

## Effects of barriers and thermal refugia on local movement of the threatened leopard darter, *Percina pantherina*

Jacob F. Schaefer<sup>a,c</sup>, Edie Marsh-Matthews<sup>a,b</sup>, Daniel E. Spooner<sup>c</sup>, Keith B. Gido<sup>a,f</sup> & William J. Matthews<sup>a,d</sup>

<sup>a</sup>Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK 73072, U.S.A.

<sup>b</sup>Department of Zoology, University of Oklahoma, Norman, OK 73072, U.S.A.

<sup>c</sup>Oklahoma Biological Survey, University of Oklahoma, Norman, OK 73019, U.S.A.

<sup>d</sup>Biological Station, University of Oklahoma, Kingston, OK 73439, U.S.A.

<sup>e</sup>Present address: Department of Biology, Southern Illinois University Edwardsville, Edwardsville, IL 62026, U.S.A. (e-mail: jacscha@siue.edu)

<sup>f</sup>Present address: Division of Biology, Ackert Hall, Kansas State University, Manhattan, KS 66506, U.S.A.

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### Synopsis

Local, short-term dispersal by the U.S. federally-threatened leopard darter, *Percina pantherina*, was examined in the field and in the laboratory to assess the possible effects of natural *versus* man-made barriers on movement. Mark-resight studies were conducted in two summers at sites in the Glover River (southeastern Oklahoma, U.S.A.). At one site, patches of 'preferred' habitat were separated by a natural riffle; at the other site, by a low-water road crossing with culverts. At the Natural Riffle site, darters moved downstream across the riffle, but also moved upstream into deeper water when water temperatures exceeded 29°C in the 'preferred' habitat. Use of deeper, cooler waters by this species in late summer suggests that thermal refugia may be important habitats for the long-term management of leopard darters. At the Road Crossing site, all documented movement was in a downstream direction, and at least two darters traversed culverts in the low-water bridge. Laboratory studies of movement across several types of culverts suggested that culverts significantly decrease the probability of movement among habitat patches.

### Introduction

Management to protect threatened and endangered stream fishes requires preservation of preferred or optimal habitat, the full spectrum of habitat types used and the corridors that allow movement among them (Schlosser 1995, Smithson & Johnston 1999). The importance of long-distance movement for spawning (e.g., Colorado pikeminnow, *Ptychocheilus lucius*, Tyus 1990) or seasonal avoidance of habitat types of diminished quality (e.g., brown trout, *Salmo trutta*, Clapp et al. 1990) is documented for many stream fishes. The significance of short-term, localized movements among habitats is becoming recognized increasingly because such movements are important for habitat assessment (Power 1984), foraging

(Clapp et al. 1990), escape from predators (Power et al. 1985, Harvey 1991), or use of thermal refugia (Kaya et al. 1977, Matthews & Berg 1997). Studies of movement in many species of stream fishes reveal that typically only a few individuals in a population move long distances (Smithson & Johnston 1999, Schaefer 2001) while most individuals tend to remain in a home pool but make exploratory forays into adjacent habitat (Smithson & Johnston 1999). These short-term, localized movements among habitats suggest that even sedentary stream fishes may require larger areas of habitat than typically assumed (Smithson & Johnston 1999).

This study examined effects of road crossings on local movements of the U.S. federally-threatened leopard darter, *Percina pantherina*, to make

recommendations about habitat management for this species in the Glover River, Oklahoma. *Percina pantherina* was listed as threatened by the U.S. Fish and Wildlife Service in 1978, and critical habitat was designated in three major tributaries of the Little River system in Oklahoma and Arkansas (U.S. Fish and Wildlife Service 1978). The historic range of this species encompasses five of the six major drainages of the Little River system in southeastern Oklahoma and southwestern Arkansas (Miller & Robison 1973, Eley et al. 1975, Zale et al. 1994). Four of the five known populations are restricted to upland reaches and are isolated from other populations by downstream reservoirs (Zale et al. 1994, Williams et al. 1999). The only unimpounded drainage with the leopard darter is the Glover River, McCurtain County, Oklahoma (Zale et al. 1994, Williams et al. 1999). The population in this drainage is relatively large (Zale et al. 1994, Williams et al. 1999), although recent surveys by U.S. Fish and Wildlife Service and United States Department of Agriculture (USDA) Forest Service suggest that the Glover River population is declining (K. Collins unpublished data). Much of the critical habitat in the Glover River currently is managed by the USDA Forest Service with goals that include maintaining thriving populations of leopard darters. To this end, the Forest Service sought information to determine the need for modifying or removing road crossings that might inhibit leopard darter passage among habitat patches (R. Standage, personal communication).

Many low-water crossings allow water to pass through round pipe or square-to-rectangular box culverts. These culverts vary in size, but all potentially reduce migration of fishes by concentrating discharge and reducing the navigable cross-sectional area fish use to move within the stream. Pipe culverts in particular significantly reduce movement of stream fishes, although other types of openings may not impact movement (Warren & Pardew 1998). Laboratory studies of swimming ability suggest that current velocity in both pipe and box culverts restricts movement of leopard darters (Toepfer et al. 1999), although rates of movement under natural conditions are unknown for this species. The degree to which leopard darters move across barriers, either natural or artificial, is likely to depend on the characteristics of the barrier itself. Current velocity, riffle length, and/or thalweg depth affect movement of various stream fish across natural riffles (Lonzarich et al. 2000, Schaefer 1999, 2001). Furthermore, different species show differential rates of movement across both natural and artificial barriers

(Warren & Pardew 1998, Schaefer 1999, Lonzarich et al. 2000).

We conducted a set of field mark-resight surveys and laboratory experiments designed to measure the effects of road crossings on leopard darter movement. In field studies, we compared movement rates across road crossings and over natural riffles in the Glover River and documented movement into deep-water habitats in response to water temperature variation. In laboratory experiments we used scaled-down culverts in a large artificial stream to assess effects of different culvert characteristics on leopard darter movement.

## Methods

### *Field survey and resight methods*

We selected two sites on the Glover River known to have large leopard darter populations based on surveys by the United States Fish and Wildlife Service (K. Collins unpublished data). Federal permit restrictions limited the 'take' of leopard darters during the 2 years of the field survey to 14 individuals. Although we considered surveying four sites (two each with and without culverts), we chose to restrict the number of sites and mark more darters, and thereby maximize sample size, at each site.

At both sites, the river channel was narrow with distinct riffle and pool structure. Substrate consisted mostly of large cobble, boulders and some reaches with bedrock. The reported 'preferred' or 'optimal' habitat for the leopard darter is large cobble to boulder substrate in low or non-flowing water less than 1 m deep (Jones et al. 1984, James et al. 1991), and it has been generally thought that the species is restricted to these habitats, except in spring when they move into gravel riffles to spawn (Jones et al. 1984, James & Maughan 1989, James 1996). Based on these published descriptions of preferred habitat, we concentrated survey and marking effort around the edges of pools where there were boulders and some emergent vegetation. One site (hereafter called Natural Riffle) had no man-made road crossing, while the second site (Road Crossing) had a low-water road crossing with two round culverts roughly 60 cm in diameter and four box culverts approximately 3 m wide (Table 1).

At each site, we identified four patches (numbered 1–4 from upstream to downstream). Patches 2 and 3 were areas known to be suitable habitat for leopard darters based on previous surveys and habitat

*Table 1.* Habitat characteristics for the Natural Riffle (NR) and Road Crossing (RC) sites on the Glover River, McCurtain County, Oklahoma. At each site, four patches were identified and numbered 1–4 from upstream to downstream. For the mark-resight study, leopard darters were captured from patches 2 and 3, marked and released into the patch of origin. Resight surveys were conducted in all four patches. Depths given in the description indicate relative depths of the patches because absolute depth varied over the course of the study.

Site	Patch	Size (length × width m)	Description
NR	1	15 × 30	Deep pool, boulder
		Habitat between patches = 20 m span of bedrock habitat	
NR	2	80 × 25	Shallow, boulder, cobble, gravel
		Natural Riffle Barrier = 20 m natural riffle	
NR	3	35 × 20	Medium depth, cobble, gravel, silt
		Habitat between patches = 10 m natural riffle	
NR	4	100 × 20	Medium depth, cobble, gravel
RC	1	25 × 30	Shallow, boulder, cobble
		Habitat between patches = 3 m natural riffle	
RC	2	85 × 20	Medium depth, boulder, cobble, gravel
		Road Crossing Barrier = road crossing with culverts	
RC	3	20 × 25	Shallow, deep in areas, boulder, cobble
		Habitat between patches = 15 m natural riffle	
RC	4	100 × 15	Shallow, boulder, cobble

characteristics (Table 1). At the Natural Riffle site, a riffle approximately 20 m long separated patch 2 from patch 3. At the Road Crossing site, the road separated patch 2 from patch 3. At each site, we captured darters for marking in patches 2 and 3. For marking, snorkelers hand-netted fish and transported them to 40 liter containers along the shore. Using a standard 27 gauge syringe, we gave fish a small subcutaneous injection of acrylic paint (Liquitex brand: red, orange, yellow and blue) following the method of Hill & Grossman (1987). To allow us to assess movement across barriers, we used different colors to mark fish from patches 2 and 3. Other studies (e.g., Schaefer 1999) demonstrated that these marks are visible for up to 2 months and that stress to fish is minimal. Only six mortalities of 266 marked fish were directly attributable to the marking procedure. After fish recovered, we released them back into the patch of origin. We released all fish from a given patch at the same point near the middle of that patch.

In the weeks following marking, we conducted snorkel and SCUBA surveys to look for marked fish. In each patch, observers swam non-overlapping transects across the stream (starting downstream and working upstream) covering the entire area. We surveyed patches 2 and 3 for 2 person-hours each and patches 1 and 4 for 1 person-hour each. Matthews et al. (1994) found that a survey time of 15–20 min by a single snorkeling observer was sufficient to detect most fishes in large pools of a south Oklahoma stream. We counted all fish, marked and unmarked, in a given patch.

In 1999 (4–5 August), we marked and released 79 individuals at the Natural Riffle site (45 from patch 2 and 34 from patch 3) and 35 individuals at the Road Crossing site (22 from patch 2 and 13 from patch 3). We conducted surveys for marked individuals on 15 August, 28 August, 18 September, 8 October, and 21 October 1999. In 2000 (30 June to 1 July), we marked and released 87 darters at the Natural Riffle site (57 from patch 2 and 30 from patch 3), and 59 at the Road Crossing site (32 from patch 2 and 27 from patch 3) (Figure 2). We conducted resight surveys on 7 July, 12 July, and 20 July 2000.

#### *Laboratory methods*

On 8 October 1999, we hand-netted 20 leopard darters (10 from each field site) and transported them to the University of Oklahoma Research Park in Norman, OK, for laboratory trials. We marked individual fish uniquely with acrylic paint injections. We maintained fish from October 1999 until August 2000 in holding pools 1.3 m in diameter and 15–20 cm deep, and fed them daily with a mixed diet of frozen brine shrimp and frozen bloodworms.

Our indoor artificial stream was 8 m long, 1.3 m wide, and averaged 40 cm water depth. The stream was made as large as possible so that habitat patches established within could be large and widely spaced. There was only room for one such stream in the available laboratory space. The stream substrate consisted of gravel, cobble, and boulders (13–26 cm in diameter)

from the Natural Riffle site on the Glover River. Three equally-spaced patches (hereafter designated Upper, Middle, Lower), designed to imitate the preferred habitat of *Percina pantherina* were established in the stream. Each patch consisted of 5 boulders in an array roughly 1.5 m in diameter. The Upper and Middle patches were separated by a barrier constructed of cement blocks with a middle section (culvert) that could be altered according to treatment. Although the barrier could be reconfigured to test effects of culvert shape on movement, the basic structure of the barrier was permanent so the position of the barrier could not be changed between trials. There was no barrier between the Middle and Lower patches. Water was pumped from one end of the stream to the other by one to three sump pumps (Little Giant Pump Company, Oklahoma City, model 6-CIM-R) each rated at 10410 liter h<sup>-1</sup>.

Treatments consisted of four different culverts (Table 2): square-narrow, square-wide, round-smooth, and round-ribbed. Culverts used in laboratory trials were smaller than those at the Road Crossing site, but they presented effects on stream flow similar to those in the Glover River. Schwalme et al. (1985) and Katapodis et al. (1991) found that even large fish (>500 mm FL) pass through Denil fishways only 30 cm wide (less than half the width of our square-wide laboratory culvert). While these culverts were smaller than many in natural streams, the thalweg depths and current velocities in our trials were similar to those measured in the field. In addition, a darter in one of our culverts took up much less than 25% of the total cross-sectional area of the culvert so that flows were not restricted. Typically, objects taking up 25% or more of this cross-sectional area will begin to restrict flow and cause compression forces (Suren et al. 2000).

Table 2. Conditions of the passage through the barrier for each of the four trials. The barrier was located between the upper and middle patches and was constructed of cement blocks. Thalweg depth remained constant at 5 cm.

Trial	Shape	Width (cm)	Length (cm)	Current (cm s <sup>-1</sup> )
1	Square-narrow	19	38	15
2	Square-narrow	19	38	30
3	Square-wide	76	38	7.5
4	Square-wide	76	38	15
5	Round-smooth	16	38	15
6	Round-smooth	16	38	30
7	Round-ribbed	16	38	15
8	Round-ribbed	16	38	30

For each culvert type, we conducted one trial with 1 pump in operation (low discharge) and a second trial with 3 pumps in operation (high discharge). For all culvert types except the square-wide, water velocity inside the culvert was 15 cm s<sup>-1</sup> for the low discharge trials and 30 cm s<sup>-1</sup> for the high discharge trials. For the square-wide culvert, which had a much larger cross-sectional area (Table 2), water velocity was 7.5 cm s<sup>-1</sup> for the low discharge trial and 15 cm s<sup>-1</sup> for the high discharge trial.

We conducted one 10-day trial for each discharge-culvert shape combination (eight total trials). Because we were limited by permit restrictions to holding only 20 leopard darters in the laboratory, individual darters were used in more than one trial. For each trial, we selected nine individuals haphazardly from the holding tank and released three fish into each of the three patches of the artificial stream. For each 10-day trial, we made daily observations and recorded the location (patch) of each fish observed. From these observations we calculated the minimum number of moves necessary to explain changes in the daily distribution of darters in the artificial stream. We classified movements as upstream or downstream, and as crossing the barrier or not. During the trials, we fed fish (about noon) by distributing equal amounts of food to each patch. We carried out surveys in the evening of the 10 days following the initiation of the trial. On average, we saw 60% of marked fish in any one survey, almost always within the boulder habitat patches and not in the space between patches. After each trial, we removed all fish and returned them to holding pools.

## Results

### Field surveys and resights

During the eight resighting surveys, we counted a total of 1251 leopard darters. At both study sites, the number of leopard darters observed and the distribution of fish among patches varied over time (Figure 1). At the Natural Riffle site (Figure 1a–d), we most often observed leopard darters in patch 2 (an area considered to be ‘optimal’ habitat based on depth, substrate, and current), but we also found darters in large numbers in patches 1 and 3. Patch 3 was similar to patch 2, but patch 1 was much deeper (4–5 m deep) than is considered ‘optimal’ for leopard darters. We recorded extensive use of patch 1 at times (15 August 1999,

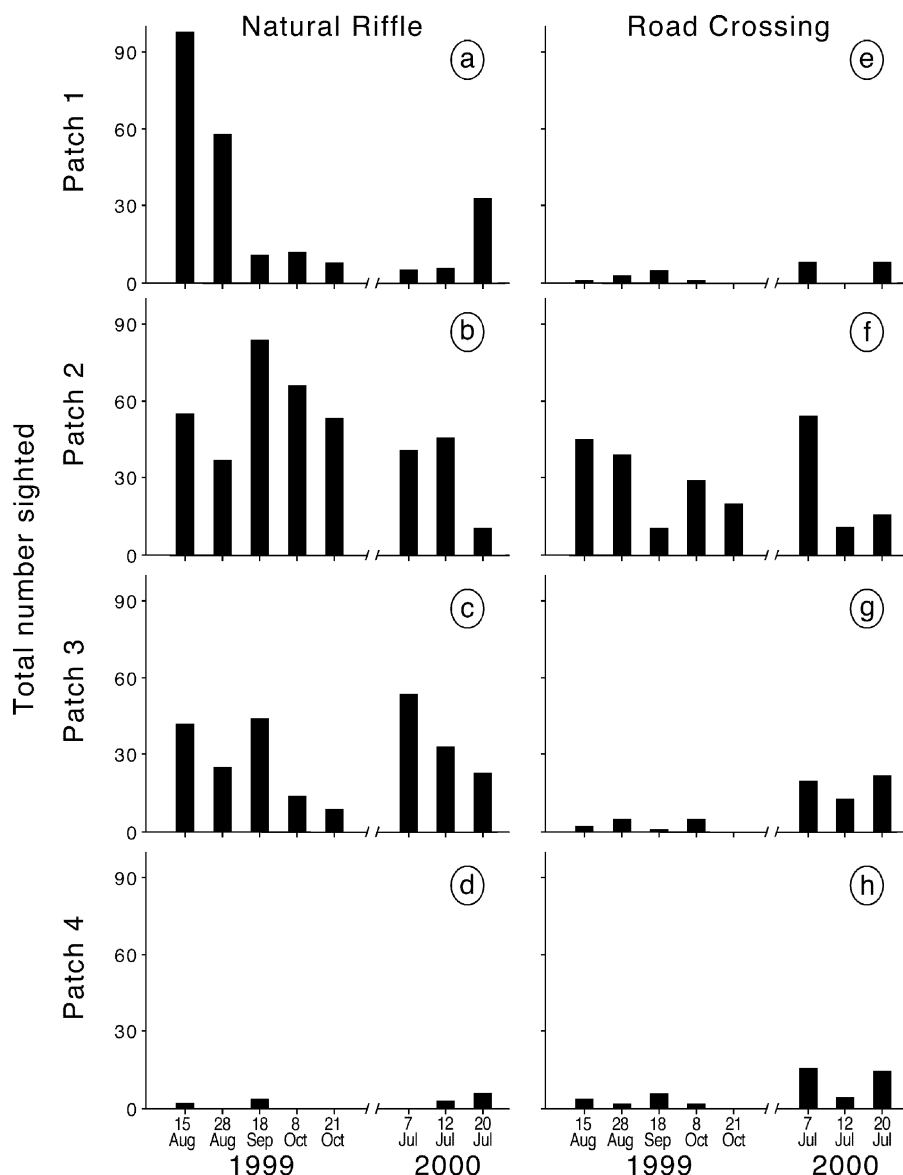


Figure 1. Total number of leopard darters seen during snorkel and SCUBA surveys in 1999 and 2000, in each patch (numbered 1–4, upstream to downstream) at the Natural Riffle site (a–d) and the Road Crossing site (e–h).

28 August 1999, and 20 July 2000) when water temperature in the shallower patches was very warm (approximately 30°C or higher) and temperatures in the deeper pool were 3–4°C lower. At the Road Crossing site, we observed most leopard darters in patch 2 (again, an area of ‘optimal’ habitat), although darters were also observed in patches 3 and 4 (Figure 1e–h). At this site, there were no available deep-water habitats in any of the four patches.

In both years combined, we marked a total of 260 leopard darters. In the eight surveys after marking, we resighted marked fish 97 times. Most marked darters were resighted in the patch where they were originally marked and released (Figure 2), but 11 resights were in patches other than the patch of origin.

At the Natural Riffle site (Figure 2a–d), we recorded movement downstream (but not upstream) across the natural barrier. At least two (and possibly three, if all

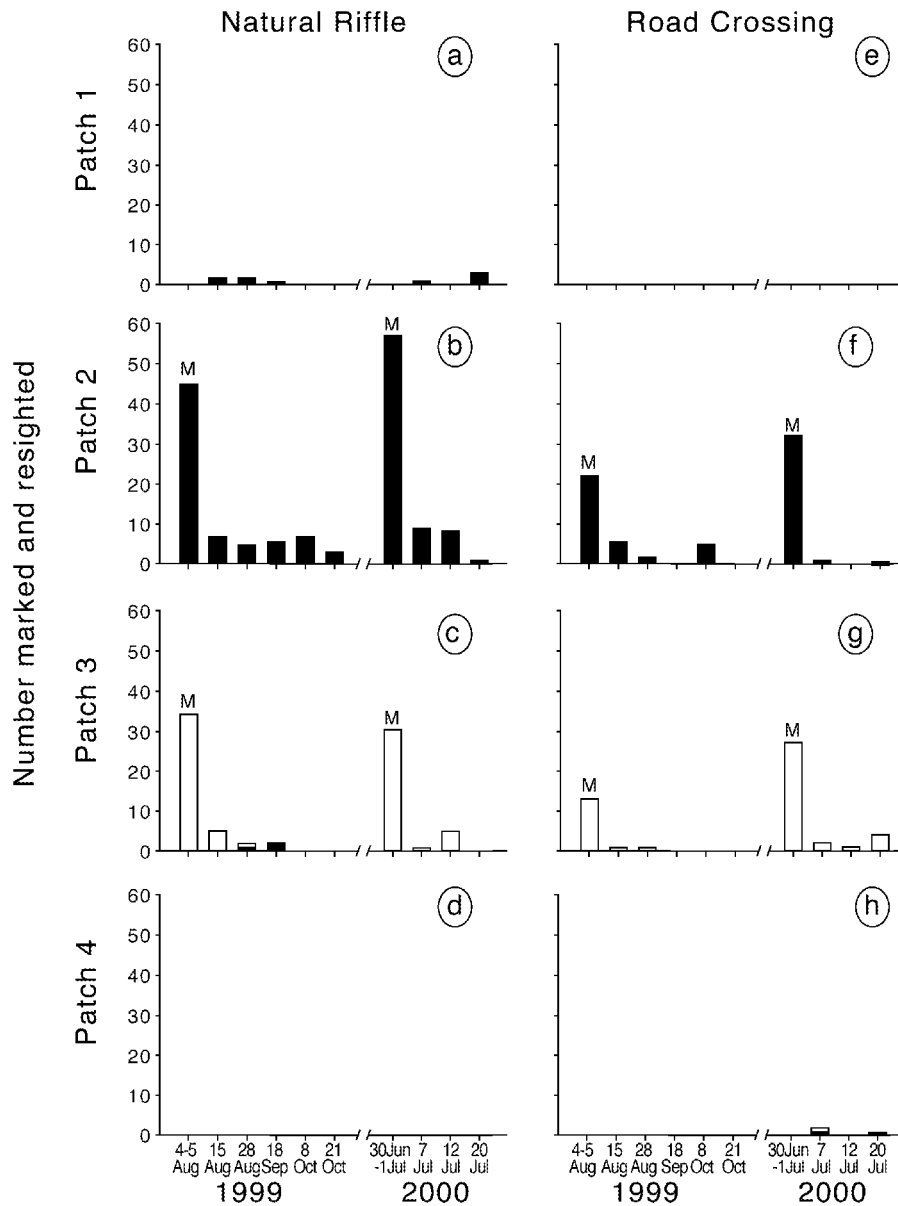


Figure 2. Number of leopard darters marked (designated by 'M' above the date of marking) and resighted in 1999 and 2000 at the Natural Riffle site (a–d) and the Road Crossing site (e–h). Darters marked in patch 2 (upstream of the barrier) indicated by black bars; darters marked in patch 3 (downstream of the barrier) indicated by open bars.

sightings on different dates represented different individuals) darters marked in patch 2 were resighted downstream below the natural riffle in patch 3 in 1999. Darters did move upstream into the deep pool habitat. In 1999, at least two (and possibly as many as five) darters moved upstream from patch 2 to patch 1. In 2000, at least three darters (and possibly four) moved

from patch 2 to patch 1. Movement from patch 2 to patch 1 required moving upstream across a 20–30 m stretch of bedrock substrate.

At the Road Crossing site (Figure 2e–h), all documented movement was in a downstream direction. In 2000, at least one (and possibly two) darters crossed the road crossing barrier as well as a natural riffle from

patch 2 to patch 4, a distance of at least 200 m. One other marked darter moved from patch 3 to patch 4.

Most movement among patches was from patch 2 to patch 1 at the Natural Riffle site. We resighted marked darters that had moved upstream into this deeper habitat at times when large numbers of unmarked leopard darters also occupied this habitat (Figures 1 and 2). Occupancy of the deeper habitat coincided with higher temperatures (32–34°C) in the surrounding shallow habitat. Temperature profiles of the deep hole and surrounding shallow habitats at the Natural Riffle site were measured on three dates in 2000: 1 July (the day that fish were marked), 7 July, and 20 July. On 1 July, temperatures of the shallow habitats (10–80 cm deep) ranged from 25.5°C to 27°C. Temperature of the deep hole ranged from 26°C at the surface to 23°C at 5–6 m deep. At this time, no leopard darters were observed in the deep hole, which was occupied by a large bass (*Micropterus* sp.). On 7 July, water temperature at shallow (<10 cm deep) areas was 32°C, but temperature in typical leopard darter habitat (ca. 80 cm deep) was 29°C. Temperature in the deep pool was 26–27°C. Four leopard darters were observed in this deep habitat on this date. On 20 July, water temperatures were much warmer than in the previous surveys. Water temperature decreased with depth in patch 1 as follows: 33°C at 0.3 m, 30°C at 1.2 m, 29°C at 3 m, 28°C at 3.3 m. Most leopard darters in patch 1 at this time were observed at a depth of 3 m.

#### Laboratory studies

Darters in all trials actively dispersed among all three patches of the artificial stream. Using combined data from all trials, movement upstream (mean = 11.1 moves per trial, SE = 1.9) did not differ from that downstream (mean = 9.6 moves per trial, SE = 0.80, Figure 3a) (paired  $t = 1.15$ ,  $p = 0.26$ ,  $df = 7$ , power = 0.19). The barrier (regardless of culvert type), however, significantly reduced movement (paired  $t = 6.25$ ,  $p < 0.001$ ,  $df = 7$ , power = 0.98). Movement between the two patches with no barrier (mean = 15.9 moves per trial, SE = 1.9) was much greater than movement between the patches divided by the barrier (mean = 4.8 moves per trial, SE = 1.2).

Discharge had minimal effect on movement detected in laboratory trials (Figure 4). Difference in number of upstream moves at the two discharge levels was marginally significant with number of moves per trial at high discharge (mean = 14.25, SE = 2.32) greater

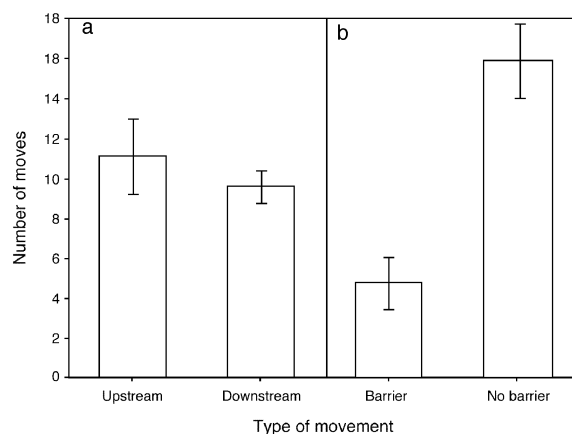


Figure 3. Number of moves per trial ( $\pm 1$  SE) for all trials ( $n = 8$ ) in the artificial stream in an a-upstream versus downstream direction and b-across a barrier versus between patches without a barrier.

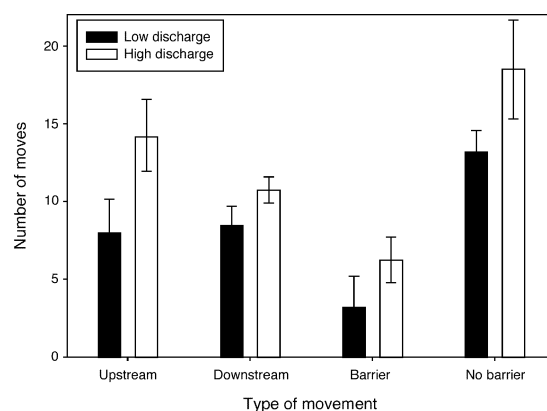


Figure 4. Number of moves per trial ( $\pm 1$  SE) under high discharge (open bars;  $n = 4$  trials) versus low discharge (black bars;  $n = 4$  trials) conditions for each of the following types of movement: upstream, downstream, across the barrier, and between barrier-free patches.

than that at low discharge (mean = 8.00, SE = 2.12) (paired  $t$  test,  $t = 2.7$ ,  $p = 0.07$ ,  $df = 3$ , power = 0.41). Discharge had no effect on downstream moves (paired  $t = 1.5$ ,  $p = 0.23$ ,  $df = 3$ , power = 0.36), movement across the barrier (paired  $t = 1.5$ ,  $p = 0.23$ ,  $df = 3$ , power = 0.35), or movement between patches with no barrier (paired  $t = 1.5$ ,  $p = 0.24$ ,  $df = 3$ , power = 0.42).

Movement rates differed marginally across the four different types of culverts (one-way ANOVA,  $F = 5.30$ ,  $p = 0.058$ , power = 0.44, black bars, Figure 5).

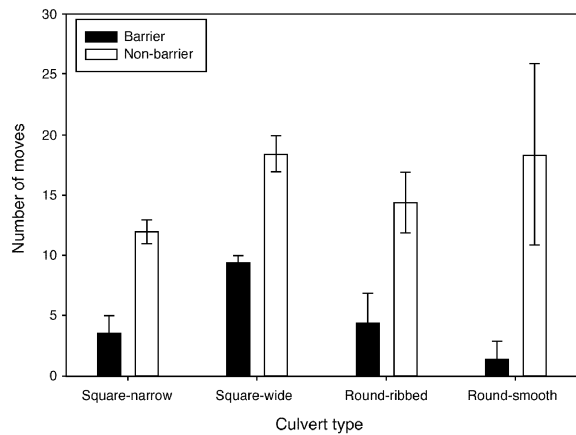


Figure 5. Number of moves per trial ( $\pm 1$  SE) in the artificial stream trials ( $n = 2$ , one at high and one at low discharge) for each of the four culvert types. Black bars represent movement across the barrier, open bars represent movement between patches with no barrier.

Movement rate across the square-wide culvert (mean = 9.5 moves per trial,  $n = 2$  trials, one at 'high' and one at 'low' discharge, see Table 2) was nearly seven times that across the round-smooth culvert (mean = 1.5 moves per trial). Movement across the other two culvert types (square-narrow, mean = 3.5 moves per trial) and round-ribbed (mean = 4.5 moves per trial) was intermediate. Although trials with different barrier types revealed differences in movement across the barrier, there were no differences in movement between patches without a barrier ( $F = 0.62$ ,  $p = 0.64$ , power = 0.10, Figure 5). This suggests that differences in barrier crossings were, in fact due to barrier type rather than to variation in movement tendencies among trials.

## Discussion

Leopard darters in the Glover River exhibited limited movement among habitat patches. Most darters marked in our field studies in both years were resighted in their patch of origin, but we did document movements of up to 200 m. Our observation concurs with those for other species which suggest that while a small portion of the population may move long distances, many individuals remain in the same area over the short term (Funk 1955, Gerking 1959, Freeman 1995; but see Hill & Grossman 1987, Gowan & Fausch 1996). Given that our surveys were conducted over a limited area at each site, we were unable to address long-distance

movements in this study. Of the limited movement observed in the field, we documented movement in both upstream and downstream directions, with most being upstream into deeper habitat when water temperatures exceeded 29°C.

Our field study compared movement across a natural versus a man-made barrier in a small river. Downstream movement across the pre-defined barriers (Natural Riffle and Road Crossing) occurred at both sites but was limited: only two (or three) individuals crossed the natural riffle, and only one (or two) individuals crossed the road barrier (and a natural riffle downstream of the road crossing). Although upstream movement was documented for darters moving into cooler waters, no upstream movement was documented across either of the pre-defined barriers (natural riffle or culvert) in this study.

Our laboratory observations suggest that leopard darters should be able to traverse some types of barriers more readily than others. In artificial stream trials, leopard darters were more likely to cross square-wide than narrow (either square or round) culverts. In our laboratory trials, lowest current speeds across any barrier type were recorded across the square-wide culvert for both the low and high discharge trials. In the field, it is also likely that wider culverts increase velocities less than narrow culverts, and present a larger corridor for fish passage. During periods of elevated flow, some of the riffles and culverts in the Glover River had higher current velocities than we were able to reproduce in the laboratory. Toepfer et al. (1999) found that both box and pipe culverts in the Glover River typically had current velocities greater than  $25 \text{ cm s}^{-1}$  (the current velocity at which they found the greatest swimming activity in the laboratory). Because the leopard darter exhibits limited swimming ability, culverts in the Glover River likely restrict passage under even moderate flow (Toepfer et al. 1999).

### *Thermal refugia*

Although study of habitat use was not an initial goal of this study, the serendipitous observation of habitat shift into deeper, cooler habitats when water temperatures are high may provide critical insight for conservation of this threatened species. Our observations suggest that leopard darters use deep-water habitats as thermal refugia when temperature in the surrounding shallow habitats exceeds 29°C. The use of thermal refugia by warmwater stream fishes (Peterson & Rabeni 1996) and specifically by darters (Smith & Fausch 1997) is a

well-studied phenomenon. During our study, flows in the Glover River (USGS data, site U07337900) varied from peaks near  $283 \text{ m}^3 \text{ s}^{-1}$  to extended periods at or near  $0 \text{ m}^3 \text{ s}^{-1}$ . During these low-water periods, temperatures commonly rose above  $34^\circ\text{C}$  in habitats where leopard darters had been very common. At these times, darters were most abundant in the deep pool habitats where temperatures were as much as  $6^\circ\text{C}$  cooler. Daily fluctuations in stream temperature are extreme in the Little River drainage. We recorded increases from  $28^\circ\text{C}$  to  $34^\circ\text{C}$  at one location in a matter of hours. This is especially true for shallow, exposed habitats that are commonly inhabited by the leopard darter. We often measured temperatures in shallow areas ( $36\text{--}38^\circ\text{C}$ ) that were at or above the thermal tolerance of other darter species (Smith & Fausch 1997). Fish were rarely seen in these shallow habitats when temperatures increased. It is interesting to note that while these individuals may move to deeper, cooler habitats to avoid heat death, the threat of predation is likely greater in these deep pools where large predators (*Micropterus* spp.) were observed on several occasions.

The presence of leopard darters in deeper water habitats has not been previously reported in the literature, and our finding suggests that population estimates may need to be reassessed. Recent analyses (Williams et al. 1999) of population viability for extant populations of leopard darters have relied on estimates of availability of suitable habitat as a basis for estimating total numbers within each of the tributaries of the Little River system. In the model of Williams et al. (1999), suitable habitat was defined according to published studies that noted leopard darters occupied pools less than 1 m deep. Our observations of leopard darters using pools up to 5 m deep during periods of warmwater temperature suggest that estimates of total numbers within tributaries may need to take this habitat into account. If survivorship in late summer depends on availability of adjacent deep pools that provide thermal refugia, inclusion of only shallow pool habitats for population estimates may substantially overestimate population size. That is, some shallow habitats lacking access to deeper pools nearby might not be suitable year-around habitat, and including such habitat as 'occupied' would inflate the total population estimate. Conversely, the use of thermal refugia by leopard darters may cause population estimates based on late-summer censuses to be too low. Reduction in population size that is often observed in late summer in habitats that are adjacent to deep pools (K. Collins unpublished data) may reflect migration into uncensused deep habitat rather than mortality.

Based on the results of field and laboratory studies and on data available in the literature, we made the following recommendations to the USDA Forest Service regarding construction/reconstruction of road crossings in the Glover River and other streams and rivers containing the leopard darter. Given that barriers appear to reduce movement of leopard darters, the number of road crossings should be kept to a minimum to facilitate local movement. However, if road crossings are necessary, they should be constructed to incorporate the widest possible box culverts, particularly if construction of Denil fishways (which are known to be used by other species of darters, Bunt et al. 2001), or more elaborate fish passages are not an option. Most importantly, habitat should be managed so that barriers to local movement do not preclude access to thermal refugia.

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