## EVALUATION OF RAINBOW TROUT STOCKING

## IN A NORTHEASTERN OKLAHOMA

## OZARK STREAM

By

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# PREFACE

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All chapters of this dissertation were written as manuscripts that will be submitted to peer-reviewed journals. Therefore, each chapter follows the style and guidelines of the respective journal in which it was intended to be submitted.

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### CHAPTER I

# PERFORMANCE OF HATCHERY RAINBOW TROUT STOCKED IN AN OZARK STREAM

Abstract: We evaluated the potential for stocked rainbow trout *Oncorhynchus mykiss* to establish a naturalized population in a small, spring-fed, northeastern Oklahoma Ozark stream. The stream was separated from the reservoir into which it drained (Lake Eucha) for the majority of the year by an area of subsurface flow. We experimentally stocked individually tagged rainbow trout into the stream from November 2000 to March 2001 and November 2001 to March 2002, and resampled trout monthly between January 2001 and October 2002 using electrofishing methods. Floy anchor and visible implant elastomer tag retention in stocked rainbow trout was greater than 90% over six months. Movement of stocked rainbow trout among pools in the stream increased with increases in stream discharge, and some stocked trout moved into Lake Eucha in the second year of stocking during high flow associated with rain events. Daily maximum temperatures in pool habitats exceeded upper lethal temperature limits for rainbow trout (25°C) in summer months. Apparent survival of stocked rainbow trout was negligible over the course of our study, which was likely due to mortality associated with high summer temperatures. Monthly survival estimates support patterns in both temperature- and flowrelated losses of stocked trout from the stream. Stocked rainbow trout generally lost weight and lipid reserves after stocking, and trout stocked earlier in the stocking season (November and December) lost less weight than trout stocked in February and March.

### **Introduction**

Rainbow trout *Oncorhynchus mykiss* are a widely stocked sport fish and have become naturalized in 35 states in which the species is not native (Rahel 2000). In Oklahoma, trout (primarily rainbow) are stocked in eight locations to support two tailrace fisheries, three seasonal stream fisheries, and three seasonal small lake fisheries (J. Vincent, Oklahoma Department of Wildlife Conservation, personal communication). Seasonal fisheries are located in southern areas of the state, where high water temperatures do not allow oversummer survival. In recent years, angling groups have requested that rainbow trout be stocked into Ozark streams of northeastern Oklahoma to create additional fisheries on leased, privately-owned land. Baseflow to these streams is provided by numerous natural springs and seeps, and water temperatures remain relatively cool throughout summer months.

Concerns have been raised by fisheries managers and conservation groups that rainbow trout could avoid lethal summer water temperatures by using springs as thermal refuge. If stocked rainbow trout were able to survive over-summer, they could have long-term impacts on native fish populations. In a coolwater Arkansas stream where rainbow trout were stocked, potential long-term impacts on native fishes were limited by low survival of stocked trout due to both intense fishing pressure and low feeding rates (Ebert and Filipek 1991). Hatchery salmonids may lose weight and decrease in condition after stocking (Ersbak and Haase 1983; Metcalf et al. 1997; Weiss and Schmutz 1999), possibly because of insufficient consumption of natural food items (Ersbak and Haase 1983) or increased energy expenditure in the stream environment (Metcalf et al. 1997). However, stocked rainbow trout have become naturalized in coldwater streams of the

southern Appalachians, and considerable research has been directed toward trying to understand effects of rainbow trout on native brook trout populations (Ensign et al. 1989; Lohr and West 1992; Larson et al. 1995; Strange and Habera 1998).

Movement of rainbow trout both within and out of the system in which they were stocked could increase potential impacts on native fishes. Movement of hatchery salmonids is often limited, with a majority of stocked fish recaptured in close proximity to the area in which they were stocked (Kendall and Helfrich 1980; Helfrich and Kendall 1982; Weiss and Schmutz 1999). However, hatchery rainbow trout have been documented to move distances over 10 km under both natural and artificial flow conditions (Moring 1993; Bettinger and Bettoli 2002).

It was unclear if stocked rainbow trout could survive the environmental conditions of Ozark streams. Our objectives were to evaluate rainbow trout performance in a small northeastern Oklahoma Ozark stream by evaluating 1) movement and habitat use of stocked rainbow trout, 2) survival of stocked rainbow trout, and 3) relationships between rainbow trout abundance and stream discharge and temperature conditions.

### **Methods**

### *Trout Stocking*

We stocked Emerson strain rainbow trout into Brush Creek at a rate of 500/mo from November 2000 to March 2001 and November 2001 to March 2002. Brush Creek is a small (mean width, 9 m), spring-fed stream in the Ozark highlands of northeastern Oklahoma (36°23' N, 94°47' W). It is about 10 km long and drains into Lake Eucha, an impoundment created to provide water to the city of Tulsa. The stream is intermittent

immediately upstream of the confluence with the lake; flow is subsurface for the majority of the year, connecting with the reservoir only during periods of high flow. The intermittent nature of Brush Creek made it an attractive study site for this project because it approximated a closed system, allowing limited emigration of rainbow trout from Brush Creek.

We designed stocking procedures (stocking density, frequency, and practice of stocking all trout into one location) to approximate methods that would be used by trout angling groups leasing private property. Prior to stocking, we anesthetized (tricaine methanesulfonate, MS-222), measured (mm total length, TL), and weighed (massing) rainbow trout at Crystal Springs Trout Farm in Cassville, Missouri. In both years of stocking, we marked trout with individually numbered Floy FD-68B anchor tags. We used a different tag color for each month of stocking to facilitate identification of stocking groups during sampling. Our phone number was printed on anchor tags in case any trout were caught by anglers, but we did not advertise stocking procedures or offer a reward for angler returns.

In the first year of stocking, we double-marked all rainbow trout with an adipose fin clip, and stocked all trout into one stocking site located 5.7 km from the confluence of Lake Eucha. In the second year of stocking, we implemented a slightly different stocking strategy. During the first year of stocking we observed that temperatures at a pool located 3.8 km upstream from the stocking site were more constant and summer temperatures were not as high as downstream sites. However, most of the habitat separating the stocking site and this upstream pool was shallow, and relatively few rainbow trout reached this upstream site in the first year of stocking. We wanted to see if

rainbow trout stocked directly into this site would have different patterns of movement and survival than trout stocked at the original stocking site. Therefore, in the second year of stocking, we repeated the stocking protocol used in the first year at the primary stocking site but also stocked about 50 individuals on each stocking into the pool located 3.8 km upstream (secondary stocking site). Both stocking sites were large, bedrockformed lateral scour pools with fractured bedrock cover.

We used visible implant elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, Washington) tags as a second mark to evaluate anchor tag loss in stocked rainbow trout in the second year of stocking. Each stocking group received unique VIE batch tags. Trout stocked from November 2001 to February 2002 received a tag on the left pectoral fin, with a different color for each stocking group (green in November, pink in December, yellow in January, and orange in February). We had only four VIE colors, so we marked the March 2002 stocking group with a green tag located on the anal fin. Rainbow trout stocked into the secondary stocking site received another mark streamside to differentiate between trout stocked into primary and secondary locations (same color as the initial VIE tag for each stocking group, located on the left ventral fin in trout stocked November 2001 to February 2002 and the lower caudal fin for trout stocked in March 2002). Presence of anchor tags also allowed us to evaluate retention of VIE tags.

After measuring and marking all rainbow trout, we held them in a raceway at the hatchery overnight and checked for mortalities the following morning. Trout were loaded into a 836 L hauling tank and transported for about 3 h to Brush Creek.

# *Field Sampling*

We initially planned to evaluate abundance and movement of stocked rainbow trout using snorkeling methods, because we were concerned that electrofishing rainbow trout at the stocking site might cause mortality and adversely affect our ability to conduct this study. We snorkeled in November and December 2000, but it became apparent that this sampling method was not efficient and could not yield individual-level information. In January 2001, we began sampling stocked rainbow trout in pool habitats approximately monthly using boat electrofishing. We continued to use these methods through the completion of our study in October 2002, and we do not believe that electrofishing contributed significantly to mortality of stocked rainbow trout (Walsh et al. in press). Some of the sampling was directed specifically at capturing trout (targeted sampling), whereas other sampling was for fish assemblage structure, or to estimate the number of trout at the stocking site. Each of these sampling procedures is described below.

We sampled the fish assemblage and stocked rainbow trout each season in riffle, glide, and pool habitats. We used an electric seine to sample riffle and glide habitats and a boat electrofisher to sample pool habitats. At all sites, we blocknetted the area prior to sampling and took two electrofishing passes (60-Hz AC, 3-4 A). In months when we did not sample assemblage structure, we conducted targeted sampling for stocked rainbow trout and native sport fish (smallmouth bass *Micropterus dolomieu* and shadow bass *Ambloplites ariommus)* in pool habitats. Two pools located upstream of the primary stocking site [0.1 and 3.8 km (secondary stocking site)] and three pools located downstream of the stocking site (0.4, 1.5, and 2.6 km) were sampled on almost all trout resampling trips. All of these pools were bedrock-formed lateral scour pools with

fractured boulder cover, except the pool located 0.1 km upstream of the primary stocking site, which was made up of a series of rootwad-formed lateral scour pools with rootwad and woody cover. In months when rainbow trout were not stocked, we sampled the primary stocking site during the targeted trout sampling.

In months when stocking took place (starting in January 2001), we sampled the primary stocking site on the day of trout stocking to estimate the number of rainbow trout from previously stocked groups that were still present in the pool. We blocknetted the site prior to stocking and waited approximately two hours after trout were stocked before electro fishing. Based on numbers of recaptures of each stocking group, we used a Lincoln-Peterson single-census mark-recapture estimate to calculate how many rainbow trout from each stocking group remained at the stocking site relative to the known number of trout that we had just stocked (about 500). During the second year of stocking, we used these procedures at the primary and secondary stocking sites. Electrofishing methods for targeted sampling and sampling to estimate the number of rainbow trout remaining at the stocking site were similar to assemblage sampling methods, except we generally took three electrofishing passes to calculate depletion estimates of trout abundance.

We used mark-recapture data from those sampling events to evaluate change in weight and lipid content of stocked rainbow trout, evaluate trout abundances over time in the six major pools, relate abundance in individual pools to patterns in discharge and temperature, and evaluate tag retention and estimate monthly survival of stocked rainbow trout.

## *Analyses*

*Change in weight and lipids.* We evaluated weight change in stocked rainbow trout by identifying trout with anchor tag numbers and calculating change in weight for each individual trout at each time period that it was recaptured. We averaged change in weight for all individuals of a stocking group recaptured at each point in time for which we had at least 10 individuals in the sample. If the number of recaptures of a given stocking group was <10, we did not include mean change in weight in our results. We did not calculate mean change in weights for trout stocked in January 2001 because we were unable to correctly weigh this group prior to stocking due to an equipment malfunction. We used linear regression to evaluate the relationship between weight change and residence time in the stream for stocking groups in both years (except January 2001). We compared slopes among stocking groups within each year (PROC GLM, SAS 2000) to evaluate if the month of stocking influenced weight change in stocked rainbow trout.

We evaluated change in lipid content of stocked rainbow trout over time in the first year of stocking. In February and March 2001, we sacrificed five rainbow trout from each stocking group captured, representing a range of residence times in the stream. We sacrificed five fish from the hatchery that were not stocked to determine baseline lipid concentration in rainbow trout at the time of stocking. We froze the carcasses for later lipid extractions following Hamilton et al. (1992). We ground individual fish using a meat grinder and extracted 2-3 samples per fish for lipid analysis. Samples (wet weights 2.5 - 3.0 g) were placed on weighed filter paper and dried in an oven at 75° C for approximately 12 hours. We recorded dry weights of each sample prior to extraction in petroleum ether for 24 hours. After extraction, samples were first air dried for 30

minutes, then dried in an oven for one hour. We recorded extracted dry weights and computed lipid content as the difference in dry weights before and after extraction. Results were expressed as the mean proportion of lipid content for each stocking group. We used linear regression to assess the relationship between lipid content and residence time in the stream.

*Rainbow trout CPUE and relationships with discharge and temperature.* We evaluated movement and survival of stocked rainbow trout using catch-per-unit-effort (CPUE, fish/min) through time at the six pools described above. We performed Spearman rank correlations (PROC CORR, SAS 2000) to evaluate if changes in rainbow trout abundance in individual pools over time were related to mean or maximum discharge  $(m^3/s)$  or mean or maximum temperatures ( ${}^{\circ}$ C) that occurred between sampling intervals. We used provisional hydrograph data to calculate mean and maximum discharge between sampling intervals. Because there was no U.S.G.S. gauge station on Brush Creek we used gauge data for Beaty Creek (U.S.G.S gauge number 07191222) as a surrogate. Beaty Creek is a stream in the same watershed that drains into Lake Eucha approximately 800 m from the confluence of Brush Creek and Lake Eucha and likely experienced similar hydrological conditions to Brush Creek. We used data from fixedpoint temperature loggers (Stowaway® Tidbit®, Onset Computer Corporation, Bourne, Massachusetts) to calculate mean and maximum temperatures between sampling intervals. We set loggers in the primary and secondary stocking sites and in pools located 1.5 and 2.6 km downstream from the primary stocking site. We used data from the primary stocking site for pools located 0.1 km upstream and 0.4 km downstream from the primary stocking site. Loggers measured temperature every four hours. We also

monitored temperature loggers in the inflow, upstream, and downstream of a large natural spring located 0.7 km downstream of the primary stocking site to evaluate how spring inputs influenced downstream temperatures.

*Survival models.* We evaluated monthly survival for each stocking group separately using recapture-only Cormack-Jolly-Seber (CJS; Cormack 1964; Jolly 1965; Seber 1965) survival models with Program MARK (White and Burnham 1999). Based on our knowledge of the system, we used a baseline model of fully time dependent survival and constant recapture probability and compared this model to constant survival and constant recapture probabilities for each stocking group in both years. We used a bootstrap goodness-of-fit test to evaluate if our baseline model was appropriate for each stocking group, and adjusted for overdispersion of data when necessary (Burnham and Anderson 1998). We accounted for known mortalities (for example, fish removed for diet evaluation) and uneven sampling intervals in the survival models. We evaluated the relative fit of models using corrected Akaike's Information Criteria (AICc) weights for each model, adjusted for overdispersion of data [quasi-AICc (QAICc) weights; Burnham and Anderson 1998].

### **Results**

#### *Field sampling*

We stocked about 500 rainbow trout per month from November 2000 to March 2001 and November 2001 to March 2002, although some mortality (1-7 fish) occurred prior to stocking in some months (Table 1). In November and December 2001, we stocked 525 rainbow trout because we removed fish for an electrofishing injury study that took place

in December 2001 (Walsh et al. in press). Mean lengths of stocking groups were close to our target stocking size of 254 mm (Table 1). Differences existed in mean lengths among stocking groups in the first (F = 24.06, df = 2,497, P < 0.0001) and second (F=111.99, df  $= 2,538$ ,  $P < 0.0001$ ) years of stocking (Table 1). This was due to the availability of fish afrom the hatchery. However, length differences were small (maximum difference of 12 mm in  $2000 - 2001$  and  $29$  mm in  $2001 - 2002$ ; Table 1), and mean length among all groups did not differ between the first ( $N = 2498$ , mean = 257, SD = 28) and second ( $N =$ 2542, mean = 257, SD = 27) years of stocking (t = 0.00, df = 4,019, P = 0.9480).

We sampled rainbow trout with electrofishing approximately monthly between January 2001 and October 2002 and obtained relatively high numbers of recaptures in all stocking groups {Table 2). Generally > 100 rainbow trout from each stocking group were recaptured at least once (Table 2). In the second year of stocking, we were able to recapture more rainbow trout because we began sampling immediately after stocking began in November. Although we sampled glide and riffle habitats during seasonal assemblage sampling, we only recaptured stocked rainbow trout in pool habitats. *Change in weight and lipids* 

We were able to obtain at least three months of recapture data for each stocking group to evaluate lipid and weight change over time (Table 3). Patterns in weight change differed among stocking groups in the first ( $F = 48.39$ ; model df = 7, error df = 968, total df = 975; P < 0.0001) and second (F = 29.83; model df = 9; error df = 1555, total df = 1564; P < 0.0001) years of stocking (Figure 1). In the first year of rainbow trout stocking, trout stocked in November and December 2000 fluctuated from a mean weight loss of about 10 g to mean weight gain of about 10 g, and we did not detect a difference

in slopes for weight change for those rainbow trout (Table 4). Trout stocked in February and March declined in weight over time, with trout stocked in March losing an average of 56.3 g/fish by August 2001 (Figure 1). Slopes for weight change in rainbow trout stocked in February and March were negative, indicating that in  $2000 - 2001$  trout stocked later lost weight compared to trout stocked earlier (Table 4). Lipid content in stocking groups from the first year decreased significantly with residence time for rainbow trout sampled in February (slope =  $-0.0014$ ,  $P < 0.0001$ ) and March (slope =  $-$ 0.0013, P <0.0001) 2001 (Figure 2).

During the second year of stocking, we sampled rainbow trout as soon as stocking began, and also sampled all six pools on all sampling trips. This resulted in more recaptures of stocked rainbow trout over a longer period of time than in the first year of stocking (Table 3), although our analyses were limited by low sample size toward the end of the summer and early autumn because few trout were left in the stream. Rainbow trout stocked in November and December 2001 overall gained weight (i.e., had significant positive slopes; Table 4), and gained an average of 37 g/fish by May 2002, but sample sizes were low (Table 3). The slope for weight change for rainbow trout stocked in December 2001 was significantly higher than other stocking groups, and we did not detect a difference in slopes among the other four stocking groups (Table 4). Rainbow trout stocked in January, February, and March 2002 lost weight immediately after stocking but maintained their weight after that point at an average of  $5 - 20$  g below stocking weights. We did not see the same pattern of steady declines in weight in trout stocked in February and March 2002 that we observed in trout stocked in February and March 2001.

#### *Rainbow trout CPUE and relationships with discharge and temperature*

Concentrations of stocked rainbow trout were highest at the primary stocking site throughout the course of our study. We estimated that 891 and 1,295 individual rainbow trout were located in this pool after stocking was completed in the first year and second years, respectively (Table 5). At the secondary stocking site, we estimated 96 individual trout remained in the pool after stocking in March 2002. We estimated that 641 rainbow trout stocked in November 2001 remained at the primary stocking site in December 2001. That estimate exceeded the actual number of rainbow trout stocked because we recaptured more rainbow trout in the December 2001 sample from the November stocking group than the December stocking group.

Our CPUE data showed a pattern of high abundance of stocked rainbow trout at the primary stocking site through March 2001, followed by gradual declines in abundance through summer (Figure 3). Rainbow trout abundance began to increase at pools located upstream and downstream from the stocking site in February 2001, although abundances at these sites were much lower than those at the primary stocking site (Figure 3). At upstream and downstream pools, CPUE increased through May 2001 and then decreased through September 2001 (Figure 3). In October 2001, shortly before the second year of stocking, we recaptured one rainbow trout among the six commonly sampled pool habitats. That individual was missing its anchor tag, so we were not able to identify the month in which it was stocked.

In the second year of stocking, rainbow trout abundance at the primary stocking site peaked in January 2002 and declined through spring and summer (Figure 3). Rainbow trout abundance among other sites was highest at the pool located 0.1 km upstream

(Figure 3). Higher abundances of rainbow trout at the pool located 3.8 km upstream in the second year were attributable to the trout that we stocked at that location rather than increased movement of trout to that location (Figure 3). Pools located downstream of the stocking site had lower abundances of rainbow trout during the second year of stocking than the first year of stocking (Figure 3). In October 2002, we recaptured one rainbow trout among the six commonly sampled pools. That individual had been stocked in March 2002. We sampled those six locations in March 2003 as part of an ongoing monitoring program and recaptured one rainbow trout missing a Floy tag. It had a green left pectoral VIE tag, indicating it was stocked in November 2001.

Hydrograph data showed that discharge differed during the two years that we stocked rainbow trout (Figure 4). In the first year of stocking, water levels remained low through the first three months of stocking. Between late January and early February, three flood events occurred, including a major flood event on 24 February 2001 in which discharge reached 89.7  $m^3$ /s. After those flood events, discharge returned to low levels and remained low through September 2001. In the second year of rainbow trout stocking, the hydrograph was characterized by more frequent, but less severe, discharge events (Figure 4). Rain occurred fairly often between February 2002 and June 2002, including a number of closely spaced rain events in late March and early April, and late May and early June. Rainbow trout abundance (CPUE) was positively related to mean  $(r_s = 0.63, P = 0.0035)$ and maximum ( $r_s = 0.54$ ,  $P = 0.0168$ ) discharge at the primary stocking site, and positively related to maximum discharge at the secondary stocking site ( $r_s = 0.0476$ ). Abundance was related to both mean  $(r_s = 0.73, P = 0.0019)$  and maximum  $(r_s = 0.52, P = 0.0019)$ 0.0450) discharge at the pool closest to the primary stocking site (0.1 km upstream),

while abundance was related only to mean discharge at the pools located 0.4 km downstream ( $r_s = 0.48$ , P = 0.0500) and 1.5 km downstream ( $r_s = 0.53$ , P = 0.0365) of the primary stocking site. At the furthest downstream pool, we did not detect any significant relationship between rainbow trout abundance and mean or maximum discharge (P > 0.1467).

Minimum and maximum temperatures recorded at the primary stocking site in the first and second year of trout stocking were 3.1 and 26.0°C, and 4.4 and 27.0°C, respectively. Mean monthly temperatures at the primary stocking site fluctuated from lows of around 8°C in January to highs of about 22°C in August in both years of our study (Figure 5). Temperatures at pools located 1.5 and 2.6 km downstream of the stocking site were similar to those observed at the primary stocking site, while temperatures at the secondary stocking site were less extreme. At this site, temperatures fluctuated from lows of about 12°C in January to highs of about 20°C in August (Figure 5). Temperatures at the natural spring that we monitored remained close to 15<sup>o</sup>C during all times of the year (Figure 5).

Among the six pools that we sampled, rainbow trout abundance was negatively related to both mean  $(r_s = -0.83, P < 0.0001)$  and maximum  $(r_s = -0.82, P < 0.0001)$ temperature between sampling intervals at the primary stocking site, and also at the pool located 0.1 km upstream (mean temperature,  $r_s = -0.61$ ,  $P = 0.0152$ ; maximum temperature,  $r_s = -0.64$ ,  $P = 0.0108$ ). At the secondary stocking site, rainbow trout abundance was negatively related to mean temperature between sampling intervals ( $r_s =$  $-0.54$ ,  $P = 0.0441$ ). We did not detect relationships between rainbow trout abundance and temperature at the three pools located downstream of the stocking site  $(P > 0.6730)$ .

#### *Tag retention and survival models*

Based on mark-recapture data from the second year of stocking, we were able to evaluate Floy anchor and VIE tag retention in stocked rainbow trout. Looking at each stocking group separately, estimated anchor tag retention was  $96 - 98\%$ , and estimated VIE tag retention was  $99 - 100\%$  (Table 6). We were able to recapture  $> 10$  rainbow trout stocked during a three to six mo period for trout stocked in November 2001 (Table 6). Actual VIE tag retention was probably higher than that estimated because the seven encounters of rainbow trout without VIE tags that were stocked in November 2001 were composed of three fish: two fish that were recaptured three times and another that was recaptured once (we were still able to individually identify rainbow trout that had lost VIE tags but retained Floy tags). The three recaptures of rainbow trout stocked in December 2001 that lost VIE tags consisted of two recaptures of one individual and one recapture of another fish. Using the actual number of rainbow trout stocked in November and December 2001 recaptured without a VIE tag, retention was 99.5% for both groups. All rainbow trout recaptured from January, February, and March 2002 stocking groups retained VIE tags.

Combining recapture information for all stocking groups, retention was > 90% for anchor tags and> 95% for VIE tags during six mo (Table 6). Retention of both anchor and VIE tags was lowest after 6 mo, but numbers of rainbow trout recaptured also were lower at this time than in earlier months.

We recaptured 13 rainbow trout that did not have either anchor or VIE tags (5 in) December 2001, 3 in January 2002, 3 in February 2002, and 2 in March 2002) and removed those fish from the stream at each sampling interval. Those fish may have lost

both anchor and VIE tags; however, a small number unmarked fish were stocked in December 2001. Those were fish that were able to move into the hatchery raceway through a gap in the holding screen. We observed those untagged fish before transporting to the stocking site; however, we felt that resorting fish would cause undue stress before stocking and decided to stock the fish with these unmarked individuals. Although those fish could have biased our estimates of tag retention and survival, our observation suggested there were very few of those fish and they probably had little influence on our estimates. After March 2002, we did not recapture any stocked rainbow trout missing both anchor and VIE tags.

We recaptured sufficient numbers of stocked rainbow trout to evaluate monthly survival using a CJS recapture-only survival model for all stocking groups in the second year of trout stocking. The model with fully time dependent survival and constant recapture for all stocking groups showed a pattern of high monthly survival through April 2002, followed by a drop in survival between April and May 2002 (Table 7). That period corresponded with a rain event in April, and we hypothesize that increased flow may have contributed to loss of rainbow trout from the system. Because we felt that increased flow in April caused trout to move from the stream, we evaluated another time-dependent model with three survival parameters, one before April, one between April and May, and one for recapture periods after May. We also evaluated a model with constant survival and constant recapture over all time periods. For stocking groups in November 2001, December 2001, and January 2002, time-dependent survival models fit the data better than the constant survival model (Table 8). For February and March 2002 stocking groups, the constant monthly survival model fit the data better due to relatively large

standard errors. Monthly survival was estimated to be 0.64 (standard error,  $SE = 0.08$ ) and  $0.53$  (SE = 0.07) for the February and March 2002 stocking groups, respectively.

Our sampling protocol in the first year of trout stocking did not obtain as much recapture information as in the second year of stocking. We did not begin electrofishing until January 2001, did not sample all sites on each trip, and were also removing individuals from each stocking group as part of a diet study (Fenner 2002). Therefore, the first recapture data that we were able to use were from sampling in April 2001. We did not have enough recapture data from the November 2000 stocking group to evaluate any survival models. For the December 2000 stocking group, a constant survival model fit the data best, and the monthly survival estimate was  $0.68$  (SE = 0.03). Fully timedependent monthly survival models fit the data best for stocking groups in January, February, and March 2002 (Table 9). We did not see the same pattern of decreased survival between April and May that we observed during the second year of stocking, but there appeared to be a decrease in survival between May and June 2002 (Table 10). *Angler returns* 

We did not receive any angler returns of stocked rainbow trout in the first year of stocking. We received one angler return from Brush Creek about 1 km upstream of the confluence of Brush Creek and Lake Eucha, and we received anecdotal accounts of rainbow trout being caught at the confluence of Brush Creek and Lake Eucha. In the second year of stocking, we received 10 tag returns between April 2002 and June 2002 from seven anglers fishing in Lake Eucha. Six of the trout were caught close to the confluence of Brush Creek and Lake Eucha, while the other two moved into the reservoir away from the confluence.

#### **Discussion**

Our results indicate it is unlikely that rainbow trout would become naturalized in Brush Creek. Apparent survival of rainbow trout in this system was very low over the course of our study. We recaptured only one rainbow trout in electrofishing samples in October 2001 and October 2002, seven months after the final stocking in each year. The six pool habitats that we sampled frequently represented a majority of large pool habitat in Brush Creek, but because we were not able to sample the entire stream it is possible that survival of stocked rainbow trout was higher than we estimated. We believe the primary mechanism causing mortality in stocked rainbow trout was an inability to survive summer temperatures, although some stocked rainbow trout moved into Lake Eucha.

Temperature influences mortality and growth of stocked fishes (Wahl et al. 1995). Optimal temperature range for rainbow trout is 12 - 18°C, and the upper lethal temperature for adults is believed to be 25°C (Raleigh et al. 1984). Mean monthly temperatures exceeded l 8°C between May and June in the first year of stocking, and June and July in the second year of stocking. Maximum daily temperatures at the primary stocking site approached or exceeded 25°C between late July and late September in both years and exceeded 26°C on several occasions in late August in both years. McMichael and Kaya ( 1991) observed kills of rainbow trout when maximum daily water temperature exceeded 27°C and also found that catchability of rainbow trout was inhibited at temperatures  $\geq 19^{\circ}$ C.

We observed a significant negative relationship between temperature and rainbow trout abundance at the sites where abundances were highest: the primary stocking site,

the closest pool to the primary stocking site, and the secondary stocking site. At other sites, abundance over time was variable, and no clear pattern between temperature and abundance was observed. Temperatures of natural springs in Brush Creek remained constant at about l 5°C and may moderate temperature in areas of the stream. However, it does not appear that rainbow trout reached and used these areas. Temperature differences that we observed between primary and secondary stocking sites are likely due to increased spring input in the headwaters of the stream and increased canopy cover. We hypothesized that rainbow trout stocked at the upstream site would have higher . survival because of lower summer temperatures, but our data do not support this hypothesis. Only one rainbow trout was recaptured at this site in October 2002, and none in March 2003.

In addition to high summer temperatures, decline of suitable available habitat during summer months also may have contributed to mortality in rainbow trout. Increased mortality in winter months has been attributed to increased population density and decreased food and habitat resources (Whitworth and Strange 1983). In our study, suitable habitat declined during summer rather than winter. Conditions of increased temperature and negligible rainfall occurred between July and October in both years of trout stocking. Many deep-water habitats became isolated, and movement of both rainbow trout and native fishes among habitats was highly restricted. Increased population densities of fish in remaining deep-water habitats may have led to increased interactions between trout and native fishes and possibly limited food resources.

Condition of hatchery fish has been shown to decline after stocking (Ersbak and Haase 1983; Metcalf et al. 1997; Weiss and Schmutz 1999), although causative

mechanisms are not well understood. Declines in condition have been attributed to insufficient natural feeding (Ersbak and Haase 1983) and higher bioenergetic demands on hatchery fish in a stream environment (Metcalf et al. 1997). A bioenergetics model of stream-dwelling rainbow trout predicted low summer growth values, likely because of increased metabolic demands at higher temperatures (Railsback and Rose 1999). In . Brush Creek, stocked rainbow trout fed on natural prey items but at low rates (Fenner 2002), and it is likely that a combination of low prey consumption and increased metabolic demands, especially as water temperatures rose, contributed to the patterns in lipid content and change in weight.

In the first year of stocking, lipid levels in rainbow trout declined steadily following stocking. Patterns in changes in weight of stocked rainbow trout were more pronounced in the first year of stocking, but we saw evidence that trout stocked in November and December of both years lost less weight than trout stocked later in the year. In the first year of stocking, it seemed that rainbow trout stocked later in the year lost weight rapidly, while trout stocked earlier did not. This may be because trout stocked earlier were able to acclimate to stream conditions while water temperatures were still cool, while trout stocked later in the year had less time to adjust before water temperatures began to rise.

In the second year of stocking, individuals stocked in November and December that survived through May 2002 actually gained weight. This pattern of increasing weight through time may be related to mortality of fish that lost weight, resulting in larger individuals left in the stream. Although rainbow trout stocked later in the second year did not show the same decline in weights as in the previous year, they did not gain in weight as did trout stocked in November and December 2001.

Movement of stocked fishes from fishable areas causes economic and recreational losses (Helfrich and Kendall 1982; Moring 1993). Movement from the stocking area becomes particularly important under conditions of privatized fishing on leased land (Weiss and Schmutz 1999). In the case of stocking nonnative fishes, escape of stocked fish may also pose ecological risks (Ham and Pearsons 2001 ). Movement of stocked rainbow trout within Brush Creek was linked to flow conditions in the stream. Rainbow trout abundance was correlated positively with discharge conditions at all locations except the pool farthest downstream. At the two stocking sites, this positive correlation may be due to stocking in spring months when discharge was generally high. However, at the other three pools, positive correlations indicate increased rainbow trout abundance following higher mean or maximum discharges. Our abundance data in the first year of stocking show that trout abundance in all pools increased following rain events in February 2001. Abundance of rainbow trout was highest at sites closer to the primary stocking site, and lowest at the farthest downstream and upstream pools.

In the second year of stocking, flow conditions were consistently higher than in the first year, and patterns in rainbow trout abundance among pools also differed from the first year of stocking. Abundances of rainbow trout increased over time at the pool 0.1 km upstream of the primary stocking site, but abundances were negligible at the three downstream pools. Low-water conditions punctuated by an extreme flood observed in the first year of stocking appeared to be more conducive to movement of rainbow trout within the stream. Conditions of more frequent, less severe rain events observed in the second year of stocking appeared to be more conducive to movement of rainbow trout out of the stream into Lake Eucha.

Inability to survive natural flow conditions has been cited as the reason for failed invasions of introduced species (Meffe 1991; Brown and Moyle 1997; Fausch et al. 2001). Native fishes are adapted to natural flow regimes and may avoid high flows by seeking natural refuges (Pearsons et al. 1992). While it has been suggested that nonnative fish would be less able to control movements under high flows than native fish, Weiss and Kummer (1999) observed that movement of stocked brown trout *Salmo trutta*  was not affected by flood conditions. This also appears to be true of stocked rainbow trout in Brush Creek. We expected that the extreme flood conditions in February 2001 would have displaced stocked trout into Lake Eucha. However, we continued to recapture relatively high numbers of rainbow trout after the flood event, and we did not receive any confirmation that stocked trout had reached the reservoir. It appears that the more consistent flow conditions and frequent rain events during the second year of stocking created higher flows over a longer time period. This may have resulted in greater movement of rainbow trout out of Brush Creek than conditions in the first year, given that we documented rainbow trout recaptures from the reservoir.

Our monthly survival estimates support temperature and flow-related losses of stocked trout from Brush Creek. In the first year of stocking, we did not obtain adequate recaptures to evaluate any possible effects of the large floods in February 2001. The lowest monthly survival was between May and June 2001, which corresponded with an increase in mean monthly temperature to  $> 18^{\circ}$ C. In the second year of stocking, we observed relatively high monthly survival of stocked trout through April 2002, followed by a decrease between April and May. Mean monthly temperatures at that time were 14 l 5°C, well within the optimal range for rainbow trout, so the decrease in apparent
survival was likely not temperature-related. However, a series of moderate rain events occurred in late March and early April, and we began to receive angler tag returns from Lake Eucha during April, indicating that some rainbow trout had been displaced from Brush Creek.

The observed decrease in survival is likely representative of rainbow trout escaping from Brush Creek rather than true mortality. However, another event occurred during this time period that may have contributed to mortality or movement. In April 2002, the landownders of the primary stocking site dredged the pool, deepening it by about 2 m. It is difficult to predict how this event may have influenced rainbow trout mortality or movement during this time period. Following decreases in monthly survival in both years, survival increased, indicating relatively high survival by those fish that remained. By mid- to late summer in both years, we were not able to recapture sufficient numbers of rainbow trout to estimate survival, but our abundance data indicated minimal apparent survival of stocked rainbow trout in Brush Creek through the summer.

In addition to losses of rainbow trout from Brush Creek due to abiotic conditions, other factors likely contributed to the mortality of stocked trout. We do not believe that our electrofishing methods caused delayed sampling-related mortality that could have biased survival estimates (Walsh et al. in press). However, we did have one significant mortality event after sampling in which 64 rainbow trout died (those losses were accounted for in the models). We were not able to measure conductivity at the time of this sample, but we hypothesize that higher conductivity resulted in our standard electrofishing settings causing increased damage to stocked fish. We adjusted electrofishing settings accordingly and did not observe any additional mortalities.

During the course of our sampling on Brush Creek, we observed evidence of both avian (healed wounds on fish) and terrestrial (Floy tags in scat) predation pressure on fish in pool habitats. Primary avian predators would most likely be great blue heron *Ardea herodias* and belted kingfisher *Ceryle alcyon,* while primary mammalian predators would be raccoon *Procyon lotor* and mink *Mustela vison.* Another possible cause of mortality that we did not estimate is fishing pressure. Landowners were aware of our study, and we asked them to return any tags to us or report trout mortality. However, most landowners were not interested in fishing for stocked trout and did not report any tags. In the second year of trout stocking, we received anecdotal evidence from landowners that poaching might be occurring. We are unable to quantify how much mortality might be attributable to illegal fishing, but we do not believe it represents a significant portion of observed mortality.

The survival model that we used does not differentiate between mortality, emigration, or tag loss when estimating survival. However, we do not believe that tag loss led to underestimates of survival in Brush Creek. Retention rates of VIE and anchor tags that we observed fell within ranges reported for salmonids. Our anchor tag retention of 91% for six months is slightly higher than the 89% anchor tags retention for four months in hatchery rainbow trout (140 – 240 mm TL) observed by Mourning et al. (1994). Brewin et al. (1995) found annual anchor tag retention rates of 85% for female and 56% for male brown trout and hypothesized that difference in retention between sexes might be due to agonistic encounters among males during spawning. Our estimate is closer to their estimate for females, and this might be expected because rainbow trout in our study did not spawn. Retention of anchor tags for stocked rainbow trout was higher than that of

native smallmouth bass in Brush Creek (48% for four months, Walsh and Winkelman in press), likely due to differences in habitat use between the two species. Smallmouth bass were closely associated with bedrock boulder or woody cover, while stocked rainbow trout were most often observed in open water. Anchor tag retention in stocked rainbow trout appeared to decrease over time, probably due to decreasing numbers of fish in the stream.

Visible implant elastomer tag retention was close to 100% in the stocked rainbow trout despite extensive handling within 24 h of tagging (hauling fish at high densities, netting fish into the stream, sampling numerous stocked trout with electrofishing on the day of stocking). Previous studies also have found high VIE tag retention in adult (Hale and Gray 1998) and juvenile (Bonneau et al. 1995) salmonids. In hatchery rainbow trout  $(80 - 314$  mm TL), Hale and Gray  $(1998)$  found  $94 - 97\%$  VIE tag retention over a 30 d period and observed that VIE tag loss may increase with time. We did not observe any pattern of increasing VIE tag loss with time, and only five fish of 1,115 fish recaptured lost VIE tags.

#### **Conclusions**

Given the low apparent survival of stocked rainbow trout, we feel there is little chance that rainbow trout would be able to establish a naturalized population in Brush Creek. Condition of stocked rainbow trout decreased after stocking; mortality was high, and would be expedited under conditions of a put-and-take seasonal fishery. However, even in this small stream where potential movement was restricted by isolation of the stream for the majority of the year, we documented movement of stocked rainbow trout

into the reservoir. It would be difficult to ensure that stocked trout would remain within fishable areas in a privatized fishing situation, and movement of stocked trout within a watershed, particularly in larger streams, seems likely.

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Table 1. Number of hatchery rainbow trout stocked, anchor tag color, mean length and length range, and mean weight and weight range for all groups of rainbow trout stocked into Brush Creek November 2000-March 2001 and November 2001- March 2002.



 $\mathcal{A}^{\mathcal{A}}$ 

Table 2. Number of times individual stocked rainbow trout from each stocking group were recaptured from Brush Creek with electrofishing methods between January 2001 and October 2002.

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Table 3. Sample sizes of recaptured rainbow trout from each stocking group in Brush Creek used to calculate mean change in weights for the first (November 2000- March 2001) and the second (November 2001 - March 2002) year of stocking.

 $\bar{\mathcal{L}}$ 



Table 4. Slopes of regressions for change in weight versus residence time in stream for each stocking group of rainbow trout in the first (November 2000 - March 2001) and second (November 2001-March 2002) years of stocking in Brush Creek. Slopes significantly different from zero  $(P < 0.05)$  are denoted with an asterisk (\*). Superscripted letters indicate significant differences in slopes from multiple comparisons ( $P < 0.05$ ).



Table 5. Estimated number of rainbow trout in Brush Creek from each stocking group remaining at the primary stocking site in the first year of stocking (November 2000 - March 2001 ), and remaining at the primary and secondary stocking sites in the second year of stocking (November 2001 - March 2002). Numbers of trout stocked in each month are indicated with an asterisk(\*).



Table 6. Number (%) of rainbow trout from each stocking group in Brush Creek in the second year of stocking (November 2001 - March 2002) that retained both anchor and visual implant elastomer (VIE) tags, lost VIE tags, and lost anchor tags  $(A)$ ; number  $(\%)$  of rainbow trout stocked into Brush Creek in the second year of stocking that retained both anchor and visible implant elastomer (VIE) tags, lost VIE tags, or lost anchor tags over time (all stocking groups combined, B).





Table 7. Cormack-Jolly-Seber monthly survival ( $\pm$  1 standard error) for each group of rainbow trout stocked into Brush Creek in the second year of stocking (November 2001-March 2002). Survival of rainbow trout stocked in January was estimated to be 1.0 between February and March, and March and April 2002 (indicated with an asterisk) because the number of recaptures increased through these time intervals.

	Monthly survival estimates							
Month stocked	Nov-01 to $Dec-01$	Dec $-01$ to $Jan-02$	Jan-01 to Feb-02	Feb-01 to $Mar-02$	Mar-01 to Apr-02	Apr-01 to May-02	May-02 to $Jul-02$	Jul-02 to Aug- $02$
$Nov-01$					$0.89(0.05)$ $0.76(0.05)$ $0.69(0.06)$ $0.74(0.08)$ $0.83(0.11)$ $0.29(0.07)$ $0.63(0.17)$			
$Dec-01$					$0.92(0.05)$ $0.57(0.06)$ $0.95(0.10)$ $0.90(0.11)$ $0.35(0.07)$ $0.65(0.14)$ $0.54(0.22)$			
$Jan-02$			0.66(0.08)	$\ast$	$\ast$	$0.48(0.11)$ 0.54 (0.17)		
$Feb-02$					$0.72(0.22)$ $0.89(0.26)$ $0.36(0.17)$ $0.56(0.33)$			
$Mar-02$						$0.66(0.20)$ $0.32(0.14)$ $0.68(0.29)$ $0.49(0.43)$		

Table 8. Quasi-Akaike's Information Criteria Weights (corrected, QAICc) weights and number of parameters for Cormack-Jolly-Seber survival probability models for all groups of rainbow trout stocked into Brush Creek in the second year of stocking. Models represent fully time-dependent survival (phi (t)), timedependent survival before and after a flood event between April and May 2002 (phi  $(t_F)$ ), and constant survival (phi(.)). Recapture probability (p) was held constant for all models.



Table 9. Quasi-Akaike's Information Criteria Weights (corrected, QAICc) weights and number of parameters for Cormack-Jolly-Seber survival probability models for groups ofrainbow trout stocked into Brush Creek in January, February, and March 2001. Models represent fully time-dependent survival (phi(t)), and constant survival (phi(.)). Recapture probability (p) was held constant for all models.



Table 10. Cormack-Jolly-Seber monthly survival (±1 standard error) estimates for groups of rainbow trout stocked into Brush Creek in January, February, and March 2001.



#### **Figure Captions**

- Figure 1. Mean change in weight over time for each stocking group of rainbow trout in the first year of stocking (A) and the second year of stocking (B).
- Figure 2. Proportion lipid content over time in stocked rainbow trout sampled in February and March 2001.
- Figure 3. Catch-per-unit-effort (CPUE, fish/min) of stocked rainbow trout between January 2001 and October 2002 at the primary stocking site (A), three pools located downstream from the primary stocking site (B), and two pools located upstream of the primary stocking site (C).
- Figure 4. Hydrograph data for Beaty Creek, Delaware County, Oklahoma, for water years October 2000- September 2001, and October 2001 - September 2002. In late February 2001 (indicated with an asterisk)., discharge reached 89.7  $m<sup>3</sup>/s$ , but is not shown to scale.
- Figure 5. Mean monthly temperature profiles at four pool locations and one natural spring located on Brush Creek between August 2000 and October 2002.







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## **CHAPTER II**

# **FISH ASSEMBLAGE STRUCTURE** IN AN **OKLAHOMA OZARK STREAM BEFORE AND AFTER RAINBOW TROUT INTRODUCTION**

**Abstract:** Rainbow trout *Oncorhynchus mykiss* have been widely stocked throughout the United States as a popular sport fish. Our study was initiated to evaluate potential effects of rainbow trout introduction on native fishes to inform future decisions about trout stocking in northeastern Oklahoma streams. We sampled fish assemblages in pools, glides, and riffles in Brush Creek, Delaware County, Oklahoma, from February 2000 to September 2002, and experimentally stocked rainbow trout into the stream from November 2000 to March 2001 and November 2001 to March 2002. We used a combination of multivariate analyses to evaluate seasonal and habitat effects on native fish assemblages and to compare assemblage structure between pre-stocking, the first. year of stocking, and the second year of stocking. Mesohabitat type significantly affected assemblage structure among years, whereas we did not detect an effect of season. We did not detect differences in assemblage structure among years in glide or riffle habitats. Relative abundance of species in pool habitats before rainbow trout introduction differed from assemblage structure in both the first and second year of stocking. Declines in seven species, including two native gamefish (smallmouth bass *Micropterus dolomieu*  and bluegill *Lepomis machrochirus),* contributed to assemblage dissimilarity in pool habitats between pre-stocking conditions and the second year of stocking. Our results indicate that stocking rainbow trout may cause local disruption in assemblage structure in pool habitats.

## **Introduction**

An estimated 2.5 billion sport fish are stocked annually in the United States and Canada (Heidinger 1999). In the U.S., 49 states' recreational fisheries programs include non-native fisheries, and more than one-third of states have fewer native game fish than non-native game fish (Horak 1995). Stocking programs are often initiated without explicit objectives or criteria to measure success or potential negative impacts of stocking (Heidinger 1999). Although it is recommended that detailed evaluation of potential ecological impacts on native fishes should be conducted before non-native fish are introduced (Waples 1999; Ham and Pearsons 2001), such evaluation has rarely occurred.

Rainbow trout *Oncorhynchus mykiss* have been widely stocked as a popular game fish, and 35 states in which this species is not native currently support populations (Rahel 2000). In Oklahoma, the Oklahoma Deparment of Wildlife Conservation (ODWC) stocks trout (primarily rainbow) in eight locations to support two tailrace fisheries, three seasonal stream fisheries, and three seasonal small lake fisheries (J. Vincent, ODWC, personal communication). Seasonal fisheries are located in southern areas of the state, where high water temperatures do not allow over-summer survival.

In recent years, angling groups have requested that rainbow trout be stocked into Ozark streams of northeastern Oklahoma to create additional fisheries on leased, privately-owned land. Baseflow in these streams is provided by numerous natural springs and seeps, and water temperatures remain relatively cool throughout summer months. If rainbow trout could avoid lethal summer water temperatures by using springs as thermal refuge, they could potentially survive over-summer and have long-term impacts on native fishes. We initiated this study in cooperation with the ODWC to

evaluate potential effects of trout introduction on native fishes and provide data on which to base future decisions about trout stocking in northeastern Oklahoma Ozark streams.

Research regarding effects of introduced salmonids generally has focused on interactions between the introduced species and one or a few other species, usually native sport fish. For example, numerous studies have addressed interactions between stocked rainbow trout and native brook trout *Salvelinus fontinalis* in eastern U. S. coldwater streams (Ensign et al. 1989; Lohr and West 1992; Larson et al. 1995; Clark and Rose 1997; Magoulick and Wilzbach 1997, 1998; Strange and Habera 1998; lsely and Kempton 2000). It is often only after introduction has been widespread and the species is well established that interactions between introduced salmonids and native fish assemblages have been evaluated (Crowl et al. 1992; Penczak 1999).

There has been little research regarding potential interactions between rainbow trout and native fishes in warmwater streams. Two studies addressed competition for food and habitat resources between introduced rainbow trout and smallmouth bass in streams in Arkansas (Ebert and Filipek 1991; Metcalf et al. 1997). To our knowledge, no evaluation of potential effects of rainbow trout introduction on warm water fishes or overall assemblage structure has been conducted before implementing stocking procedures. Our objective was to evaluate assemblage structure of native fishes in Brush Creek, Delaware County, Oklahoma, before rainbow trout introduction and in the first and second year of rainbow trout stocking to compare assemblages to pre-stocking conditions.

## **Methods**

#### *Field sampling*

We sampled fish assemblages in mesohabitats (pools, glides, and riffles) in Brush Creek from February 2000 to September 2002. Brush Creek is a small (mean width, 9 m), spring-fed stream in Delaware County, Oklahoma (36°23' N, 94°47' W). Brush Creek is about 10 km long and drains into Lake Eucha, a small impoundment used to provide water to the city of Tulsa. Immediately upstream of the confluence with Lake Eucha, the flow is subsurface for the majority of the year and the stream connects with the lake only during brief periods of high flow following rain events. This feature of made Brush Creek an attractive study site for our project because it approximated a closed system, which we presumed would limit escapement of stocked rainbow trout from Brush Creek. We originally proposed a before-after-control-impact (BACI) experimental design and planned to collect data on Beaty Creek, a stream in the same watershed that drains into Lake Eucha about 800 m from the confluence of Brush Creek and Lake Eucha. Trout were not stocked in Beaty Creek, and this site would have provided control data for comparison to Brush Creek data. However, due to limited access to private property on Beaty Creek, we were unable to consistently collect data for making comparisons with Brush Creek.

We classified mesohabitats in Brush Creek according to McCain et al. (1990). We used an electric seine (ES) to sample riffle and glide habitats and a boat electrofisher to sample pool habitats. The same Smith-Root 2.5 GPP electrofishing system powered both gears. We constructed the ES according to design specifications of Bayley et al. (1989) and Angermeier et al. ( 1991 ), making only minor structural modifications (Walsh et al.

2002). At all sites, we blocknetted the sample area and made two electrofishing passes (60-Hz AC, 3-4 A).

For each pass, we identified and counted all fish collected. After each site had been sampled, we released all fish. We next evaluated habitat by taking three to five measurements (depending on the width of the site) of flow, depth, and substrate size along evenly-spaced transects (usually four to six) throughout the site. We used a Marsh-McBirney model 2000 portable flow meter and wading rod to measure flow and depth, and a U.S. Geological Survey US SAH-97 gravelometer to measure substrate size. Averages of all measurements for each variable were used to characterize flow, depth, and dominant substrate size at each site. We also measured site length and widths to estimate habitat volume.

#### *Trout stocking*

We stocked rainbow trout into Brush Creek at a rate of 500 per month from November 2000 to March 2001, and November 2001 to March 2002. Before stocking we anesthetized (tricaine methanesulfonate, MS-222), measured (mm total length, TL), and weighed (g) the rainbow trout at Crystal Springs Trout Farm in Cassville, Missouri. We individually marked each trout with Floy FD-68B anchor tags (a different color for each month of stocking). In the first year of stocking, we double-marked all trout with an adipose fin clip, and in the second year, we used visible implant elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, Washington) tags as a second mark. We held trout in the raceway at the hatchery overnight and checked for mortalities the following morning. Trout were loaded into a 836 L hauling tank and transported about 3

h to Brush Creek. The stocking site was a 710-m2 bedrock-formed lateral scour pool with fractured bedrock cover, located about in the middle of Brush Creek (Figure 1).

# *Analytical approach*

For all analyses, we converted abundance data to catch-per-unit-effort (CPUE, fish/min). We used three multivariate approaches to analyze fish assemblages: Canonical correspondence analysis (CCA), partial CCA (pCCA, CANOCO 4; ter Braak and Smilauer 1998), and Similarity Percentages (SIMPER; PRIMER 5, Clarke and Gorley 2001). We used these analyses to investigate patterns in assemblage structure for the stream as a whole and changes in assemblage structure among mesohabitats and within individual pool habitats.

Canonical correspondence analysis is a direct gradient technique used to relate variation in assemblage structure to environmental variation (ter Braak 1986; Palmer 1993). While both direct and indirect gradient analyses rely on the investigator's interpretation of the influence of environmental variables based on knowledge of species' life histories, distributions, and environmental preferences, direct gradient analyses are constrained to evaluate only measured environmental gradients (ter Braak 1986). Canonical correspondence analysis is robust to potential problems with ecological data, including skewed species distributions, "noisy" data, and correlations among environmental variables (Palmer 1993). Nominal and continuous environmental variables can be evaluated; nominal variables are generally represented as centroids in ordination plots, while continuous variables are represented as vectors (Jongman et al. 1997).

Partial CCA is a type of CCA in which covariables are specified to remove effects of variables suspected of influencing a species' distribution so as to isolate effects of variable( s) of interest. If variables are carefully chosen *a priori,* CCAs and pCCAs can be used to test hypotheses using Monte Carlo permutation tests (Hallgren et al. 1999). For all CCAs and pCCAs, we used a square-root transformation to dampen effects of abundant species, performed 999 permutations, and evaluated significance at  $\alpha = 0.05$ . To evaluate relative abundances of native stream fishes we excluded rainbow trout abundance in all post-stocking assemblage analyses.

The SIMPER procedure is primarily descriptive, and identifies the relative contribution of each species to average Bray-Curtis dissimilarity between groups (Clarke and Gorley 2001; Clarke and Warwick 2001). We included in our analysis species that contributed  $\geq$  5% to dissimilarity between assemblages in our results. We originally used analysis of similarity (ANOSIM; Clark and Gorley 2001) to quantitatively evaluate similarity among groups, but those results were similar to pCCA results, so we do not present ANOSIM results herein.

*Stream-level assemblage structure.* We performed a pCCA to determine if there were differences in assemblage structure in Brush Creek among seasons. In the pCCA, we used season (winter, spring, summer, fall) as a nominal environmental variable and distance from the confluence of Brush Creek and Lake Eucha (creek kilometer, CKM), mesohabitat type (pool, glide, riffle), and year (pre-stocking, first year of stocking, second year of stocking) as covariables. We used CKM as a covariable to account for longitudinal variation in assemblages. Mesohabitat and year were used as covariables to factor out possible effects of variation in assemblage structure among habitats or within

years. We constrained permutations within year (pre-stocking, first year of stocking, second year of stocking).

We also performed a pCCA to determine if there were differences in assemblage structure among mesohabitat types (pool, glide, riffle). We used mesohabitat as a nominal environmental variable and CKM and year as covariables, again constraining permutations within year. Season was not used as a covariable because we did not find any influence of season on assemblage structure.

*Mesohabitat-level assemblage structure.* To evaluate if assemblage structure differed within mesohabitats among years, we performed pCCAs for each mesohabitat separately using a nominal environmental variable to represent sampling that occurred before trout stocking (Pre), during the first year of stocking (Post 1 ), or during the second year of stocking (Post 2). For pool and glide habitats, we used CKM and a nominal environmental variable to represent location (for example, stocking site) as covariables. We constrained permutations within location to factor out effects of sampling the same sites on multiple occasions. For riffle habitats, we sampled various locations based on water levels, so we only used CKM as a covariable. If we detected a significant effect of year on assemblage structure in a mesohabitat type, we conducted pCCAs between years (e.g., Pre vs. Post 1, etc.) to further evaluate changes in assemblage structure between the three years. We investigated which species contributed to significant differences between assemblages using SIMPER.

*Pool-level assemblage structure.* In small streams, it is appropriate to evaluate species interactions at the individual pool level, particularly when pools are separated by shallow water habitats (Matthews et al. 1994). We used pCCAs and SIMPER to evaluate

differences in assemblage structure among the five most commonly sampled pools (Figure 1) prior to rainbow trout introduction. We also used CC As to evaluate the relationship among pool assemblages and environmental gradients of depth, flow, substrate size, and creek kilometer (CKM) prior to trout introduction. We used CCAs to· evaluate changes in assemblage structure within individual pools among years (prestocking, the first year of stocking, and the second year of stocking). If we detected a significant effect of year on assemblage structure in an individual pool, we conducted pCCAs between year pairs to further evaluate changes in assemblage structure among the three years. We investigated which species contributed to significant differences between assemblages using SIMPER. We used Spearman rank correlations (PROC CORR, SAS 2000) to evaluate relationships between individual species' CPUE and rainbow trout CPUE in individual pools.

#### **Results**

#### *Field sampling*

We conducted assemblage sampling before trout introduction (February, May, and September 2000), during the first year of trout stocking (December 2000, March, May, and September 2001), and during the second year of trout stocking (December 2001, March, July, and September 2002). We sampled pool, glide, and riffle habitats before trout introduction and in the first year of stocking and pool and glide habitats in the second year of stocking (Tables 1-2). We were unable to sample riffle habitats in September 2000 and 2001 due to low water conditions and discontinued riffle sampling for the second year of trout stocking because we did not recapture rainbow trout in riffle

habitats during the first year of stocking. We sampled bedrock-formed lateral scour pools with bedrock boulder and/or woody cover. Five individual pools, including the stocking site, were sampled on a majority of sampling trips (Table 3; Figure 1).

#### *Stream-level assemblage structure*

Prior to trout stocking, the native fish assemblage was composed of 25 species belonging to seven families (Table 4). We collected all native species on at least one occasion in the first year of trout stocking, and all species except spotted sucker in the second year of trout stocking. There was no evidence of seasonal structuring in the fish assemblage within years (F-ratio = 1.809,  $P = 0.2620$ ). We did not include season in any further analyses.

Mesohabitat did affect assemblage structure within years (F-ratio = 53.857,  $P =$ 0.0100). Thirteen species, including all centrarchids and catostomids, and both ictalurid species, were collected only from pool habitats (Table 4; Figure 2). No species were unique to glide or riffle habitats (Table 4; Figure 2). Pool and riffle habitats were most dissimilar from each other (average dissimilarity  $= 96\%$ ). Pools had greater abundances of stonerollers and cardinal shiners, and riffles had greater abundances of banded sculpins and fantail darters (Table 5). Pool and glide habitats had an average dissimilarity of 82%. Dissimilarity was influenced by higher abundances of stonerollers and cardinal shiners in pools and higher abundances of banded sculpin, fantail darters, southern redbelly dace, slender madtoms, and orangethroat darters in glide habitats (Table 5). Glide and riffle habitats had an average dissimilarity of 70%. Dissimilarity in these habitats was influenced by higher abundances of cardinal shiners, stonerollers, slender madtoms, and
orangethroat darters in glides, and higher abundances of banded sculpins and fantail darters in riffles (Table 5).

#### *Mesohabitat-level assemblage structure*

Due to significant effects of mesohabitat type on assemblage structure, we evaluated each mesohabitat separately to evaluate effects of rainbow trout introduction. We sampled a total of eight species from riffle habitats (Table 4). We did not detect differences in assemblage structure in riffle habitats between pre-stocking and the first year of trout stocking (pCCA: F-ratio =  $0.627$ , P =  $0.8260$ ); we did not sample riffles in the second year of trout stocking. We sampled a total of eleven species from glide habitats (Table 4). We also did not detect significant differences in assemblage structure in glide habitats among pre-stocking, the first year of stocking, and the second year of stocking (pCCA: F-ratio = 1.464,  $P = 0.2800$ ).

All native species in Brush Creek were collected from pool habitats during our sampling (Table 4). Partial CCAs indicated that assemblage structure in pools differed among pre-stocking, the first year of stocking, and the second year of stocking (F-ratio = 3.074,  $P = 0.0010$ . Partial CCAs between years detected differences between prestocking and the first year of stocking (F-ratio = 1.951,  $P = 0.0100$ ) and between prestocking and the second year of stocking  $(F = 2.675, P = 0.0010)$ . Between pre-stocking and the first year of stocking, central stonerollers, southern redbelly dace, banded sculpin, and white suckers were more abundant in pre-stocking samples, while cardinal shiners and smallmouth bass were more abundant in the first year of stocking ( average dissimilarity =  $39\%$ ; Table 6). Seven species, including smallmouth bass, were more abundant before stocking than in the second year of stocking (average dissimilarity  $=$ 

43%; Table 6). We did not detect differences in assemblage structure in pools between the first and second year of stocking (F-ratio = 1.141,  $P = 0.3940$ ).

#### *Pool-level assemblage structure*

Before rainbow trout stocking, native fish assemblage structure differed among the five most frequently sampled pools (F-ratio = 3.193,  $P = 0.0070$ ). The assemblage at the farthest upstream site was  $57 - 72\%$  dissimilar to assemblages at the four downstream pools, generally because of higher abundances of southern redbelly dace and white suckers at the upstream site. Factoring out effects of CKM indicated that assemblages differed among individual pools (F-ratio = 2.750,  $P = 0.0040$ ) prior to stocking. Assemblage structure at the stocking site was  $57 - 60\%$  dissimilar to assemblages at pools located 0.4 km and 1.5 km downstream and the pool located 3.8 km upstream. The stocking site assemblage prior to stocking was least dissimilar (32%) to the assemblage at the pool located 2.6 km downstream.

Canonical correspondence analysis including the environmental variables of depth, flow, substrate size, and CKM parelleled patterns based on Bray-Curtis similarity and added insight to relationships between assemblage structure in individual pools and environmental gradients. Assemblage structure among the five pools was most strongly influenced by a longitudinal gradient; the first canonical axis was significant (F-ratio  $=$ 10.624,  $P = 0.0010$ ), and highly correlated with CKM ( $r = 0.90$ ; Figure 3). Samples among years at the furthest upstream site were separated from downstream sites along the first axis. Factoring out effects of CKM, the first canonical axis was still significant (Fratio  $= 5.968$ ,  $P = 0.0010$ ) and was correlated positively with depth and correlated negatively with flow (Figure 4). The stocking site and pool 3.8 km upstream generally

were deeper with lower flow, while the pool located 0.4 km downstream was shallower with higher flow. Almost all sites (except the pool located 0.4 km downstream from the stocking site) showed a general trend of decreased depth and increased flow during the study.

To further investigate assemblage structure within pool habitats before and after trout introduction, we performed CCAs to evaluate if assemblages differed among years in each of the five individual pools that we sampled frequently (Figure 1; Table 3). We did not detect differences in assemblage structure among pre-stocking, the first year of stocking, and the second year of stocking in pools located 0.4 km downstream and 3.8 km upstream from the stocking site (F-ratio = 1.058,  $P = 0.6000$ ; F-ratio = 1.433,  $P =$ 0.1400), respectively.

Assemblage structure in the three other pools differed among pre-stocking, the first year of stocking, and the second year of stocking. The pool 2.6 km downstream from the stocking site differed among years with the CCA (F-ratio = 2.230,  $P = 0.0240$ ). However, at that pool, we were able to conduct only one sample before rainbow trout introduction, so we did not conduct any further analyses on the species assemblage. Assemblage structure differed among years at the stocking site (F-ratio = 1.873,  $P =$ 0.0270) and the pool 1.5 km downstream from the stocking site (F-ratio = 1.871,  $P =$ 0.0160). We performed CCAs to evaluate pairwise comparisons between years for these two pools, and also calculated Spearman rank correlations to determine if individual species' abundances (based on species that made up at least 1% CPUE) in each pool were related to rainbow trout abundance at the same location.

At the pool 1.5 km downstream from the stocking site, we did not detect significant differences in assemblage structure between pre-stocking and the first year of stocking  $(F\text{-ratio} = 1.273, P = 0.1450)$ , but detected differences between pre-stocking and the second year of stocking (F-ratio = 2.289,  $P = 0.0070$ ) and the first year of stocking and the second year of stocking (F-ratio = 1.816,  $P = 0.0280$ ). Comparing pre-stocking to the second year of stocking, the average dissimilarity was 41%. Eight of the nine species that contributed  $\geq 5\%$  to dissimilarity, including smallmouth bass and shadow bass, were more abundant in the pool before stocking; only creek chub showed an increase in abundance after trout were introduced (Table 7). Average dissimilarity was 36% between the first and second years of stocking. Six of the seven species that contributed  $\geq$  5% to dissimilarity were more abundant in the first year of stocking, and again creek chubs were more abundant in the second year of stocking (Table 7). Despite differences in the assemblage as a whole among years, no individual species' abundances at this location were related to rainbow trout abundance  $(-0.35 < r_s < 0.28; P > 0.2960)$ .

At the stocking site, we did not detect significant differences in assemblage structure between pre-stocking and the first year of stocking (F-ratio =  $1.498$ , P = 0.0620), although this is a conservative interpretation given that nosignificance was marginal and changes in assemblage structure may have occurred. There were no differences in assemblages between the first year of stocking and the second year of stocking (F-ratio = 0.770,  $P = 0.7700$ ). Assemblage structure differed between pre-stocking and the second year of stocking (F-ratio = 1.557,  $P = 0.0390$ ; average dissimilarity = 32%). In April 2002, landowners dredged this pool, deepening it about 2 m. Two of our seasonal sampling trips in the second year of stocking followed the dredging (July 2002,

September 2002). Lack of detectable differences between assemblages in the first year of stocking and the second year of stocking  $(P = 0.7700)$  indicated that the disturbance did not significantly alter the fish assemblage. Cardinal shiners and central stonerollers were more abundant after stocking, while seven other species, including smallmouth bass and shadow bass, were more abundant before stocking (Table 8). Abundances of smallmouth bass ( $r_s = -0.82$ ; P = 0.0070) and shadow bass ( $r_s = -0.80$ ; P = 0.0109) were correlated negatively with rainbow trout abundance in the stocking site. A negative association between creek chub abundance and rainbow trout abundance was marginally nonsignificant ( $r_s = -0.65$ ; P = 0.0566); no other species' abundances were related to trout abundance at this location  $(-0.53 < r_s < 0.33; P > 0.1414)$ .

### **Discussion**

We detected differences in assemblage structure in pool habitats among years that may be interpreted as evidence that presence of rainbow trout in Brush Creek influenced assemblage structure. Trout abundances in pools were highest following stocking in both years, with a general pattern of decline in trout abundance through summer, and only isolated individuals were located by the following October of both years (after October 2001 stocking began again, and our study concluded in October 2002; Walsh et al. in preparation). Despite low rainbow trout survival, we detected changes in the assemblage structure of native fishes in pools between pre-stocking and the first and second year of trout stocking, although differences that we observed among years may be due rainbow trout introduction, other environmental factors, or both.

Among pools in Brush Creek, decreases in abundance of central stonerollers, cardinal shiners, and southern redbelly dace primarily accounted for the dissimilarity in assemblage structure between pre-stocking and the second year of stocking, although that was not the pattern at the stocking site. In the southwestern U.S., presence of rainbow trout influenced behavior and habitat use (Blinn et al. 1993) and decreased activity patterns (Bryan et al. 2002) of the native cyprinid Little Colorado spinedace *Lepidomeda vittata.* Rainbow trout also were a predatory threat to spinedace (Blinn et al. 1993) and prey on humpback chub *Gila cypha* and other native fishes, although fish was a small percentage of the overall diet (Marsh and Douglas 1997). We do not believe that predation by stocked rainbow trout had a significant effect on native fishes in Brush Creek; however, rainbow trout did consume fish prey in low quantities (Fenner 2002). Fish in trout diets were usually unidentifiable or cyprinids. Presence of unidentifiable fish in trout diets indicates that trout naturally consumed fish prey on some occasions. However, many observed cases of fish consumption by trout were from rainbow trout collected after our first electrofishing pass, and it was apparent that the fish prey had been recently ingested (personal observation), indicating that trout were targeting stunned fish from the first electrofishing pass.

In addition to declines in cyprinids in pool habitats between pre-stocking and the second year of stocking, declines in smallmouth bass, white sucker, and bluegill each accounted for 5% of the dissimilarity between time periods. We do not know why those species declined, but it was associated with presence of high densities of trout in the stocking site. The potential for exploitative competition for food resources between rainbow trout and smallmouth bass and bluegill was low (Fenner 2002), and it seems

unlikely that competition for food was occurring. Diet overlap between white suckers and trout was not evaluated.

Matthews et al. (1994) found the species assemblage in a series of 14 pools in a spring-fed Oklahoma stream to be consistent over time, while assemblages in individual pools were more variable. The authors concluded that it is appropriate to evaluate species interactions at the pool level, particularly when pools are separated by shallow water habitats (Matthews et al. 1994). At the stocking site, assemblage structure differed between pre-stocking and the second year of stocking, and there was some evidence of differences between pre-stocking and the first year of stocking. The stocking site had the highest trout densities among all pools sampled in both years of stocking. Observed changes in the assemblage at this site are likely linked to high abundances of rainbow trout or may be due to interactions between rainbow trout abundance and environmental changes.

Assemblage structure at the stocking site showed increases in stonerollers and cardinal shiners and declines in seven large-bodied species, including smallmouth bass, between pre-stocking and the second year of stocking. *Micropterus* spp. have been shown to influence stoneroller abundance (Power and Matthews 1983; Power et al. 1985) in Oklahoma streams. It is possible that abundance of stonerollers and cardinal shiners increased because of decreases in abundance of potential predators, such as smallmouth bass and creek chub. Smallmouth bass and shadow bass, two popular game fish in the Ozark region, appeared to be negatively associated with high densities of rainbow trout at this site. Based on the interactions in this individual pool, there seems to be evidence that high densities of rainbow trout could negatively affect these fisheries.

The pool closest to the stocking site that we sampled (0.4 km downstream) contained relatively high densities of rainbow trout, particularly in the first year of stocking, and we expected to see similar patterns in assemblage structure to those observed at the stocking site. However, we did not detect any changes in assemblage structure among years at this site. Differences in assemblages between these pools that existed before rainbow trout stocking may have influenced whether presence of rainbow trout affected assemblage structure. The pool 0.4 km downstream had the smallest pool volume of all pools that we sampled and did not contain as many large-bodied species as other sites, particularly smallmouth bass. Additionally, before stocking, higher abundances of stonerollers and cardinal shiners at this site 0.4 km downstream contributed 66% to dissimilarity between this site and the stocking site. If presence of smallmouth bass influenced cyprinids as we speculated for the stocking site, then we would not expect similar changes at this site because of initially lower abundances of this important predator.

The other pool with no difference in assemblage structure was located 3.8 km upstream from the stocking site. That site was physically similar to the stocking site, and also had a large pool volume and supported large-bodied species, particularly catostomids. However, we never collected smallmouth bass or shadow bass at this site. This may be due to cooler temperatures at the upstream site (Walsh et al. in preparation), or the relatively long distance of shallow-water habitats that is present between the stocking site and this site. Shallow water also likely prevented rainbow trout from reaching that site, and consequently that pool had low densities of trout relative to the stocking site.

The pool 1.5 km downstream of the stocking site showed differences in assemblage structure between pre-stocking and the second year of stocking but had low densities of rainbow trout compared with the stocking site. Abundance of cardinal shiners and central stonerollers decreased at this location between these two time periods compared with the stocking site where abundance of those species increased. As in the stocking site, smallmouth bass and bluegill abundance declined in this pool, but creek chub abundance increased. That was the only pool where differences in assemblage structure were detected between the first year of stocking and the second year of stocking, and patterns were very similar between these time periods and between pre-stocking and the second year of stocking. No individual species' abundances were related to rainbow trout abundance at this site. Changes observed at this site may have been more related to environmental changes than to rainbow trout introduction.

While both rainbow trout introduction and environmental changes may have influenced assemblages in pool habitats, neither of these factors appear to have affected assemblage structure of native fishes in shallow water (riffle and glide) habitats. Assemblages in these habitats remained stable throughout the course of our study. Stocked rainbow trout used pool habitats exclusively in Brush Creek (Walsh et al. in preparation), so it is unlikely that trout had much interaction with shallow-water fishes. Additionally, depth and flow conditions in these habitats vary with water level, and riffle and glide habitats often experience complete drying in summer months. These habitats would be less likely to experience the type of gradual changes that we observed in permanent pool habitats.

While we did not detect any seasonal changes in the assemblage structure of native fishes in Brush Creek, seasonal variation in fish assemblages has been observed in upland streams (Dewey 1981; Bart 1989; Pezold et al. 1997). Changes in abundance of individual species over time has been attributed to addition of young-of-year fish (YOY; Dewey 1981; Bart 1989) or movement of juvenile (Gelwick 1990) or adult (Pezold et al. 1997) fishes. Addition ofYOY fish in Brush Creek either did not influence individual species' overall abundances or influenced species' abundances in a similar way and did not affect species composition and relative CPUE. In our analyses, we did not subdivide species into size classes and evaluated only overall assemblage structure using CPUE as a measurement of abundance. It is possible that additional patterns in size-specific habitat use or seasonal variation would be detected with further analyses. Seasonal movements of adult centrarchids and cyprinids observed by Pezold et al. (1997) would not be expected in this system. Brush Creek is not a tributary of another stream but rather drains directly into an impoundment, and it is not connected to the impoundment during most of the year. Significant movement of fish in and out of Brush Creek (e.g., to spawn) is unlikely.

The native fish assemblage in Brush Creek is fairly typical of other Ozark streams (Matthews 1982, McNeely 1986, Bart 1989; Gelwick 1990), although Brush Creek had a lower number of cyprinid species than most other streams. Shallow-water species assemblages were composed of subsets of pool fish assemblages. Bart (1989) also found that many Ozark species showed generalized habitat use, occurring across the range of environmental conditions available among habitat types. The pattern in Brush Creek is common in small streams that experience drying during summer (Gelwick 1990; Taylor

2000); species entirely dependent on shallow or fast-flowing habitats would be unlikely to persist in such a system. Species' distributions among habitats in Brush Creek also are similar to other studies, with pool habitats supporting higher number of species than shallow-water habitats (Gelwick 1990, Taylor 2000). In a similar Oklahoma Ozark stream, Gelwick (1990) found that only three species were more common in riffle than pool habitats (slender madtom, fantail darter, and banded sculpin), while cyprinids, catostomids, and centrarchids were more abundant in pool habitats. In Brush Creek, greater abundance of banded sculpins and fantail darters in riffle habitats and greater abundance of cardinal shiners and central stonerollers in pool habitats accounted for 81% of the dissimilarity between these two habitats.

While no species were specific to shallow-water habitats, 13 species were collected only from pool habitats ( centrarchids, catostomids, *Ameiurus* spp.) in Brush Creek. These species tended to be the largest present in the stream. Pools, such as the ones that we sampled, offer the largest available habitat in the stream. Additionally, it has been suggested that larger species may associate with deeper water to avoid avian or terrestrial predation (Power 1984; Gelwick 1990). During the course of our sampling on Brush Creek, we observed evidence of avian (healed wounds on fish) and terrestrial (Floy tags in scat) predation pressure on fish in pool habitats.

Variability inherent in stream environments makes it extremely difficult to directly relate changes in assemblage structure to presence of an introduced species. Differences in native fish assemblage structure that we observed among pool habitats and in individual pools may be related to factors other than rainbow trout introduction. Even in this small stream, we observed pre-existing differences in assemblage structure among

pools and environmental changes that may have interacted with presence of rainbow trout to influence native fish assemblages. It seems most likely that rainbow trout stocking contributed to local disruption of the native fish assemblage at the stocking site, where high abundances of rainbow trout were present during and immediately following stocking. We were not able to evaluate assemblage structure in a stream in which rainbow trout were not stocked. Patterns of assemblage change in pool habitats in a stream without rainbow trout would have given us more insight as to whether differences that we observed in Brush Creek were driven by rainbow trout introduction or environmental changes.

Introduction of nonnative fish species has been implicated in extirpation or reduction of native species (Moyle and Light 1996; Penczak 1999) and is most likely when the introduced species is piscivorous (Moyle and Light 1996). Additionally, changes in assemblage structure generally have been assessed after the introduced species has become naturalized within a system (Moyle and Light 1996; Penczak 1999). Stocked rainbow trout showed low survival in Brush Creek, and an inability to survive abiotic conditions of the stream (Walsh et al. in preparation). It is unlikely that rainbow trout would become naturalized in Brush Creek and we do not believe that stocking caused localized extirpation of any native species. The only species not collected after rainbow trout were introduced, spotted sucker, was a relatively rare fish (0.20% CPUE in pools before trout stocking and 0.06% CPUE in pools in the first year of stocking). In Ozark streams, spotted suckers usually are less common than other sucker species and have been collected only sporadically even in frequently sampled streams (Pflieger 1997).

Intensive study at multiple scales has been necessary to elucidate effects of brown trout *Salmo trutta* on native communities in New Zealand (reviewed in Townsend 1996). Based on our results alone, it is not possible to state conclusively if rainbow trout stocking negatively affected native fish assemblages in Brush Creek. However, we did observe changes in assemblage structure in pool habitats that may be linked to rainbow trout introduction. However, variation in assemblage structure among individual pools made effects of trout introduction difficult to interpret. Additionally, most of the pools showed a trend of decreased depth and increased flow during our study, and those environmental changes may have contributed to assemblage changes. We feel that conservative decision-making is warranted until additional information can be collected. Longer-term monitoring of this system could help to determine how trout introduction and environmental changes contributed to assemblage changes. Future work could also include mesocosm experiments to observe interactions between rainbow trout and native fish species to create a greater understanding of whether biotic interactions could explain mechanisms behind observed changes in assemblage structure.

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		Depth	Flow	Substrate Size
Sample	N	(m)	(m/s)	(mm diameter)
Feb-00	8	0.89(0.39)	0.01(0.03)	98 (38)
May-00	5	1.00(0.16)	0.05(0.03)	125 (26)
Sep-00	7	0.83(0.23)	0.01(0.01)	106 (23)
$Dec-00$	3	0.94(0.16)	0.14(0.12)	77 (57)
$Mar-01$	5	1.05(0.25)	0.08(0.03)	114 (20)
	5			
May-01 $^a$		0.78(0.33)	0.06(0.03)	73 (43)
	5	1.04(0.17)	0.00(0.02)	94 (11)
$Sep-01$				
Dec-01 $b$	4	0.60(0.10)	0.16(0.08)	83 (43)
Mar-02 $\degree$	5	0.53(0.14)	0.24(0.05)	96 (41)
$Jul-02$	5	0.71(0.29)	0.07(0.07)	47 (21)
Sep-02 $d$	5	0.49(0.07)	0.02(0.01)	71 (21)

Table 1. Sample sizes and mean (SD) depth, flow, and substrate size for bedrock-formed lateral scour pools sampled between February 2000 and September 2002 in Brush Creek.

<sup>a</sup> Habitat measurement taken on 3 of 5 pools sampled in May 2001

 $<sup>b</sup>$  Habitat measurement taken on 2 of 4 pools sampled in December 2001</sup>

 $\degree$  Habitat measurement taken on 4 of 5 pools sampled in March 2002

 $d$  Habitat measurement taken on 3 of 5 pools sampled in September 200.

			Depth Flow		Substrate Size
Sample	Mesohabitat	${\bf N}$	(m)	(m/s)	(mm diameter)
Feb-00	Glide	5	0.26(0.08)	0.05(0.05)	68 (33)
	Riffle	3	0.12(0.02)	0.24(0.17)	61(35)
May-00	Glide	5	0.24(0.07)	0.16(0.08)	65(9)
	Riffle	5	0.15(0.02)	0.46(0.07)	72(16)
$Sep-00$	Glide	3	0.20(0.08)	$-0.02(0.02)^{a}$	50(13)
$Dec-00$	Glide	4	0.24(0.06)	0.03(0.02)	66 (27)
	Riffle	4	0.12(0.03)	0.29(0.15)	83(27)
$Mar-01$	Glide	$\overline{\mathcal{L}}$	0.23(0.06)	0.16(0.05)	54 (9)
	Riffle	$\overline{2}$	0.11(0.02)	0.43(0.18)	74(7)
May-01	Glide	3	0.20(0.10)	0.06(0.03)	61(21)
	Riffle	3	0.08(0.02)	0.50(0.06)	68(9)
$Sep-01$	Glide	3	0.17(0.06)	0.01(0.04)	66 (23)
Dec-01	Glide	3	0.29(0.03)	0.26(0.09)	57(9)
Mar-02	Glide	3	0.21(0.07)	0.42(0.10)	60(7)
Jul-02	Glide.	3	0.20(0.07)	0.17(0.09)	49(7)
Sep-02	Glide	$\overline{2}$	0.18(0.07)	0.02(0.01)	52(4)

Table 2. Sample sizes and mean (SD) depth, flow, and substrate size for glide habitats sampled between February 2000 and September 2002 and riffle habitats sampled between February 2000 and May 2001 in Brush Creek.

<sup>a</sup> Backwater flow

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Table 3. Sample sizes and mean (SD) depth, flow, substrate size, and pool volume for the five most frequently sampled pool habitats between February 2000 and September 2002. Locations are identified by distance and direction from the stocking site.



Table 4. Native fish species, species' codes (used in ordinations), and distribution among mesohabitats in Brush Creek between February 2000 and September 2002. Each species has a unique code, except largemouth and spotted bass, which are grouped as black bass (BLK).

Family	Species	Species Code			Pool Glide Riffle
Cyprinidae	Cardinal Shiner, Luxilus cardinalis	<b>CRD</b>	X	$\bf{X}$	X
	Central Stoneroller, Campostoma anomalum	STN	X	X	X
	Creek Chub, Semotilus atromaculatus	CRK	X	X	
	Redspot Chub, Nocomis asper	<b>RSP</b>	X	X	
	Southern Redbelly Dace, Phoxinus erythrogaster	<b>RBD</b>	X	X	
Catostomidae	Northern Hogsucker, <i>Hypentilium nigricans</i>	NHS.	X		
	Spotted Sucker, Minytrema melanops	SPT	X		
	White Sucker, Catostomus commersoni	<b>WHT</b>	X.		
Ictaluridae	Black Bullhead, Ameiurus melas	BBH	X		
	Slender Madtom, Noturus exilis	SMT	X.	X	X
	Yellow Bullhead, Ameiurus natalis	YBH	X		
Fundulidae	Northern Studfish, Fundulus catenatus	NSF	X	X	
Cottidae	Banded Sculpin, Cottus carolinae	<b>BSC</b>	X	X	X
Centrarchidae	Bluegill, Lepomis macrochirus	BGL	X		
	Green Sunfish, Lepomis cyanellus	<b>GRN</b>	$\mathbf{x}$		
	Largemouth Bass, Micropterus salmoides	<b>BLK</b>	X		
	Longear Sunfish, Lepomis megalotis	LNG.	X		
	Shadow Bass, Ambloplites ariommus	SHD.	X		
	Smallmouth Bass, Micropterus dolomieu	SMB	X		
	Spotted Bass, Micropterus puctulatus	<b>BLK</b>	X		
	Warmouth, Lepomis gulosus	WRM	X		
Percidae	Fantail Darter, Etheostoma flabellare	FTD.	X	X	X
	Orangethoat Darter, Etheostoma spectabile	<b>OTD</b>	X	Х	X
	Logperch, Percina caprodes	LOG.	X		X
	Stippled Darter, <i>Etheostoma puctulatum</i>	STD	X	X	X

Table 5. Species contributing  $\geq 5\%$  dissimilarity between pool and glide, pool and riffle, and glide and riffle habitats in Brush Creek. The mesohabitat in which each species was more abundant is indicated in bold.

		Abundance (fish/min)		Contribution to	Average	
Comparison	Species	Pool	Glide	Riffle		Dissimilarity (%) Dissimilarity (%)
Pool vs. glide	Central stoneroller	5.48	4.82		22	82
	Cardinal shiner	4.37	3.41		17	
	Banded sculpin	0.33	3.72		16	
	Fantail darter	0.04	3.27		13	
	Southern redbelly dace	0.63	1.75		$\overline{7}$	
	Slender madtom	0.04	1.57		6	
	Orangethroat darter	0.10	1.45		6	
Pool vs. riffle	Banded sculpin	0.33		8.93	29	96
	Central stoneroller	5.48		0.09	20	
	Fantail darter	0.04		4.93	17	
	Cardinal shiner	4.37		0.04	15	
	Glide vs. riffle Banded sculpin		3.72	8.93	30	70
	Fantail darter		3.27	4.93	23	
	Cardinal shiner		3.41	0.04	12	
	Central stoneroller		4.82	0.09	11	
	Slender madtom		1.57	0.93	8	
	Orangethroat darter		1.45	0.08	7	

Table 6. Species contributing  $\geq$  5% dissimilarity in pool habitats between pre-stocking (Pre) and the first year of trout stocking (Postl ), and pre-stocking and the second year of trout stocking (Post2) in Brush Creek. The year in which each species was more abundant is indicated in bold.

		Abundance (fish/min)		Contribution to	Average	
Comparison	Species	Pre	Post1	Post2	Dissimilarity (%)	Dissimilarity (%)
Pre vs. post1	Cardinal shiner	4.41	5.84		17	39
	Central stoneroller	6.99	5.29		15	
	Southern redbelly dace	1.26	0.32		10	
	Smallmouth bass	0.33	0.44		6	
	Banded sculpin	0.54	0.19		5	
	White sucker	0.30	0.21		5	
Pre vs. post2	Central stoneroller	6.99		4.09	16	43
	Cardinal shiner	4.41		2.94	14	
	Southern redbelly dace	1.26		0.27	10	
	Smallmouth bass	0.33		0.27	5	
	Banded sculpin	0.54		0.24	5	
	White sucker	0.30		0.14	5	
	Bluegill	0.35		0.15	5	

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 $\Delta \sim 10^4$ 

Table 7. Species contributing  $\geq 5\%$  dissimilarity in a pool located 1.5 km downstream from the stocking site between pre-stocking (Pre) and the second year of trout stocking (Post2), and the first year of stocking (Postl) and second year of stocking in Brush Creek. The year in which each species was more abundant is indicated in bold.



Table 8. Species contributing  $\geq 5\%$  dissimilarity in the stocking site between prestocking (Pre) and the second year of trout stocking (Post2) in Brush Creek. The year in which each species was more abundant is indicated in bold.



### **Figure Captions**

- Figure 1. Location of pool sampling sites on Brush Creek, and relative distance from the pool in which trout were stocked November 2000-March 2001 and November 2001 - March 2002.
- Figure 2. Biplot of species and environmental variables (mesohabitat types) in Brush Creek. Species' codes are located in Table 4.
- Figure 3. Biplot of species and nominal environmental variables in pool habitats. Continuous environmental variables flow, depth, substrate size, and creek kilometer (CKM) are represented as vectors. Nominal environmental variables representing location  $[SS =$  stocking site (triangle);  $0.4$  DS = pool 0.4 km downstream from the stocking site (right triangle);  $1.5 \text{ DS} = \text{pool} 1.5 \text{ km}$ downstream of the stocking site (left triangle); 2.6 DS = pool 2.6 km downstream of the stocking site (down triangle);  $3.8 \text{ US} = \text{pool } 3.8 \text{ km}$ upstream of the stocking site (box)] and year of the study (1 = pre-stocking,  $2 =$ first year of stocking, 3 = second year of stocking) are represented as centroids. Species' codes are located in Table 4.
- Figure 4. Biplot of species and nominal and continuous environmental variables for pCCA to factor out effects of CKM. Variables and symbols are the same as those used in Figure 3. Species' codes are located in Table 4.









#### CHAPTER III

# CHARACTERISTICS OF SMALLMOUTH BASS AND SHADOW BASS POPULATIONS IN AN OZARK STREAM BEFORE AND AFTER RAINBOW TROUT INTRODUCTION

Abstract: We evaluated characteristics of smallmouth bass *Micropterus dolomieu*  and shadow bass *Ambloplites ariommus* populations in a small, northeastern Oklahoma Ozark stream from February 2000 to March 2003 to evaluate potential effects of rainbow trout *Oncorhynchus myldss* introduction on these species. We experimentally stocked rainbow trout into the stream from November 2000 to March 2001 and November 2001 to March 2002. Abundances of smallmouth bass and shadow bass were correlated negatively with high densities of rainbow trout at the stocking site, but we did not detect similar relationships at other pools. Both species showed patterns of limited movement among pool habitats, and presence of rainbow trout in Brush Creek did not appear to influence movement patterns. We documented recruitment by smallmouth and shadow bass over the during our study, indicating that rainbow trout introduction did not inhibit spawning. Mean relative weight  $(W_r)$  of smallmouth bass ranged from  $77 - 80$ , and we did not detect differences in relative weight among pre-stocking, the first year of stocking, and the second year of stocking.

### **Introduction**

Recreational stream fisheries in eastern Oklahoma are economically important to the state, and the estimated economic benefits of eastern Oklahoma fisheries were about \$24 million in 1993 (Fisher et al. 2002). Black bass *Micropterus* spp., and particularly smallmouth bass M. *dolomieu,* populations support active recreational fisheries in eastern Oklahoma streams with about 12% of Oklahoma anglers targeting black bass species in this region (Fisher et al. 2002). Characteristics of smallmouth bass populations including age and growth, survival, and relative weight have been documented for Oklahoma upland and Ozark streams (Orth et al. 1983; McClendon and Rabeni 1987; Reed and Rabeni 1989; Balkenbush and Fisher 1999). Smallmouth bass are the dominant predator in most Ozark streams (Pflieger 1997) and play an important ecological role in structuring stream community dynamics (Power and Matthews 1983; Power et al. 1985).

*Ambloplites* spp. (rock bass *A. rupestris,* shadow bass *A. ariommus,* and Ozark bass *A. constellatus)* also are popular sportfish in the Ozarks (Pflieger 1997). Rock bass and shadow bass generally are differentiated by geographic distribution and morphologic characteristics; however, the genetic relationship between these two species is unresolved (Koppelman et al. 2000). Rock bass populations in Missouri Ozark streams have been studied in conjunction with smallmouth bass (Probst et al. 1984; McClendon and Rabeni 1987), but little research has focused on shadow bass.

In recent years, angling groups have requested that rainbow trout be stocked into Ozark streams of northeastern Oklahoma to create additional fisheries on leased, privately-owned land. Rainbow trout *Oncorhynchus mykiss* are a widely stocked game fish that have established naturalized populations in 35 states (Rahel 2000). Baseflow to

Ozark streams is provided by numerous natural springs and seeps, and water temperatures remain relatively cool throughout summer. If rainbow trout could avoid lethal summer water temperatures by using springs as thermal refuge, they could potentially survive over-summer and have long-term impacts on native fishes. Research regarding potential interactions between rainbow trout and native game fishes in warmwater streams is limited. Two studies have addressed competition for food and habitat resources between introduced rainbow trout and smallmouth bass in Arkansas (Ebert and Filipek 1991; Metcalf et al. 1997). Although shadow bass were present in the system studied by Ebert and Filipek (1991) and Metcalf et al. (1997), they did not evaluate shadow bass populations or possible interactions between shadow bass and introduced rainbow trout.

We initiated this study in cooperation with the Oklahoma Department of Wildlife Conservation (ODWC) to evaluate potential effects of rainbow trout introduction on native fisheries and provide them with data on which to base future decisions about trout stocking in northeastern Oklahoma Ozark streams. Our objectives were to 1) evaluate abundance, movement patterns, and condition of smallmouth and shadow bass populations in Brush Creek, Delaware County, Oklahoma before and after rainbow trout introduction and 2) evaluate relationships between rainbow trout abundance and smallmouth and shadow bass abundance in pool habitats.
## **Methods**

### *Field sampling*

We sampled smallmouth bass and shadow bass from Brush Creek between February 2000 and March 2003. Brush Creek is a small (mean width, 9 m), spring-fed stream in Delaware County, Oklahoma (36°23' N, 94°47' W). It is about 10 km long and drains into Lake Eucha, a small impoundment used to provide water to the City of Tulsa. Immediately upstream of the confluence with Lake Eucha, the flow is subsurface for the majority of the year and the stream connects with the lake only during brief periods of high flow following flood events.

We originally proposed a before-after-control-impact (BACI) experimental design and planned to collect data on smallmouth and shadow bass from Beaty Creek, a stream in the same watershed that drains into Lake Eucha about 800 m from the confluence of Brush Creek and Lake Eucha. Trout are not present in Beatty Creek, so those data would have provided a control site for comparison to Brush Creek data. However, due to limited access to private property on Beaty Creek, we were unable to consistently collect data for making comparisons with Brush Creek.

We collected smallmouth and shadow bass during seasonal assemblage sampling and during sampling directed specifically at capturing smallmouth bass, shadow bass, and stocked rainbow trout. We sampled the fish assemblage four times a year using electrofishing methods to evaluate abundance of all native fish species and stocked rainbow trout in riffle, glide, and pool habitats. We blocknetted sample areas before sampling and took two electrofishing passes (60-Hz AC, 3-4 A). Before the beginning of stocking in November 2000, we sampled smallmouth and shadow bass only during

assemblage sampling. After stocking began, we conducted monthly targeted sampling for stocked rainbow trout and native sport fish (smallmouth bass and shadow bass) in pool habitats in months when we did not sample assemblage structure. Electrofishing methods for targeted sampling were similar to assemblage sampling methods, except we generally took three electrofishing passes to allow us to calculate depletion estimates of trout abundance. We sampled six pool habitats on almost all trips: the stocking site, two pools located upstream of the primary stocking site [0.1 and 3.8 km], and three pools located downstream of the stocking site (0.4, 1.5, and 2.6 km). All of those pools were bedrock-formed lateral scour pools with fractured boulder cover, except the pool located 0.1 km upstream of the primary stocking site comprised of a series of rootwad-formed lateral scour pools with rootwad and woody cover.

We measured (mm total length, TL) and weighed (mass in g) all smallmouth and shadow bass collected. Beginning in May 2000, we marked smallmouth and shadow  $bass \ge 150$  mm TL with individually numbered Floy FD-68B anchor tags. On subsequent sampling trips, we continued to mark individuals that did not have a tag at the time of capture. Originally, we were not able to determine anchor tag retention because we marked fish at many different time periods and did not use a double-mark. To evaluate anchor tag retention in both species, we marked all smallmouth and shadow bass collected in October 2001 with new individually-numbered anchor tags and removed the third anal fin spine as a double-mark. We did not mark any smallmouth or shadow bass after that time. At all capture periods, we recorded anchor tag and double-mark information for all smallmouth and shadow bass.

## *Trout stocking*

We stocked rainbow trout into Brush Creek at a rate of 500 per month from November 2000 to March 2001 and November 2001 to March 2002. Before stocking we anesthetized (tricaine methanesulfonate, MS-222), measured (mm total length, TL), and weighed (mass in g) the rainbow trout at Crystal Springs Trout Farm in Cassville, Missouri. We individually marked each trout with Floy FD-68B anchor tags ( a different color for each month of stocking). We held trout in the raceway at the hatchery overnight and checked for mortality the following morning. Trout were loaded into a 836 L hauling tank and transported approximately 3 h to Brush Creek. The stocking site was a large  $(710 \text{ m}^2)$  bedrock-formed lateral scour pool with fractured bedrock cover, located 5.7 km upstream of the confluence of Brush Creek and Lake Eucha.

#### *Telemetry*

In October 2000, we surgically implanted radio-telemetry transmitters in five adult smallmouth bass from Brush Creek and five adult smallmouth bass from Beaty Creek. We collected smallmouth bass using standard electrofishing procedures and anesthetized fish (MS-222) before surgery. Water temperatures at the time of surgery were  $16 - 17$ °C. We inserted transmitters (MBFT-4;  $11 \times 43$  mm,  $7.7 \text{ g}$  in air; Lotek Engineering, Inc., Newmarket, Ontario, Canada) into the abdominal cavity through a 3-cm ventral incision. We left the antenna trailing from the incision and closed the incision with three absorbable sutures [2-0 PDS II (polydioxanone), CP-1 reverse cutting needle; Ethicon, Inc., Somerville, New Jersey] as recommended by Walsh et al. (2000). Transmitters were programmed for a duty cycle of  $1$  week on  $/1$  week off to prolong battery life. We located telemetered fish in both streams once a month using an SRX-400 low-frequency

radio receiver (Lotek Engineering, Inc., Newmarket, Ontario, Canada) and portable Yagi antenna.

### *Analyses*

*Movement.* We evaluated movement of smallmouth and shadow bass among pools by using mark-recapture data to create individual recapture histories. We examined recapture histories of individual fish to identify how many times fish were recaptured, how many pools were used by individual fish, distance between pools used, and timing of movement between pools. Telemetry relocations provided additional information on movement patterns of smallmouth bass.

*Length-frequency and relative weight.* We evaluated length-frequency distributions of smallmouth and shadow bass among years for all individuals sampled throughout the stream, and individuals sampled specifically from the stocking site. We evaluated relative weight  $(W_r)$  for smallmouth bass among years for all individuals  $>150$  mm sampled throughout the stream. We used the standard weight  $(W_s)$  equation for smallmouth bass  $\geq 150$  mm developed by Kolander et al. (1993):  $\log_{10}(W_s) = -5.329 +$ 3.200 $\lceil \log_{10}(TL) \rceil$  and  $W_r = (W/W_s)^*100$  (where  $W =$  weight of an individual). We evaluated mean *Wr* among 50-mm length groups using a two-way analysis of variance (ANOVA; PROC GLM, SAS 2000) to determine if  $W_r$  differed among pre-stocking, the first year of stocking, and the second year of stocking.

*Relationships with rainbow trout abundance.* We performed Spearman rank correlations (PROC CORR, SAS 2000) to evaluate relationships between each species' CPUE and rainbow trout CPUE in six pools: the stocking site, pools located 0.4, 1.5, and

2.6 km downstream of the stocking site, and pools located 0.1 and 3.8 km upstream of the stocking site.

### **Results**

### *Field sampling*

We sampled smallmouth and shadow bass from five of the six frequently sampled pools; they were never collected from the pool located 3.8 km upstream of the stocking site. We collected 153 smallmouth bass  $(45 - 360 \text{ mm}, 1 - 710 \text{ g})$  and 123 shadow bass  $(87 - 250 \text{ mm}, 15 - 258 \text{ g})$  during sampling before rainbow trout stocking in February, May, and September 2000. During the first year of stocking, we conducted sampling in December 2000, March, May, and September 2001 and targeted sport fish in January, February, June, August, and October 2001. We collected 467 smallmouth bass  $(49 - 380)$ mm,  $1 - 812$  g) and 350 shadow bass  $(31 - 267$  mm,  $1 - 264$  g). In the second year of stocking, we conducted assemblage sampling in December 2001, March, July, September 2002, and March 2003, and targeted game fish sampling in January, February, April, May, August, and October 2002. We collected 480 smallmouth bass (54 – 387 mm, 1-865 g) and 357 shadow bass  $(40-285 \text{ mm}, 2-390 \text{ g})$ . Smallmouth and shadow bass were only sampled in pool habitats and were frequently associated with boulder or woody cover.

## *Movement*

Mark-recapture data showed a pattern of limited movement among pool habitats by smallmouth and shadow bass, and presence of rainbow trout did not appear to influence movement of either species. We recaptured 49% of tagged smallmouth bass and 45% of

tagged shadow bass on at least one occasion, and documented multiple recaptures of some individuals over approximately two years (Table 1). Of the 19 smallmouth bass recaptured seven or more times (time period from  $1 - 2.4$  years), two fish were recaptured in three different pools, eight fish were recaptured in two different pools, and nine fish were recaptured only in the pool in which they were tagged. Movement between pools was most common between pools that were close to each other. For example, several tagged smallmouth bass showed movements between the stocking site and the series of rootwad-formed pools 0.1 km upstream. In general, when we recaptured smallmouth bass in more than one pool, movements between pools occurred in late spring to early summer. The longest movement that we observed by smallmouth bass was 1.5 km. One individual marked at the stocking site in May 2000 that was recaptured in a pool 1.5 km downstream on four additional occasions. We marked another individual in the pool 1.5 km downstream of the stocking site in March 2001 and recaptured it in that pool until December 2001; we did not recapture it until July 2002, when it was located at the stocking site.

Telemetry data also suggested limited movement by smallmouth bass in Brush Creek. Our radio-transmitters lasted from October 2000 to August 2001. In October, about a week after transmitter implantation and before the first rainbow trout stocking in November 2000, all five tagged smallmouth bass  $(325-374 \text{ mm}; 422-720 \text{ g})$  were located at the stocking site. After stocking began, one smallmouth bass moved from the stocking site to the pool 0.1 km upstream in mid-November 2000 and remained in this location through February 2001. By March it moved further upstream to a rootwad pool (0.25 km upstream from the stocking site) and remained in that location through July

2001. Between July and August, it moved 0.15 km back downstream to the pool 0.1 km upstream of the stocking site. We were able to relocate this fish after transmitter life expired with mark-recapture data. We recaptured it in October 2001 at the stocking site, in December 2001 at the pool 0.1 km upstream, April and May 2002 at the stocking site, and July, September, October 2002 and March 2003 in the pool 0.1 km upstream of the stocking site.

Three of the telemetered smallmouth bass remained at the stocking site through January 2001. After January 2001, one bass was located at the pool 0.4 km downstream February and remained in that location through August 2001. We were able to continue to locate this individual after the transmitter life expired by using its' anchor tag number. We recaptured that bass in the pool 0.4 km downstream from the stocking site again in December 2001 but recaptured it at the stocking site in January, May, September, and October 2002. The second fish that moved out of the stocking site in January was recaptured in February at the upper end of the stretch of continuous rootwad pools located 0.1 km upstream of the stocking site. It remained in that location through August 2001. The third bass remained in the stocking site through January, and then moved to the pool 0.1 km upstream. It was located in close proximity to the previous fish through May 2001, but we were unable to locate it in subsequent trips in July and August 2001.

The final telemetered smallmouth bass remained at the stocking site through March 2001, and we were not able to relocate it after that time. We believe that our inability to locate those two bass was attributable to either fishing mortality or transmitter failure because of our ability to search the stream thoroughly.

The smallmouth bass telemetered in Beaty Creek  $(282 - 355$  mm;  $350 - 650$  g) were all located in one bedrock pool after release in October 2001. In general, timing and pattern of movement were similar to smallmouth bass in Brush Creek, but movements were on a larger scale. One smallmouth bass remained in the pool in which it was released through December 2000, but we were unable to relocate it in any subsequent trips. Another tagged smallmouth remained in the pool in which it was released through January 2001. By February 2001, it had moved downstream (about 1.8 km) and was located from the road due to limited stream access. In March 2001 it was located back in the pool in which fish were originally released, but we could not locate it in April 2001. In May 2001, we located this fish in a bedrock pool 6.4 km downstream from where it was last located (the release pool), but we were again unable to locate it in June-July 2001.

The other three tagged bass moved from the release pool in March - April 2001. One smallmouth bass remained in the pool in which it was released through February 2001. We were unable to locate it in March but located it in the original pool in April 2001, where it remained through July 2001. Another smallmouth bass followed a similar pattern. It remained in the pool in which it was released through March 2001, and we were unable to locate it in April. We located it back in the release pool in May 2001, and it remained there through July 2001. The last smallmouth bass remained in the pool in which it was released through March 2001. We located it slightly downstream from this pool in April 2001, and were unable to locate it in May. By June 2001, it had returned to the pool in which fish were released, and it remained there through our last location in July 2001. The two fish that we were unable to locate may have moved to parts of the

stream where we did not have property access, or may have been caught by anglers. Overall, larger stream size and limited property access made relocation of fish in Beaty Creek more difficult than in Brush Creek.

Shadow bass in Brush Creek showed a similar pattern of affiliation with one or two pools throughout the course of our study. The four shadow bass that we recaptured the most  $(6 - 9$  recaptures) were all recaptured from one pool over a one to two year period: one from the stocking site, one from the pool 0.4 km downstream of the stocking site, and two from the pool 2.6 km downstream of the stocking site.

Similar to smallmouth bass, shadow bass movement between closely located pools was common, and 18 shadow bass used both the stocking site and the pool 0.1 km upstream. Two fish moved between the stocking site and the pool 0.4 km downstream. Five shadow bass moved distances  $>1$  km between pools, and we did not encounter four of these fish for periods of about a year, indicating that they were using habitat that we did not sample during this time. For example, one shadow bass moved from the pool 2.6 km downstream of the stocking site to the stocking site between August 2001 and August 2002, with no recaptures between these times. One shadow bass was captured at the stocking site in August 2001, recaptured in the pool 1.5 km downstream in April 2002, and recaptured a second time in the pool 0.1 km upstream of the stocking site in July 2002, moving a minimum of 1.6 km upstream in a three month period.

We planned to evaluate anchor tag retention in shadow bass as we had for smallmouth bass (Walsh and Winkelman in press), but we did not obtain sufficient recapture data. We tagged 68 shadow bass with anchor tags and removed the third anal spine as a double-mark in October 2001. In sampling trips during December 2001, and

January - April 2002 we collected large numbers of shadow bass, but recaptured low numbers  $(4-14)$  of tagged shadow bass, and only identified two fish with the doublemark that had lost their anchor tag.

#### *Length-frequency and relative weight*

Length-frequency distributions indicated that size structure of smallmouth bass in Brush Creek was similar between pre-stocking and post-stocking (Figure 1 ). At the stocking site, length-frequency distributions also were generally similar among years, although there appeared to be more smallmouth bass between  $200 - 250$  mm in the first year of stocking than in the other two years (Figure 1 ). We sampled young-of-year smallmouth bass throughout the stream and in the stocking site in all three years, indicating that presence of rainbow trout did not inhibit spawning or recruitment. We saw a similar pattern for shadow bass, with little difference between the length-frequency distributions for all sample sites and the stocking site (Figure 2). We sampled young-ofyear shadow bass in pools in the second year of stocking, indicating that spawning occurred.

Relative weights of smallmouth bass among 50-mm length increments ranged from 73 - 81 (Table 2). We did not detect a difference in mean  $W_r$  among pre-stocking [77  $\pm$  8] (mean  $\pm$  SD); N = 135], the first year of stocking (80  $\pm$  10; N = 420), and the second year of stocking (79  $\pm$  13; N = 357) (F = 1.24; model df = 14, error df = 897; total df = 911; P  $= 0.2431$ ) for all length groups combined. We also did not detect any differences in  $W_r$ for length groups among years (F = 0.49; df = 8; P = 0.8631).

#### *Relationships with rainbow trout abundance*

Based on CPUE data for smallmouth bass and rainbow trout at all sampling periods between February 2000 and March 2003, smallmouth bass abundance was correlated negatively with rainbow trout abundance at the stocking site ( $r = -0.48$ ,  $P = 0.0199$ ; Figure 3). We did not detect a relationship between smallmouth bass and rainbow trout abundance at the other four pool locations  $(-0.40 \le r_s \le 0.18, P > 0.1217)$ . Shadow bass abundance was also negatively correlated with rainbow trout abundance at the stocking site ( $r_s = -0.73$ , P = 0.0001; Figure 3), but not at the other pool sites (-0.35 <  $r_s$  < 0.33, P > 0.1117).

#### **Discussion**

In Brush Creek, abundance of both smallmouth and shadow bass was correlated negatively with rainbow trout abundance at the stocking site, but we did not detect similar relationships at other pools. The stocking site generally had the highest densities of rainbow trout during, and in the months following, stocking (Walsh et al. in preparation). Additionally, presence of rainbow trout in Brush Creek did not appear to influence movement patterns or spawning and recruitment of smallmouth or shadow bass and had no detectable effect on relative weights of smallmouth bass.

Ebert and Filipek (1991) recommend limiting trout stocking to winter months to minimize potential competition for invertebrate food items between stocked rainbow trout and young-of-year smallmouth bass. Our data did not indicate any influence of rainbow trout on recruitment in Brush Creek. Smallmouth bass spawning in Ozark streams generally begins in mid-April, and may extend into June and July (Pflieger

1997). In Brush Creek, this time period corresponded with a change in mean temperature from about 15°C to about 20°C and with a decline in rainbow trout abundance (Walsh et al. in preparation). Stocked rainbow trout were unable to survive these temperatures in Brush Creek, and we collected only isolated individuals through summer in both years of stocking (Walsh et al. in preparation). However, in other Ozark streams with cooler temperatures, rainbow trout that survive through spring and early summer may compete for food resources with young-of-year smallmouth bass.

Telemetry and mark-recapture data indicated limited movements of smallmouth bass among pool habitats in Brush Creek, and differences in movement patterns before and after rainbow trout stocking were not apparent. Most between-pool movements that we observed occurred in late spring to early summer and probably were related to spawning activity. These patterns agree with other research on smallmouth bass in the Ozarks (Todd and Rabeni 1989) and correspond with regional patterns for movement in small streams with mild winters (Lyons and Kanehl 2002).

Smallmouth bass and shadow bass were frequently recaptured in the pool in which they were tagged and used more than one pool when pool habitats were close to each other. Some smallmouth bass and shadow bass probably used more pool habitats than we were able to sample. About one-half of tagged fish of both species were never recaptured after they were tagged. Those fish may have moved into habitats that we did not sample, lost their tags, or died. We did not obtain sufficient data to evaluate anchor tag retention by shadow bass in Brush Creek, but we believe it is probably similar to retention that we observed for smallmouth bass (48%; Walsh and Winkelman in press). Due to low recaptures, we could not estimate survival for either species.

Length and age structure of the smallmouth bass population in Brush Creek appears similar to that observed in the Baron Fork River, a tributary to the Illinois River located in the northeastern Oklahoma Ozark highlands (Balkenbush and Fisher 1999). Balkenbush and Fisher (1999) estimated maximum age of smallmouth bass at 6 yrs, with 93% age 5 or less. They back-calculated length at age five to be 388 mm. Maximum length of smallmouth bass that we collected was 387 mm, indicating that the largest smallmouth bass we collected were about 5 years in age. Balkenbush and Fisher (1999) noted that preferred length smallmouth bass (350 mm; Gabelhouse 1984) were rare in the Baron Fork River. Fish did not achieve memorable (430 mm) or trophy (510 mm) lengths in either the Baron Fork River (Balkenbush and Fisher 1999) or Brush Creek.

We did not detect differences in relative weights in smallmouth bass from Brush Creek before and after rainbow trout stocking. Relative weights that we observed  $(73 -$ 81) were similar to those calculated for similar streams using the  $W_s$  equation of Wege and Anderson (1978) (Orth 1983; McClendon and Rabeni 1987; Reed and Rabeni 1989), and we did not detect differences in mean *Wr* among years of our study. Orth (1983) found that  $W_r$  ranged from 75 - 89 for smallmouth bass between ages 2 - 6. McClendon and Rabeni (1987) observed relative weights ranging from 84- 88, and Reed and Rabeni (1989) observed a mean  $W_r$  of 83 in an unexploited stream-dwelling smallmouth bass population. Fishing pressure on smallmouth bass in Brush Creek is probably light because there is no public access on the stream.

Introduction of naturalized populations of nonnative fish species has been implicated in extirpation or reduction of native species (Moyle and Light 1996; Penczak 1999). An additional conservation concern in this region is that smallmouth bass in the southwestern

Ozarks, including streams in the Illinois River drainage, have been documented as genetically distinct from other interior U.S. populations (Neosho smallmouth, Stark and Echelle 1998). However, it is unlikely that rainbow trout would become naturalized in Brush Creek (Walsh et al. in preparation), and rainbow trout introduction did not appear to adversely affect some characteristics of native smallmouth bass or shadow bass populations during the course of our study. The ability of smallmouth and shadow bass to successfully reproduce is likely linked to declines in rainbow trout abundance as water temperatures increased during the spawning season. The negative relationship between these species and rainbow trout at the stocking site indicate potential for high densities of rainbow trout to cause local population disruptions.

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Table 1. Numbers of tagged smallmouth bass and shadow bass recaptured from Brush Creek between May 2000 and March 2003.



 $\sim 10^{-1}$ 

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Table 2. Mean (SD) relative weights  $(W_r)$  for smallmouth bass  $> 150$  mm in Brush Creek among 50-mm length increments before rainbow trout stocking (February 2000 - September 2000; Pre), and in the first (November 2000- October 2001; Post1) and second (November 2001 - March 2003; Post2) years of stocking.



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## **Figure Captions**

- Figure 1. Length-frequency distributions for smallmouth bass among the five pools sampled on Brush Creek (A) and at the rainbow trout stocking site (B) prior to rainbow trout introduction (February- September 2000, Pre), in the first year of trout stocking (November 2000- October 2001, Postl) and in the second year of trout stocking (November 2001 - March 2003, Post2).
- Figure 2. Length-frequency distributions for shadow bass among the five pools sampled on Brush Creek (A) and at the rainbow trout stocking site (B) prior to rainbow trout introduction (February- September 2000, Pre), in the first year of trout stocking (November 2000-0ctober 2001, Postl) and in the second year of trout stocking (November 2001-March 2003, Post2).
- Figure 3. Scatterplot of stocked rainbow trout catch-per-unit-effort (CPUE, fish/min) versus smallmouth bass CPUE (A) and shadow bass CPUE (B) at the stocking site in Brush Creek.



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# VITA<sup>2</sup>

Maureen Graham Walsh

Candidate for the Degree of

Doctor of Philosophy

#### Dissertation: EVALUATION OF RAINBOW TROUT STOCKING IN A NORTHEASTERN OKLAHOMA OZARK STREAM

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