |
| Total | 1078 | 1103 | 955 | |

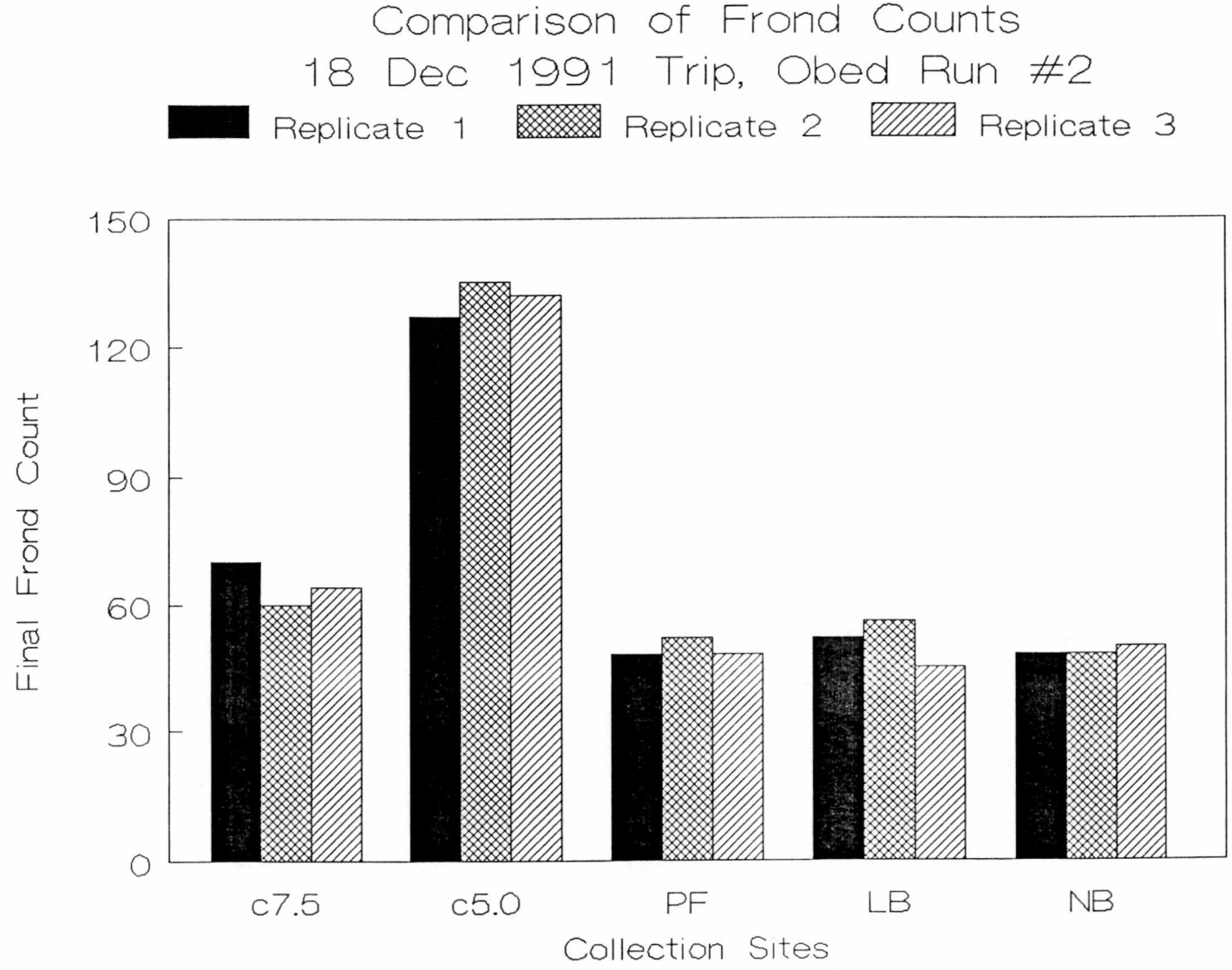
2. Comparison of Frond Counts
18 December 1991 Trip, Obed River, Run 1

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 88 | Lab media |
| C7.5B | 99 | |
| C7.5C | 92 | |
| Total | 279 | |
| | | |
| C5.0A | 100 | Lab media |
| C5.0B | 124 | |
| C5.0C | 119 | |
| Total | 343 | |
| | | |
| PF-A | 31 | Potters Ford, TN |
| PF-B | 34 | |
| PF-C | 34 | |
| Total | 99 | |
| | | |
| NB-A | 28 | Nemo Bridge, TN |
| NB-B | 27 | |
| NB-C | 23 | |
| Total | 78 | |
| | | |
| LB-A | 32 | Lilly Bridge, TN |
| LB-B | 31 | |
| LB-C | 32 | |
| Total | 95 | |


Comparison of Frond Counts
18 Dec 1991 Trip, Obed Run #1
Replicate 1
Replicate 2
Replicate 3
Final Frond Count
150
120
90
60
30
0
c7.5
c5.0
PF
LB
NB
Collection Sites

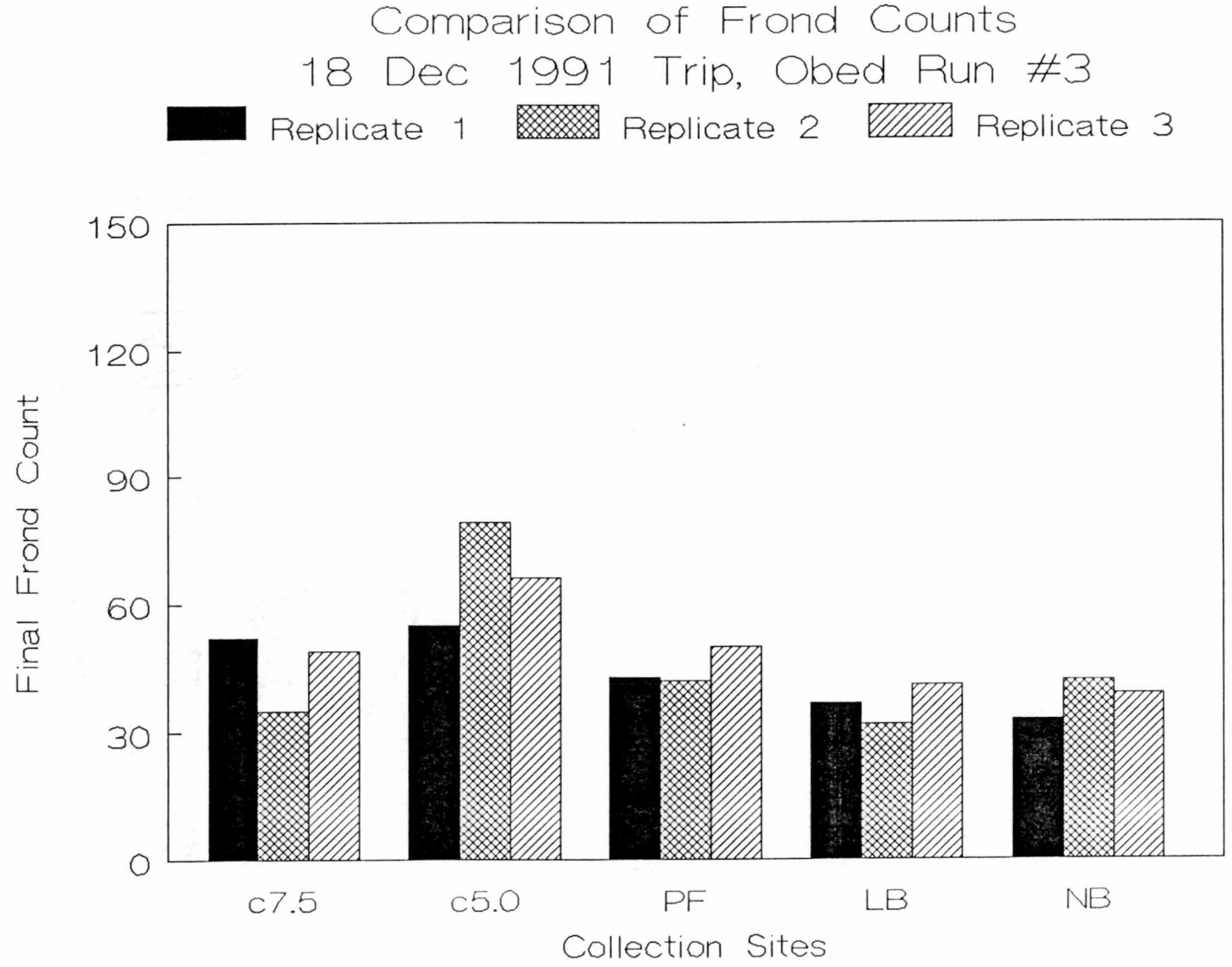
3. Comparison of Frond Counts
18 December 1991 Trip, Obed River, Run 2

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 70 | Lab media |
| C7.5B | 60 | |
| C7.5C | 64 | |
| Total | 194 | |
| | | |
| C5.0A | 127 | Lab media |
| C5.0B | 135 | |
| C5.0C | 132 | |
| Total | 394 | |
| | | |
| PF-A | 48 | Potters Ford, TN |
| PF-B | 52 | |
| PF-C | 48 | |
| Total | 148 | |
| | | |
| NB-A | 48 | Nemo Bridge, TN |
| NB-B | 48 | |
| NB-C | 50 | |
| Total | 146 | |
| | | |
| LB-A | 52 | Lilly Bridge, TN |
| LB-B | 56 | |
| LB-C | 45 | |
| Total | 153 | |


Comparison of Frond Counts
18 Dec 1991 Trip, Obed Run #2
Replicate 1
Replicate 2
Replicate 3
Final Frond Count
150
120
90
60
30
0
c7.5
c5.0
PF
LB
NB
Collection Sites

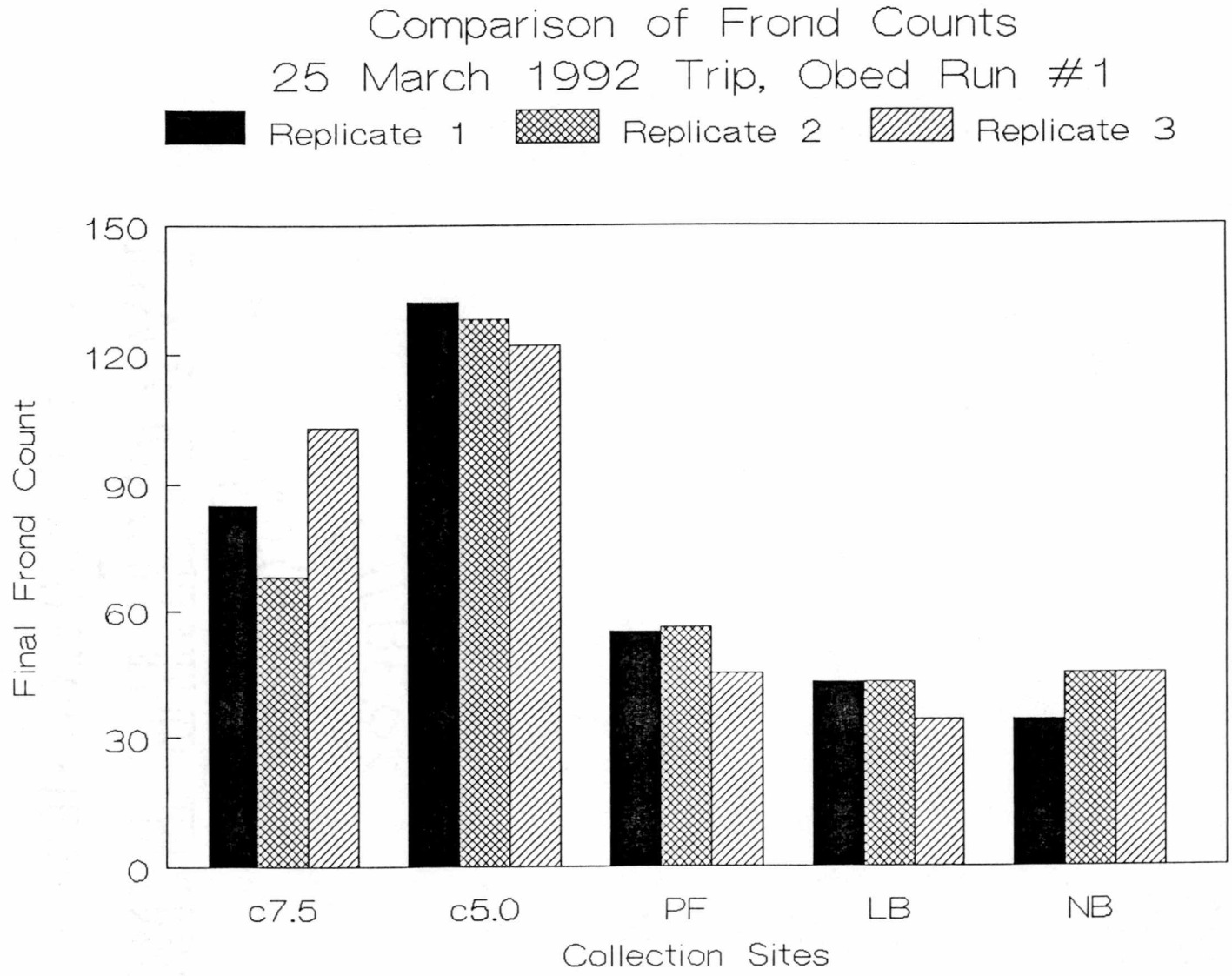
4. Comparison of Frond Counts
18 December 1991 Trip, Obed River, Run 3

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 52 | Lab media |
| C7.5B | 35 | |
| C7.5C | 49 | |
| Total | 136 | |
| | | |
| C5.0A | 55 | Lab media |
| C5.0B | 79 | |
| C5.0C | 66 | |
| Total | 200 | |
| | | |
| PF-A | 43 | Potters Ford, TN |
| PF-B | 42 | |
| PF-C | 50 | |
| Total | 135 | |
| | | |
| NB-A | 33 | Nemo Bridge, TN |
| NB-B | 42 | |
| NB-C | 39 | |
| Total | 114 | |
| | | |
| LB-A | 37 | Lilly Bridge, TN |
| LB-B | 32 | |
| LB-C | 41 | |
| Total | 110 | |


Comparison of Frond Counts
18 Dec 1991 Trip, Obed Run #3
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

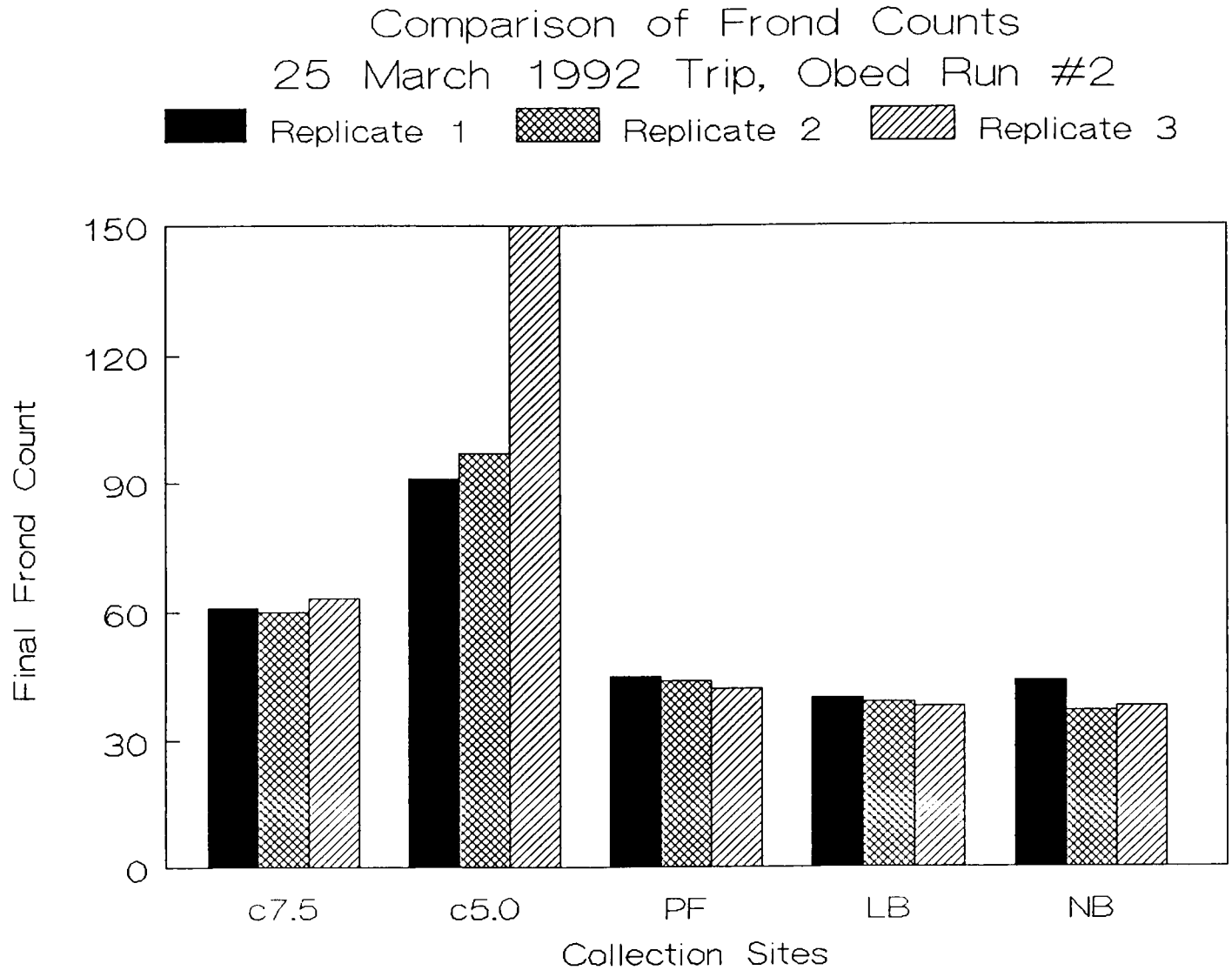
## 5. Comparison of Frond Counts
## 25 March 1992 Trip, Obed River, Run 1

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 85 | Lab media |
| C7.5B | 68 | |
| C7.5C | 103 | |
| Total | 256 | |
| | | |
| C5.0A | 132 | Lab media |
| C5.0B | 128 | |
| C5.0C | 122 | |
| Total | 382 | |
| | | |
| PF-A | 55 | Potters Ford, TN |
| PF-B | 56 | |
| PF-C | 45 | |
| Total | 156 | |
| | | |
| NB-A | 34 | Nemo Bridge, TN |
| NB-B | 45 | |
| NB-C | 45 | |
| Total | 124 | |
| | | |
| LB-A | 43 | Lilly Bridge, TN |
| LB-B | 43 | |
| LB-C | 34 | |
| Total | 120 | |


Comparison of Frond Counts
25 March 1992 Trip, Obed Run #1
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

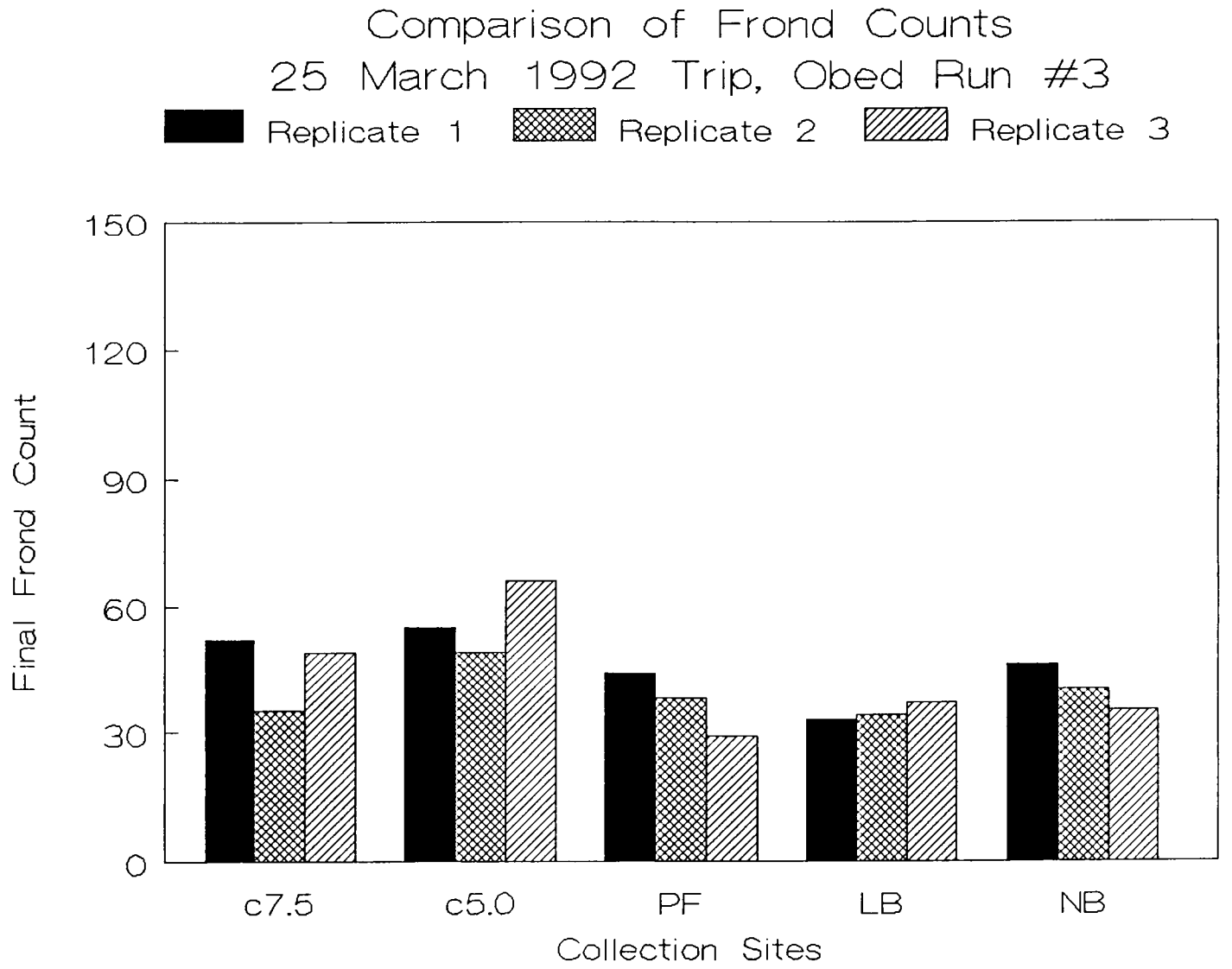
## 6. Comparison of Frond Counts
## 25 March 1992 Trip, Obed River, Run 2

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 97 | |
| C5.0C | 150 | |
| Total | 338 | |
| | | |
| PF-A | 45 | Potters Ford, TN |
| PF-B | 44 | |
| PF-C | 42 | |
| Total | 131 | |
| | | |
| NB-A | 44 | Nemo Bridge, TN |
| NB-B | 37 | |
| NB-C | 38 | |
| Total | 119 | |
| | | |
| LB-A | 40 | Lilly Bridge, TN |
| LB-B | 39 | |
| LB-C | 38 | |
| Total | 117 | |


Comparison of Frond Counts
25 March 1992 Trip, Obed Run #2
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

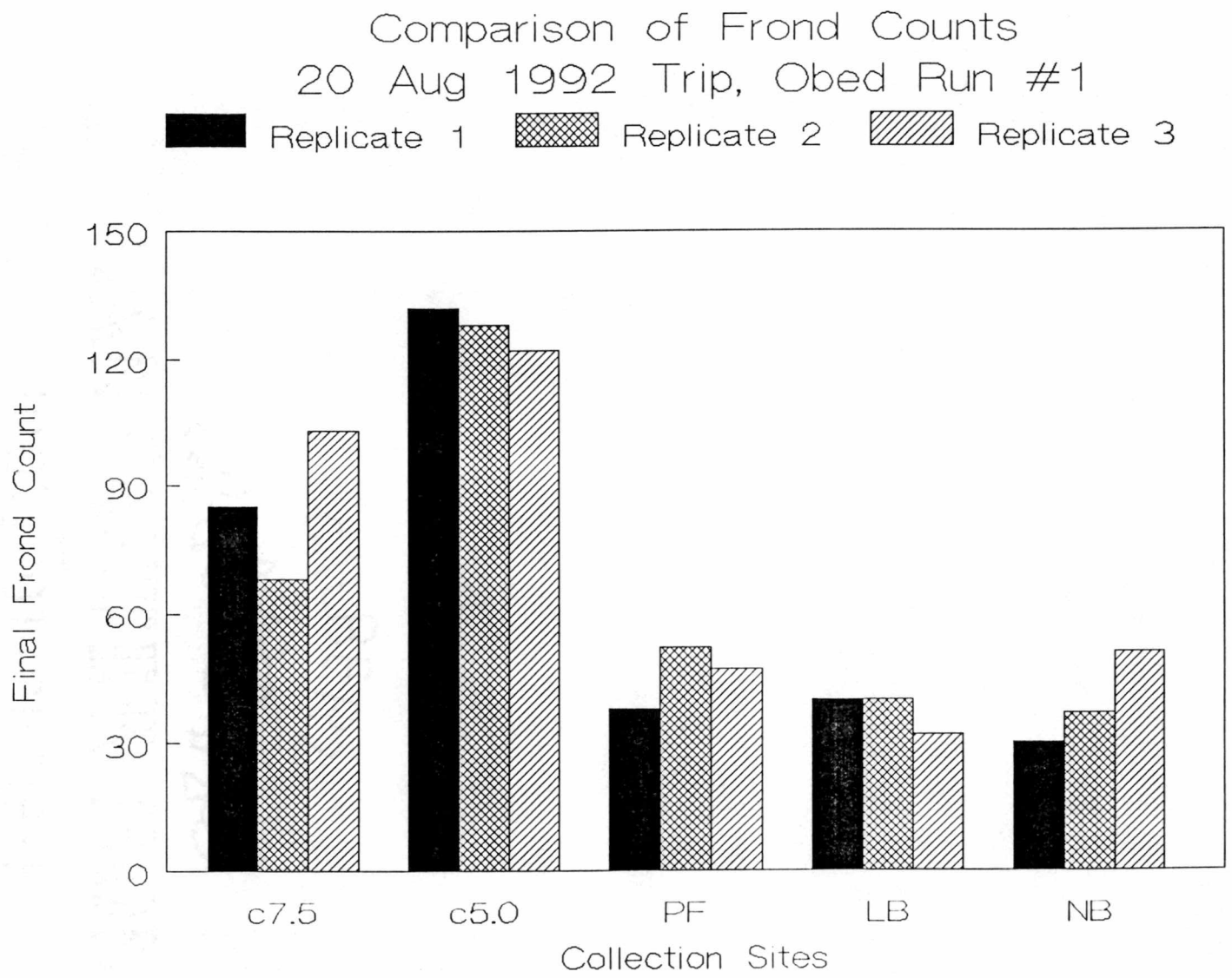
## 7. Comparison of Frond Counts
## 25 March 1992 Trip, Obed River, Run 3

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 52 | Lab media |
| C7.5B | 35 | |
| C7.5C | 49 | |
| Total | 136 | |
| | | |
| C5.0A | 55 | Lab media |
| C5.0B | 49 | |
| C5.0C | 66 | |
| Total | 382 | |
| | | |
| PF-A | 44 | Potters Ford, TN |
| PF-B | 38 | |
| PF-C | 29 | |
| Total | 111 | |
| | | |
| NB-A | 46 | Nemo Bridge, TN |
| NB-B | 40 | |
| NB-C | 35 | |
| Total | 121 | |
| | | |
| LB-A | 33 | Lilly Bridge, TN |
| LB-B | 34 | |
| LB-C | 37 | |
| Total | 104 | |


Comparison of Frond Counts
25 March 1992 Trip, Obed Run #3
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

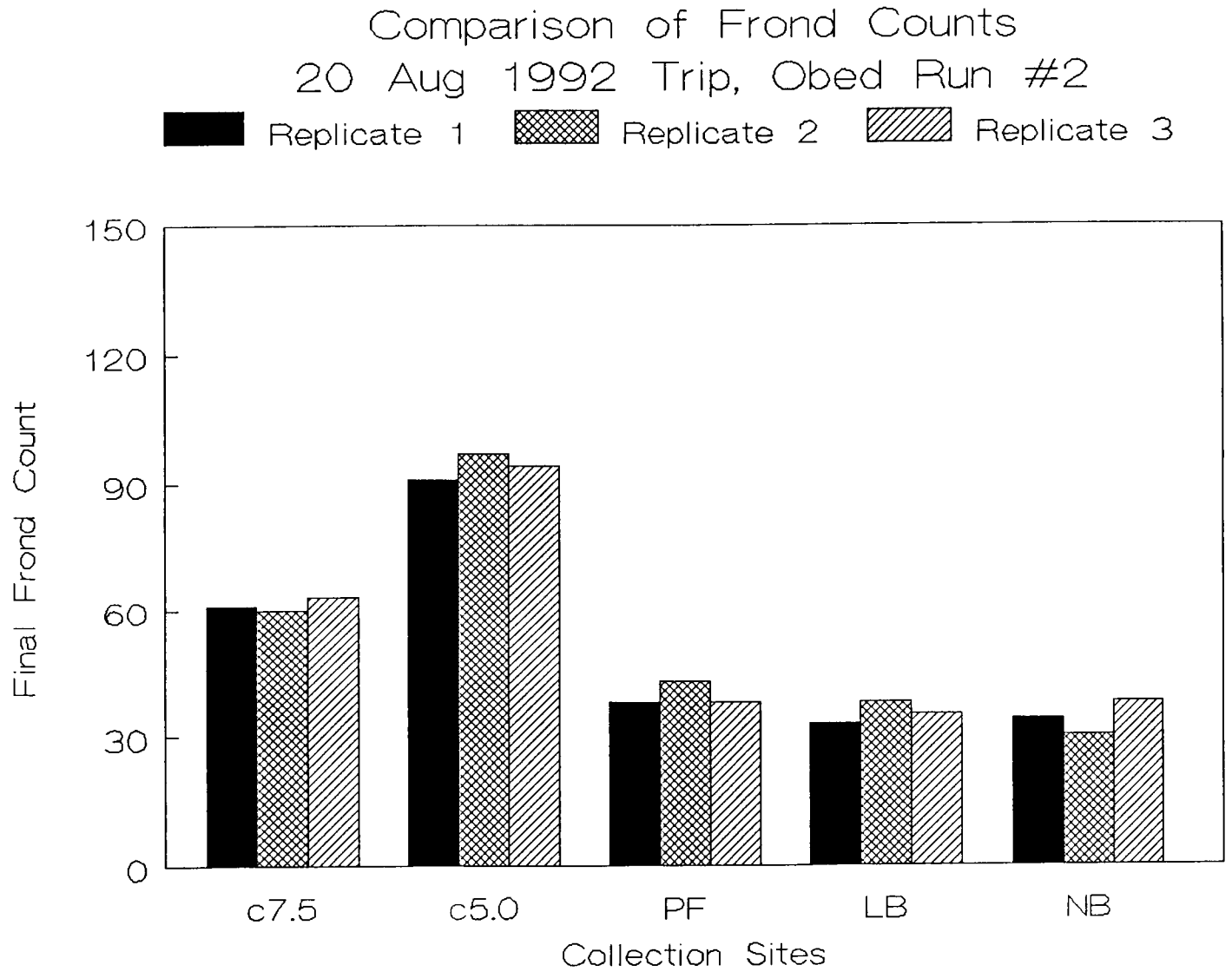
## _____ 8. Comparison of Frond Counts 20 August 1992 Trip, Obed River, Run 1

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 85 | Lab media |
| C7.5B | 68 | |
| C7.5C | 103 | |
| Total | 256 | |
| | | |
| C5.0A | 132 | Lab media |
| C5.0B | 128 | |
| C5.0C | 122 | |
| Total | 382 | |
| | | |
| PF-A | 38 | Potters Ford, TN |
| PF-B | 52 | |
| PF-C | 47 | |
| Total | 137 | |
| | | |
| NB-A | 30 | Nemo Bridge, TN |
| NB-B | 37 | |
| NB-C | 51 | |
| Total | 118 | |
| | | |
| LB-A | 40 | Lilly Bridge, TN |
| LB-B | 40 | |
| LB-C | 32 | |
| Total | 112 | |


Comparison of Frond Counts
20 Aug 1992 Trip, Obed Run #1
Replicate 1
Replicate 2
Replicate 3
Final Frond Count
150
120
90
60
30
0
c7.5
c5.0
PF
LB
NB
Collection Sites

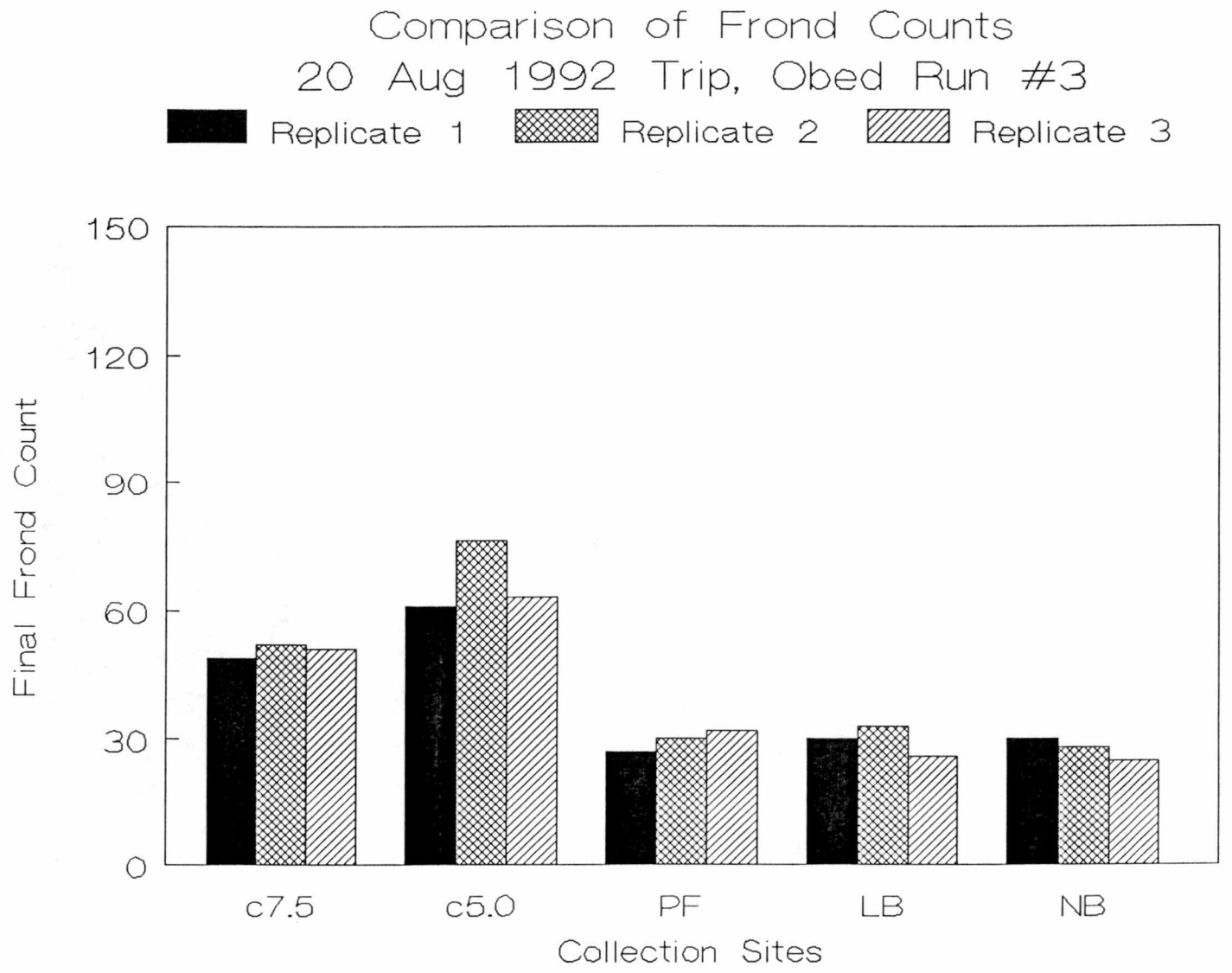
## 9. Comparison of Frond Counts
## 20 August 1992 Trip, Obed River, Run 2

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 61 | Lab media |
| C7.5B | 60 | |
| C7.5C | 63 | |
| Total | 184 | |
| | | |
| C5.0A | 91 | Lab media |
| C5.0B | 97 | |
| C5.0C | 94 | |
| Total | 282 | |
| | | |
| PF-A | 38 | Potters Ford, TN |
| PF-B | 43 | |
| PF-C | 38 | |
| Total | 119 | |
| | | |
| NB-A | 34 | Nemo Bridge, TN |
| NB-B | 30 | |
| NB-C | 38 | |
| Total | 102 | |
| | | |
| LB-A | 33 | Lilly Bridge, TN |
| LB-B | 38 | |
| LB-C | 35 | |
| Total | 106 | |


Comparison of Frond Counts
20 Aug 1992 Trip, Obed Run #2
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

## _____ 10. Comparison of Frond Counts 20 August 1992 Trip, Obed River, Run 3

| Sample | Endpoint Frond Count | Site |
|---|---|---|
| C7.5A | 49 | Lab media |
| C7.5B | 52 | |
| C7.5C | 51 | |
| Total | 152 | |
| | | |
| C5.0A | 61 | Lab media |
| C5.0B | 76 | |
| C5.0C | 63 | |
| Total | 200 | |
| | | |
| PF-A | 27 | Potters Ford, TN |
| PF-B | 30 | |
| PF-C | 32 | |
| Total | 89 | |
| | | |
| NB-A | 30 | Nemo Bridge, TN |
| NB-B | 28 | |
| NB-C | 25 | |
| Total | 83 | |
| | | |
| LB-A | 30 | Lilly Bridge, TN |
| LB-B | 33 | |
| LB-C | 26 | |
| Total | 89 | |


Comparison of Frond Counts
20 Aug 1992 Trip, Obed Run #3
Replicate 1
Replicate 2
Replicate 3
150
120
90
60
30
0
Final Frond Count
c7.5
c5.0
PF
LB
NB
Collection Sites

## Appendix G: Geological Formations
## TN and KY Geologic Maps, (U.S.G.S. 1981 and 1966)

Mp Pennington Formation, Mississipian age. Highly variegated clay shale, very distinctive; contains siltstone and locally gray, fine-grained sandstone. Thickness from 400 - 700feet. Lower river bed.

Pf Fentress Fornation, Pennsylvanian age. Mostly dark gray to light brown shale, with minor siltstone and sandstone. Wilder coal near the middle of the formation. Thickness to 340 feet. Upper river bed.

Pco Crab Orchard Mountains Group, Clear Fork area. Conglomerate, with sandstone, siltstone, shale and coal. Includes the Rockcastle Conglomerate (Pr), Vandever Formation, Newton Sandstone, Whitwell Shale and Sewanee Conglomerate.

Rockcastle Conglomerate. Conglomeratic sandstone and sandstone, gray to brown, fine- to coarse-grained. Thin coal-bearing shale locally present near middle of formation. Thickness 150-220 feet.

Vandever Formation. Mostly shale and siltstone, dark gray to light brown, conglomerate or sandstone in middle to south of formation. Lantana and Morgan Springs coals near base and top. Thickness up to 450 feet, with an average of 300 feet.

Newton Sandstone. Sandstone, gray to brown or pink, fine- to medium-grained, locally conglomeratic. Thickness as much as 200 feet, with an average of 90 feet.

Whitwell Shale. Mostly dark gray to light brown shale, with minor siltstone; locally the middle part is sandstone. Richland coal near base, with Sewanee coal in upper part. Thickness to 220 feet, with an average of 75 feet.

Sewanee Conglomerate. Conglomeratic sandstone and sandstone, gray to brown, fine- to coarse-grained. Thickness to 200 feet, with an average of 100 feet.

Psl Slateston Formation, Pennsylvanian age. Located near Oneida, TN. Shale, sandstone, siltstone and several important coals, from the Jellico coal to Poplar Creek coal. Thickness 500 - 720 feet.

Pcf Crooked Fork Group, New River area. Shale, sandstone, conglomerate, siltstone and coal; from Poplar Creek coal to the top of the Rockcastle Conglomerate. Thickness 320-455

feet. Includes the Wartburg Sandstone, Glenmary Shale, Coalfield Sandstone, Burnt Mill Shale, Crossville Sandstone and Dorton Shale.

Wartburg Sandstone. Sandstone, gray to brown, fine- to medium-grained, locally conglomeratic. Poplar Creek coal and then shale at top. Thickness 0 - 50 feet.
Glenmary Shale. Mostly dark gray to light brown shale with minor siltstone and sandstone. This coal near base locally. Thickness 50-150 feet.

Coalfield Sandstone. Sandstone, gray to brown, fine- to medium-grain. Thickness 0 - 80 feet.

Burnt Mill Shale. Mostly dark gray to light brown shale, with minor siltstone; thin sandstone locally present near base. Hooper coal just above the base. Thickness to 110 feet.

Crossville Sandstone. Sandstone, gray to pink or brown, fine- to medium-gran, thinly and evenly bedded. Thickness 30-70 feet.
Dorton Shale. Mostly dark gray to light brown shale, with minor siltstone and sandstone. Thin coal near top. Rex coal as much as 70 feet above base. Thickness as much as 150 feet.

Pennsylvanian strata to east of Oneida, TN is very fragmented, and includes the Cross Mountain Formation, Vowell Mountain Formation, Redoak Mountain Formation, Graves Gap Formation, Indian Bluff Formation and Slatestone Formation.

Pcm Cross Mountain Formation. Mostly shale, interbedded with sandstone, siltstone, and thin coal beds; base at top of Frozen Head Sandstone. Maximum thickness 554 feet.

Pvm Vowell Mountain Formation. Shale, sandstone, siltstone and coal; from Frozen Head Sandstone Member to Pewee coal. Thickness 230 - 375 feet.

Prm Redoak Mountain Formation. Shale, sandstone, siltstone and several important coals; from Pewee coal to Windrock coal. Thickness 340 - 420 feet.

Pgg Graves Gap Formation. Shale, sandstone, siltstone and coal; from Windrock coal to top of Pioneer Sandstone. Thickness 275 - 385 feet.

Pib Indian Bluf Formation. Shale, sandstone, siltstone and thin coal beds; from Pioneer Sandstone Member to Jellico

coal. Thickness 150 - 415 feet.

Psl Slatestone Formation. Shale, sandstone, siltstone and several important coals; from Jellico coal to Poplar Creek coal. Thickness 500 - 720 feet.

Kentucky geology Middle Pennsylvanian overlaying Lower Pennsylvanian and Upper Mississippian age strata. Pennington Formation riverbed.

Mp Pennington Formation. Rocks of Chester age (Mcl) in the lower part; includes sandstone member of Mooretown Formation (SS). Overlies Hartselle Formation.

Mbk Bangor Limestone, thickness 0 - 160 feet; Hartselle Formation, thickness 0 - 50 feet; and Kidder Limestone, 90 - 230 feet. Member of Monteagle Limestone, which lies below Hartselle Formation.

Mn Newman Limestone. Upper member, on east side of gorge, overlies the Ste. Geneviere Limestone Member.

Mgl Ste. Geneviere Limestone, thickness 30 - 90 feet; overlies the St. Louis Limestones, thickness 70 - 160 feet.

East of the river, there are fragments of the Lee Formation and the Breathitt Formation, with the Corbin Sandstone Member near Route 92.

Pbm Breathitt Formation. Middle part of formation, with Pbl underneath.

Pca Caseyville Formation.

Plc Lee Formation. Includes the Corbin Sandstone Member, the Rockcastle Sandstone Member and the Livingstone Conglomerate Member.

Plc Corbin Sanstone Member, thickness 0 - 200 feet.

Plr Rockcastle Sandstone Member, thickness 0 - 210 feet.

Pll Livingstone Conglomerate Member.

# VITA

Margaret Dianne Parks was born in Washington, D. C. on June 24, 1952. She attended elementary and intermediate schools in the Fairfax County (Northern Virginia) school district and graduated from W. T. Woodson High School in May 1970. The following September she entered the University of Virginia at Charlottesville and in May 1974 received the degree of Bachelor of Science in Environmental Science, with a specialty in Ecology. She took graduate level coursework while working at the University of Texas in Austin and the University of North Carolina at Chapel Hill. In August 1990 she entered the University of Tennessee at Knoxville, and in December 1993 received a Master of Science degree in Botany.