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The effects of rainbow trout (*Oncorhynchus mykiss*) on the use of spatial resources and behavior of rosyside dace (*Clinostomus funduloides*)

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With 4 figures and 4 tables in the text

Abstract: Rainbow trout (*Oncorhynchus mykiss*) and rosyside dace (*Clinostomus funduloides*) exhibit substantial overlap in microhabitat use in Coweeta Creek, North Carolina, USA. We conducted a replicated experiment in an artificial stream to assess the effects of both the presence of rainbow trout and dace density on: 1) microhabitat use, 2) agonistic behavior, 3) social behavior and 4) feeding rates of dace. Our results demonstrated that microhabitat use of dace was highly variable both among competition treatments within a replicate and for the same competition treatment among replicates. Moreover, the pattern of the differences between competition treatments was not consistent among replicates. As a result, we detected a significant effect of experimental replicate on dace microhabitat use but no significant competition effect. Trout had little effect on the behavior of dace and intraspecific aggression was much more common than interspecific aggression. There were strong inter-individual differences in the agonistic behavior of dace, and 94 of the 127 aggressive displacements observed were initiated by one or two highly aggressive individuals per replicate. We detected few significant acclimation or time of day effects. The significant differences in microhabitat use and behavior observed within replicates appeared to be a consequence of differential initial settlement patterns of dace or the presence of one or two highly aggressive dace in treatments. Hence, we do not believe that interspecific competition with rainbow trout has a strong effect on microhabitat use by dace in Coweeta Creek.

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Introduction

Environmental variation may have a strong effect on a variety of ecological processes within animal assemblages (PICKETT & WHITE 1985, MENGE & SUTHERLAND 1987). Of all North American freshwater habitats, temperate streams exhibit some of the greatest variability with respect to physico-chemical conditions. For example, many streams experience unpredictable increases and decreases in flow levels (e.g. floods and droughts), and hence, can exhibit substantial physico-chemical variability over time scales ranging from days to years (RESH et al. 1988, POFF & WARD 1989, POFF 1996). This variability may affect a variety of ecological characteristics within stream fish assemblages including: 1) assemblage structure (STARRETT 1951, HORWITZ 1978, GROSSMAN et al. 1997, SCHLOSSER 1985, POFF & ALLAN 1995), 2) resource use (GROSSMAN et al. 1996, 1997), and 3) interspecific interactions such as competition and predation (POWER et al. 1985, GREENBERG 1988, HARVEY & STEWART 1991).

For the last fifteen years we have been studying the effects of environmental variation on resource use by fishes residing within Coweeta Creek, North Carolina, USA. During our study period, water levels in this system have fluctuated markedly, encompassing some of the highest and lowest annualized mean daily flows recorded in the last 58 years (GROSSMAN et al. 1995 a, GROSSMAN et al. 1997). In fact, GROSSMAN et al. (1997) demonstrated that variation in flows had a stronger impact on both the structural and functional relationships within this assemblage, than other potential mechanisms of community organization (e.g. resource limitation, or predation). For example, assemblage members utilized spatial resources in a flexible and highly overlapping manner, and seasonal and annual variation in microhabitat availability had a much greater effect on microhabitat use than either potential competitors or predators (GROSSMAN & FREEMAN 1987, GROSSMAN et al. 1997, GROSSMAN & RATAJCZAK 1997). These findings are not unusual for organisms residing within fluctuating environments, and similar results have been obtained for other stream fish assemblages (ANGERMEIER 1987, GORMAN 1988 a, GROSSMAN & DE SOSTOA 1994 a, b, BROWN et al. 1995).

Despite the environmental variability present in many stream systems, several investigators have demonstrated that interspecific competition has a strong effect on microhabitat use by stream fishes (CADWALLADER 1975, FAUSCH & WHITE 1981, BALTZ et al. 1982, ALLAN 1986, GORMAN 1988 b, GREENBERG 1988, FAUSCH et al. 1994). Consequently, as a more rigorous test of our descriptive findings, we conducted an experimental analysis of the effects of rainbow trout (*Oncorhynchus mykiss* WALBAUM) on microhabitat use and behavior of rosyside dace (*Clinostomus funduloides* GIRARD): two species that co-occur in Coweeta Creek and many other southern Appalachian streams.

Rosyside dace, a native cyprinid, and rainbow trout, an introduced salmonid which has replaced the native brook trout (*Salvelinus fontinalis,* MITCHILL), are the two most abundant members of a guild of mid-water column fishes occupying the upper reaches of Coweeta Creek, North Carolina, USA, (GROSSMAN & FREEMAN 1987). In a previous study, GROSSMAN et al. (1997) found that dace and trout exhibited high overlap in microhabitat use over a ten-year period despite variations in both microhabitat availability and diversity, and changes in the abundance of potential competitors and predators. These investigators concluded that competition for space between the two species was unlikely (GROSSMAN et al. 1997), a result in concordance with those of an earlier but less extensive field study (GROSSMAN & FREEMAN 1987).

The findings of GROSSMAN et al. (1997) were complemented by several other observational and experimental studies: the first of which (GROSSMAN & BOULE 1991) demonstrated that rosyside dace do not affect microhabitat use or behavior of rainbow trout in an artificial stream. The second investigation indicated that trout and dace in Coweeta Creek, generally occupied focal-point velocities that were not significantly different (trout 8 of 8 cases, dace 6 of 8 cases) from optimal velocities predicted by an energy-maximization model of microhabitat use (HILL & GROSSMAN 1993). Nonetheless, HILL & GROSSMAN (1993) suggested that one of the two deviations by dace from optimal microhabitat use may have been caused by competitive displacement of dace by other species. In a subsequent observational study, FREEMAN & GROSSMAN (1992 a) found that dace which occurred in foraging groups containing both dace and rainbow trout had: 1) lower feeding rates, and 2) higher group departure rates than dace which foraged in monospecific groups. However, agonistic interactions between trout and dace were extremely rare, and dace did not differ significantly in the frequency with which they joined aggregations containing only dace or both dace and trout (FREEMAN & GROSSMAN 1992 a). Hence, given the results of previous studies, it is conceivable that the presence of trout may influence either microhabitat use or behavior of dace. To examine this possibility, we conducted an experiment in a laboratory – housed artificial stream. These experiments addressed whether the presence of trout at natural densities influenced: 1) microhabitat use, 2) agonistic behavior, 3) social behavior, or 4) feeding rates of rosyside dace.

Materials and methods

We captured dace in pools of Coweeta Creek using dip nets. Rainbow trout were obtained by electrofishing (DC) areas of Shope Fork near its confluence with Coweeta Creek. We measured all fish (fork length ± 1 mm) with a straight-edge prior to their use in experiments (Table 1). We then either placed fish in the artificial stream for a repli-

Table 1. Mean lengths of fish and initiation dates for each competition treatment in each replicate. Fish groups are E = experimental subjects and T = treatment fish. Treatment fish in DT treatments are rainbow trout. Treatments are D = 5 dace, DD = 10 dace, DT = 5 dace + 5 trout.

Replicate	Treatment	Start Date	Fish Group	length ± sd (mm)	Range (mm)
1	D	11/1/94	E	58 ± 5	66–52
	DD	10/18/94	E	63 ± 10	79–48
			T	61 ± 9	75–49
	DT	10/25/94	E	61 ± 8	72–50
			T	77 ± 5	85–69
2	D	18/5/95	E	69 ± 10	81–55
	DD	29/4/95	E	68 ± 8	79–57
			T	67 ± 8	78–55
	DT	10/5/95	E	67 ± 7	75–55
			T	95 ± 11	103–82
3	D	4/8/95	E	65 ± 8	76–54
	DD	17/7/95	E	70 ± 9	83–57
			T	68 ± 9	80–55
	DT	27/7/95	E	61 ± 4	67–54
			T	61 ± 5	70–56

cate or held them in an adjacent tank. Specimens were used in only one treatment segment of a single experimental replicate.

Our experimental design required the identification of individual subjects. In the first replicate of the experiment we individually marked all dace with subcutaneous injections of acrylic paint (Hill & Grossman 1987), but in subsequent replicates, we identified individual dace by their size and markings (e.g. color patterns, spots, scars, etc.). Hill & Grossman (1987) showed that dace marked with latex paint did not exhibit reduced survivorship and there were no visually detectable differences in the behavior of marked and unmarked individuals. Individual trout were identified using natural markings and unique morphological characteristics.

We fed fishes using automatic feeders that released trout chow pellets (typically 1 mm wide × 1.5 mm long; range 0.5 × 0.75 to 2 × 4 mm) at the surface of the stream at intervals ranging from 40 to 80 minutes (average = 60 minutes) during dawn, daytime and dusk (Grossman & Boule 1991). The total daily ration approximated 12 g of food, which represented at least a maintenance ration for these fishes (see below). We placed feeders above locations with the highest surface velocities, and food was rapidly dispersed throughout the stream. Neither trout nor dace aggregated under feeders. Pellets remained on the water column for at least 10–15 minutes, and provided a reasonable simulation of the drifting prey consumed by these species in Coweeta Creek (Stouder 1990, Freeman & Grossman 1992 a).

In concordance with the findings of Grossman & Boule (1991), all dace and trout in experiments either gained or maintained their initial wet weights (± 0.01 g) through the end of experiments. Mortality in our replicates was limited to two dace that jumped out of the stream at the beginning of the first replicate and were subsequently replaced by fish of identical size. We prevented additional mortalities by placing a sheet of transparent plastic approximately 50 cm above the surface of pools.

Experimental procedures

A thorough description of the indoor artificial stream was provided by Grossman & Boule (1991) and Grossman et al. (1995 b). In brief, the stream is a 10 m long oval, 50–75 cm wide, with two pools (maximum depth ≈50 cm) and two riffles (maximum depth = 10 cm). Pools and riffles are connected by gently sloping areas that simulate runs. Water temperature was controlled by a cooling system equipped with a thermostat and during the three replicates it was 15 °C (± 0.5 °C). Photoperiod was 14.5 h light: 9.5 h dark with 1 h phases of gradually increasing and decreasing illumination that simulated dawn and dusk. These conditions approximate those found in Coweeta Creek during early to mid-summer.

We also attempted to replicate experiments using spring and fall conditions (i.e., 10 °C, 13 h light: 11 dark) during both March and October 1995. However, during these attempts dace bid under stones and were not visible for periods of several days. Subsequent field observations indicated that dace in Coweeta Creek also were inactive at these temperatures, and hidden beneath cover (large cobbles, boulders, undercut banks, roots, snags). Hill & Grossman (1993) have reported similar behaviors, though at lower water temperatures. Consequently, we discontinued our attempt to conduct experiments under spring and fall environmental regimes, and based our conclusions on summer conditions when space is potentially most limiting (Grossman et al. 1997).

Our experimental design resembled that of Grossman & Boule (1991) in that we had three treatments which were used to test for the effects of both fish density and interspecific competition (i.e., trout). We initiated a replicate releasing 10 dace into the stream (high density dace treatment – DD). We began replicates with the high density treatment because at times it was difficult to capture sufficient numbers of dace to complete all three segments of a replicate. Five dace were then randomly selected as experimental subjects and we obtained microhabitat use and behavioral data from these specimens. We recorded data for only five dace to ensure that sample sizes would remain equal among treatments (see below). Observations were begun after a 48 h acclimation period and we collected data three times daily (morning: 08:00–10:00, afternoon: 13:00–15:00, and evening: 18:00–20:00) for five days. We then removed all specimens, and replaced them with five different dace and five trout (interspecific competition treatment – DT). These fish also were allowed to acclimate for 48 hours, prior to the initiation of a second five-day period of observations. We concluded each replicate by replacing trout and dace with five new dace (low density dace treatment D) for a final five-day observation period.

The relative sizes of dace and trout in DT treatments differed among replicates (Table 1). Nonetheless, we utilized trout size classes that were most abundant in Coweeta Creek at the time experiments were conducted, and hence, our experiments approximated natural conditions. The sizes of "subject" and "treatment" dace in the DD treatments always were similar (Table 1). The densities of dace used throughout the experiments (1–2 individuals/m^2) are well within those recorded within a variety of habitats in Coweeta Creek (Grossman & Freeman 1987, Freeman et al. 1988, Freeman & Grossman 1993).

We collected data using an observational protocol similar to that of Grossman & Boule (1991). Each of the three daily observation periods lasted for 1 h. At 15 min intervals during this period, we located subject fish and recorded their positions using X (longitudinal position, nearest cm), Y (distance from the channel wall, nearest cm), and Z (distance from the bottom, nearest cm) coordinates, as well as microhabitat type (pool, run or riffle). In addition, once a treatment segment was finished, we measured total depth (cm), distance from shelter, (i.e., a structure capable of concealing at least 50% of the fish's body, nearest cm), focal-point velocity (cm/s), average water column velocity (cm/s), and the percentage of bedrock, boulder, cobble, gravel, sand, silt and debris in a 20×20 cm quadrat directly below the fish for each of the previously recorded fish positions. However, only data corresponding to the first fish location for each 1 h observation period were used in the analyses presented here. All measurements were made using the techniques of GROSSMAN & FREEMAN (1987). We made linear measurements using a straight edge, velocity measurements with an electronic velocity meter, and estimated substratum composition visually (GROSSMAN & FREEMAN 1987). To estimate microhabitat availability we recorded microhabitat type, total depth, average velocity and substratum composition in 40 randomly selected points at the end of each replicate. We did not make repeated microhabitat availability measurements because GROSSMAN & BOULE (1991) and GROSSMAN et al. (1995 b) have shown that microhabitat availability in the artificial stream did not change over a 15-day period.

During each 1 h observation set we also made 3 min of behavioral observations for each specimen. We recorded the following data: 1) number of strikes at food pellets, 2) the time spent within 15 cm of a conspecific, 3) number of displacements performed by the focal specimen, 4) number of displacements of the focal individual by other specimens, 5) number of chases by the focal individual, and 6) number of times the focal individual was chased, and 7) the sizes and identities of all specimens in interactions. Chases and displacements were identified using the criteria of FREEMAN & GROSSMAN (1992 a, b). We initiated behavioral observations 3 min after food was released into the stream. The subject fish generally formed a single aggregation or were close enough to each other that they could be observed simultaneously. In the few cases where all fish were not observable at the same time, we randomly chose a group of specimens for measurement until observations had been completed on all fish. Even when this occurred, it rarely took more than six minutes to complete all observations. Consequently, it is likely that food availability during behavioral observations probably did not differ among subject fish.

Statistical design

To quantify non-random microhabitat use by fishes, we first transformed availability measurements to either their ln (linear measurements) or arcsine (substratum composition) and performed a principal component analysis (PCA) on the transformed data using the correlation matrix. We only interpreted axes with eigenvalues >1. We then calculated scores for each fish observation on each component by multiplying the vector for the fish data by the scoring coefficient matrix of the microhabitat availability PCA (see GROSSMAN & FREEMAN 1987). Raw data for fish observations were transformed prior to the PCA using transformations identical to those utilized for microhabitat availability data. Finally, for each component we derived frequency distributions for both microhabitat use and availability scores and compared them with a partitioned chi-square test for independence (ZAR 1984). Alpha levels were adjusted for multiple comparisons with the Dunn-Sidak procedure (URY 1976). These statistical techniques have been successfully employed to elucidate non-random microhabitat use by stream fishes in several previous descriptive and experimental studies (GROSSMAN & FREEMAN 1987, GROSSMAN & BOULE 1991, GROSSMAN et al. 1995).

Our experimental design was a full factorial design with four factors: block (i.e., replicate), competition, acclimation, and time of day. The block factor controlled for possible differences among replicates and, therefore, had three levels. Competition consisted of three treatments representing different levels of intra-and interspecific competition (i.e., low density dace, D = 5 dace, high density dace, DD = 10 dace, and interspecific competition, DT = 5 dace + 5 rainbow trout). Acclimation included five treatments representing the number of days since the beginning of an observation period, and time of the day possessed three treatments (i.e., morning, afternoon, evening). The last two factors included repeated measurement of the same specimen (WINER et al. 1991). This design enabled us to assess the relative significance of intra- and interspecific competition while controlling for variations in the responses of individual dace to both the artificial stream and time of day. In addition, we examined the significance of all interaction terms, although we only report results for interaction terms that possessed at least one significant main effect.

Following ENDE (1993) we tested for significant differences among factors using a repeated-measures analysis of variance (ANOVAR). Because the data were not normally distributed we transformed the dependent variables to ranks as suggested by POTVIN et al. (1990) (also see CONOVER & IMAN 1981, and KEPNER & ROBINSON 1988). ANOVAR assumes sphericity of the variance-covariance matrix, when this assumption is not met the F-statistics of factors with repeated measures are inflated and it is necessary to decrease their degrees of freedom according to the magnitude of the violation (WINER et al. 1991). Huynh-Feldt's ϵ is an appropriate metric for quantifying departures from sphericity, and values of $\epsilon < 1$ are indicative of non-sphericity (WINER et al. 1991). For our data, values of ϵ were often 1 and always >0.85 indicating only mild deviations from sphericity. Therefore, we adjusted significance levels with the Huynh-Feldt correction by multiplying the nominal degrees of freedom by ϵ (POTVIN et al. 1990, ENDE 1993). Finally, when significant effects were detected by ANOVAR, we used Tukey's HSD test for a posteriori comparisons. Statistical analyses were carried out with the SAS 6.0 and Statistica 4.5 computer packages. We have presented average values of the untransformed variables when they would be more informative to the reader than average ranks (e.g., focal-point velocity, distance from shelter and behavioral variables).

Results

Microhabitat use

Rosyside dace almost always occurred in pools. We never observed dace in riffles, though in 4 of 675 total observations they did occupy transitional areas between runs and pools. The distribution of dace among habitat types always was significantly non-random with specimens under-represented in riffles and runs and significantly over-represented in pools (partitioned chi-square, $P<0.0001$).

The PCA of microhabitat use data from replicates 1–3 (henceforth R1–R3) produced three to four components with eigenvalues higher than 1. These components explained between 68 and 73 % of the variance in the data set. The first component (PC1) accounted for much of this variance (i.e., 27–37 %), and displayed similar variable loadings among all three replicates (Fig. 1). These loadings indicated that component 1 depicted a pool-riffle gradient which contrasted shallower areas with higher average velocities and greater amounts of erosional-substrata (cobble and bedrock) against deeper locations with lower average velocities, possessing high amounts of depositional substrata (gravel, silt and debris). The remaining components did not extract consistent patterns of microhabitat availability among replicates. They appeared to reflect particular substratum combinations within pools or riffles or were ecologically uninterpretable and hence, are not discussed further.

Dace exhibited non-random microhabitat use on PC1 in all replicates (Chi-square, $P<0.05$). Although differences existed, dace always avoided shallow, high velocity locations and preferred deeper, depositional areas (Fig. 1). The ANOVAR detected significant differences in PC1 scores among replicates (i.e., significant block effect) and a significant replicate x competition interaction term (Table 2), but the effect of the competition factor alone was not significant ($F = 1.25$, df = 2, $P = 0.23$). These results reflected the variability in microhabitat use exhibited by dace. Tukey tests did detect significant differences between competition treatments within a block for all three blocks, but the pattern was not consistent. Thus, in R1 dace at both low density (D) and in

Table 2. Significant treatments (R = replicate, C = competition, A = acclimation, T = time of the day) and interaction terms for the ANOVAR on microhabitat use (i.e. PC1 score) by dace in. Treatments are: D = 5 dace, DD = 10 dace, DT = 5 dace + 5 trout. Average ranks of levels with the same superscript are not significantly different (Tukey test, $P>0.05$). Note that Huynh-Feldt (H-F) correction only applies to factors that have repeated measurements and their interaction terms.

Effect	d.f.	adjusted d.f.	*F*	Tukey test
R	2	–	8.6***	$1^{1,2}\ 2^{2}\ 3^{1}$
A	4	4	2.6*	$1^{1}\ 2^{2}\ 3^{1,2}\ 4^{1,2}\ 5^{1,2}$
R × C	4	–	20.0***	–
C × A	8	8	3.3**	–
R × C × A	16	16	2.8**	–
R × A × T	16	8	2.75*	–

* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

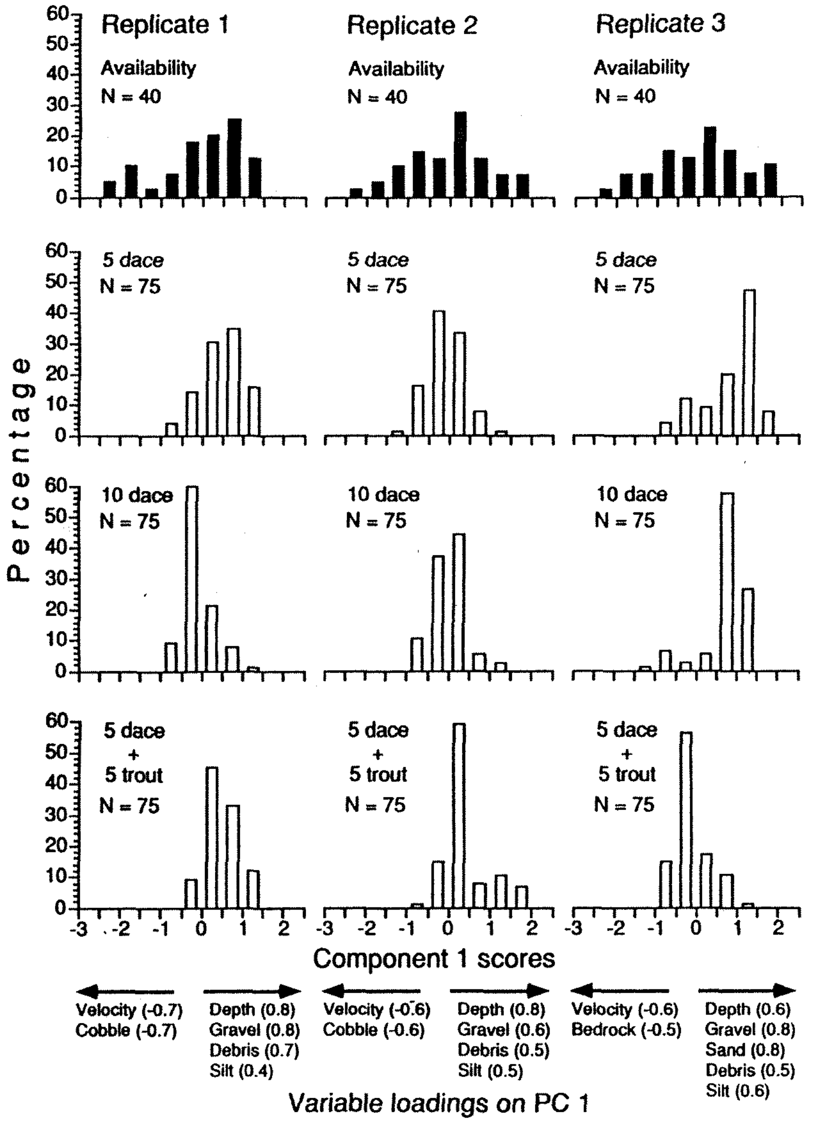

Fig. 1. Component 1 variable loadings (>0.4) and score distributions for both microhabitat availability and microhabitat use by the 5 subject dace for all blocks (= replicates – R1, R2 and R3) and competition levels.

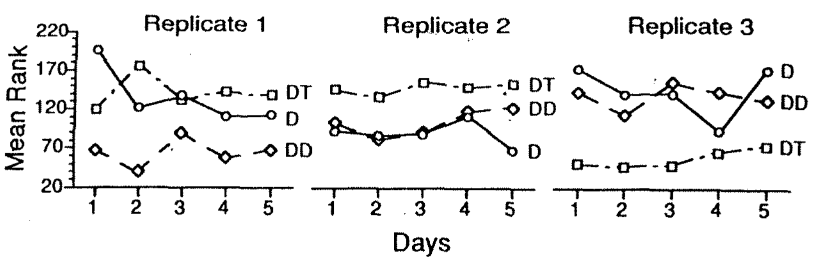

Fig. 2. Daily average ranks of PC1 scores for each competition treatment (D = 5 dace, DD = 10 dace, DT = 5 dace + 5 trout) and block.

the presence of trout (DT) occupied deeper areas with more depositional substrata than in the high density treatment (DD). In R2, however, dace in DD did not utilize microhabitats that were significantly different from specimens in either D or DT. Nonetheless, dace in DT did occupy deeper microhabitats with greater quantities of depositional substrata than dace in D. Finally, in contrast to R1 and R2, during R3 dace in DT occupied shallower areas with more boulders and lower quantities of depositional substrata than dace in either D or DD, which showed no significant differences beween them (Figs. 1 and 2).

Moreover, the Tukey tests also demonstrated significant differences in microhabitat use between dace under the same competition treatment in different blocks for all three competition treatments. For example, dace at high density (DD) in R1 and R2 occupied shallower areas with more erosional substrata than during R3. Additionally, dace at low density (D) used deeper areas with more depositional substrata in R2 than in either R1 or R3 as did dace with trout (DT) during R3 relative to dace in DT during R1 or R2. (Fig. 2). Given the significant block (i.e., replicate) effect and the lack of a significant competition effect, the treatments differences detected probably are a consequence of a high inter-individual variability in dace microhabitat use rather than competitive interactions. Because dace in all cases occupied pools almost exclusively, the observed differences in microhabitat use appear to be related to the use of differing velocities and depths within pools.

The significant acclimation effect that the ANOVAR detected (Table 2) appeared to be due to a shift by dace to microhabitats with higher velocities and greater amounts of erosional substrata on day 2 (Fig. 2). This shift produced a significant difference between PC1 scores of days 1 and 2 (Tukey test, $P = 0.047$). After day 2, dace again occupied locations with characteristics similar to those used on day 1.

The focal velocities used by dace showed significant differences between replicates and also a significant replicate x competition interaction, but no significant competition effect (Table 3). As with PC scores, this appeared to be related to the high inter-individual variability in microhabitat use displayed by dace. Thus, Tukey tests detected significant differences between the focal velocities occupied by dace in the same competition treatment of different repli-

Table 3. Significant treatments (R = replicate, C = competition, A = acclimation, T = time of the day) and interaction terms for the ANOVAR on univariate microhabitat variables (FV = focal velocity, SHD = distance from shelter). Competition treatments are described in Table 2. Acclimation treatments represent days 1 to 5. Average ranks of levels with the same superscript are not significantly different. Note that Huynh-Feldt (H-F) correction only applies to factors that have repeated measurements and their interaction terms.

Variable	Effect	d.f.	adjusted d.f.	*F*	Tukey test
FV	R	2	–	45.74***	$1^1\ 2^2\ 3^1$
	A	4	4	5.25***	$1^1\ 2^1\ 3^{2,3}\ 4^{1,2}\ 5^3$
	R×C	8	4	22.49***	–
	R×A	4	8	2.09*	–
	R×C×A	16	16	3.57***	–
	R×C×T	8	8	3.24**	–
SHD	R	2	–	3.31*	$1^1\ 2^2\ 3^{1,2}$
	R×A	8	8	3.40**	–
	R×C×A	16	16	3.87***	–

* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

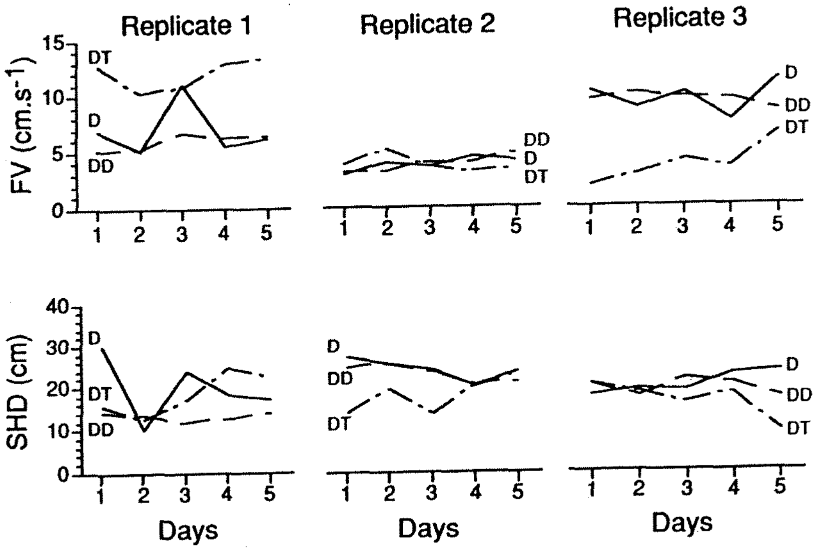

Fig. 3. Daily mean focal-point velocities (FV, $cm \cdot s^{-1}$) occupied by dace in R1–R3 and daily mean distance of dace from shelter (SHD, cm) for R1–R3. Significant effects and interactions are presented in Table 3.

cates. There also were significant differences among competition treatments within a replicate in R1 and R3. However, in R1 dace in DT used significantly higher velocities than in D or DD, whereas, in R3 dace in DT used significantly lower focal velocities than in the other two treatments (Fig. 3). The apparent lack of concordance with our findings for the PCA (Figs. 1 and 3) stems from the fact that within pools (where dace microhabitat shifts occurred) depth and velocity were not inversely correlated as they were for the stream as a whole (i.e., pools versus riffles).

We also detected significant acclimation effects and replicate x acclimation interaction terms in the use of focal-point velocities (Table 3). These results appear to have been produced by a trend for dace to use significantly higher velocities in days three and five than in days one or two (Table 3, Fig. 3).

Dace also displayed a significant replicate effect for distance from shelter, with dace being significantly farther from cover in R2 than in R1 (Fig. 3, Table 3). There were no other significant single factors, but the interactions between replicate and acclimation and between replicate, competition and acclimation were significant (Table 3). It is likely that these results were due to a shift in the distance to cover of dace between days one and two of the D treatment in R1 (Fig. 3) that did not occur in other replicates.

Behavior

The presence of trout (DT) or dace at high density (DD) did not elicit consistent responses in the agonistic (i.e., displacements or chases), feeding, or social behavior of dace (Table 4, Fig. 4). Most significant effects were attributable to either: 1) variation in the behavior of dace either on a particular day or during a particular time of day, which resulted in significant main effects or interactions terms. These produced effects that were significant but biologically uninterpretable (e.g., displacements performed or received, time near conspecifics, feeding rate, Table 4, Fig. 4), or 2) significant interaction terms without significant main effects (i.e., chases received, Table 4). For brevity, these results will not be discussed further.

Both feeding rate and the number of chases received showed significant replicate effects and significant replicate x competition interactions (Table 4) that appeared to reflect a relatively large intrinsic variability in dace behavior. Feeding rate was significantly lower in R1 than in R2 or R3 and dace received a marginally significant higher number of chases in R1 than in R2 (Tukey test, P = 0.045) or R3 (P = 0.044). For both variables, the pattern of differences among competition treatments within a replicate (all non-significant, Tukey tests) changed between replicates (Fig. 4, Table 4).

Displacements were the most common agonistic interaction (dace performed a total of 256 displacements and 50 chases and received 243 displacements and 51 chases, respectively) Dace received significantly more displacements in R1 than in R3, whereas R2 was not significantly different from either R1 or R3. The number of displacements received was the only variable that showed a significant competition effect (Table 4). However, even this result could not be readily interpreted as a competitive effect, because it was attributable to the fact that dace in DD treatments were displaced significantly fewer times than dace in D or DT. There was no significant difference between these last two treatments. In R1 dace were displaced more in DT, than in D, but the reverse was true for R2 and R3. Although these differences were not significant (Tukey tests, P>0.05), this resulted in a significant replicate x competition interaction (Fig. 4, Table 4). Furthermore, 73 of the 103 displacements observed in the three DT treatments occurred in R1 and of these only 9 were performed by trout (3 of 19 in R2 and 1 of 11 in R3). In addition, dace displayed high inter-individual variability in agonistic behavior. For example, in R1 59 of the 73 displacements in DT were instigated by two particularly aggressive dace (dace 1 – 42 displacements, dace 2 – 17 displacements). Similar results were observed during D in R3 where one individual produced 35 of 54 displacements and during DD in R1 with two fish responsible for 17 of 20 chases.

Table 4. Significant treatments (R = replicate, C = competition, A = acclimation, T = time of the day) and interaction terms for the ANOVAR on behavioral traits of dace (DP = displacements performed, DR = displacements received, CR = chases received, FE = feeding rate, TNC = time near a conspecific). Competition and acclimation levels are described in Tables 2 and 3. Average ranks of levels with the same superscript are not significantly different. Note that Huynh-Feld (H-F) correction only applies to factors that have repeated measurements and their interaction terms.

Variable	Effect	d.f.	adjusted d.f.	*F*	Tukey test
DP	A	4	4	3.03*	$1^{1}\ 2^{1,2}\ 3^{1,2}\ 4^{1,2}\ 5^{2}$
	R×A×C	16	16	1.93*	
DR	R	2	–	4.08*	$1^{1}\ 2^{1,2}\ 3^{2}$
	C	2	–	4.50*	$D^{1}\ DD^{2}\ DT^{1}$
	A	4	4	4.22**	$1^{1}\ 2^{1,2}\ 3^{1,2}\ 4^{1,2}\ 5^{2}$
	R×C	4	–	3.50*	–
	R×A	8	8	2.00*	–
	C×A	8	8	2.33*	–
CR	R	2	–	4.14*	$1^{1}\ 2^{2}\ 3^{2}$
	R×C	4	–	2.99*	
	R×A	8	8	3.05**	
TNC	A	4	4	3.38*	$1^{1,2}\ 2^{1}\ 3^{1,2}\ 4^{1,2}\ 5^{2}$
	C×A	8	8	2.69**	–
	R×C×A	16	16	3.32***	–
FE	R	2	–	17.69***	$1^{1}\ 2^{2}\ 3^{2}$
	R×C	4	–	3.52*	–
	R×C×A	8	8	1.95*	–

* = P<0.05; ** = P<0.01; *** = P<0.001.

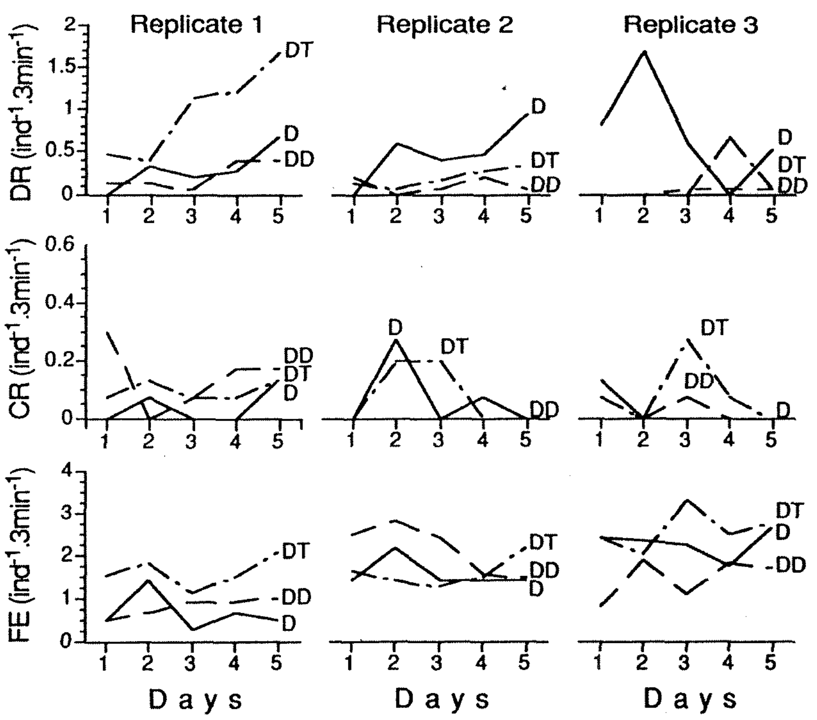

Fig. 4. The effects of competition treatment and time of day on: 1) mean number of displacements received (DR) by dace, 2) mean number of chases received (CR) by dace, and 3) mean feeding rate (FE). Only significant results are presented (see Table 4).

Discussion

Animals that reside in variable environments commonly exhibit substantial flexibility in their use of ecological resources. Rosyside dace are no exception to this pattern, and microhabitat use by this species varies on the basis of 1) the individual, 2) season, 3) hydrologic regime, and 4) fish length (GROSSMAN & FREEMAN 1987, FREEMAN & GROSSMAN 1992 b; GROSSMAN & RATAJCZAK 1997, GROSSMAN et al. 1997). Hence, it is not unexpected that the presence of trout or additional conspecifics did not produce consistent responses in microhabitat use and behavior of dace and that the ANOVAR did not detect significant competition effects. For example, the addition of trout elicited: 1) a lack of microhabitat shifts by dace (R1), 2) a shift to deeper, microhabitats with greater quantities of depositional substrata (R2), and 3) a shift to shallower, microhabitats with higher amounts of erosional substrata (R3). It is possible that the disparate microhabitat shifts by dace in R2 and R3 are a result of the fact that trout in R2 were substantially larger than those in R3 (Table 1). However, GROSSMAN & FREEMAN (1987) and GROSSMAN & RATAJCZAK (1997) have shown that 9 cm trout (R3) occupy deeper microhabitats than 6–7 cm trout (R2), which suggests that if anything, the response of dace to trout may have been attraction rather than avoidance. Unfortunately, we did not record the exact positions of trout during replicates, so we cannot explicitly distinguish between these two possibilities (i.e., attraction versus avoidance). Nonetheless, behavioral observations of dace yielded no indication that dace were either strongly attracted to, or repelled by trout (RINCÓN & GROSSMAN, pers. observation). Hence, even if the presence of trout influenced microhabitat use by dace, the results of this interaction were not consistent across replicates.

Instead, the microhabitat shifts exhibited by dace appeared to be a consequence of the settlement patterns of individual dace after placement in the artificial stream (i.e., although dace in each competition treatment were introduced into the stream in the same spot, they quickly aggregated in one of the pools, and maintained this aggregation throughout the treatment). Because new subject dace were used for each competition treatment in each replicate (total dace groups = 9), it is reasonable to suggest that chance differences in settlement affected our results, especially given the lack of consistency in the responses of dace to trout. We used new dace in each competition segment because it enabled us to analyze our data as a full factorial design. It is now clear that the substantial inter-individual variability observed in the behavior of dace may have reduced our ability to clearly demonstrate either the presence or absence of interspecific competition or dace density effects on microhabitat use or behavior of dace. Nonetheless, if these effects were present, they were not strong enough to override the natural variability inherent in the behavior of individual dace. Finally, very few rainbow trout in the Coweeta drainage attain a size large enough to become potentially piscivorous (GROSSMAN et al. 1997). In addition, both GROSSMAN & FREEMAN (1987) and GROSSMAN et al. (1997) found that predators had no significant effects on microhabitat use by fishes in Coweeta Creek. Consequently, it seems reasonable to suggest that dace and trout are probably "weak interactors"; a relationship similar to that of many intertidal invertebrates and algae studied by PAINE (1980).

In contrast to our results, FREEMAN & GROSSMAN (1992 a) found that the presence of trout within foraging groups of dace in Coweeta Creek elicited both higher departure rates and lower feeding rates from dace in comparison to those for dace in monospecific aggregations. We did not measure arrival or departure rates of dace from foraging groups, but we did find that the addition of trout to the stream produced no significant effect in dace foraging rates (the increase of the latter in R3 did not reach significance). In addition, FREEMAN & GROSSMAN (1992 a) also demonstrated that despite the aforementioned negative interactions between dace and trout, dace still joined aggregations containing trout with frequencies that were not significantly different from those for monospecific aggregations of dace. The differences between our results and those of Freeman & Grossman (1992 a) may have been caused by a variety of factors including: local food abundance, food type and quantity (maintenance versus unknown), season (mid-summer versus autumn), and year.

In other systems, however, salmonids typically are competitively dominant over a variety of cyprinid species (Li 1975, Symons 1976, Reeves et al. 1987). In fact, we did observe interspecific interactions in which trout displaced or chased dace, or dace avoided trout that appeared to be unaware of their presence. Nonetheless, agonistic interactions between trout and dace were uncommon, especially when compared to aggressive interactions between dace (overall, in DT treatments we only observed 3 chases and 13 displacements of dace by trout, whereas dace-dace interactions yielded 16 chases and 90 displacements). Freeman & Grossman (1992 a) described an identical pattern for dace in Coweeta Creek, with respect to the relative frequencies of interspecific (dace-trout) versus intraspecific agonistic interactions.

It is possible that the disparities between our results and those of other investigators regarding the dominance of trout in trout-cyprinid interactions are a function of differing biological conditions. Many stream salmonids (including rainbow trout) exhibit higher levels of aggression and territoriality in areas with higher velocities (e.g., riffles) than in lower velocity habitats (e.g., pools, Kalleberg 1958, Hartman 1965, Gibson 1978, Puckett & Dill 1985, Grant & Noakes 1988 and references therein). In fact, salmonids in pools generally display low levels of intra- and interspecific aggression and may even form aggregations (e.g., Wankowski & Thorpe 1979). Thus, it is not surprising that the salmonids studied by both Symons (1976) and Reeves et al. (1987) held feeding positions in riffles or higher velocity areas, and also exhibited interspecific territoriality. Rainbow trout in Coweeta Creek inhabit deep, intermediate velocity areas that include runs, tails of riffles and heads and tails of pools (Grossman & Freeman 1987, Grossman et al. 1997), but trout in our experiment almost always occupied heads and tails of pools, and displayed low levels of both intra- and interspecific aggression. In addition, rather than exclude dace, trout frequently occurred in foraging groups with members of this species. Similar behaviors were observed by Grossman & Boule (1991) in their study of the effects of dace on microhabitat use and behavior of rainbow trout, as well as by other researchers who have examined patterns of microhabitat use by dace and trout in Coweeta Creek (Grossman & Freeman 1987, Freeman & Grossman 1992 a, b, Grossman & Ratajczak 1997, Grossman et al. 1997).

Our results also illustrate the high level of inter-individual variability in agonistic behavior among dace (i.e., the majority of agonistic interactions were instigated by one or two highly aggressive dace per replicate). In fact, it

appeared that a few highly aggressive dace were responsible for many of the significant effects observed in replicates and that neither the presence nor the behavior of these individuals were linked with dace density or the presence of trout. Freeman & Grossman (1992 b) also observed highly aggressive dace in Coweeta Creek, and found that these individuals had a disproportionate effect on both arrival and departure rates of dace from foraging groups. As with our results, Freeman & Grossman (1992 b) could not correlate the presence or behavior of these specimens with either dace density or abundance of potential competitors. High inter-individual variability in agonistic behavior has been reported by other researchers working with different species of drift-feeding cyprinids (Symons 1975, Reeves et al. 1987, Tyler 1993, Katano 1996).

Although interspecific competition clearly affects resource use in some species pairs (Cadwallader 1975, Baltz et al. 1982, Nakano & Furukawa-Tanaka 1994) our results indicate that under summer temperatures and above-maintenance food rations, the addition of rainbow trout did not produce consistent changes in either microhabitat use or behavior of rosyside dace. In contrast, the significant differences observed were more likely a consequence of the initial settlement patterns of dace or the chance presence of one to two highly aggressive dace in treatments, although differences in the size of the fishes used in the experiments or seasonal effects also may have affected our findings. In addition, it is worth noting that the abundance of both dace and trout in Coweeta Creek is strongly affected by variations in flow levels (i.e., floods and droughts, Freeman et al. 1988). These findings, coupled with the fact that dace failed to exhibit strong behavioral or consistent microhabitat responses in the presence of trout, suggest that interspecific competition may have less of a role in determining resource use by dace than unpredictable environmental variation (Grossman et al. 1997). The wide behavioral flexibility exhibited by dace may increase the individual fitness of members of this species in Coweeta Creek: an environment with strongly fluctuating physicochemical and biological characteristics (Freeman et al. 1988, Grossman et al. 1997).

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