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# AN ECOLOGICAL STUDY ON THE INTRODUCTION OF THE BANDED SCULPIN INTO A COAL FLYASH IMPACTED STREAM



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February 1996





Prepared for
J. A. Hodgins
Environmental Restoration Program
Y-12 Plant
Oak Ridge, Tennessee 37831

Prepared by the
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831-6285
managed by
LOCKHEED MARTIN ENERGY RESEARCH CORPORATION
for the
U.S. DEPARTMENT OF ENERGY
under contract DE-AC05-96OR22464

MANAGED BY
LOCKHEED MARTIN ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

UCN-13673 (36 6-95)

ORNL/TM-13175

ENVIRONMENTAL SCIENCES DIVISION

# AN ECOLOGICAL STUDY ON THE INTRODUCTION OF THE BANDED SCULPIN INTO A COAL FLYASH IMPACTED STREAM.

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Environmental Sciences Division
Publication No. 4521

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Date Published – February 1996

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## ACKNOWLEDGEMENTS

I thank my mentor and friend Mike Ryon for his boundless patience and for all the advice and inspiration he provided me with during the course of this study. I thank Drs. David Etnier, Arthur Stewart, Marshall Adams, and Walter Farkas for serving on my committee, and for all the guidance they provided. I thank my employer Jaycor for all the financial and technical support they provided me over the course of this study, the personnel in the Benthic Lab for providing their taxonomic expertise, and J. Fred Heitman for all his advice and editorial suggestions. I express thanks to Keith Weaver, Randy Hoffmeister, Russ Redden, Chris Mcall, Kelly Roy, Beth Schilling, Denny Smith, and Jason Khym for their invaluable help in collecting all my field data. I also thank Jackie Richmond and Ken Ham for all of their help with my graphs and charts. I thank the Scates family, the Hansard family, and the Evars family for kindly allowing my intrusion on their land during the course of this study. I thank my wife Michelle, and my daughter, Hope for putting up with the major changes caused in our lives, and for providing love and encouragement during this thesis study. I especially thank my mother, Joan, and my father, Allan, for always having faith in me and for providing the inspiration to embark upon and successfully complete this project.

This work was funded by the Y-12 Plant Environmental Restoration Program. The Y-12 Plant and Oak Ridge National Laboratory are managed by Lockheed Martin Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

## ABSTRACT

A number of banded sculpins [*Cottus carolinae* (Gill)] were obtained from a population in stream, marked with subcutaneous acrylic paint injections, and introduced into McCoy Branch, a small second-order stream located on the Oak Ridge Reservation in eastern Tennessee, which was inhabited by only a few banded sculpins prior to the study. McCoy Branch had received deposits of coal ash slurry for a prolonged period, however, there were some indications of recovery in the macroinvertebrate community due to improvements in water quality (Tolbert and Smith 1992). Stream habitat characteristics and water chemistry parameters were monitored in McCoy Branch and a reference stream (Hansard Mill Branch) for a three-year period. Feeding patterns and reproductive activities of the banded sculpins were also monitored during the study. Sculpin population parameters including density, condition factor, and young-of-year (YOY) abundance and survival were studied.

The results of the study show that the introduced fish have survived and appear to be in good condition. The sculpins have maintained a density of approximately 0.12 fish per $m^2$ of stream, a figure similar to that found in other headwater streams located in the region. Colonization rates and sculpin densities in McCoy Branch were lower than expected, perhaps due to physical habitat degradation and reduced macroinvertebrate abundance. Evidence of sculpin reproduction in McCoy Branch was seen in the presence of gravid female sculpins (1994 and 1995) and YOY fish (1993 through 1995 year classes). This study indicates that McCoy Branch continues to recover from past perturbations to the point where it can now support a viable population of banded sculpins.

TABLE OF CONENTS

SECTION PAGE

I. INTRODUCTION ........ 1

II. METHODS

Study Sites ........ 5

Stream Habitat ........ 9

Water Quality ........ 12

Fish Introduction ........ 16

Fish Community ........ 17

Food Resources - Feeding Habits ........ 19

Reproduction ........ 21

III. RESULTS

Stream Habitat ........ 23

Water Quality ........ 28

Fish Introduction ........ 39

Fish Community ........ 42

Food Resources - Feeding Habits ........ 55

Reproduction ........ 61

IV. DISCUSSION ........ 66

SECTION PAGE

REFERENCES CITED . . . . . . . . . . 72

APPENDIXES . . . . . . . . . . 85

VITA . . . . . . . . . . 95

LIST OF TABLES


TABLE PAGE

1. Streambank Soil Alteration Rating .......... 10
2. Streambank Vegetative Stability Rating .......... 11
3. Substrate Analysis Coding System .......... 13
4. Embeddedness Ratings for Substrate Material .......... 14
5. Stream Habitat Parameters: McCoy Branch and Hansard Mill Branch .......... 24
6. Substrate and Embeddedness Analysis:
   McCoy Branch and Hansard Mill Branch .......... 26
7. Substrate Type Frequencies: McCoy Branch and Hansard Mill Branch .......... 27
8. Results of GLM Comparing Substrate and Embeddedness:
   McCoy Branch to Hansard Mill Branch .......... 29
9. Water Chemistry Analysis: McCoy Branch and Hansard Mill Branch 1992-1994 .......... 30
10. Results of GLM Comparing Water Chemistry:
    McCoy Branch to Hansard Mill Branch .......... 32
11. Mean Weekly Water Temperatures:
    McCoy Branch and Hansard Mill Branch 1992-1994 .......... 34
12. Results of GLM Comparing Water Temperature:
    McCoy Branch to Hansard Mill Branch .......... 35
13. ICP Sample Results: McCoy Branch .......... 37
14. ICP Sample Results: Hansard Mill Branch .......... 38
15. Results of Banded Sculpin Marking and Introductions .......... 40

LIST OF TABLES

TABLE PAGE

16. Banded Sculpin Population Samples: McCoy Branch 1993-1995 . . . . . 43

17. Banded Sculpin Population Samples - Species Associates:
Hansard Mill Branch 1993-1995 . . . . . 45

18. Banded Sculpin Densities at Other Streams Within 8 km of McCoy Branch 1993-1995 . . . . 48

19. Abundance Estimates for YOY Banded Sculpins:
McCoy Branch and Hansard Mill Branch 1993-1995 . . . . . 52

20. Survival Rates for YOY Banded Sculpins:
McCoy Branch and Hansard Mill Branch 1993-1995 . . . . . 53

21. Banded Sculpin Condition Factors:
McCoy Branch and Hansard Mill Branch 1993-1994 . . . . . 54

22. Results of GLM Comparing Banded Sculpin Condition Factor:
McCoy Branch to Hansard Mill Branch . . . . . 56

23. Food Resources - Taxa Richness: McCoy Branch and Hansard Mill Branch . . . . . 58

24. Banded Sculpin Food Item Selectivity:
McCoy Branch and Hansard Mill Branch . . . . . 60

25. Comparison of Spawning Habitat Available to Banded Sculpins:
McCoy Branch and Hansard Mill Branch . . . . . 65

## LIST OF FIGURES

FIGURE PAGE

1. McCoy Branch Watershed, Anderson County, Tennessee ........................... 6

2. Hansard Mill Branch Watershed, Knox County, Tennessee .......................... 8

3. Mean Weekly Water Temperatures:

McCoy Branch and Hansard Mill Branch 1992-1994 .............................. 33

4. Banded Sculpin Densities:

McCoy Branch and Hansard Mill Branch 1993-1994 .............................. 46

5. Banded Sculpin Length Frequencies:

McCoy Branch and Hansard Mill Branch 1993 .................................. 49

6. Banded Sculpin Length Frequencies:

McCoy Branch and Hansard Mill Branch 1994 .................................. 50

## ACRONYMS

| | |
|---|---|
| AA | Atomic Absorptions |
| DO | Dissolved Oxygen |
| DOE | Department of Energy |
| ER-M | NOAA Effects Range Median |
| GLM | General linear Models procedure |
| HMK | Hansard Mill Branch Kilometer |
| ICP | Inductively Coupled Plasmaspectroscopy |
| MCK | McCoy Branch Kilometer |
| NAS | National Academy of Science |
| NOAA | National Oceanographic and Atmospheric Administration |
| NRCC | National Research Council of Canada |
| SAS | Statistical Analysis System |
| SD | Standard Deviation |
| SPR POOL | Spring Pool located on upper McCoy Branch |
| YOY | Young of Year (Sculpin) |

# I. INTRODUCTION

McCoy Branch is a second-order headwater stream located in eastern Tennessee. The McCoy Branch watershed was used as a depository for coal flyash from the Y-12 Plant from 1955 to 1993. Coal flyash slurry was pumped south over Chestnut Ridge via an 8-inch pipe into a retention pond located in the McCoy Branch watershed (CDM 1994). The retention pond was constructed in 1955 and was used to contain coal flyash until maximum capacity was reached in 1967. After 1967, the flow from McCoy Branch was used to transport the slurry through the valley into Rogers Quarry (Murphy 1988, CDM 1994). In November, 1989, the flow of coal flyash slurry to upper McCoy Branch was terminated by extending the pipeline directly to Rogers Quarry (Murphy, 1988; M.A. Kane, Y-12 Plant, personal communication). Coal flyash discharges to Rogers Quarry ceased in July, 1993 (R. Ahl, personal communication to R.P. Hoffmeister, ORNL).

Historical coal flyash disposal practices in upper McCoy Branch had catastrophic effects on the fish community. Previously, flyash byproduct contaminants such as aluminum, copper, iron, and zinc were at sufficiently high levels to impact fish communities and perhaps extirpate fish species (DOE 1994a). Evidence for ecological recovery of the stream after termination of flyash disposal included improved water quality and changes in benthic invertebrate communities (Hinzman 1992, Tolbert and Smith 1992). The rate at which streams are recolonized by fishes after natural or anthropogenic extirpation events is dependent upon the severity and duration of the event, the seasonality of the event, and the distance from and quality of nearby sources of colonist species (Olmstead and Cloutman 1974). The severity of the effects of the coal flyash on the resident fish community, the duration of the contamination event (over 34 years), and the lack of access to nearby refugia of fish species, indicated a long recovery time for the upper McCoy

Branch watershed.

Even though water quality in upper McCoy Branch has improved over time, there was no access to this portion of the watershed to potential fish species colonizers. Griswold et al. (1982) found that the fish community in a section of channelized stream that was decimated due to drought conditions was repopulated by fish from small feeder streams and upstream sections once the water had returned. That study described how the presence of a weir downstream from the depopulated section of stream prevented some fish species from recolonizing the channelized upstream section. In my study, Rogers Quarry served as a barrier, effectively denying access to downstream refugia, thereby limiting the recolonization of upper McCoy Branch by fish. Barriers were also reported to have slowed the recolonization rates of various species of fish by Niemi et al. (1990). It was not evident whether the fish present in the system at the time of the study constituted a viable propagule (MacArthur and Wilson 1967), able to repopulate the upper section of McCoy Branch. Therefore, a number of banded sculpins, a common member of fish communities found in headwater streams in eastern Tennessee, were collected from a reference stream, marked with subcutaneous injections of acrylic polymers, and introduced into McCoy Branch.

At the inception of this study, there was no evidence of a fish population in upper McCoy Branch. As part of the biomonitoring plan for the McCoy Branch watershed, I introduced a number of banded sculpins to upper McCoy Branch in an attempt to repopulate that stream. However, during the two final electrofishing surveys made in 1992, before the sculpin introduction, four adult (8.7-12.0 cm TL) sculpins were found. These fish presumably were from refugia such as deeply undercut banks, springs, or places where the stream flows underground. These types of refugia are common in upper McCoy Branch. No sculpins or fish of any kind had been found in six previous surveys of upper McCoy Branch above Rogers Quarry from 1987 to 1992.

The introduction of sculpins to upper McCoy Branch provided a recolonization source similar to the "rescue effect" discussed by Brown and Gibson (1983) and Smith (1980). This describes a situation where a population of animals in danger of possible extinction in one area is "rescued" through continual immigration from a nearby source, or as in the present case, repeated introductions of the same species. It was not possible to know whether the sculpins present at the onset of the study would have been able to repopulate McCoy Branch without the aid of the introductions. The fact that these adult sculpins had been in the system for some time, and had not yet repopulated this system, was a possible indication that the coal flyash related perturbations to the system prevented these resident fish from achieving the level of fecundity or reproductive success necessary to fully colonize upper McCoy Branch. In any event, the introductions of additional banded sculpins to McCoy Branch increased the potential gene pool and rate of colonization by fish in this system.

The literature reviewed for this study concentrated on two topics: (1) natural or anthropogenic extirpation of fish species or populations and the recolonization of streams, and (2) colonization rates and success of introduced populations of fish. Some studies on streams where fish populations had been extirpated naturally or artificially, and where these streams were recolonized from nearby refugia include Larimore et al. (1959), Gunning and Berra (1969), Berra and Gunning (1970), Cairns et al. 1971, Olmstead and Cloutman (1974), Cherry et al. (1979), Griswold et al. (1982), Ross et al. 1985, and Meffe and Sheldon (1990). In studies concerning short-term disturbances where fish species are extirpated in areas and then recolonize those same areas, total biomass and species richness of fish had equaled or exceeded pre-disturbance figures within one year (Gunning and Berra 1969, Berra and Gunning 1970, Olmstead and Cloutman 1974).

Examples of studies considering colonization rates and success of introduced fish species or populations include Larimore (1954), Hocutt and Hambrick (1973), Gwinner et al. (1975), Avery (1979), Courtenay and Hensley (1979), and Ryon (1987). In general, introductions of fish species fall into one of two categories, planned or unplanned. Both types of introductions can reveal important information about fish colonization patterns and rates. Hocutt and Hambrick

(1973) discussed the accidental introduction of the Roanoke darter (*Percina roanoka*) to the New River and this species subsequent colonization of that system. These darters were believed to have been transferred in a bait bucket mixed in with some madtoms (*Noturus* sp.) which are commonly used for bait in that region. The study by Hocutt and Hambrick (1973) is relevant because it is an example of a colonization theory (MacArthur 1960) where a species newly entering a system containing few competitors may rapidly colonize that system via an initial population explosion.

Colonization rates of introduced fish species are constrained by the same kinds of factors that affect natural recolonization processes. The accidental introduction of the fathead minnow (*Pimephales promelas*) to the White Oak Creek system in eastern Tennessee, and this species' subsequent colonization of that system is described in Ryon (1987). These fish were first found in the system in the fall of 1985 at densities of 0.01 to 0.03 fish per $m^2$. By the summer of 1986, fathead minnow densities had increased to 0.10 to 0.15 fish per $m^2$, a five- to ten-fold increase in density in one year. This shows that rates of colonization by fish in eastern Tennessee streams can be very rapid.

The objectives of this study were to (1) determine if upper McCoy Branch could support a population of banded sculpins, and if so, (2) how might that population differ from a "successful" population in a non-contaminated reference stream (e.g., Hansard Mill Branch). Description of the introduced banded sculpin population in upper McCoy Branch included dietary analysis, and monitoring of fish densities, condition and reproductive activities. A comparison of the sculpin populations in the two streams included an assessment of the differences in habitat including substrate and embeddedness, water chemistry, and temperature. The two streams were also compared with respect to other factors potentially influencing sculpin populations, including food resources, and fish community structure. This information was used to more accurately assess the status of the banded sculpin populations in the two streams.

## II. METHODS

### Study Sites

McCoy Branch is a second-order stream of moderate gradient located on the Oak Ridge Reservation, near Oak Ridge, Tennessee. This stream drains Fanny's Knob on the southern slope of Chestnut Ridge. The McCoy Branch watershed has an area of approximately 1.48 $km^2$ and contains mainly forested ridges and marshy second-growth floodplain. McCoy Branch flows approximately 0.9 km from an earthen dam at the head of the valley before entering Rogers Quarry, a steep sided reservoir with a surface area of about 4 ha. Water exiting Rogers Quarry comprises the lower section of McCoy Branch; this segment of stream is approximately 0.8 km long and discharges into Melton Hill Reservoir (Murphy and Loar 1988) (Figure 1).

Two reaches on upper McCoy Branch, each approximately 50 m in length, were selected for banded sculpin introduction sites. The site located furthest upstream, referred to as MCK 2.12, had a slightly sinuous configuration and it contained moderately shallow riffles separated by medium sized pools. The average pool-riffle ratio was 0.7. Substrate was moderately embedded, and composed of mainly cobble, with some leaf litter, woody debris, and root wads. This site had a dense riparian cover. The downstream site, referred to as MCK 1.97, also had a slightly sinuous configuration, with moderately deep runs separated by narrow, shallow riffles. The mean pool-riffle ratio was 0.5. Substrate at this site was moderately embedded, and composed mainly of small cobbles, gravel, and root wads. MCK 1.97 had slightly less riparian canopy cover than MCK 2.12.

An important feature of MCK 1.97 is a moderate sized spring-pool (194 $m^2$) which feeds into McCoy Branch about 10 m below the bottom boundary of the site. The substrate in this pool consisted of a thick layer of silty mud (25 to 48 cm deep), with a few small boulders and large woody debris. Much of the mud surface (45 to 70%) was covered with an algal mat, and dead

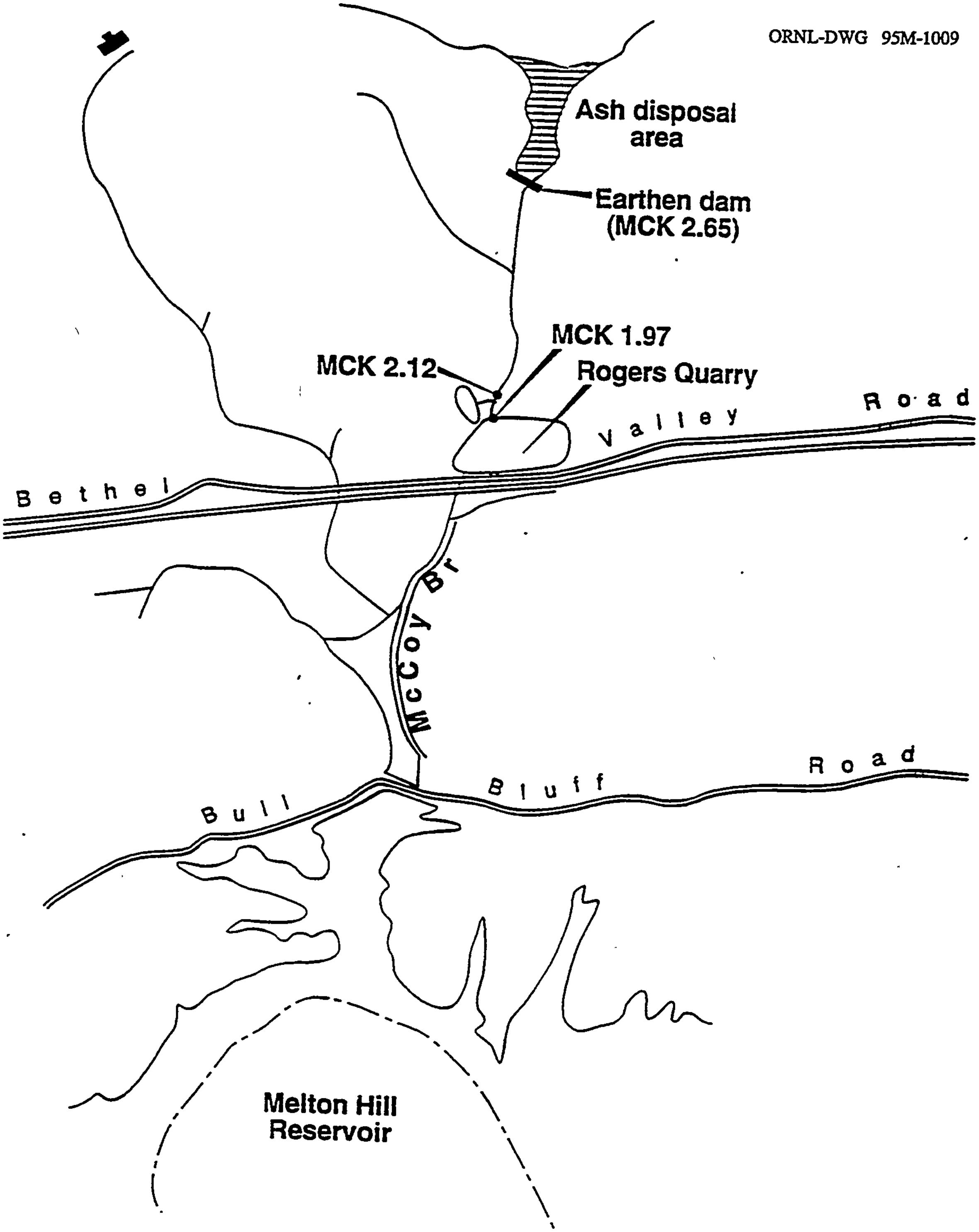


Figure 1.

McCoy Branch Watershed, Anderson County, Tennessee.

leaves were commonly found in the upper portion of the pool near the spring. Approximately 100 m of stream separates the two study sites in McCoy Branch.

Hansard Mill Branch was chosen as a source of sculpins for introduction into McCoy Branch because it (1) contained a large existing population of banded sculpins, and (2) was similar to McCoy Branch with respect to drainage area and stream habitat characteristics. Also, the study sites in Hansard Mill Branch appeared to be relatively unimpacted despite the stream's proximity to a road and the areas of rural development found within the watershed. Hansard Mill Branch is located in Knox County approximately 32 km northeast of McCoy Branch; it is a second-order, spring-fed stream of low to moderate gradient, and drains a 2.32 $km^2$ watershed on the southern slope of Chestnut Ridge. The Hansard Mill Branch watershed includes mainly forested ridges, grassland and small-scale rural development. This stream is about 2.2-km long and discharges into Bull Run Creek (Figure 2).

Two reaches, similar to those on McCoy Branch, were chosen as study sites in Hansard Mill Branch. The site located farthest upstream, referred to as HMK 1.23, was slightly sinuous, and had moderately wide, shallow riffles bordered by medium sized pools. The mean pool-riffle ratio was 0.3. At HMK 1.23, the substrate was slightly to moderately embedded, and was composed mainly of small gravel and cobbles, with some woody debris. There was less riparian cover at HMK 1.23 than at either of the two McCoy Branch sites. HMK 1.23 was located about 800 m downstream from five springs that form the headwaters of the stream. The downstream site, referred to as HMK 0.41, was slightly sinuous and contained long, moderately deep riffles bordered by medium sized pools. The pool-rifle ratio at this site was 0.4. The substrate was moderately embedded, and included bedrock outcrops, large flat cobbles and coarse gravel. HMK 0.41 had less riparian cover than any of the other sites. HMK 0.41 was approximately 1 km downstream from the HMK 1.23 site and about 410 m upstream from the stream's confluence with Bull Run Creek.

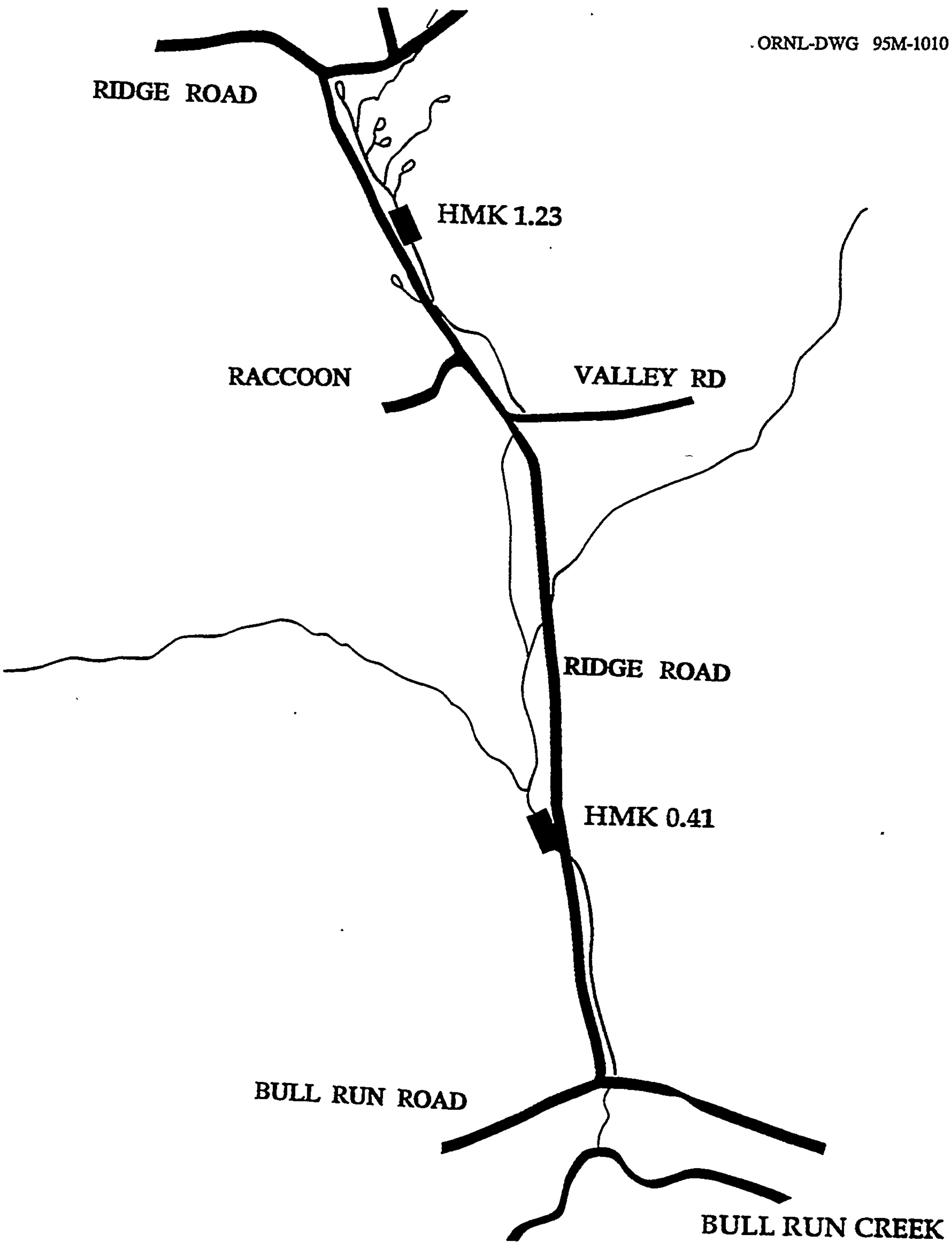


Figure 2.

Hansard Mill Branch Watershed, Knox County, Tennessee.

**Stream Habitat**

Stream habitat at each of the four sites was assessed three times during the study: in August 1992, August 1993, and September 1994. Samples used to characterize the spring-pool near MCK 1.97 were collected in June, 1995. All samples were collected using methods described by Platts et al. (1983) and Bain et al. (1985), as modified by Ryon et al. (1992). The parameters that were studied and the equipment used are as follows: flow rate, Marsh McBirney model 201D flow meter; stream width, pool-riffle ratio, and channel sinuosity, 100-m tape; stream shore depth, vegetative overhang, and stream bank undercut, meter stick; substrate type and embededness analysis, weighted rope marked off in 10-cm increments; stream bank angle, Suunto clinometer and meter stick; canopy cover, convex mirror with 100-square grid; aquatic vegetation, stream bank alteration and bank vegetational stability, by direct observation.

All stream habitat measurements were taken at 5-m intervals in each study reach. Five measurements of water velocity were made at each transect (one at each bank and three evenly distributed along the cross-stream transect). The sinuosity values were calculated once for each study reach. Sinuosity is a ratio of the length of the stream channel to the straight-line distance between two points in a section of stream. Stream bank angle is the angle of the bank in relation to the stream-bottom, with undercut banks being less than 90°, and sloping banks greater than 90°. The bank angle was read directly from a clinometer positioned on a meter stick held parallel to the bank. Angles less than 90° were read directly, and angles greater than 90° were subtracted from 180°. The Streambank Soil Alteration rating system includes five classes used to asses the streambank stability (Table 1). Streambank Vegetative Stability is a 4-class rating of the ability of vegetation on the stream bank to resist erosion (Table 2). Means for pool-riffle ratio, stream width, water depth, water velocity, canopy cover, stream shore depth, streambank angle, amount of undercut bank, vegetative overhang, streambank soil alteration, and streambank vegetative stability were calculated for each site.

Table 1.

Streambank Soil Alteration Rating.[a]

| Rating | Description |
|---|---|
| 0 | Streambanks are stable and are not being altered by water flows or animals. |
| 1 to 25 | Streambanks are stable, but are being lightly altered along the transect line. Less than 25% of the streambank is receiving any kind of stress, is false, broken down, or eroding. |
| 26 to 50 | Streambanks are receiving only moderate alteration along the transect line. At least 50% of the streambank is in a natural, stable condition. Less than 50% of the streambank is false, broken down, or eroding. |
| 51 to 75 | Streambanks have received major alteration along the transect line. Less than 50% of the streambank is in stable condition. Over 50% of the streambank is false, broken down, or eroding. |
| 76 to 100 | Streambanks along the transect are severely altered. Less than 25% of the streambank is in stable condition. Over 75% of the streambank is false, broken down, or eroding. |

a. Modified from Platts et al. (1983).

Table 2.

Streambank Vegetative Stability Rating.[a]

| Rating | Description |
|---|---|
| 4 (excellent) | Over 80% of the streambank surfaces are covered by vegetation in vigorous condition or by boulders and rubble. If the streambank is not covered by vegetation, it is protected by materials that do not allow bank erosion. |
| 3 (good) | Fifty to 79% of the streambank surfaces are covered by vegetation or by gravel or larger material. Those areas not covered by vegetation are protected by materials that allow only minor erosion. |
| 2 (fair) | Twenty five to 49% of the streambank surfaces are covered by vegetation or by gravel or larger material. Those areas not covered by vegetation are covered by materials that give only limited protection. |
| 1 (poor) | Less than 25% of the streambank surfaces are covered by vegetation or by gravel or larger material. That area not covered by vegetation provides little or no control over erosion and banks are eroded each year by high water flows. |

a. Modified from Platts et al. (1983).

The predominant substrate types and the degree of embeddedness were major components of substrate analysis for each site. Substrate type measurements were made at 10-cm intervals along cross-stream transects at 5-m intervals in each site annually during the study. The substrate values were based on a coded scale ranging from 1 to 12 adapted from Platts et al. (1983) and Bain et al. (1985) (Table 3). Embeddedness ratings (Table 4) were recorded using the same transects that were used for substrate analyses. In this study, embeddedness refers to the degree to which the dominant substrate type was covered with fine sediments. Substrate and embeddedness analyses for the HMK 0.41 site were done using only one years' worth of data (1993). Samples were not collected from this site for 1992 or 1994. Site characterization data were analyzed with SAS software and procedures (SAS 1985a, b). Comparisons of the study reaches based on substrate types and embeddedness ratings were done using General Linear Models (GLM) procedure, since many of the comparisons were made from unequal data sets. A lower limit $R^2$ value of 0.20 was used as the cut-off level for biological importance (Yoccoz 1991). When comparisons did not exceed the cut-off value, it was considered impractical to look further into the model. Site-wise comparisons were made with Tukey's standardized range test, at an alpha level of 0.05. These same procedures were followed with all of the GLM analysis of variance comparisons.

**Water Quality**

Water from both streams was analyzed to describe and compare the study reaches with respect to water chemistry, temperature regimes, and levels of contaminants. Water quality in McCoy Branch was impacted due to the deposition of coal flyash into the McCoy Branch watershed from 1955 to 1989 (Hinzman 1992). Coal ash sluice water discharged into the McCoy Branch system had elevated levels of arsenic, aluminum, barium, boron, cadmium, iron, magnesium, sodium, strontium, potassium, total suspended solids, total phosphorus, sulfide, and sulfate when compared to background water concentrations (Turner et al. 1986). Other studies of coal flyash show that it also includes copper, chromium, lead, molybdenum, nickel, selenium, and

Table 3.

Substrate Analysis Coding System.[a]

| Code | Substrate index | Particle size range (mm) |
|---|---|---|
| 1 | Bedrock, smooth | >2000 |
| 2 | Clay | <.004 |
| 3 | Silt | .004-.062 |
| 4 | Sand-fine sediment | .062-2 |
| 5 | Gravel | 2-64 |
| 6 | Cobble-rubble | 64-250 |
| 7 | Small boulder | 250-610 |
| 8 | Large boulder | 610-2000 |
| 9 | Bedrock, rough | >2000 |
| 10 | Plant detritus | NA[b] |
| 11 | Woody debris | NA |
| 12 | Root wads | NA |
| 13 | Trash of human origin | NA |

a. Substrate coding system modified by Ryon et al. (1992): from Platts et al. (1983) and Bain et al. (1985).

b. NA = size rankings not applicable.

Table 4.

Embeddedness Ratings for Substrate Material.[a]

| Rating | Rating description |
|---|---|
| 5 | Less than 5% of the surface of the dominant substrate type is covered by fine sediments. |
| 4 | Five to 25% of the surface of the dominant substrate type is covered by fine sediments. |
| 3 | Twenty five to 50% of the surface of the dominant substrate type is covered by fine sediments. |
| 2 | Fifty to 75% of the surface of the dominant substrate type is covered with fine sediments |
| 1 | More than 75% of the surface of the dominant substrate type is covered with fine sediments. |

a. Modified from Platts et al. (1983).

zinc (El-Mogazi et al. 1988). At elevated levels, these elements can harm fish and other aquatic organisms (DOE 1994a, b). The presence of metals such as arsenic (As) and selenium (Se) was deemed important in this study because of their effects on the fish communities that once existed in McCoy Branch and their potential to affect the introduced sculpins. Uptake of Se by fish can result from either exposure through the water or by biomagnification through the food chain (Lemly 1985, 1993). High concentrations of Se can cause tissue damage, reproductive failure and the extirpation of entire fish communities (Cumbie and Van Horn 1978, Garrett and Inman 1984, Lemly 1985, Sorenson 1986). Arsenic uptake in fish is caused by direct contact with the water and bottom sediments: arsenic uptake through food chain magnification does not seem to occur (Woolson 1975, NAS 1977, NRCC 1978, Duke Power Co. 1980, Hallacher et al. 1985, Hood 1985, Coughlan and Velte 1989). The toxicity of As to fish varies with the species of arsenic (e.g., $As^{3+}$ or $As^{5+}$), physical factors (water temperature, pH, organic content, phosphate concentration, suspended solids), and the presence of other toxicants (Eisler 1994).

In the present study, water quality assessments consisted of monthly measurements of pH, dissolved oxygen and conductivity, and continuous temperature monitoring. Periodic analyses for metals were made by inductively coupled plasmaspectroscopy (ICP), and atomic absorptions (AA) were also used in the description of water quality at the two streams. Measurements of pH, dissolved oxygen content, and conductivity were made with a Horiba model U-7 water quality meter. Water samples to be analyzed for those parameters were taken in mid-stream near the downstream end of each site. Water temperature was monitored in situ at 1-h intervals with Ryan Tempmentors. The water temperature data were obtained at MCK 1.92, MCK 2.17, and HMK 1.28 from August, 1992 to August, 1994. Water samples for analysis of metals were collected on three occasions (30 October 1992, 27 May 1993, and 30 November 1993) during the study. For this purpose, samples were taken from a mid-stream location at the downstream end of the site. The samples were collected in 60-mL plastic jars and preserved with 0.01 mL of nitric acid. The preserved samples were sent to the Y-12 Analytical Services Organization laboratory for ICP analysis (EPA method 200.7). Atomic absorption analysis (EPA method 270.2) was used for As and Se in the May and November 1993 samples because AA has lower detection levels than ICP for these constituents. Data were analyzed with SAS software and procedures (SAS 1985a, b). Comparisons of temperature and water chemistry among sites and years were made with GLM procedure, followed by Tukey's standardized range test.

**Fish Introduction**

Banded sculpins were collected from Hansard Mill Branch using a Smith-Root model 15-A electrofisher on 25 August 1992 (110 individuals), 28 August 1992 (75 individuals), and 2 October 1992 (52 individuals). Twenty three banded sculpins were also collected from the area above HMK 1.23 on 15 July 1993 for a second introduction. Each batch of fish was taken to the laboratory and kept in a 620-L flow-through tank supplied with dechlorinated tap water (0.13 L/s) to allow them to recover from the electrofishing process. Water temperature in the tank ranged from 13.5 °C to 15.0 °C. Water depth was set at 10 cm. A submersible pump was placed at one end of the tank to create a current effect. The fish were fed every other day with frozen brine shrimp and red worms.

In this study, the sculpins were first anesthetized with FINQUEL Tricane Methanesulfonate, and then injected, as per Lotrich and Merideth (1974), with Liquitex non-toxic acrylic polymer emulsions. To mark the fish, I used a 3-$cm^3$ syringe with a number 23 needle. The syringe contained an aqueous mixture of Liquitex (2 $cm^3$ water to 5 g paint). Each sculpin was injected in the loose subcutaneous area on the mid-ventral portion of the caudal peduncle. For the first introduction, fish slated for release at MCK 1.97 were injected with Brilliant Orange, and the fish slated for release at MCK 2.12 were injected with Vivid Lime Green. Different colors were used to mark sculpins used in the second introduction: fish to be introduced at the MCK 1.97 area were marked with Brilliant Blue, whereas the fish introduced at the MCK 2.12 area were marked with Liquitex Medium Magenta. Using different colors for each group of sculpins aided in the monitoring of movement patterns connected with the dispersal of the fish introduced into upper McCoy Branch. When used to mark fish in this manner, Liquitex paints are reported to last 4 to 16 months, depending on color (Lotrich and Meredith 1974). After marking was completed, the fish were held in the tank for a brief period (1-10 d) to determine the extent of latent mortality associated with the injection process. The fish were then introduced into upper McCoy Branch. A subsample of the marked fish (25) from the first batch of injections was kept in the laboratory during the study as a control for describing any long-term effects of the subcutaneous markings.

**Fish Community**

Fish communities were sampled quarterly by blocking off 50-m segments of stream with 5-mm mesh nylon nets and electroshocking these sections with Smith-Root model 15-A electrofishers. The electrofishers were set at 400-600 V DC, with frequencies of 60-90 pulses per second. The cathode and anode probes were fitted with circular rings; the anode probe ring was covered with 5-mm nylon mesh to allow the electrofisher operator to assist in netting stunned fish. Within each site, three electrofishing passes were made in an upstream direction covering all habitat types. All stunned fish were captured and anesthetized with FINQUEL, identified to species, measured to the nearest mm TL, and weighed to the nearest 0.1 g. At Hansard Mill Branch, fish species other than banded sculpins were tallied only; no lengths or weights were taken. The fish were then returned to the same section of stream from which they had been collected, after they had recovered from the anesthetic.

During the course of the study, possible under-sampling of the sculpins in the McCoy Branch sites became a concern because the number of sculpins recovered during 3-pass population samples was lower than expected, based on results from other mark-recapture studies. Mark-recapture studies involving sculpins reported fish recovery rates of 27 to 58% (Bailey 1952, Brown and Downhower 1982, Greenberg and Holtzman 1987). Therefore, a new method of sampling was sampling was employed at both streams to minimize possible undersampling bias. More electrofishing passes were performed, and in some instances stream sampling reaches were lengthened. At least 4 passes were made in each site, and sampling passes were continued until no more fish were recovered, as modified from Riley and Fausch (1992). The numbers of fish captured and the estimated densities of fish showed slight increases when the more intense sampling regime was used.

The populations of banded sculpins were estimated using data from the multiple pass replacement method as described by Carle and Strub (1978). Fish densities and length frequencies were calculated using a FORTRAN program with a weighted likelihood method (Railsback et al. 1989). The density measurements estimated from this program provided a better representation of the sculpin populations in the two streams. Length-frequency data also were used to track size classes of fish and to describe the demographic makeup of the sculpin populations in both streams. This information, in addition to the YOY sculpin survival and abundance data, helped in evaluating the colonization of McCoy Branch by the introduced sculpins.

Survival and abundance estimates for YOY sculpins were made on a annual basis with fish population data from each stream. Abundance of YOY sculpins was calculated as the number of YOY sculpins divided by the total number of sculpins captured on an annual basis for each stream. The YOY survival percentage was calculated as the number of YOY individuals surviving until the next spawning season. Condition factors (Hile 1936) of sculpins in McCoy Branch and Hansard Mill Branch were calculated using the formula given below.

$$\text{Condition factor (K)} = [\text{weight(g)} \;/\; \text{length(cm)}^3]100$$

The condition factor values were compiled and analyzed using SAS software and procedures (SAS 1985a, b). Comparisons between streams were done using GLM procedure, followed by Tukey's standardized range test. Condition factors for sculpins collected during the quarterly population samples were also separated into three seasons for comparisons of site and year; those fish collected from January to April were grouped in spring, May to August in summer, and September to December in fall.

## Food Resources - Feeding Habits

The food resources available to the sculpins at the four sites were estimated from two semi-quantitative Sürber samples of benthic macroinvertebrates. This information provided a baseline assessment of the food resources available at the two streams and was used to reveal how the sculpins introduced into McCoy Branch utilized the different resources in their new habitat as they attempted to colonize that stream. The diets of banded sculpins have been reported to consist mainly of immature aquatic insects including trichoptera, plecoptera, ephemeroptera, diptera (chironomids), and small fish (Starnes 1977); and megaloptera, coleoptera, isopoda, amphipoda, decopoda, ostracoda, and oligocheata (Craddock 1965). In this study, terrestrial inputs to sculpin diets were deemed relatively insignificant, based on results from preliminary stomach samples from sculpins in both streams. The benthic macroinvertebrate sampling locations within each site were chosen by first dividing the reaches up into pool and riffle cells. These cells were approximately 1 m long and half the stream in width (2 cells per 1-m length of stream). One cell for each pool and riffle habitat type was selected randomly within each study reach. The data were used to produce a simple, non-statistical food resource assessment at the four sites.

A 30-cm$^2$ Sürber sampler with a 360-micron net was placed in the center of the cell, facing into the current, directly on the substrate, and then the substrate within the sampler was dislodged causing the macroinvertebrates to drift into the net. All the substrate in the square was dislodged in this process. Large rocks (>15 cm diam.) in the square were scrubbed with a test-tube brush within the net to dislodge attached macroinvertebrates. The samples were then preserved in 850-mL jars with 90% ethanol. Benthic samples were processed in the laboratory with the aid of an illuminated Electrix 2x magnifier. Benthic macroinvertebrates were identified to the family level with a dissecting microscope using keys by Brigham et al. (1982) and Merrit and Cummins (1978).

The amounts and types of food consumed by the sculpins were estimated by examining the stomach contents of sculpins collected from both streams. Sampling within 50-m of the four sites was avoided to prevent biasing the fish community studies within the sites. Three to 15 banded sculpins of various size classes were taken from the two Hansard Mill Branch sites quarterly, beginning in August 1992. These fish were preserved in a 10% formalin solution for subsequent analysis. In January 1993, three banded sculpins were collected for stomach content analysis at each of the two McCoy Branch sites. These were the only whole fish samples taken from McCoy Branch.

To reduce the impact of stomach content sampling on the introduced sculpins in McCoy Branch, I began using a non-destructive method of stomach sampling, beginning in June of 1993 at both streams. A stomach pump, consisting of a 10-$cm^3$ hypodermic syringe and a piece of electrical wire casing (7 cm long, 3 mm diam.) was constructed as per Baker and Fraser (1976). A smaller model of the pump (3 $cm^3$ syringe; 4 cm long, 1.5 mm wide tube) was used for fish less than 7.0 cm TL. The syringe with the casing attached to the needle base was filled with water and inserted into the fish's mouth, and advanced through the esophagus until it reached the stomach. The fish was then inverted over a 250-micron screen, and water was pumped into the fish's stomach flushing the contents onto the screen. The stomach contents were preserved in 20-mL vials with 90% ethanol. Fish mortality associated with this method was minimal (1.3%). The efficiency of this method was tested by checking the stomach contents of 6 fish after their stomachs had been pumped. Fish were preserved in 10% formalin solution immediately after stomach pumping for subsequent examination. The examination of the stomachs and intestinal tracts of these fish showed that the pumping method had evacuated over 98% (by number) of the contents.

Two sculpins per site per quarter were used for the stomach content analyses. The contents of the stomachs were identified to the lowest taxon level possible. The types of food items found in sculpin stomach samples were analyzed by examining frequency of occurrence (i.e., the proportion of a population that feeds on a particular food item), and the percentage composition by number (i.e., the relative abundance of a food item in the diet) (Windell and Bowen 1971).

**Reproduction**

Because there is little information on banded sculpin breeding habits, I conducted surveys in McCoy Branch and Hansard Mill Branch to locate and describe nests, nest sites, egg types, egg numbers, egg depositional patterns, larval and post-larval fish numbers, and nursery locations. Nest surveys consisted of careful searches of the stream-bed in likely spawning areas. During these surveys, rocks were overturned and a fine-meshed net was positioned directly downstream to catch sculpin eggs or larval fish drifting from nest sites. All observations of banded sculpin nesting activities were recorded at the time of the survey. Post-larval fish and nursery searches were done by lifting up likely nest rocks and quickly scooping under the rock with a fine-meshed net to capture the fish. Banded sculpin nursery sites were also located during population surveys. The resulting information was used to help evaluate the colonization efforts of the introduced sculpins in McCoy Branch.

Spawning habitat area measurements were taken in March of 1995. Two types of areas were used in calculating the percentages of available spawning habitat, including (1) areas with large rocks (25-35 cm diameter) on fine gravel to sandy substrates, which were located in side areas of the stream near spring outlets, in water approximately 7 to 15 cm in depth, and (2) undercut banks on the outside turns of streams with root wads hanging down into the water, with low to moderate flow, and water depths that ranged from 5 to 15 cm. Substrates at these areas were composed of small to medium cobbles situated on fine gravel to coarse sandy bottoms. These types of areas were frequented by gravid female sculpins during the spawning season in late January to mid February. Observations of ripe sculpins in these types of spawning habitat were made at McCoy Branch, Hansard Mill Branch, the laboratory stream, and at other reference streams.

During the breeding season, observations of the two streams were combined with observations of banded sculpins in an artificial stream set up in the laboratory to resemble conditions found in McCoy Branch. The artificial stream used for this purpose was the same 620-L tank described previously in the **Fish Introduction** section. Fifteen banded sculpins from Chestnut Branch, a stream draining the same ridge as McCoy Branch, but approximately 1.5 km farther northeast, were stocked in the tank in February 1993. These fish were monitored daily during the spawning season.

Surveys to describe banded sculpin fecundity were conducted in McCoy Branch and Hansard Mill Branch during January and February, the reported breeding season for banded sculpins in the Cahaba River System in Alabama (Williams and Robins 1970). Gravid female sculpins were collected by electroshocking areas that contained suitable spawning habitat as described above. Captured female sculpins were transported alive to the laboratory, then euthanized using a solution of FINQUEL. Ovaries were removed from the sculpins, weighed to the nearest mg with a Mettler balance, and preserved in Karnovsky's fixative for subsequent analysis. Processing of ovaries began with soaking the preserved ovaries in water overnight to remove excess fixative. The oocytes were then removed from the ovaries and counted with the aid of a dissecting microscope equipped with an ocular micrometer. Counts on all eggs larger than 0.3 mm in diameter were made for both of the ovaries, and maximum egg sizes were recorded.

## III. RESULTS

### Stream Habitat

Stream habitats in upper McCoy Branch and in the reference sites on Hansard Mill Branch were relatively similar with respect to riparian composition, based on measured parameters such as stream shore depth, stream bank angle, stream bank undercut, vegetation overhang, streambank alteration, and vegetational stability (Table 5). Some trends in these data can be explained by simple downstream progression; in other cases, trends were apparently related to anthropogenic factors. MCK 1.97 and HMK 0.41, located downstream of MCK 2.12 and HMK 1.23, respectively, had greater shore depths and amounts of bank undercut; the two downstream sites also had lower bank angles compared to their upstream counterparts. The increase in stream order corresponded to a general increase in depth and an increase in the eroding and undercutting of banks. Channel width, water depth and velocity all increase as mean discharge of a river increases in the downstream direction (Bloom 1978). At HMK 1.23, many of the anomalies seen in the habitat data are due to the close proximity of the road to the stream at this point. Almost the entire western bank is made up of asphalt and stone wall shoring, which accounts for the relatively low ratings for bank undercut, vegetative overhang, and bank soil alteration. The low percentage of riparian canopy cover and the low bank vegetational stability ratings for HMK 0.41 were due to the overgrown pasture-land that borders the stream in this area. One of the important differences in the riparian area between the two streams was in the amount of riparian shading. The McCoy Branch sites had much higher canopy cover averages than the Hansard Mill Branch sites. In Murphy et al. (1981) and Hawkins et al. (1983), the amount of riparian shading was shown to effect macroinvertebrate abundances and sculpin densities in small coastal drainage northwestern streams, especially in areas of high sedimentation. These authors revealed that

Table 5.

Stream Habitat Parameters: McCoy Branch and Hansard Mill Branch.[a]

| Parameter | MCK 2.12 | Spring-Pool | MCK 1.97 | HMK 1.23 | HMK 0.41 |
|---|---|---|---|---|---|
| Stream width (m) | 1.6 | 5.8 | 1.2 | 1.1 | 1.6 |
| Stream depth (cm) | 6.8 | 20.0 | 7.8 | 5.8 | 7.4 |
| Flow rate ($m/s^2$) | 0.07 | 0.02 | 0.11 | 0.16 | 0.11 |
| Pool/riffle ratio (m) | 0.7 | NA | 0.5 | 0.3 | 0.4 |
| Sinuosity (ratio) | 1.20 | NA | 1.21 | 1.11 | 1.24 |
| Canopy (%) | 98.3 | 50.9 | 98.0 | 90.8 | 59.7 |
| Shore depth (cm) | 4.0 | 16.3 | 4.4 | 3.2 | 4.4 |
| Bank angle (°) | 127.7 | 133.6 | 106.8 | 120.3 | 78.1 |
| Bank undercut (cm)[b] | 112.5 | 44.0 | 119.0 | 49.0 | 197.0 |
| Veg. overhang (cm)[b] | 478.0 | 385.0 | 534.3 | 494.7 | 653.0 |
| Soil alteration (%) | 30.8 | 31.9 | 42.3 | 46.5 | 45.2 |
| Bank veg. stability[c] | 2.2 | 2.6 | 2.1 | 2.4 | 2.0 |

a. Means for each site (1992-1994).

b. Figures are means for site-wide totals.

c. Stability figures are from coded scale; see Table 2.

sedimentation of stream bottoms in conjunction with dense riparian canopies had significant negative effects upon macroinvertebrate abundance and sculpin densities.

Means and standard deviations for substrate and embeddedness were calculated for each of the sites (Table 6). In combination with other physical habitat factors such as depth, water velocity, and cover, substrate type can serve as an indirect indicator of fish habitat quality (ASCE Task Committee 1992). The substrate at the McCoy Branch sites was generally more heterogenous than the substrate at the Hansard Mill Branch sites. HMK 1.23 and HMK 0.41 were dominated by gravel-cobble substrate types; these substrate types also predominated at the McCoy Branch sites. Embeddedness was higher in McCoy Branch than in Hansard Mill Branch during the study period. The percent embeddedness decreased at McCoy Branch each year of the study, from 73.6% in 1992 to 45.9% in 1994. However, the embeddedness rating at Hansard Mill Branch (from 60.3 to 41.8%) was still lower. The percentage of silty substrate types at the McCoy Branch sites was 2 to 3 times greater than that found at sites in Hansard Mill Branch (Table 7).

Stream habitat can be an important determinant of fish distributions and abundance. McClendon and Rabeni (1987) found that physical habitat characters were linearly related to biomass and densities of rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*). I compiled data for substrate type frequencies as part of a stream habitat analysis using annual mean values for each site (Table 7). Root wads, woody debris, and large rock substrates were important factors in this analysis, because they provide cover and spawning habitat for banded sculpins. The stream habitat data revealed that the Hansard Mill Branch sites contained much more large cobble and small boulder substrates (Substrate codes 6 and 7; Table 7) than the McCoy Branch sites. Larger-sized rock substrates were shown to be utilized extensively by banded sculpins in a study by Greenburg and Holtzman (1987). In upper McCoy Branch, banded sculpins consistently inhabited areas containing root wads, undercut banks and woody debris, while at Hansard Mill Branch this trend was not as evident. The percentages of root wads and woody debris at McCoy Branch equalled or exceeded those for Hansard Mill Branch, but large rock

Table 6.

Substrate and Embeddedness Analysis: McCoy Branch and Hansard Mill Branch.[a]

| Site | Substrate (Mean ± SD) | Embeddedness (Mean ± SD) | Embeddedness (%)[a] |
|---|---|---|---|
| MCK 2.12 | 5.96 ± 2.33 | 2.29 ± 0.85 | (67.0) |
| SPR POOL | 7.08 ± 3.05[b] | 2.53 ± 0.52[b] | (62.7)[b] |
| MCK 1.97 | 7.04 ± 3.03 | 2.60 ± 0.82 | (59.6) |
| HMK 1.23 | 5.71 ± 1.88 | 2.87 ± 0.88 | (53.1) |
| HMK 0.41 | 4.95 ± 1.76[c] | 2.95 ± 0.81[c] | (51.2)[c] |

a. Annual mean percent embeddedness.

b. These data represent only one sampling period, May 1995.

c. These data represent only one sampling period, August 1993.

Table 7.

Substrate Type Frequencies: McCoy Branch and Hansard Mill Branch.[a]

| Code[b] | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41[c] |
|---|---|---|---|---|
| 13 | — | — | 0.4 | — |
| 12 | 3.7 | 16.8 | 2.9 | — |
| 11 | 4.9 | 6.7 | 4.1 | — |
| 10 | 9.0 | 9.5 | 0.8 | — |
| 9 | — | — | 0.6 | — |
| 8 | — | — | — | — |
| 7 | 0.4 | 1.5 | 3.0 | 15.9 |
| 6 | 13.6 | 7.2 | 21.5 | 19.6 |
| 5 | 57.8 | 52.1 | 59.5 | 50.5 |
| 4 | 1.9 | 1.4 | 3.4 | — |
| 3 | 8.7 | 4.8 | 2.9 | — |
| 2 | — | — | 0.9 | — |
| 1 | — | — | — | 14.0 |

a. Values are mean annual percent frequencies.

b. Numbers are codes for substrate types; see Table 3.

c. Data from one sample; August 1993.

substrate was less common in McCoy Branch than at Hansard Mill Branch. Probst et al. (1984) found that rock bass and smallmouth bass showed a preference for root wads and submerged logs over other substrate types.

The results of the GLM procedures used to compare mean substrate values were not deemed biologically important, due to the extremely low $R^2$ value (0.07). I did find significant differences between the means using Tukey's test in comparisons of mean substrate types among the sites. MCK 2.12 and HMK 1.23 substrates remained similar throughout the study. The results of the GLM comparisons of embeddedness were deemed biologically important, with an $R^2$ of 0.28; this procedure revealed significant differences in mean embeddedness between the four sites. The Tukey's test revealed that the mean embeddedness was higher at the two McCoy Branch sites than at the Hansard Mill Branch sites (Table 8).

**Water Quality**

Results from the water quality monitoring of McCoy Branch indicate that the stream has improved somewhat from past disturbances. The stream chemistry and water temperatures were similar to the conditions found at Hansard Mill Branch. In both streams, pH increased with distance downstream and at the two downstream sites (MCK 1.97 and HMK 0.41) larger fluctuations in pH were evident. Concentrations of dissolved oxygen (DO) were generally higher at the two upstream sites, MCK 2.12 and HMK 1.23, than at the two downstream sites. Conductivity measurements provided the only detectable differences in the basic water quality parameters measured at the two streams, with conductivity at Hansard Mill Branch slightly higher and somewhat less variable than at McCoy Branch (Table 9).

The GLM procedures used to compare sites with respect to pH, dissolved oxygen, and conductivity all had relatively high $R^2$ values indicating they were of potential biological significance. I found no significant differences in pH, dissolved oxygen, or conductivity between sites or among years using the GLM procedure. Application of Tukey's test revealed significant

Table 8.

Results of GLM Comparing Substrate and Embeddedness:

McCoy Branch to Hansard Mill Branch.

| Parameter | $R^2$ | N | F | P | Site*Year (P) | Tukey's Test |
|---|---|---|---|---|---|---|
| SUBSTRATE: | | | | | | |
| | 0.072 | 1335 | 11.54 | <0.0001 | NA | S1>S2,S3,S4,S2=S3>S4[a] |
| EMBEDDEDNESS: | | | | | | |
| | 0.276* | 1342 | 56.47 | <0.0001 | <0.0001 | E1<E2>E3,E4,E3=E4 |

a. Number 1 corresponds to MCK 1.97, 2 is MCK 2.12, 3 is HMK 1.23, and 4 is HMK 0.41.

*. Indicates $R^2$ is above cut-off value (0.20) for potential biological significance.

Table 9.

Water Chemistry Analysis: McCoy Branch and Hansard Mill Branch 1992-1994[a].

| | McCoy Branch | | Hansard Mill Branch | |
|---|---|---|---|---|
| Parameter | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| pH (su) | 7.61 ± 0.29 | 7.70 ± 0.37 | 7.74 ± 0.29 | 7.81 ± 0.32 |
| Min-Max | 7.2 - 8.1 | 7.0 - 8.5 | 7.3 - 8.4 | 7.1 - 8.2 |
| DO (ppm) | 10.6 ± 2.16 | 10.1 ± 1.75 | 10.3 ± 1.67 | 10.0 ± 1.77 |
| Min-Max | 7.3 - 15.3 | 7.2 - 13.5 | 8.0 - 14.2 | 7.1 - 13.8 |
| Cond. (u/L) | 195.0 ± 80.82 | 199.6 ± 71.79 | 222.1 ± 39.25 | 264.1 ± 52.37 |
| Min-Max | 80.0 -319.0 | 110.0 - 303.0 | 132.0 - 266.0 | 134.0 - 350.0 |

a. Water chemistry values are from monthly readings (1993-1994).

differences in mean conductivity, with the two Hansard Mill Branch sites having higher mean values than the two McCoy Branch sites. No significant differences (alpha = 0.05) were found between the sites, for either pH or dissolved oxygen (Table 10). Increases in particular cations, such as sodium and calcium, correlated to downstream progression in both streams, but the degree of increase in concentrations of these constituents was much greater between the two Hansard Mill Branch sites. The increase in stream size and order can explain the high conductivity readings observed at the HMK 0.41 site. A 1-km distance separates the two Hansard Mill Branch sites, whereas the two McCoy Branch sites are separated by only 100 m.

Temperature fluctuations were greater at MCK 1.92 than at the other two sites (Figure 3). Temperatures at the two McCoy Branch sites was more variable than at the Hansard Mill Branch site, probably due to the fact that the spring sources at Hansard Mill Branch were more persistent than those feeding McCoy Branch. The low mean standard deviation for water temperature (0.89) at HMK 1.28 was indicative of the constant temperature environment provided by the springs that are the source of Hansard Mill Branch (Table 11). Flow in McCoy Branch was intermittent in some sections during the summer months (June to September) from 1992 to 1994, but was continuous in Hansard Mill Branch during the study period.

A comparison of mean weekly temperatures between sites with the GLM procedure revealed significant differences with respect to site and year. This test was robust, with an $R^2$ value of 0.93, indicating the differences were substantial. Tukey's standardized range test revealed that mean water temperatures differed significantly between the MCK 2.17, and the MCK 1.92 and HMK 1.28 sites. No significant differences in mean water temperature were found between MCK 1.92 and HMK 1.28 (Table 12).

The ICP analyses showed that surface water levels of flyash constituents including arsenic, aluminum, chromium, and iron, were below detection limits. They were also below the Lowest Chronic Values (LCVs) for Fish (DOE 1994b) in both streams. While LCVs for Fish were not available for flyash elements such as barium, boron, magnesium, molybdenum, potassium, sodium

Table 10.

Results of GLM Comparing Water Chemistry: McCoy Branch to Hansard Mill Branch.

| Parameter | $R^2$ | N | F | P | Site*Year (P) | Tukey's Test |
|---|---|---|---|---|---|---|
| pH | 0.896* | 47 | 1.77 | 0.1997 | 0.1538 | pH1=pH2=pH3=pH4[a] |
| DO | 0.922* | 47 | 2.42 | 0.0940 | 0.6123 | DO1=DO2=DO3=DO4 |
| Cond. | 0.939* | 47 | 3.14 | 0.0459 | 0.5724 | C1<C2<C3<C4 |

b. Number 1 corresponds to MCK 1.97, 2 is MCK 2.12, 3 is HMK 1.23, and 4 is HMK 0.41.

*. Indicates $R^2$ is above cut-off value (0.20) for potential biological significance.

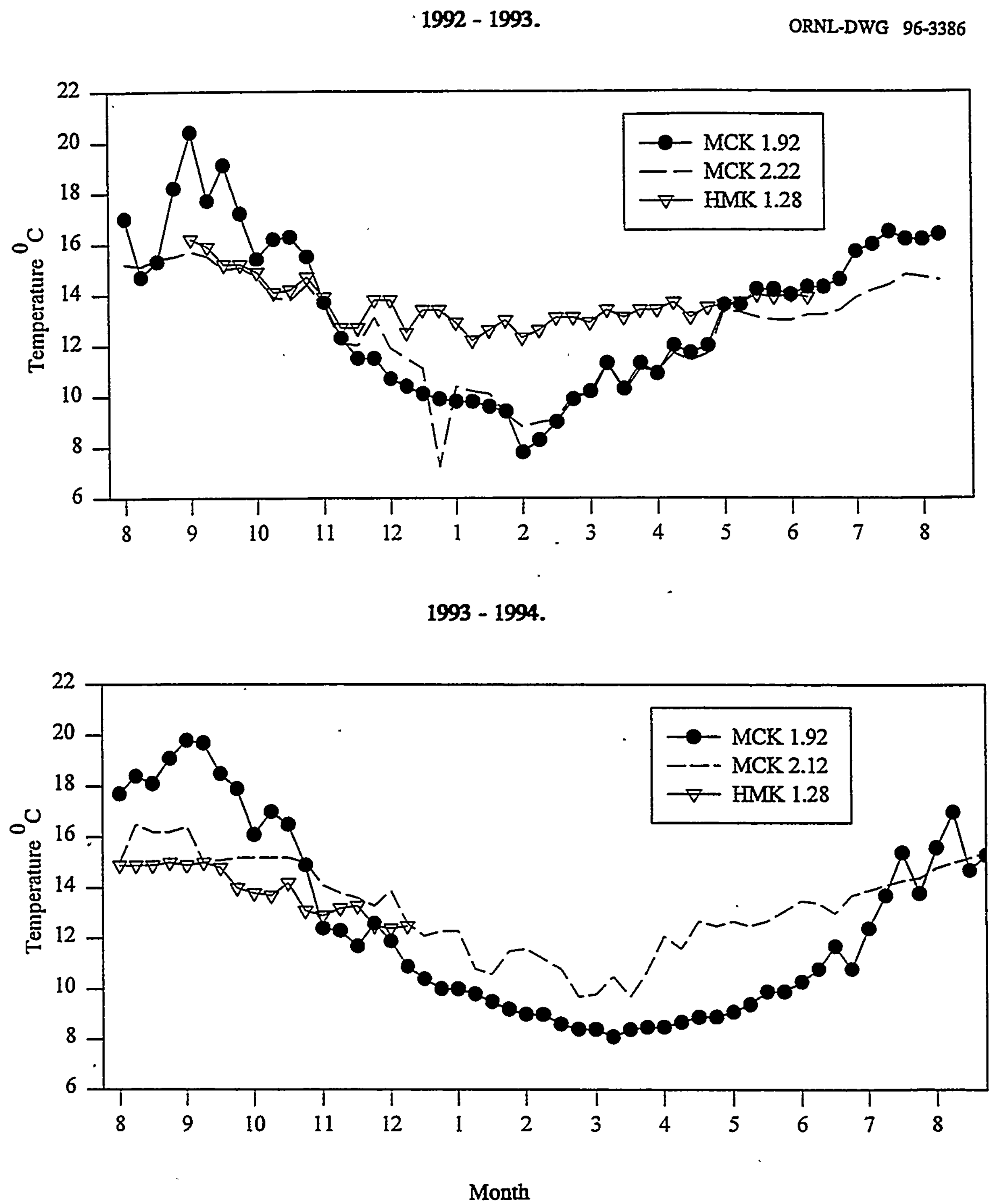


**Figure 3.**

**Mean Weekly Water Temperatures: McCoy Branch and Hansard Mill Branch 1992-1994.**

Table 11.

**Mean Weekly Water Temperatures: McCoy Branch and Hansard Mill Branch 1992-1994.**

| | | Site | | |
|---|---|---|---|---|
| | | **MCK 1.92** | **MCK 2.17** | **HMK 1.28** |
| **Year** | **Parameter** | | | |
| 1992[a] | Temp. (°C) | 12.7 ± 3.39 | 13.5 ± 1.57 | 13.9 ± 0.95 |
| | Min-Max | 7.1 - 21.6 | 9.4 - 16.2 | 11.6 - 17.1 |
| 1993[b] | Temp. (°C) | 11.9 ± 3.52 | 12.8 ± 2.24 | 14.0 ± 1.18 |
| | Min-Max | 6.4 - 22.3 | 5.9 - 17.2 | 11.1 - 21.9 |
| 1994[c] | Temp. (°C) | 12.6 ± 2.69 | 12.0 ± 1.92 | 13.3 ± 0.53 |
| | Min-Max | 6.4 - 19.0 | 5.6 - 19.6 | 10.4 - 16.0 |

a. The 1992 temperature data contains approximately 5 months of readings (Aug.-Dec.).

b. Temperature data for January to July, 1993, was not available for HMK 1.28

c. The 1994 temperature data contains approximately 7 months of readings (Jan.-Jul.).

Table 12.

Results of GLM Comparing Water Temperature: McCoy Branch to Hansard Mill Branch.

| Parameter | $R^2$ | N | F | P | Site*Year (P) | Tukey's Test |
|---|---|---|---|---|---|---|
| TEMPERATURE: | | | | | | |
| | 0.931* | 186 | 56.75 | <0.0001 | 0.0013 | T1<T2, T1=T3, T2>T3 |

a. T1 is MCK 1.92 Tempmentor, T2 is MCK 2.17, and T3 is HMK 1.28.

*. Indicates $R^2$ is above cut-off value (0.20) for potential biological significance.

and strontium, the levels of these flyash elements were below the LCV levels for "all species" (DOE 1994b) in both streams. At both McCoy Branch and Hansard Mill Branch, the levels of other flyash constituents including cadmium, copper, lead, nickel, selenium and zinc, were below detection limits set higher than the LCV for Fish (DOE 1994b). While historical deposition of coal flyash into the McCoy Branch watershed caused the flushing of high concentrations of flyash constituents such as As and Se through upper McCoy Branch, water concentrations have decreased over time until the differences between the two streams are barely discernible. Between 1986 and 1990, the mean concentration of Se declined from 0.019 mg/L to 0.004 mg/L and mean concentrations of As declined from 0.21 mg/L to 0.04 mg/L at Rogers Quarry outfall (Hinzman 1992). Atomic absorption was used to analyze for As and Se concentrations in water from the May 1993 and November 1993 samples; as indicated in my data both elements were below detection limits (0.005 mg/L and 0.002 mg/L, respectively) at all four sites (Tables 13, 14).

Comparisons of water sample results for the two streams revealed some large differences in the concentrations of certain elements commonly found in coal flyash. The concentrations of boron and strontium in McCoy Branch, for example, were an order of magnitude greater than those at Hansard Mill Branch. Concentrations of barium and sulfur also were higher than those found in the Hansard Mill Branch samples. In the November 1993 sample, the measured (but unverified) concentration of zinc at HMK 0.41 was 0.29 mg/L. Zinc concentrations at the other sites were all below the detection limit (<0.04 mg/L). Calcium concentrations were much higher at HMK 0.41 than at the other three sites for all three sampling periods.

Sediment samples were not taken as part of this study, but those data were included because of the potential effects of sediments on the introduced banded sculpins. Fish may be exposed to flyash constituent elements in sediments by direct consumption of sediments, or secondarily, through consumption of macroinvertebrates (DOE 1994a). Banded sculpins are at a greater risk of exposure to sediment-based contaminants, compared to organisms which inhabit the upper portion of the water column, due to their benthic feeding habits. Chemicals can be directly

Table 13.

ICP Sample Results: McCoy Branch.

| | MCK 1.97 | | | MCK 2.12 | | | |
|---|---|---|---|---|---|---|---|
| | 10/92 | 5/93 | 11/93 | 10/92 | 5/93 | 11/93 | |
| Element[a,d] | | | | | | | LCVF[c] |
| Arsenic | <0.3 | <0.3[b] | <0.3[b] | <0.3 | <0.3[b] | <0.3[b] | 0.89 |
| Barium | 0.09 | 0.08 | 0.08 | 0.09 | 0.08 | 0.09 | NA |
| Boron | 0.14 | 0.12 | 0.13 | 0.15 | 0.13 | 0.13 | NA |
| Calcium | 44.1 | 41.2 | 45.8 | 42.8 | 39.6 | 44.2 | NA |
| Magnesium | 16.5 | 15.1 | 17.1 | 16.9 | 15.5 | 17.4 | NA |
| Selenium | <0.6 | <0.6[b] | <0.6[b] | <0.6 | <0.6[b] | <0.6[b] | 0.088 |
| Sodium | 1.5 | 1.3 | 1.5 | 1.5 | 1.3 | 1.4 | NA |
| Strontium | 0.36 | 0.29 | 0.37 | 0.37 | 0.30 | 0.38 | NA |
| Sulfur | 4.6 | 4.8 | 4.7 | 4.6 | 4.8 | 4.6 | NA |
| Zinc | <0.06 | <0.06 | <0.06 | <0.06 | <0.06 | <0.06 | 0.036 |

a. All chemical concentrations are in mg/L.

b. Atomic absorptions were also run on arsenic and selenium for these sample dates.

c. LCVF stands for Lowest Chronic Value for Fish (DOE 1994b).

d. Elements not included in chart below detection limits at all sites (mg/L); aluminum (0.3), cadmium (0.03), iron (0.4), lead (0.1), and nickel (0.05).

Table 14.

ICP Sample Results: Hansard Mill Branch.

| | HMK 1.23 | | | HMK 0.41 | | | |
|---|---|---|---|---|---|---|---|
| | 10/92 | 5/93 | 11/93 | 10/92 | 5/93 | 11/93 | |
| Element[a,d] | | | | | | | LCVF[c] |
| Arsenic | <0.3 | <0.3[b] | <0.3[b] | <0.3 | <0.3[b] | <0.3[b] | 0.89 |
| Barium | 0.04 | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 | NA |
| Calcium | 34.7 | 36.4 | 37.5 | 49.6 | 45.0 | 68.5 | NA |
| Magnesium | 19.2 | 19.8 | 20.3 | 16.9 | 17.5 | 13.3 | NA |
| Selenium | <0.6 | <0.6[b] | <0.6[b] | <0.6 | <0.6[b] | <0.6[b] | 0.008 |
| Sodium | 1.0 | 1.0 | 1.7 | 2.7 | 1.5 | 2.6 | NA |
| Strontium | 0.03 | 0.02 | 0.02 | 0.06 | 0.05 | 0.10 | NA |
| Sulfur | 1.2 | 0.8 | 1.2 | 2.6 | 1.6 | 4.0 | NA |
| Zinc | <0.06 | <0.06 | <0.06 | <0.06 | <0.06 | 0.29 | 0.036 |

a. All chemical concentrations are in mg/L.

b. Atomic absorptions were also run on arsenic and selenium for these sample dates.

c. LCVF stands for Lowest Chronic Value for Fish (DOE 1994b).

d. Elements not included in chart below detection limits at all sites (mg/L); aluminum (0.3), cadmium (0.03), iron (0.4), lead (0.1), and nickel (0.05).

transferred from sediments to many types of organisms, including fish and benthic macroinvertebrates (Adams et al. 1992a). Flyash elements including arsenic, barium, iron, molybdenum, and nickel were shown to be elevated above background levels in sediments from upper McCoy Branch. These samples were taken in May through September of 1993 from springs adjacent to upper McCoy Branch and from the spring at the base of the filled coal ash pond, a primary source of flow to McCoy Branch (DOE 1994a). The only one of these sediment based elements which was found at elevated levels considered likely to be harmful to benthic macroinvertebrates and fish was arsenic. The As concentration in sediments at the base of the filled coal ash pond was 774.0 mg/kg, about 11 times greater than the NOAA Effects Range Median (ER-M) for sediment toxicity (DOE 1994c).

**Fish Introduction**

Of the 237 banded sculpins taken to the lab for the first McCoy Branch introduction, 13 were died to electroshocking and handling stresses, 19 fish died after the injection procedure, and 25 were kept in the lab for observation. The remainder, 180 marked sculpins, were introduced into McCoy Branch. Four of the 23 fish collected for the second McCoy Branch introduction perished before injections, due to electroshocking and handling stresses, and one fish was lost after the injection procedure (Table 15).

Electrofishing and handling stresses caused more mortality in sculpins than the polymer injection process in this study. Barrett and Grossman (1988) found that mortality of mottled sculpins (*Cottus bairdi*), which were obtained by electrofishing and subcutaneously injected with acrylic polymers, ranged from 0 to 10.5% thirty days after treatment, and that neither single nor multiple electrofishing exposures had significant effects upon mortality of mottled sculpins. The study by Barrett and Grossman (1988) also suggested that handling stresses played an important role in the mortality of mottled sculpins. My study was similar with respect to the low rates of inadvertent mortality during the injection process, even though the fish were first electroshocked

Table 15.

Results of Banded Sculpin Marking and Introductions.

| Date | No. Marked | Color | Marking Mortality | Other Mortality[a] |
|---|---|---|---|---|
| 9/23/92 | 159 | Orange/Green | 12 | 8 |
| 10/2/92 | 65 | Orange/Green | 7 | 5 |
| 7/22/93 | 20 | Blue/Magenta | 1 | 2 |
| Totals | 244 | | 20 | 15 |

a. Mortality due to electroshocking and handling stress.

and then handled repeatedly within a short period of time.

The subcutaneous injections allowed me to determine dispersal patterns of the sculpins that were introduced into upper McCoy Branch. The mortality rates due to the injection process were low and the durability of the marks compared favorably to previous studies. Mortality rates in the first round of tagging were relatively low (8.5%), and were even lower in the second round of injections (5.3%). In Lotrich and Meredith (1974), a 4% mortality rate was reported for a two week period following injection. Hill and Grossman (1987) reported no statistically significant effects of subcutaneous marking on survival of mottled sculpins. As mentioned previously, a control group of 25 marked sculpins was kept in the laboratory for observation during the study. Twenty three of these fish survived at least 15 months, when a high temperature pulse in the source water caused them all to perish. One of the marked control fish died after approximately one month, and another at 4 months after injection. Fish paint markings were detected up to 33 months after injection in McCoy Branch sculpins. The average duration of the acrylic paint marks was from 12 to 14 months. This is similar to the 4 to 16 month persistence reported by Lotrich and Merideth (1974).

The first group of 180 marked sculpins was introduced into McCoy Branch on 3 October, 1992. The marked sculpins were stocked into the two 50-m sites on McCoy Branch; 90 fish ranging in size from 3.5 to 11.1 cm TL were released at each site. A second group of marked sculpins (18) was introduced into McCoy Branch on 2 August 1993; nine marked sculpins ranging in size from 5.0 to 11.6 cm TL were introduced into both sections of stream. A different method of dispersal was employed in the second introduction to dilute the short-term high density situations created by the first introduction. Half of the fish were spread out evenly over the entire lower 200-m of upper McCoy Branch, which encompassed MCK 1.97, and the other half over the upper 200-m section, which included MCK 2.12. A smaller number of fish was used in the second introduction to alleviate the potential overcrowding problems caused by the larger number of fish used in the first introduction.

**Fish Community**

Fish populations in both streams were surveyed quarterly from January 1993 to March 1995. The number of banded sculpins in McCoy Branch initially declined following their introduction, with only 55 marked fish being recaptured during the first population sample in January 1993, followed by 27 and 38 fish in subsequent samples at MCK 1.97 and MCK 2.12 combined (Table 16). The fish introduced in October of 1992 dispersed from the original two 50-m study reaches to occupy a 400-m segment of upper McCoy Branch. At least initially, the net movement of the sculpins was upstream. Fish marked with orange paint and released at MCK 1.97 were recovered as far as 300 m upstream. Movement of sculpins downstream was not as prevalent. During the first three census periods, 24 orange-marked sculpins (13.3% of those released) from the MCK 1.97 site were collected more than 50 m upstream, while 11 green-marked sculpins (6.1%) had moved more than 50 m downstream from the MCK 2.12 site. A movement of banded sculpins to deeper pools in Flint Creek, Delaware County, Oklahoma during the summer months (April-September) was reported by Todd and Stewart (1985). A similar response in McCoy Branch could explain some of the initial movements by the banded sculpins I introduced to this stream. These fish may have been trying to access the deeper pool areas found between the two sites and above MCK 2.12 in the fall of 1992. The pattern of movement to the deeper pool areas in late summer occurred each year of the study at both streams, and appeared particularly prevalent in McCoy Branch. Sculpins introduced at MCK 2.12 also dispersed upstream, with green-marked fish being collected as far as 180 m upstream from the release site.

The initial recovery of sculpins from McCoy Branch was low, but most of the fish collected the first year after introduction still had legible paint marks. Mark-recapture numbers dropped from 31.0% in the initial population sample to 15.0% and 21.1% respectively in the two subsequent samples. These figures were relatively low compared to the 58% recovery of marked sculpins reported by Greenberg and Holtzman (1987). Non-marked banded sculpins that were

Table 16.

Banded Sculpin Population Samples: McCoy Branch 1993-1995.

| Sample date | No. sculpins collected[a] | Marked sculpins recaptured[b] | Non-marked sculpins collected | Stream area sampled ($m^2$) |
|---|---|---|---|---|
| 1/93 | 55 | 18 O, 37 G | 0 (0%)[c] | 240.0 |
| 4/93 | 27 | 17 O, 10 G | 0 (0%) | 246.0 |
| 6/93 | 38 | 23 O, 14 G, | 1 (2.6%) | 540.0 |
| 10/93 | 25 | 9 O, 7 G, 1 M, 4 B | 4 (16.0%) | 270.0 |
| 2/94 | 44 | 1 G, 1 M | 42 (95.5%) | 560.0 |
| 6/94 | 24 | 0 marked | 24 (100%) | 280.0 |
| 8/94 | 11 | 0 marked | 11 (100%) | 200.0 |
| 12/94 | 67 | 0 marked | 67 (100%) | 272.0 |
| 4/95 | 84 | 1 O | 83 (98.8%) | 272.0 |

a. Banded sculpins collected during quarterly fish population surveys. Totals include both MCK 1.97 and MCK 2.12 (1/93-8/94) or MCK 1.97 and the spring-pool (12/94-4/95).

b. Numbers of marked sculpins recaptured after introduction into McCoy Branch. ( O = Orange, G = Green, M = Magenta, and B = Blue).

c. Percent of non-marked fish recovered in McCoy Branch population samples.

collected in the first year after the introduction were most likely members of the small group of fish present before the first introduction. The absence of marked fish and the increased numbers of non-marked fish collected in McCoy Branch population samples after October 1993 can be attributed to a combination of three factors: (1) natural mortality of introduced marked sculpins, (2) the fading of paint marks over time, and (3) the recruitment of YOY sculpins into the population through in situ reproduction (Table 16).

Comparison of banded sculpin densities between the two streams was complicated due to the relatively diverse fish communities extant at the two Hansard Mill Branch sites. At HMK 1.23, three fish species including banded sculpin, blacknose dace (*Rhinichthys atratulus*), and creek chub (*Semotilus atromaculatus*) were collected. Banded sculpins averaged 68.2% of the fish community at HMK 1.23. There were only 2 creek chubs collected from this site, both in July 1993. Due to its low numbers, this species was basically a non-factor in fish community distributions at this site. Banded sculpins came to increasingly dominate the fish community at HMK 1.23 over the course of the study. At HMK 0.41, seven fish species were collected including banded sculpin, blacknose dace, creek chub, stoneroller (*Campostoma anomalum*), striped shiner (*Luxilus chrysocephalus*), northern hogsucker (*Hypentelium nigricans*), greenside darter (*Etheostoma blennioides*), stripetail darter (*Etheostoma kennicotti*), and snubnose darter (*Etheostoma simoterum*). Banded sculpins averaged 15.3% (by number) of the fish community at this site. The predominant fish in this community were blacknose dace and stonerollers, together comprising over 75% of the total numbers of fish found at HMK 0.41 (Table 17).

Average fish density at McCoy Branch over the study period was approximately 0.12 sculpins/$m^2$ of stream. These figures while somewhat lower than expected, remained relatively constant after the initial population decline following the first introduction. While fish densities remained low in McCoy Branch proper, the spring-pool population was relatively dense in comparison, with a mean of 0.46 sculpins/$m^2$ (Figure 4).

Mean densities of sculpins in McCoy Branch were lower than those at Hansard Mill

Table 17.

Banded Sculpin Population Samples - Species Associates: Hansard Mill Branch 1993-1995.

| Fish species | HMK 1.23 | Percent of community[a] | HMK 0.41 | Percent of community[a] |
|---|---|---|---|---|
| Banded sculpin | 1-9[b] | 68.2 | 1,2,6,7 | 15.3 |
| Stoneroller | NF[c] | 0 | 1,2,6,7 | 22.6 |
| Striped shiner | NF | 0 | 2,6 | 2.2 |
| Blacknose dace | 1-9 | 31.6 | 1,2,6,7 | 53.4 |
| Creek chub | 4 | 0.2 | 6 | 0.4 |
| N. hogsucker | NF | 0 | 1,7 | 0.6 |
| Greenside darter | NF | 0 | 6 | 0.9 |
| Stripetail darter | NF | 0 | 1,2 | 1.2 |
| Snubnose darter | NF | 0 | 1,6,7 | 3.4 |

a. Mean percentages from combined sampling periods.

b. Numbers correspond to fish found on sampling dates: 1 = 1/93, 2 = 5/93, 3 = 7/93, 4 = 11/93, 5 = 2/94, 6 = 6/94, 7 = 9/94, 8 = 12/94, and 9 = 4/95.

c. NF indicates none of that species were collected at that site during any sampling period.

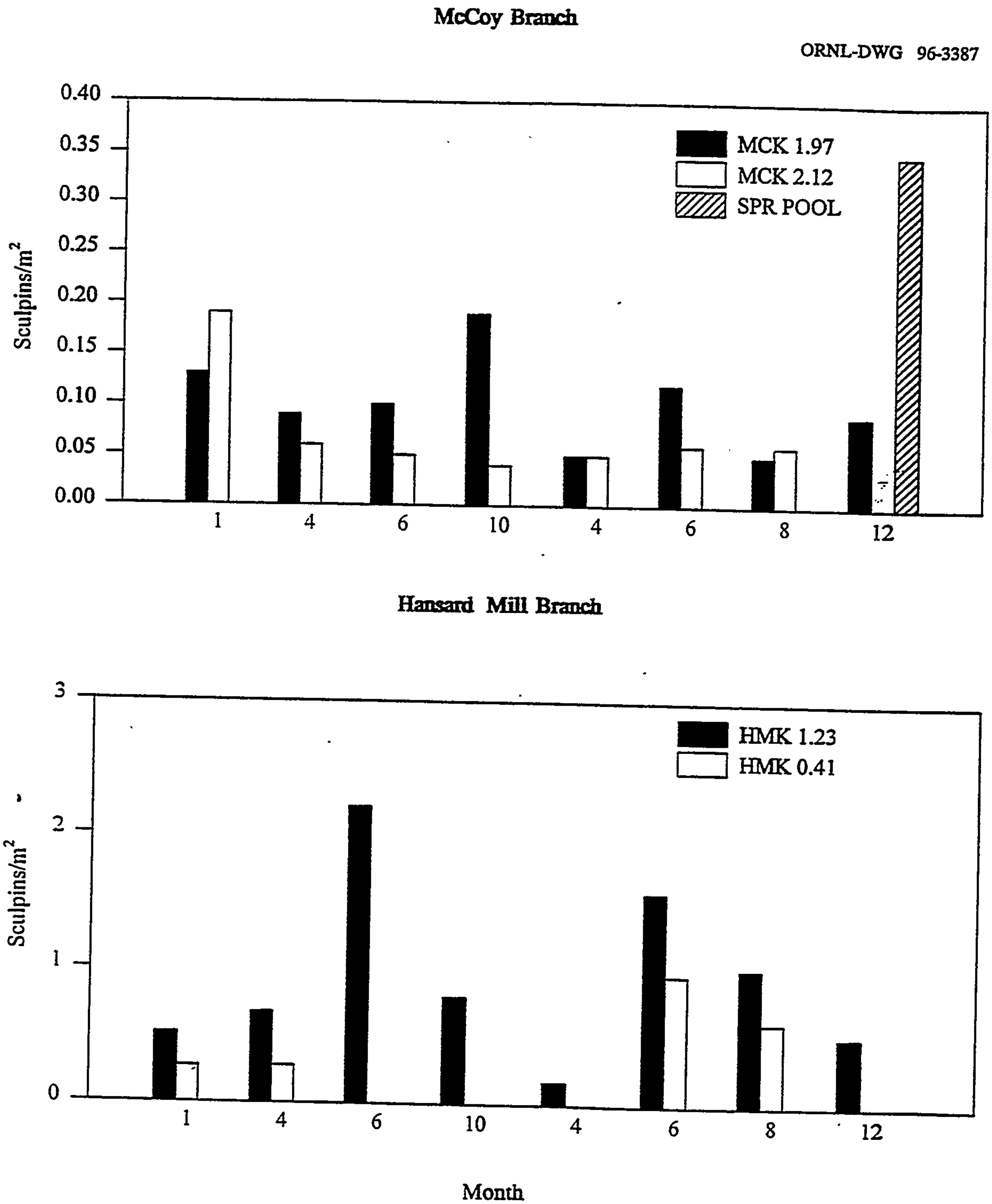


Figure 4.

**Banded Sculpin Densities: McCoy Branch and Hansard Mill Branch 1993-1994.**

Branch, but were still comparable with those observed in other geographically related streams (Table 18). Greenburg and Holtzman (1987) reported banded sculpin densities of 0.4 to 0.9 fish/m$^2$ from the Little River, Blount County, Tennessee. At Hansard Mill Branch, banded sculpins were found in relatively high densities, especially at the HMK 1.23 site. The mean sculpin density for the study period at Hansard Mill Branch was 0.80 sculpins/m$^2$ (Figure 4).

Few YOY size class sculpins (e.g.,0+ to 4+) were found in McCoy Branch population samples taken during 1993 and 1994, suggesting little reproduction of sculpins in that stream (Figures 5, 6). However, in December 1994 I collected 57 banded sculpins from a spring-pool which feeds into McCoy Branch 10 m below MCK 1.97. Twenty nine of those fish were 4.0 to 5.9 cm TL, indicative of the 1994 year class, and 24 were 6.5 to 7.9 cm TL, indicative of the 1993 year class. The spring-pool was accessible to the fish in McCoy Branch in all but the driest portions of the year. A marked sculpin from the October 1992 introduction was collected from the spring-pool in January of 1995, revealing that the introduced sculpins had accessed the pool. Therefore, the juvenile fish found in the spring-pool were likely progeny from the original introductions. Analysis of length-frequency data for Hansard Mill Branch sculpins revealed relatively abundant year classes for the 1993 and 1994 spawns (Figures 5, 6). The sculpins in this stream exhibited a more normal distribution among sizes classes in 1993, while the 1994 data revealed an apparent saturation of YOY and 1+ sculpins at both sites in Hansard Mill Branch.

Annual mean percentages of YOY sculpin abundance and survival were calculated for all sites where YOY sculpins were collected. Data for different sites on each stream were combined to calculate a stream-wide annual percentage. Mean first year growth rates (5.67 cm TL) of the sculpins were calculated by averaging growth rates from all sites. Maximum first year growth was estimated at 6.6 cm TL. Craddock (1965), reported banded sculpin growth in the first full year at 7.6 to 8.4 cm TL, while Small (1975) estimated maximum growth rates ranging from 5.0 to 6.2 cm TL. This information was used to help establish cut-off levels for first year growth, an important factor in sorting out different year classes of fish which were used in determining YOY sculpin

Table 18.

Banded Sculpin Densities at Other Streams Within 8 km of McCoy Branch 1993-1995.

| Site | Banded sculpin density (Sculpins/$m^2$) | Total density (Fish/$m^2$) | Percent of community[a] |
|---|---|---|---|
| First Creek (km 0.8) | 0.14 | 2.92 | 4.8 |
| Fifth Creek (km 1.0) | 2.56 | 7.32 | 35.0 |
| Fifth Creek (km 0.2) | 0.11 | 2.61 | 4.2 |
| White Oak Cr. (km 6.8) | 0.26 | 1.21 | 21.5 |
| Scarboro Creek (km 2.2) | 0.72 | 3.99 | 18.0 |
| Total[b] | 0.76 | 3.61 | 16.7 |

a. Percentage of sculpins in the community totals (by number).

b. Mean totals for sculpins/$m^2$ and total fish/$m^2$, and percent of community, respectively.

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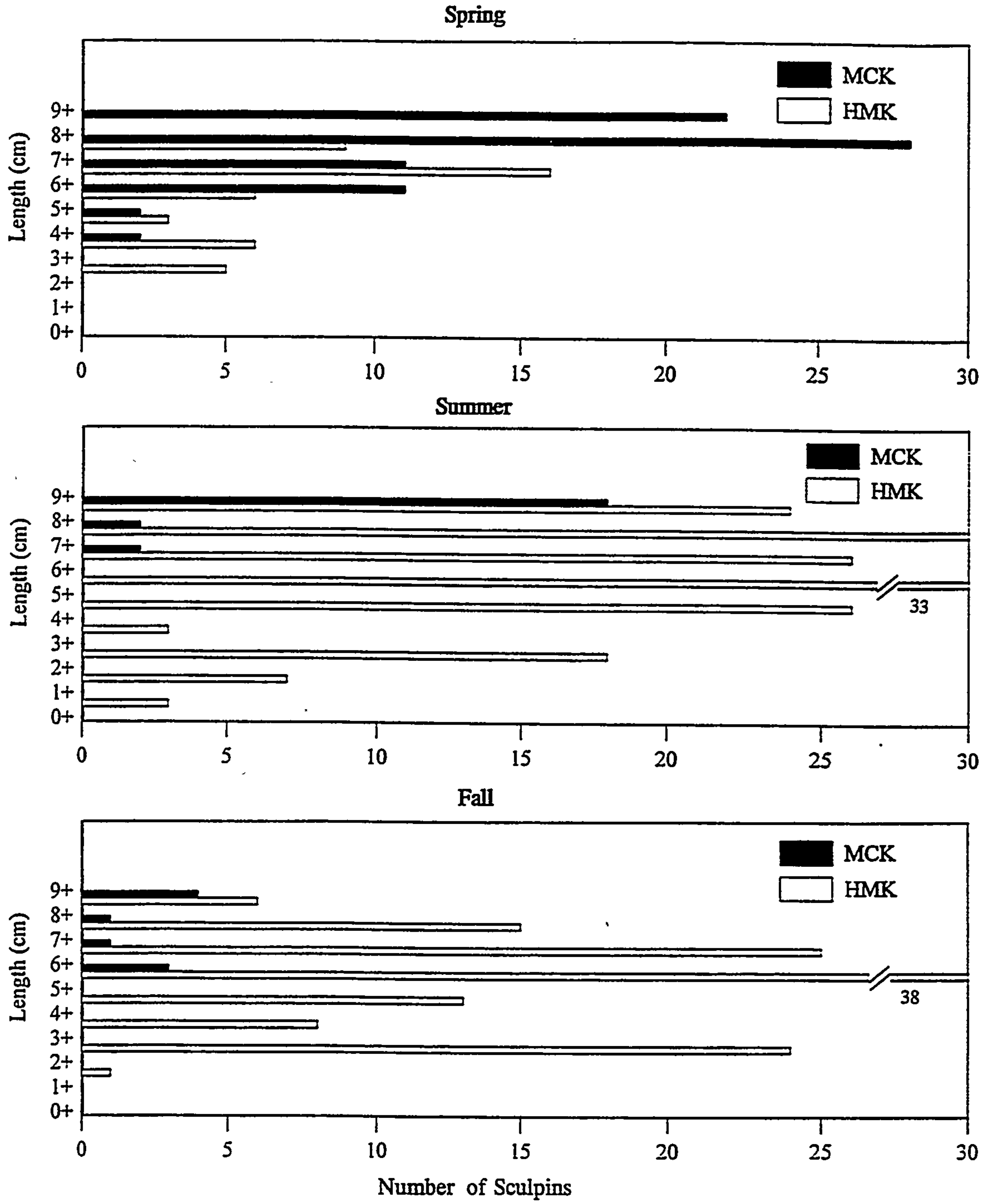


**Figure 5.**

**Banded Sculpin Length Frequencies: McCoy Branch and Hansard Mill Branch 1993.**

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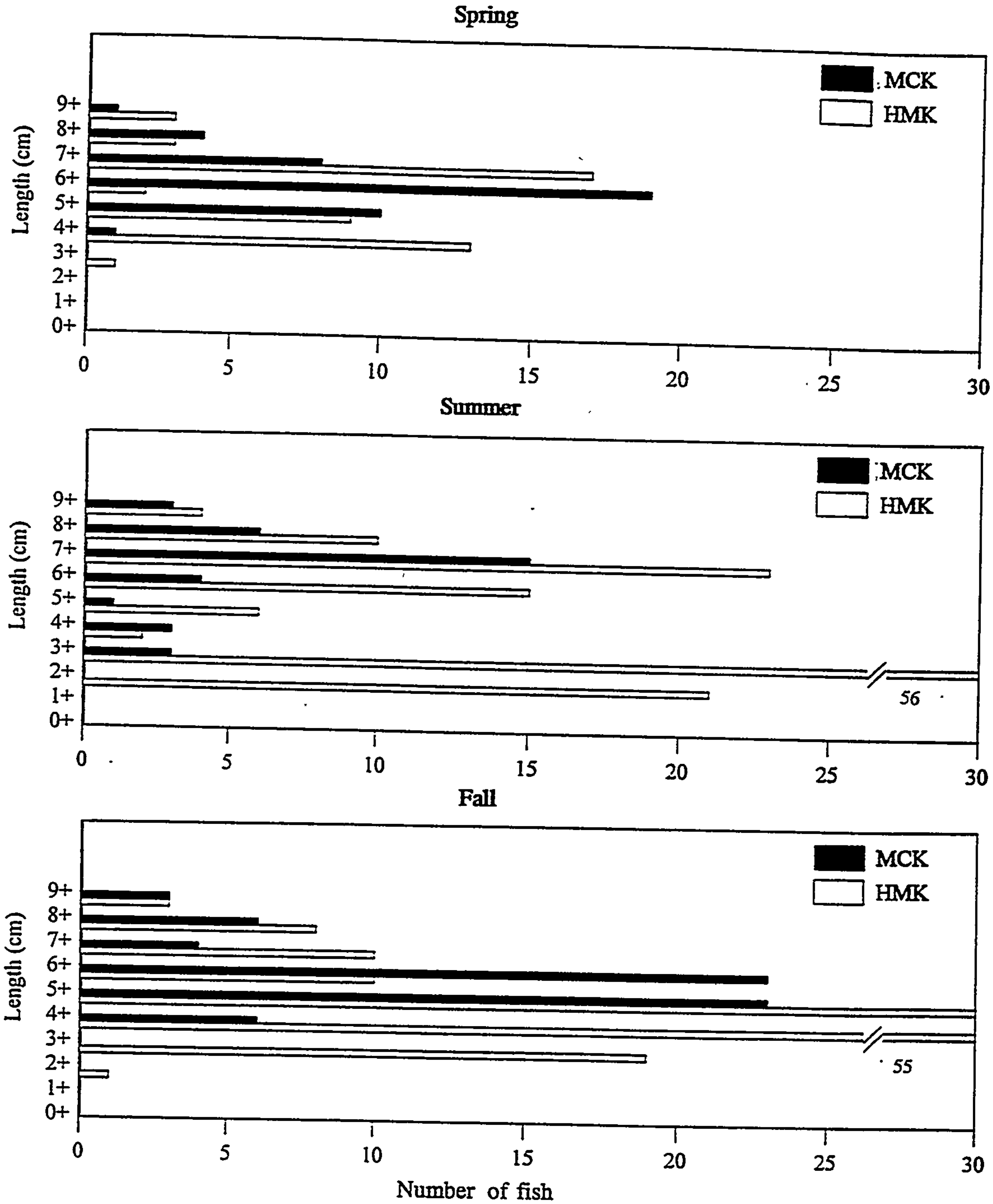


**Figure 6.**

**Banded Sculpin Length Frequencies: McCoy Branch and Hansard Mill Branch 1994.**

abundance and survival percentages. Mean abundance percentages for YOY banded sculpins were slightly higher in Hansard Mill Branch than in McCoy Branch from 1993 to 1995 (Table 19). In both McCoy Branch and Hansard Mill Branch, YOY sculpins were more abundant in 1994 than in 1993. The abundance percentages of YOY sculpins in 1994 in both streams indicated that a good spawn had occurred that year. Figures for 1995 YOY sculpin abundance do not represent a full year; it appears that they will most likely fall between the 1993 and 1994 mean percentages at both streams.

The YOY survival percentage was calculated as the number of YOY individuals surviving until the next spawning season. I could not calculate YOY survival percentages for McCoy Branch because the sculpins collected from the spring-pool were already in the 1+ and 2+ size classes. There was no way to estimate the number of sculpins originally spawned in the 1993 and 1994 breeding seasons, and therefore no way of determining mortality of YOY sculpins. Survival percentages for YOY sculpins in Hansard Mill Branch were relatively high, averaging near 50% (Table 20).

The condition factor of sculpins in McCoy Branch and Hansard Mill Branch remained relatively constant throughout the study. McCoy Branch sculpin condition factors increased slightly from 1993 to 1994, while the sculpins were undergoing a small decline in density. Hansard Mill Branch banded sculpin condition factor annual means decreased slightly, while densities remained at about the same level during the study period. Standard deviations were similar for both streams during the study, but those for Hansard Mill Branch were slightly higher than for McCoy Branch in 1993 and 1994 (Table 21). The increase in condition factors for fish in McCoy Branch over time may possibly be correlated with the sediment related depression of sculpin densities in that watershed. The low densities created a situation where fewer individuals existed, and those individual fish had somewhat higher biomasses due to the release from interspecific competition. A reduction of competition for resources such as food and habitat can be correlated with an increase in growth rate and lipid storage (Adams et al. 1992b).

Table 19.

Abundance Estimates for YOY Banded Sculpins:

McCoy Branch and Hansard Mill Branch 1993-1995.

| | McCoy Branch[a] | | | Hansard Mill Branch[a] | | |
|---|---|---|---|---|---|---|
| Year | Total sculpins[b] | YOY sculpins | YOY (%)[c] | Total sculpins[b] | YOY sculpins | YOY (%)[c] |
| 1993 | 103 | 26 | 25.2 | 295 | 62 | 21.0 |
| 1994 | 92 | 35 | 38.0 | 217 | 124 | 57.1 |
| 1995 | 107 | 28 | 26.2 | 72 | 26 | 36.1 |

a. Stream-wide data contain combined totals for McCoy Branch (MCK 1.97, MCK 2.12, and Spring-pool) and Hansard Mill Branch (HMK 1.23, and HMK 0.41).

b. Total number of sculpins based on all samples in which at least one YOY sculpin was collected.

c. Annual abundance of YOY sculpins.

Table 20.

Survival Rates for YOY Banded Sculpins: McCoy Branch and Hansard Mill Branch 1993-1995.

| | McCoy Branch | | | Hansard Mill Branch | | |
|---|---|---|---|---|---|---|
| Year | YOY sculpins[a] | 1+ sculpins[b] | YOY survival (%)[c] | YOY sculpins[a] | 1+ sculpins[b] | YOY survival (%)[c] |
| 1993 | NA[d] | 32 | NA | 33 | 14 | 42.4 |
| 1994 | NA | 38 | NA | 58 | 32 | 55.2 |

a. YOY banded sculpins collected after the spawn.

b. Number of 1+ year-class sculpins collected during spawning season at each stream.

c. Percentage of individuals surviving out of the year-class.

d. McCoy Branch YOY initial reproduction and survival data not available.

Table 21.

**Banded Sculpin Condition Factors: McCoy Branch and Hansard Mill Branch 1993-1994.**

| Site | Year | Condition factor (mean ± sd) | N |
|---|---|---|---|
| McCoy Branch | 1993 | 1.207 ± 0.14 | 107 |
| Hansard Mill Br. | 1993 | 1.232 ± 0.22 | 237 |
| McCoy Branch | 1994 | 1.245 ± 0.22 | 131 |
| Hansard Mill Br. | 1994 | 1.126 ± 0.26 | 282 |

a. Annual mean condition factors, using data from all sculpins captured during population surveys for that year.

The GLM procedure comparing condition factors for sculpins from McCoy Branch and Hansard Mill Branch was not significant ($R^2 = 0.14$); therefore this analysis of variance was not a good indicator of differences in condition factor by site and year between streams. No significant differences between sites were found using Tukey's test, but a significant difference in overall condition factors of sculpins between years was found. Condition factors were also compared by stream and season (Table 22). Both streams exhibited the similar seasonal trends with respect to condition factor; the summer group had the highest mean condition factors, followed by spring and fall respectively. Condition factors were slightly higher for fish from McCoy Branch for all three seasons.

**Food Resources - Feeding Habits**

Benthic macroinvertebrate samples taken in January and May of 1993 were used to provide an estimate of the food resources available to banded sculpins in the streams and augment the description of the colonization efforts of the banded sculpins introduced into McCoy Branch. The macroinvertebrate data were analyzed by simple non-statistical comparisons of the sites based on taxa richness and abundance. Macroinvertebrate taxa present and their relative densities at each site are listed in Appendix 1.

McCoy Branch benthic samples were dominated by two or three taxa, with the two most abundant taxa (chironomids and hydropsychids) comprising on average, 69.2% of the total number of taxa collected. The two most abundant taxa (elmids and amphipods) in Hansard Mill Branch benthic samples averaged 50.2% of the total number of taxa collected.

In January, chironomids were the dominant taxon at MCK 2.12, averaging 36.5% of the total number of taxa collected. Ephemerellids and hydropsychids were also abundant at this site. At MCK 1.97, chironomids were the dominant taxon (52.0%), but hydropsychids and elmids were also abundant. Elmids were the dominant taxa in Hansard Mill Branch, averaging 35.3% at HMK 1.23 and 23.1% at HMK 0.41. After elmids, the most abundant taxa at HMK 1.23 were

Table 22.

Results of GLM Comparing Banded Sculpin Condition Factor:

McCoy Branch to Hansard Mill Branch.[a]

| Parameter | $R^2$ | N | F | P | Site*Year (P) | Tukeys' Test |
|---|---|---|---|---|---|---|
| CONDITION FACTOR: | | | | | | |
| | 0.146* | 886 | 21.46 | <0.0001 | NA | CF1=CF2 CF93>CF94 |

a. Sites were combined for each stream and examined on an annual basis.

*. Indicates $R^2$ is above cut-off value (0.20) for potential biological significance.

amphipods and oligochaetes. At HMK 0.41, leptophlebiid mayflies and chironomids were the predominant taxa other than elmids.

In May, chironomids again were the dominant taxon at both McCoy Branch sites, comprising 46.3% of the total taxa at MCK 2.12, and 85.0% at MCK 1.97. After chironomids, the predominant taxa included other types of dipterans and leuctrid stoneflies at MCK 2.12, and elmids and oligochaetes at MCK 1.97. Elmids comprised 44.7% of the total taxa at HMK 1.23, and 30.4% at HMK 0.41. After elmids, baetid mayflies and amphipods predominated at HMK 1.23. At HMK 0.41, chironomids and leptophlebiid mayflies were also abundant (Appendix 1).

Comparisons of food resources between McCoy Branch and Hansard Mill Branch were based mostly on taxa richness. The mean number of taxa per site in the January samples were similar at the two streams. However, relatively large differences in total taxa for the two streams are evident from comparisons of the May samples (Table 23). Plecopterans were scarce in McCoy Branch; comprising less than 2.0% of the total taxa in January and 5.9% in May, when compared to Hansard Mill Branch plecopteran totals (10.6% and 20.3%, respectively). There seemed to be a negative association between the degree of embeddedness and the low numbers of plecopterans and certain trichopterans in McCoy Branch. The degree of embeddedness was much higher, and the percentage of silt substrate types were two to three times greater in McCoy Branch compared to Hansard Mill Branch. In McCoy Branch, average plecopteran densities were $9.2/0.1 m^2$ compared to 32.7 plecopterans/0.1 $m^2$ in Hansard Mill Branch. Overall trichopteran densities were higher in McCoy Branch, $41.2/0.1 m^2$ compared to $19.5/0.1 m^2$ in Hansard Mill Branch. However, the more sediment-sensitive trichopterans were found at densities of $2.6/0.1 m^2$ at Hansard Mill Branch, while they were not found in McCoy Branch at all. Chironomid densities were much higher in McCoy Branch ($1057.3/0.1 m^2$) than in Hansard Mill Branch ($207.1/0.1 m^2$), as were the densities of most other dipterans (Appendix 1). As cited earlier, McClelland and Brusven (1980) found that plecopterans and some trichopterans (free-living forms such as Rhyacophilidae) were more sensitive to siltation of stream substrates than ephemeropterans, and that dipterans,

Table 23.

Food Resources - Taxa Richness: McCoy Branch and Hansard Mill Branch.[a]

| | Number of Taxa Collected | | | |
|---|---|---|---|---|
| Time period | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| January, 1993 | 20 | 22 | 23 | 22 |
| May, 1993 | 13 | 13 | 23 | 24 |
| Total | 33 | 35 | 46 | 46 |

a. Total number of benthic macroinvertebrate taxa from 4 Sürber samples at each site each period.

especially burrowing forms such as chironomids and tipulids, were least affected.

The predominant food items consumed by banded sculpins in McCoy Branch were baetid mayflies, *Pycnopsyche* sp., and unidentified trichopterans. Salamanders and crayfish were frequently found in the sculpin stomach samples from McCoy Branch (Appendix 2, 3). The latter two taxa often accounted for over 40% of the stomach contents by volume. At MCK 2.12, baetid mayflies were the food resource consumed most often, comprising 36.4% of the total number of food items consumed. Tipulids and *Pycnopsyche* sp. were also consumed in large quantities at this site. Trichopterans comprised 16.2% of the total food items consumed by sculpins at MCK 1.97. Other food items found in fish stomach samples from this site included crayfish, chironomids, and *Pycnopsyche* sp.. In Hansard Mill Branch, the predominant food items found in sculpin stomachs were elmids, baetid mayflies, and unidentified ephemeropterans. Crayfish and fish were also an important part of the diets of Hansard Mill Branch sculpins, more in volume than in numbers. The main fish species consumed by sculpins in Hansard Mill Branch was the blacknose dace. Elmids (15.5%) were the most abundant food type found in sculpin stomach samples at HMK 1.23, followed by isopods and baetid mayflies. The most common food items found in sculpin stomach samples from HMK 0.41 were unidentified ephemeropterans (16.9%). Baetid mayflies and isopods also were also commonly found in stomach samples from HMK 0.41 (Appendix 2, 3).

Selectivity of the sculpins was estimated by comparing the types of food found in their stomachs relative to the food resources available at the four sites (Table 24). In McCoy Branch, many chironomids and ephemerellids were available as food, but were not consumed by the introduced sculpins in large quantities relative to their abundance. In Hansard Mill Branch, the number of ephemerellids consumed was almost equal to the amount available as food, but chironomids were consumed at about half the rate available. In McCoy Branch, beatid mayflies were present in low to moderate numbers but were disproportionally consumed by the sculpins. This trend for consuming large quantities of baetid mayflies was also apparent in Hansard Mill Branch, but they were much more readily available as a food resource in that system. *Pycnopsyche*

Table 24.

**Banded Sculpin Food Item Selectivity: McCoy Branch and Hansard Mill Branch.**

| | McCoy Branch[a] | | Hansard Mill Branch[a] | |
|---|---|---|---|---|
| **Prey item** | **Availability (%)** | **Consumption (%)** | **Availability (%)** | **Consumption (%)** |
| Baetidae | 6.6 | 20.0 | 5.8 | 11.2 |
| Ephemerellidae | 15.2 | 0.0 | 3.2 | 3.9 |
| Hydropsychidae | 6.9 | 1.0 | 1.8 | 4.9 |
| *Pycnopsyche* sp. | 6.8 | 13.9 | 0.0 | 0.0 |
| Elmidae | 8.0 | 0.0 | 30.4 | 10.1 |
| Chironomidae | 52.7 | 6.0 | 10.7 | 4.2 |
| Tipulidae | 0.6 | 7.3 | 0.8 | 0.9 |
| Isopoda | 0.0 | 0.0 | 6.0 | 10.5 |

a. Numbers represent mean percentages for two samples for each site at each stream.

sp. were consumed at a high level in relation to their availability in McCoy Branch, but they were not found in benthic macroinvertebrate or sculpin stomach samples from Hansard Mill Branch. In McCoy Branch, hydropsychids were available at moderate levels, but represented a very small portion of the sculpins' diets. The inverse was true for hydropsychids in Hansard Mill Branch. Elmids were relatively rare in McCoy Branch, and were not found in any of the sculpin stomach samples. In Hansard Mill Branch elmids constituted a large percentage of the available food resources, but were found in only a small portion of the stomach samples. Tipulids were consumed in disproportionally large amounts relative to their availability as a food resource at McCoy Branch. In Hansard Mill Branch, tipulids were not readily available and represented a very small portion of the sculpins' diet. Isopods were not found in any benthic samples or sculpin stomach content samples from McCoy Branch; in Hansard Mill Branch isopods were found in low to moderate numbers in the benthic samples, and in moderate to large numbers in the stomach samples.

The diet of the sculpins inhabiting Hansard Mill Branch appeared much more diverse than that of the introduced sculpins in McCoy Branch. There were 16 different types of prey items found in sculpin stomach samples from Hansard Mill Branch, compared to 12 found in McCoy Branch samples. Dipterans (chironomids and tipulids), ephemeropterans (mainly baetids), and *Pycnopsyche* sp. accounted for over 52% of the banded sculpins diet at McCoy Branch, while the diets of the sculpins at Hansard Mill Branch were not dominated by any group of macroinvertebrates.

**Reproduction**

Banded sculpins in the flow-through laboratory tank were surveyed repeatedly during February and March, 1993. On a March 10 survey, three nests were observed. Eggs were laid in semi-clear gelatinous matrices in circular patterns on the undersides of flat rocks, 10-25 cm in diameter, which were lying flat on a pea gravel substrate. The egg masses were laid on the upstream underside edges of the rocks, and contained from 30 to 86 eggs. All of the eggs and small larval sculpins which had already hatched were dead, perhaps due to the trace amounts (0.03 mg/L) of total residual chlorine found in the source water. On March 12 and March 17 1993, two possible nest sites were discovered in McCoy Branch; one in the lowest riffle of MCK 1.97, and the other approximately 220 m upstream of MCK 2.12. These nests were in similar habitats; both were under flat rocks 15 to 30 cm diameter, near spring outlets in 8 to 15 cm of water, where water velocity was low to moderate. Eggs (26-86) were laid in a circular pattern contained in a clear gelatinous matrix on the underside of the rocks. All of the eggs were opaque and partially deteriorated. It was not possible to determine whether the nests were of banded sculpin or salamander origin. On March 18 1993, three post-larval sculpins (0.5-0.8 cm TL) were found in Hansard Mill Branch upstream of HMK 1.23. These sculpins were all found in similar habitats; under large rocks (25-35 cm diameter), on fine gravel to sandy substrates, in side areas of the stream near spring outlets. At these locations water velocity was low to moderate, and water depth was approximately 7 to 15 cm. The nests were found in the same areas utilized by northern two-lined salamanders (*Eurycea bislineata*), sometimes under the same rocks.

Nest surveys were not conducted in McCoy Branch or Hansard Mill Branch in 1994 due to large rain events that occurred in February and March of that year. No nests were detected in the 1995 surveys despite numerous trips to McCoy Branch, Hansard Mill Branch, and other nearby reference streams. Sculpins in the laboratory stream were monitored during the 1994 and 1995 breeding seasons, but no spawning activity was noted.

The total area of spawning habitat available to banded sculpins in McCoy Branch and Hansard Mill Branch was very similar, 1.21% and 1.14% in McCoy Branch and Hansard Mill Branch, respectively (Table 25). Types of spawning habitat used by banded sculpins are described in the **METHODS: Reproduction** section. Probable banded sculpin nesting sites were found in McCoy Branch and Hansard Mill Branch, and in the flow-through laboratory tank in 1993, fitting the description of the first type of spawning area mentioned previously. Gravid female sculpins

Table 25.

**Comparison of Spawning Habitat Available to Banded Sculpins: McCoy Branch and Hansard Mill Branch.**

| Stream | Spawning Habitat ($m^2$) | Total Stream Area[a] ($m^2$) |
|---|---|---|
| McCoy Branch | 9.6 | 791.1 |
| Hansard Mill Branch | 9.2 | 806.3 |

a. Total stream area incorporates the sample sites on both streams, and the surrounding areas.

were found utilizing the undercut areas described above at both streams, and at other reference streams during the 1994 and 1995 spawning seasons. Similar types of habitats on Doe Run Creek, Kentucky were proposed as spawning sites by Craddock (1965).

The time of spawning for the spring of 1993 was estimated as February through early March, when water temperature was 9.8 to 10.7 °C in McCoy Branch, and 9.9 to 13.0 °C in Hansard Mill Branch. This estimate of spawning time was based on the size of juvenile sculpins recovered in Hansard Mill Branch in mid March, and the developmental stage of eggs and larval sculpins found in the in-stream laboratory tank in early March. The spawning season was difficult to pinpoint in 1994 due to the previously mentioned large rain events in February and March of that year. In 1995, spawning was estimated to occur during February to mid-March, when water temperature was 9.5 to 10.8 °C in McCoy Branch. Water temperature data were not available for Hansard Mill Branch during this period. Spawning estimates for 1995 were based mainly on the size of juvenile sculpins recovered from the McCoy Branch spring-pool in early March, and from the ripeness of female sculpins collected from McCoy Branch and other reference streams in January and early February. The spring-pool survey conducted in March 1995 resulted in the capture of 10 small juvenile sculpins (1.5 to 1.8 cm TL). Williams and Robins (1970) reported that banded sculpins spawned in the Cahaba River System, Alabama during January to early February, when water temperature was 9.0 to 13.0 °C. A date of February through early April, at water temperatures of 10.0 to 13.5 °C in Doe Run Creek, Kentucky, was reported by Craddock (1965).

Sampling to determine sculpin fecundity was relatively ineffective due to the difficulty in obtaining gravid sculpins. Only two gravid sculpins were collected from Hansard Mill Branch and three from McCoy Branch during sampling from 1993 to 1995. Fish were not taken from McCoy Branch for ovary counts until December of 1994 due to low population densities and low numbers of gravid females. A sample size of five sculpins was deemed insufficient to render any reliable quantitative analysis of banded sculpin fecundity for this study. Craddock (1965), reported an average of 477 eggs per female for banded sculpins in Doe Run Creek, Kentucky.

# IV. DISCUSSION

The goals of this study were (1) to determine whether McCoy Branch had recovered sufficiently from over thirty years of coal flyash deposition to sustain a viable population of fish, and (2) to collect life history data for the banded sculpin which would augment the description of this species colonization efforts in that system. Initially, these goals were achieved by the survival of the introduced banded sculpins, and finally, in the description of their successful colonization of upper McCoy Branch. This study was not designed to assess cause and effect, but sufficient circumstantial evidence indicated that coal flyash sedimentation was an important contributor to the low survival rates for banded sculpins during the first year of the study and the slow rate of colonization of upper McCoy Branch. The abundance patterns of macroinvertebrates, selectivity patterns in the diets of the sculpins, and the extensive embeddedness associated with stream substrates most likely resulted in deficiencies in energy stores, limited habitat availability, and a possible lowering of reproductive capacities for the banded sculpins introduced into McCoy Branch.

Banded sculpins introduced in October 1992 rapidly dispersed throughout upper McCoy Branch, covering distances up to 300 m by early January 1993. These were large-scale movements for a fish reported to have a maximum home range of approximately 6 m of stream (47 $m^2$ surface area, Greenberg and Holtzman 1987). Relatively large numbers of sculpins (90 fish/55 $m^2$) were introduced to McCoy Branch, and the fish may have dispersed in an attempt to reduce densities at the two release sites. Stauffer (1983) indicated that dispersal is one of the mechanisms employed by fish species to decrease the effects of high densities. Wide dispersal is not uncommon for species colonizing a new ecosystem, especially in areas where few competitor species are present. While there was a significant dispersal of the banded sculpins throughout upper McCoy Branch, I found no evidence of the accompanying population explosion common to invasive species referred to by MacArthur (1960) and Hocutt and Hambrick (1973).

Some indications of recovery in the upper McCoy system were noted, including improvements in water quality and changes in the macroinvertebrate community (Hinzman 1992, Tolbert and Smith 1992). The timing of the banded sculpin introductions was intended to coincide with the apparent recovery of upper McCoy Branch, but the low sculpin densities and colonization rates were an indication that one or more important factors continued to retard the recovery of this system. An indication of the causative factor in the low rate of recovery of this system can be seen by examining the migration of a considerable number of sculpins to the spring-pool. Even though the habitat for banded sculpins appeared to be less suitable than other areas of the stream, these fish populated this area in deference to the main stem of McCoy Branch. The spring-pool was not subject to the large-scale influxes of sediments that were common in main-stem McCoy Branch, and the flow regimes and water temperature were probably not as variable. While there was some sedimentation associated with the substrate in the spring pool, there was much less canopy cover at the spring-pool (50.9%) when compared to McCoy Branch (98.2%). In streams with large amounts of fine sediment deposits, increased percent riparian canopy resulted in lower macroinvertebrate abundance and sculpin densities (Hawkins et al. 1983). Thus, it is plausible to conclude that the initial low survival and colonization rates of the introduced sculpins could have been due at least in part to the persistently high sediment loads in McCoy Branch.

The coal flyash sedimentation problems that persist in upper McCoy Branch affected the introduced sculpins by filling in areas used for cover by these fish. In Greenburg and Holtzman (1987), banded sculpins in an eastern Tennessee stream used rock substrate as cover, especially during the daylight hours. Stream bottom sediments can fill up the interstitial areas between rocks and limit access to this type of cover to banded sculpins. This phenomenon occurred with regularity in upper McCoy Branch, especially in depositional areas such as pools and deeper runs. Niemi et al. (1990) revealed that alterations to physical habitat require the longest recovery periods, and that the recovery process involves adjusting to changes in the system's carrying capacity. Loss of physical habitat in upper McCoy Branch was related to the silt accumulation in the interstitial areas of the substrate. The siltation of substrates such as large cobbles and small boulders denied access for banded sculpins to these areas, which other studies have shown to be used extensively by banded sculpins (Greenberg and Holtzman 1987). McCoy Branch was shown to be already deficient in these types of substrate when compared with Hansard Mill Branch, and the siltation of these areas only increased this deficiency. After the introduction, the sculpins were recaptured predominantly from areas containing root wads, undercut banks, and woody debris; utilization of these areas for cover in McCoy Branch represents a shift from the types of cover frequented by the sculpins in Hansard Mill Branch. Thus it is likely that the introduced banded sculpins, in adapting to the new environment, were using root wads and undercut banks for cover due to the paucity of large rock substrate in upper McCoy Branch.

Numerous papers indicate the negative effects of sedimentation on macroinvertebrate densities and abundance (Brusven and Prather 1974, Luedtke and Brusven 1976, McClelland and Brusven 1980, Murphy et al. 1981, Lemly 1982, Hawkins et al. 1983). High sediment loads are known to effect macroinvertebrates, a major food resource of the banded sculpin, by accumulating particles on respiratory structures and body surfaces (Lemly 1982), restricting access to interstitial areas in substrate (Lemly 1982, McClelland and Brusven 1980), and causing difficulty in locomotion and foraging activities (Luedtke and Brusven 1976). In the study by McClelland and Brusven (1980), certain species of trichopterans were most sensitive to the effects of sediment loading, followed by plecopterans and ephemeropterans, respectively. Specialized burrowing forms of dipterans, such as chironomids and tipulids, were not affected by sedimentation of the intersticial areas in stream substrates (Brusven and Prather 1974). These sensitivity relationships correlate directly with the ratios of the different types of macroinvertebrates found in the food resource studies at the four sites. The different types of food resources available at McCoy Branch forced the introduced sculpins to adapt their diet to include the more sediment-tolerant species found in that system.

Of the flyash metals found at elevated concentrations in upper McCoy Branch sediments in 1993, such as arsenic, barium, iron, molybdenum, and nickel, only arsenic was detected at levels high enough to affect benthic macroinvertebrates or fish. It is likely that the somewhat depressed population densities of sculpins in McCoy Branch (when compared to the spring-pool and Hansard Mill Branch) are mostly due to the high sediment loads and to a lesser extent, the presence of contaminants in stream sediments. The siltation of a stream in eastern Kentucky caused an immediate decline in fish that fed on aquatic macroinvertibrates, and a decline in YOY numbers in all fish species (Lotrich 1973). Also, it was predicted that continued siltation of the stream would cause a gradual decline in all fish species. Body burden analyses of McCoy Branch fish could be used to determine the extent of bioaccumulation of coal flyash elements, but these methods were beyond the scope of this study.

The survival and subsequent dispersal of the banded sculpins throughout the upper McCoy Branch system indicated the eventual success of the introduction effort. It was evident that the introduced sculpins had adapted to the changes in physical habitat and the differences in food resources presented by the new ecosystem. However, until the fall of 1994, evidence of reproduction was not seen in McCoy Branch even though the fish were observed in gravid condition and suitable spawning habitat was available. The spawning habitat ratio was slightly higher for McCoy Branch (1.21%), than for Hansard Mill Branch (1.14%), yet little production of YOY fish was recorded for the McCoy Branch system.

Macroinvertebrate abundances and banded sculpin selectivity patterns in upper McCoy Branch indicated that there may have been deficiencies in diets of the introduced sculpins in McCoy Branch. It appeared that the introduced sculpins in adapting to their new habitat used the more sediment-tolerant macroinvertebrates which were available in greater densities as their primary food resources. Chironomids constituted a major portion of the diets of the introduced sculpins. These aquatic macroinvertebrates are generally much smaller than other groups, such as trichopterans and plecopterans, and therefore are lower in food value to the fish. Consuming smaller food items such as chironomids possibly led to a decrease in the amount of energy available for spawning. Petrosky and Waters (1975) found a significant reduction in YOY slimy sculpin production in response to a large increase in the silt load the previous year. The introduced sculpins may have been faced with an ecological dilemma, whether to allocate an already reduced energy supply to growth or reproduction. The good individual condition of McCoy Branch fish and the apparent lower reproductive capacity, as reflected by the reduced colonization rates, suggest allocation of energy to growth rather than to reproduction. Craddock (1965) reported that most banded sculpins do not spawn until their second year, a K-selected strategy which would favor early growth as opposed to supplying that energy for reproduction.

The discovery of banded sculpins in the spring-pool indicated that the previously missing 1993 and 1994 year classes were present, and that YOY production rates while low, were similar to those found at Hansard Mill Branch. Small juvenile sculpins (1.5 to 1.8 cm TL) collected from the spring-pool in upper McCoy Branch in March of 1995 provided the final proof that the introduced sculpins were successfully reproducing in McCoy Branch.

The introduction of sculpins to upper McCoy Branch provided a recolonization source, where none was previously available, to the few sculpins that existed in that system prior to the study. Courtenay and Hensley (1980) reported that multiple introductions offer a higher degree of success in the colonization of new ecosystems. Stauffer (1980) stated that: "If a species successfully colonizes a particular area, then the new habitat must obviously contain conditions that are within the physiological tolerances of the organism and posses the essential requirements for its survival and propagation." Banded sculpins are a K-selected species, and while the introduced fish are making progress in adapting to the coal flyash sedimentation related alterations of the physical habitat and macroinvertebrate community, it will require more time than allotted for this study for the introduced sculpins to fully realize their reproductive potential and significantly increase their densities in the upper McCoy Branch system. In Niemi et al. (1990), the need for long term studies is suggested as a method for better understanding recovery processes in aquatic systems. In their review of over 150 case studies of recovery in aquatic systems, projected recovery times for the genus *Cottus* (with a source for recolonization) ranged from one to seven years. A continuation of this study would be required to describe the complete recovery of this system from past perturbations.

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# APPENDIXES

Appendix 1.

Food Resources: McCoy Branch and Hansard Mill Branch.

| Family | Density (#/0.1m²) | | | |
| --- | --- | --- | --- | --- |
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Oligochaeta | 15.6 | 59.9 | 328.3 | 44.3 |
| Ephemeroptera sp. | 2.6 | 10.4 | 2.6 | 20.8 |
| Baetidae | — | 13.0 | 302.1 | 18.2 |
| Ephemerellidae | 205.7 | 13.0 | 2.6 | 67.7 |
| Ephemeridae | — | — | — | 26.0 |
| Heptageniidae | — | — | — | 23.4 |
| Leptophlebidae | 5.2 | 5.2 | 13.0 | 210.9 |
| Oligoneuridae | — | — | — | 2.6 |
| Gomphidae | — | — | 2.6 | — |
| Anisoptera sp. | 2.6 | 5.2 | — | — |
| Cordulegasteridae | 7.8 | — | — | — |
| Plecoptera sp. | 13.0 | 2.6 | 20.8 | 18.2 |
| Chloroperlidae | — | — | — | 2.6 |
| Leuctridae | 26.0 | 18.2 | 33.9 | — |
| Nemouridae | 2.6 | 5.2 | 2.6 | — |
| Peltoperlidae | 2.6 | — | 26.0 | — |

Appendix 1.

Food Resources: McCoy Branch and Hansard Mill Branch.

| Family | Density (#/0.1m²) | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Perlidae | — | — | — | 5.2 |
| Perlodidae | 2.6 | — | 88.5 | 96.4 |
| Taeniopterygidae | 10.4 | — | — | — |
| Hebridae | — | — | — | 2.6 |
| Corydalidae | 5.2 | 2.6 | — | 5.2 |
| Sialidae | — | — | 2.6 | — |
| Trichoptera sp. | 7.8 | 2.6 | — | 2.6 |
| Glossostomatidae | 7.8 | 5.2 | 54.7 | 5.2 |
| Hydropsychidae | 130.2 | 161.5 | 54.7 | 26.0 |
| Limnephillidae | 93.8 | 109.4 | 2.6 | 10.4 |
| *Pycnopsyche* sp. | 13.0 | 10.4 | — | — |
| Polycentropodidae | — | — | 33.9 | — |
| Psychomyiidae | — | — | — | 2.6 |
| Rhyacophilidae | — | — | 2.6 | — |
| Sericostomatidae | 5.2 | — | — | — |
| Elmidae | 80.7 | 224.0 | 1593.8 | 398.4 |
| Psephenidae | — | — | — | 65.1 |
| Ptilodactylidae | 2.6 | 10.4 | 13.0 | — |
| Diptera sp. | 18.2 | 91.1 | 36.5 | 31.3 |

Appendix 1.

Food Resources: McCoy Branch and Hansard Mill Branch.

| Family | Density (#/0.1m²) | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Ceratopogonidae | 7.8 | 7.8 | 72.9 | 7.8 |
| Chironomidae | 432.3 | 1682.3 | 187.5 | 226.6 |
| Simulidae | 7.8 | 26.0 | 10.4 | 5.2 |
| Tabanidae | 20.8 | 2.6 | 2.6 | — |
| Tipulidae | 5.2 | 20.8 | 13.0 | 18.2 |
| Lepidoptera sp. | — | — | 2.6 | — |
| Amphipoda sp. | — | 10.4 | 539.1 | — |
| Isopoda sp. | — | — | 419.3 | 18.2 |
| Decopoda sp. | — | — | — | 5.2 |

Appendix 2.

Stomach Contents: Frequency of Occurrence.

McCoy Branch and Hansard Mill Branch.

| Family | Frequency of Occurrence[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Oligochaeta | 16.7 | 37.5 | 25.0 | — |
| Ephemeroptera sp. | 16.7 | 25.0 | 12.5 | 66.7 |
| Baetidae | 33.3 | 25.0 | 25.0 | 50.0 |
| Ephemerellidae | — | — | — | 16.7 |
| Heptageniidae | — | — | — | 16.7 |
| Oligoneuridae | — | — | — | 16.7 |
| Anisoptera sp. | — | 37.5 | 12.5 | 16.7 |
| Calopterigidae | — | 25.0 | 12.5 | — |
| Zygoptera sp. | — | 12.5 | — | 16.7 |
| Cordulegasteridae | — | 12.5 | — | — |
| Plecoptera sp. | 33.3 | — | 12.5 | 50.0 |
| Leuctridae | 16.7 | — | — | — |
| Perlidae | — | — | 12.5 | — |
| Perlodidae | — | — | — | 16.7 |
| Corydalidae | 16.7 | 12.5 | — | 16.7 |
| Sialidae | — | — | — | 16.7 |
| Trichoptera sp. | 33.3 | 75.0 | 37.5 | 66.7 |
| Glossostomatidae | — | — | 25.0 | — |

Appendix 2.

Stomach Contents: Frequency of Occurrence.

McCoy Branch and Hansard Mill Branch.

| Family | Frequency of Occurrence[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Hydropsychidae | — | 12.5 | 37.5 | 50.0 |
| Limnephillidae | — | 25.0 | — | — |
| *Pycnopsyche* sp. | 33.3 | 25.0 | — | — |
| Philopotamidae | 16.7 | — | — | — |
| Psychomyiidae | — | — | — | 16.7 |
| Elmidae | — | — | 62.5 | 33.3 |
| Psephenidae | — | — | 12.5 | 16.7 |
| Diptera sp. | 16.7 | 12.5 | 37.5 | — |
| Chironomidae | 16.7 | 37.5 | 25.0 | 33.3 |
| Simulidae | — | — | 12.5 | — |
| Tipulidae | 33.3 | 12.5 | 12.5 | — |
| Lepidoptera sp. | 16.7 | — | 12.5 | — |
| Amphipoda sp. | — | — | 37.5 | — |
| Isopoda sp. | — | — | 62.5 | 50.0 |
| Decopoda sp. | — | 25.0 | — | 16.7 |
| Caudata sp.[b] | — | 25.0 | — | — |

Appendix 2.

Stomach Contents: Frequency of Occurrence.

McCoy Branch and Hansard Mill Branch.

| Family | Frequency of Occurrence[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Daphnia sp. | — | — | 25.0 | — |
| Cyprinidae sp.[c] | — | — | 12.5 | — |

a. Proportion of the population ingesting a particular prey item.

b. Species of salamander: (*Eurycea bislineata*).

c. Species of fish: (*Rhinichthys atratulus*).

Appendix 3.

Stomach Contents: Percent Compostion by Number.

McCoy Branch and Hansard Mill Branch.

| Family | Percent Compostion by Number[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Oligochaeta | 3.0 | 7.2 | 6.9 | — |
| Ephemeroptera sp. | 6.1 | 3.7 | 1.7 | 16.9 |
| Baetidae | 36.4 | 3.7 | 8.6 | 13.8 |
| Ephemerellidae | — | — | — | 7.7 |
| Heptageniidae | — | — | — | 1.5 |
| Oligoneuridae | — | — | — | 1.5 |
| Anisoptera sp. | — | 7.2 | 1.7 | 1.5 |
| Calopterigidae | — | 7.2 | 1.7 | — |
| Zygoptera sp. | — | 1.8 | — | 3.1 |
| Cordulegasteridae | — | 1.9 | — | — |
| Plecoptera sp. | 6.1 | — | 1.7 | 7.8 |
| Leuctridae | 3.0 | — | — | — |
| Perlidae | — | — | 1.7 | — |
| Perlodidae | — | — | — | 1.5 |
| Corydalidae | 3.0 | 1.9 | — | 1.5 |
| Sialidae | — | — | — | 1.6 |
| Trichoptera sp. | 6.1 | 16.2 | 5.2 | 12.3 |
| Glossostomatidae | — | — | 5.2 | — |

Appendix 3.

Stomach Contents: Percent Compostion by Number.

McCoy Branch and Hansard Mill Branch.

| Family | Percent Compostion by Number[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Hydropsychidae | — | 1.9 | 5.2 | 4.6 |
| Limnephillidae | — | 3.7 | — | — |
| *Pycnopsyche* sp. | 15.2 | 12.5 | — | — |
| Philopotamidae | 3.0 | — | — | — |
| Psychomyiidae | — | — | — | 1.5 |
| Elmidae | — | — | 15.5 | 4.7 |
| Psephenidae | — | — | 1.7 | 1.5 |
| Diptera sp. | 3.0 | 1.9 | 5.2 | — |
| Chironomidae | 3.0 | 8.9 | 5.2 | 3.1 |
| Simulidae | — | — | 3.5 | — |
| Tipulidae | 9.1 | 5.5 | 1.7 | — |
| Lepidoptera sp. | 3.0 | — | 1.7 | — |
| Amphipoda sp. | — | — | 6.9 | — |
| Isopoda sp. | — | — | 8.7 | 12.3 |
| Decopoda sp. | — | 10.8 | — | 1.6 |
| Caudata sp.[b] | — | 7.2 | — | — |

Appendix 3.

Stomach Contents: Percent Compostion by Number.

McCoy Branch and Hansard Mill Branch.

| Family | Percent Compostion by Number[a] | | | |
|---|---|---|---|---|
| | MCK 2.12 | MCK 1.97 | HMK 1.23 | HMK 0.41 |
| Daphnia sp. | — | — | 6.9 | — |
| Cyprinidae sp.[c] | — | — | 1.7 | — |

a. Percent Compostion by Number is the relative abundance of a food item in the fishes diet.

b. Species of salamander: (*Eurycea bislineata*).

c. Species of fish: (*Rhinichthys atratulus*).

VITA

Brian Allan Carrico was [redacted] He graduated in June of 1979 from Washington and Lee High School, Arlington, Virginia. In September 1990 he enrolled in Northern Virginia Community College, and after attending that institution intermittently until August 1986, transferred to the University of Tennessee, Knoxville. In December 1989, he received a Bachelor of Science degree in Wildlife and Fisheries Science from the University of Tennessee. Mr. Carrico re-entered the University of Tennessee in January of 1991 and subsequently received a Master of Science degree in Life Sciences in December 1995.

Mr. Carrico is presently employed as an aquatic ecologist with Jaycor Inc., in the Environmental Sciences Division at Oak Ridge National Laboratory, Oak Ridge, Tennessee. At the time of this writing, Mr. Carrico has a wife, Michelle, of six years, and a four and a half year old daughter named Hope.

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