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New Applications of Radio Frequency Identification Stations for Monitoring Fish Passage through Headwater Road Crossings and Natural Reaches

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Running Title: RFID Stations for Monitoring Fish Passage

Abstract

Within the Ouachita National Forest, roads and streams intersect each other thousands of times. Many of these road crossings alter stream hydrology and potentially limit longitudinal fish movement. To investigate the potential impacts of these road crossings on fish passage, we monitored movements of 3 native fish species (n = 2,171) individually tagged with radio frequency identification (RFID) tags in 2012 and 2013. We installed solar-powered RFID stations in 2 streams with road crossings and 2 reference streams without road crossings. Each of the 4 monitoring stations included a pair of antennas bracketing a road crossing (or similarly-sized natural reach) to continuously detect upstream or downstream passage. To monitor natural reference streams, we avoided full-duplex RFID technology, which would have required rigid in-stream structures. Alternatively, we utilized new applications of RFID technology such as direct in-stream installation of half-duplex wire antennas and figure-eight crossover antenna designs. These techniques appear promising, but technical difficulties limited the consistency of fish passage detection and consequently limited the strength of ecological conclusions. Even so, we report evidence that fish passed at significantly higher rates across reference reaches than reaches with road crossings. Furthermore, Creek Chub (*Semotilus atromaculatus*) passed reference reaches at significantly higher rates than Highland Stonerollers (*Camptostoma spadiceum*), which passed at higher rates than Longear Sunfish (*Lepomis megalotis*). Stream intermittency appeared to exacerbate reduced passage rates associated with the road crossings.

Introduction

Stream fragmentation can disrupt life history strategies of even non-migratory fishes, which rely upon

dispersal to maintain population connectivity (Fagan *et al.* 2002; Labonne and Gaudin 2006; Cook *et al.* 2007). This issue threatens the biodiversity and persistence of fish communities in the central and southeastern United States (Sheldon 1988; Bessert and Ortí 2008; Kashiwagi and Miranda 2009; Perkin and Gido 2011). In North America, road crossings in headwater streams commonly obstruct fish passage and fragment fish habitat (Gibson *et al.* 2005; Hendrickson *et al.* 2008; Park *et al.* 2008; Price *et al.* 2010; Peterson *et al.* 2016).

Road crossings in the Ouachita National Forest reduce fish passage by altering stream hydrology in 2 principal ways: 1) reducing water depth to levels too shallow for efficient swimming (Blank *et al.* 2005) and 2) increasing water velocity so that it exceeds fish swimming abilities (Belford and Gould 1989; Warren and Pardew 1998; Burford *et al.* 2009; Norman *et al.* 2009; Bourne *et al.* 2011). Road crossings often act as semipermeable barriers (Bouska and Paukert 2009) and passability varies with fluctuations in discharge (Connolly *et al.* 2008), road crossing hydraulics, and fish species and size (Norman *et al.* 2009). Road crossing designs that more severely constrict natural stream flow appear more detrimental to fish passage (Warren and Pardew 1998) and multiple barriers along a drainage network may lead to cumulative effects (Helfrich *et al.* 1999).

Researchers assess potential impacts of aquatic fragmentation via several methods including hydraulic modeling software (Bourne *et al.* 2011), analysis of population genetic structuring (Wofford *et al.* 2005; Bessert and Ortí 2008; Schanke *et al.* 2017), direct observation of individuals (Cahoon *et al.* 2007), and recapture of marked specimens (Belford and Gould 1989; Morita and Yamamoto 2002). Researchers also use radio frequency identification (RFID) tags, also known as passive integrated transponder (PIT) tags, in conjunction with RFID detection stations to study fish movement in streams (Bond *et al.* 2007; Horton *et al.*

2007; Connolly *et al.* 2008), and monitor passage through road crossings (Blank *et al.* 2005), fishways (Castro-Santos *et al.* 1996; Thiem *et al.* 2011), and hydroelectric dams (Axel *et al.* 2005).

Traditional RFID systems for fish detection were based on full-duplex (FDX) transmission technologies, which are sensitive to small antenna movements and therefore require installation of rigid, watertight housings on structures such as dams, fishways, or weirs (Bond *et al.* 2007). Alternatively, systems based on newer half-duplex (HDX) equipment do not require rigid antennas and are considerably less expensive than FDX systems (Burnett *et al.* 2013; Roghair *et al.* 2014).

The ability of an RFID system to detect a tagged fish passing its antenna array is called fish detection efficiency and has 2 components: 1) path efficiency- the proportion of tags that physically pass through the antenna array, rather than around it and 2) antenna efficiency- the proportion of tags that are detected of those tags passing through the antenna (Zydlowski *et al.* 2006). Whereas HDX systems benefit from less stringent antenna design requirements, they have lower antenna efficiency than comparably-sized FDX designs. Small tags, such as the compact 12-mm tags used for small stream fish, have a smaller read range (the maximum distance at which a tag can be detected) and result in lower antenna efficiency than larger tags (Zydlowski *et al.* 2006; Burnett *et al.* 2013).

The Ouachita National Forest, located primarily in Arkansas and managed by the USDA Forest Service, has a high density of flow-constricting road crossings and a high diversity of warmwater fishes. However, researchers have published few studies assessing the effects of road crossings on fish movement in this region (apart from: Warren and Pardew 1998 Standage and Gagen 2007; Schanke *et al.* 2017). Our initial ecological question focused on the potential impact of road crossings and hydrologic regimes (i.e., water level fluctuations, including intermittency) on individual fish movement in headwater streams.

We chose to monitor fish with RFID detection stations because they increase reencounter probabilities over other techniques (Hewitt *et al.* 2010; Roghair *et al.* 2014). Although many researchers have installed RFID systems on the upstream and downstream edges of existing instream structures (for example: Blank *et al.* 2005; Burford *et al.* 2009; Roghair *et al.* 2014), we sought to monitor fish movement through not only road crossings, but also unaltered natural reaches (reference streams). Therefore, to minimize environmental alteration and hydraulic disruption to the natural reference reaches in headwater streams, we installed

HDX systems with light-weight wire antennas directly in the stream without rigid supporting structures. However, the methodology of HDX, RFID technology to monitor small-bodied native fishes in unaltered natural reaches with small RFID tags was largely untested. Hence, to address our ecological questions, we explored new applications of RFID detection stations in remote, natural stream reaches devoid of man-made structures capable of supporting antennas. Our study thus shifted focus towards the development of new RFID applications while also showcasing the potential for ecological observations.

Materials and Methods

Field-site description

We examined fish movements in 4 similarly-sized, low-order, warmwater streams in the Ouachita National Forest within the Ouachita Mountains, an ecoregion of approximately 4.8 million hectares in Arkansas and Oklahoma (Table 1; Fig. 1). We selected 2 *culverted* streams (each intersected by multiple engineered road crossings within the study reach) and paired each with a nearby *reference* stream without road crossings. To represent the diverse hydrologic regimes of the region, these 4 streams consisted of 2 *intermittent* streams in the northeastern portion of the Ouachita Mountains and 2 *perennial* streams towards the south. We established 2-kilometer study zones (1 km upstream and downstream) centered on the study reach in each stream.

The 2 intermittent streams drained into the Fourche La Fave River in the Arkansas River watershed. Bear Creek had 3 total road crossings within the 2-km study zone (including another vented ford and a slab ford). Alternatively, Crystal Prong was located in the Flatside Wilderness Area and had no road crossings. We studied a vented ford (also known as a pipe culvert) road crossing on Bear Creek (Fig. 2). However, prior to this study, the culvert pipes had filled in with gravel and cobble so that they conveyed only trickles of water and presumably precluded fish passage. This type of flow restriction is common to this road crossing design in the Ouachita National Forest and affects stream hydrology much like a slab ford or low head dam. These intermittent

Table 1. The locations of the 4 study reaches.

Stream	Latitude	Longitude
Bear Creek	34.788809°	-93.169854°
Crystal Prong	34.861274°	-92.934850°
Long Creek	34.399824°	-93.934639°
Little Missouri River	34.430157°	-93.944220°

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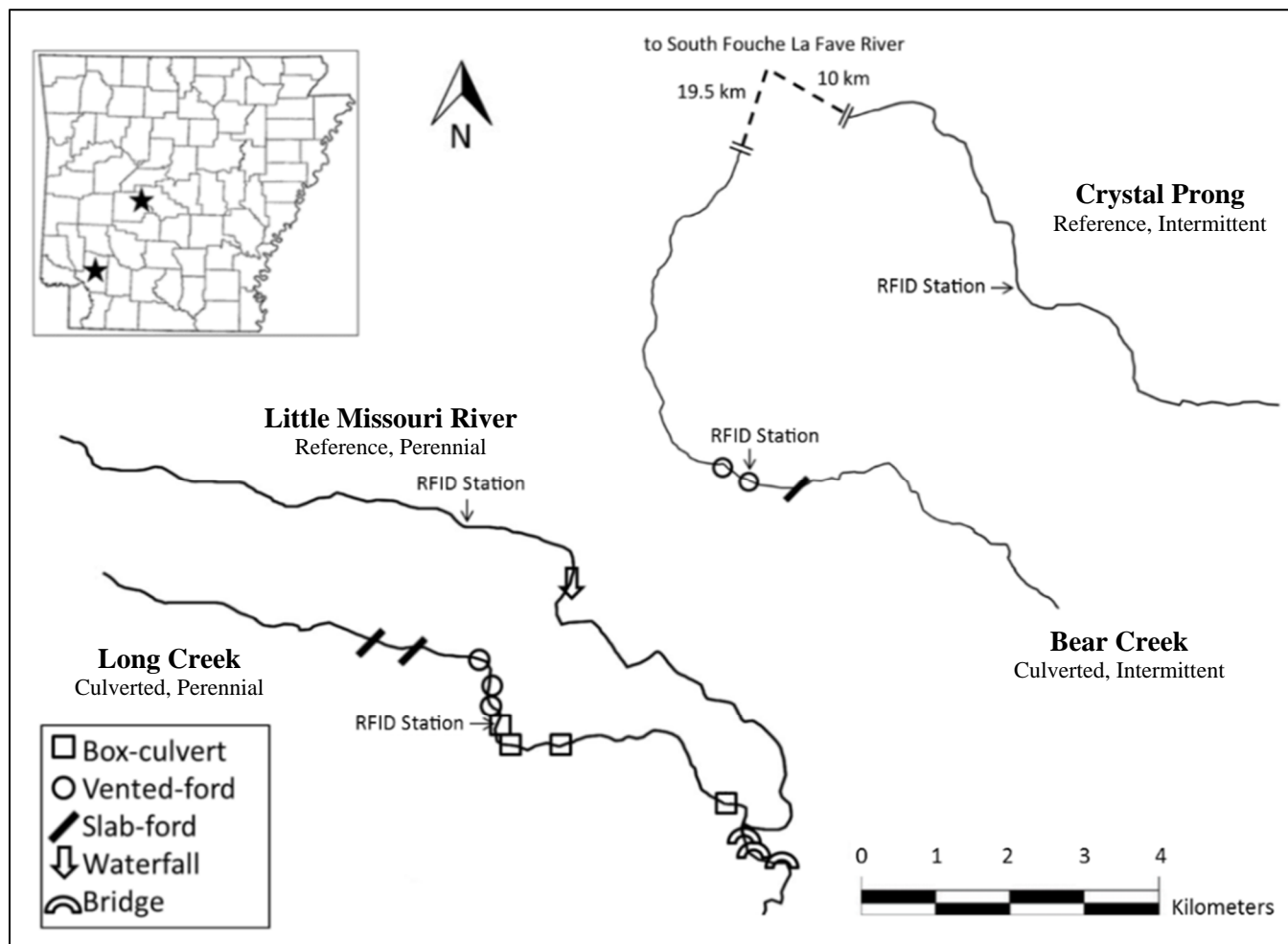


Figure 1. Map of the 4 study streams located in the Ouachita National Forest, Arkansas. Two intermittent streams, Bear Creek (culverted) and Crystal Prong (reference), were located in the central part of the state. To the southwest were 2 perennial streams, Long Creek (culverted) and Little Missouri River (reference). Bear Creek and Crystal Prong flow downstream to the South Fouche La Fave River (adapted from Schanke 2013).

streams show reduced surface flow during the summer such that wetted portions become isolated pools.

The 2 perennial streams drained to the Ouachita River watershed. Long Creek had 6 total road crossings, including vented fords and box culverts, within the 2-km study zone. The adjacent Little Missouri River had no road crossings within the study zone (though some existed in the headwaters and farther downstream). On Long Creek, we focused on a box culvert (constructed in 2008; Fig. 2) to contrast with the older style vented ford on the other culverted stream, Bear Creek. This box culvert consisted of five 2.4-m-wide boxes and spanned 6.2 m of longitudinal stream distance. The bottoms of the boxes were constructed below stream grade, resulting in a natural gravel and cobble substrate bottom (covering the concrete) that contributed to roughness and slowed water velocity. Neither of the 2 road

crossings we studied had measurable outlet drops that would impede fish movement via outlet drop height or outlet pool depth barriers.

Fish collection and marking

We encountered and tagged 9 fish species (additional details in MacLeod 2013)—however we restricted analysis and reporting to 3 species that we commonly encountered in all 4 of the streams to enable comparisons across streams. Target species, in order of captured abundance, included: *Semotilus atromaculatus* (Mitchill) (Creek Chub), *Campostoma spadiceum* (Girard) (Highland Stoneroller), and *Lepomis megalotis* (Rafinesque) (Longear Sunfish; Table 2).

Between May 2012 and February 2013, we tagged 2,171 fish in the 2-km study-zones of the 4 streams. Beginning downstream, we proceeded upstream in 50-m



Figure 2. Water moves from right to left in both photographs of the 2 culverted streams. At the clogged **vented ford** on intermittent Bear Creek, water pooled upstream and flowed over the concrete slab. The turbulent water in the left foreground indicates the 2 culvert outlets. At the **box culvert** on perennial Long Creek, water flowed easily through the road crossing, even during summer low-flow conditions.

sections and collected fish via single pass, backpack electrofishing (Smith-Root LR 20- Smith-Root, Vancouver, Washington), in continuous sweeps without block-nets. We repeated this procedure on subsequent visits to meet nominal tagging quotas (approximately 500 fish per stream). Fish were more abundant in the perennial streams and we met quotas in 2 complete sweeps, whereas the intermittent streams required additional sweeps (average 3.9 sweeps/section for Bear Creek and 4.3 sweeps/section for Crystal Prong).

We held fish in screen-bottom buckets or mesh baskets for processing and released them immediately after tagging. We injected fish larger than 85 mm total length with 12.0 mm x 2.2 mm half-duplex RFID tags (Oregon RFID, Portland, Oregon). We inserted the syringe-style implanter (MK7 Implanter- Biomark,

Table 2. The number of fish tagged and their distribution relative to the RFID station within each stream.

Species	Downstream	Upstream	Total
Bear Creek- vented ford, intermittent			
Creek Chub	303	251	554
Highland Stoneroller	25	17	42
Longear Sunfish	15	11	26
Total	343	279	622
Crystal Prong- reference, intermittent			
Creek Chub	137	150	287
Highland Stoneroller	81	120	201
Longear Sunfish	52	20	72
Total	270	290	560
Long Creek- box culvert, perennial			
Creek Chub	8	40	48
Highland Stoneroller	82	85	167
Longear Sunfish	93	158	251
Total	183	283	466
Little Missouri River- reference, perennial			
Creek Chub	104	178	282
Highland Stoneroller	102	31	133
Longear Sunfish	64	44	108
Total	270	253	523

Boise, Idaho) subcutaneously between the dorsal fin and the lateral line and deposited the tags at least 5 mm distant from the incision to reduce chances of tag loss. We recorded RFID tag numbers with a handheld RFID reader (APR 350- Agrident, Barsinghausen, Germany).

Fish detection

We began installing the autonomous RFID detection stations in January 2012. The stations recorded the timing and direction of fish passage across the study reaches (Fig. 3). In the culverted streams, the middle of the RFID station spanned the target road crossings; whereas, in reference streams, we positioned the middle of the station across a riffle (devoid of human structures). Each station included an RFID reader (Multi-antenna, Half-duplex Reader- Oregon RFID) and 2 in-stream antennas (1 upstream and 1 downstream). To accommodate the large road crossings and locate suitable locations for antenna installation, we placed antennas ~60 m apart. We installed antennas in pools, runs, and riffles.

The station's RFID reader recorded the RFID tag number of passing fish coupled with a timestamp. When a fish was detected by both antennas, the timing of detection events indicated the direction of upstream or downstream movement. We powered the RFID reader with a 12-volt, 205-watt photovoltaic solar panel that charged four, 6-volt batteries (216 amp-hour, heavy-

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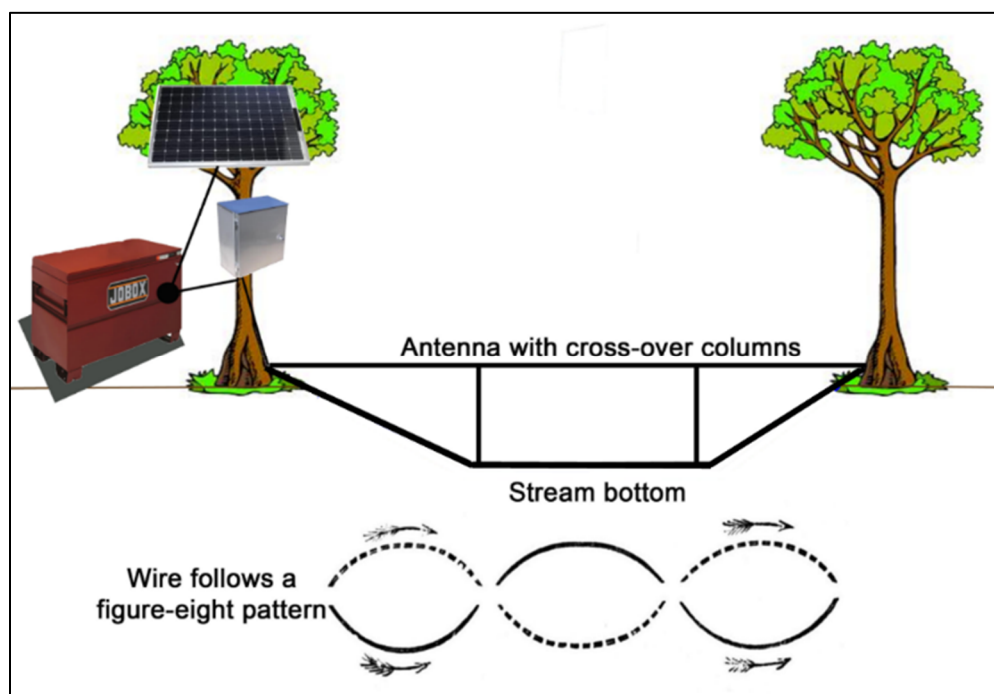


Figure 3. Each RFID station operated 2 wire antennas on either side of the road crossing (on culverted streams) or riffle (on reference streams). This figure shows a cross-section of 1 antenna spanning a stream (2nd antenna not shown). We designed antennas with cross-over columns (in a figure-eight pattern) to improve antenna efficiency. We installed the RFID reader, batteries, and solar charge controller in a locked in a steel box, utilized a solar panel to charge the station, and mounted an antenna tuner on nearby trees for each antenna.

duty, deep-cycle; Interstate Batteries, Dallas, TX) via a solar charge controller (ProStar 30M- MorningStar, Newton, PA). The batteries supplied the RFID reader with 12 volts and ~1 amp of direct current electricity (see MacLeod 2013 for additional technical details).

We built pass-through antennas that encompassed as much of the stream cross-section as possible (maximizing path efficiency) and oriented the antenna plane perpendicular to stream flow and tag direction (maximizing antenna efficiency; for more discussion of pass-through versus pass-over antennas, see Armstrong *et al.* 1996 and Zydlewski *et al.* 2006). The antenna widths ranged between 5 and 10 m and heights ranged from 0.2 m in riffles to 0.6 m in deeper pools. We constructed antennas from common stranded household electrical wire, specifically 10 or 12 American Wire Gauge (AWG) and supported the antennas with rope. We mounted antenna tuners (Oregon RFID) to nearby trees so that we could adjust the antenna inductance and ensure antennas effectively transmitted and received radio communication with the RFID tags (see MacLeod 2013 for additional installation details). The RFID stations began operating on all 4 streams by February 2012 and the 8 antennas operated for an average of 372 days (SE 10).

We continually adjusted (weekly or biweekly) the

antennas to maximize antenna efficiency. We assessed antenna efficiency during site visits by manipulating a test tag perpendicularly through the antenna plane at ~1 m/s. Antennas demonstrating “good performance” detected all tags: (1) within 5 cm (upstream or downstream) of the antenna wire plane and (2) within 10 cm above or to the side of the antenna loop. This study required large antennas at large distances from the readers, which exceeded dimensions reported in other RFID studies (e.g., Bond *et al.* 2007; Aymes and Rives 2009) and reduced antenna efficiency. Additionally, high stream flows periodically damaged the antennas and technical challenges (including electrical noise and equipment failure) periodically contributed to failure or decreased performance of one or both antennas leading to gaps in the monitoring data, sometimes for several months (more details in MacLeod 2013; Table 3).

In August 2012, we developed a crossover antenna design that dramatically improved antenna efficiency and read range relative to our initial efforts with single-loop designs (Fig. 3). By dividing the antenna loop into smaller cells in a figure-eight pattern, we reversed the electrical polarity within the cells thereby minimizing electrical noise and improving antenna efficiency (Warren Leach, Oregon RFID, *personal communication*). We formed the antenna cells by

Table 3. The percentage of time that each RFID station operated at different performance levels.

Stream	RFID Station Performance		
	Excellent or Good	Fair	Poor or Off
Bear Creek	48%	25%	27%
Crystal Prong	28%	19%	53%
Long Creek	57%	15%	28%
Little Missouri River	28%	25%	48%
Mean	41%	21%	38%

crossing the top and bottom strand of wire in opposing directions through a column made of either 1/2" (1.2 cm) plastic irrigation hose or PVC pipe. We optimized antenna designs on a site-by-site basis and constructed crossover columns every 1.5–3 m, with more crossovers needed on taller antennas.

While electrofishing, we recorded the locations of recaptured individuals that had been previously tagged, which we termed "incidental recaptures". By spring 2013 we had already met our tagging quotas, but we performed additional electrofishing to expand the spatial scale of detected fish (some of which were distant from the fixed RFID stations) and assess the efficacy of the detection stations. Specifically, in March and April 2013 we detected "intentional recaptures" by operating 2 electrofishing units simultaneously in a single pass through the 2-km study zone of each stream.

Hydrology at crossings

To characterize the hydrologic conditions associated with fish passage, we measured water levels on the culverted streams from July 2012–April 2013 and estimated the highest water level occurring during fish passage events (more detail in MacLeod 2013). We installed continuous water level recorders (Vented WL-16– Global Water Instrumentation, Dallas, TX) on the 2 culverted streams. We did not install water level recorders on the 2 reference streams, but we installed staff gauges on all 4 streams and estimated water level fluctuations on the reference streams based on the respective water level recorders on the culverted streams (the pairs of intermittent and perennial streams responded similarly to precipitation events). To estimate water levels on the study streams for May and June 2012, prior to installation of the water level recorders, we consulted USGS stream gauge data for nearby streams (more details in MacLeod 2013).

We assessed the road crossings for barrier effects caused by high velocity and low water depth and

searched for the presence of favorable passage conditions at a wide range of water levels. We used an electronic flow meter (Flo-Mate, Model 2000– Marsh-McBirney, Loveland, CO) and wading rod to measure depth and velocity along transects at the inlet and outlet of the box culvert on perennial Long Creek and along multiple transects across the trapezoidal vented ford on intermittent Bear Creek. Minimum swimming depth varies with species and length (Schaefer 2001; Rodríguez *et al.* 2006) but Blank *et al.* (2005) determined 3 cm to be the minimum swimming depth permitting passage for several species of trout. Hence, we identified areas with sufficient water depth for swimming based on the presence (or absence) of a water column equal to or greater than 3 cm. Additionally, we measured water velocity at 3 cm above the substrate to represent the lowest velocity path for fish movement, following Belford and Gould (1989) and Rajput (2003).

Data analyses

We concluded that a fish had passed a study reach when we could confirm its location at least once on both the upstream and downstream side of the RFID station. We used all available data sources, including the RFID detection stations and locations of tagged fish, "incidental recaptures", and "intentional recaptures" to detect passage. Some fish passed across the RFID station more than once, resulting in multiple detected passages. The number of fish tagged varied across streams and species, so we generated a normalized *passage rate*—dividing the number of *detected passages* by the number of *tagged fish* that did not pass.

We used chi-squared analyses (χ^2) and log-linear modeling to elucidate relationships between the passage rate and the following design variables: 3 species and 4 streams, which represented different crossing types (culverted versus reference) and hydrologic regimes (intermittent versus perennial).

We studied 2 crossing types and 2 hydrologic regimes; however, because we were limited to 4 RFID stations, we were not able to replicate the design. Additionally, variability among the 4 streams (e.g., the operating time of the RFID antennas was not uniform, the streams supported different fish communities, etc.) created additional complications for direct comparisons. Thus, the experiment was a comparative mensurative experiment (*sensu* Hurlbert 1984), and although we were able to investigate RFID technology, we did not design the study to make strong inferences regarding fish movements or ecology.

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Results

RFID detection efficiency

The RFID stations recorded more than 260,000 detection events (i.e., instances when the reader logged an RFID tag). This resulted in detection of 290 fish (of 2171 tagged individuals; average detection rate per stream 13%; SE 2.3%; Table 4). In March and April 2013, we detected 47 tagged individuals (of 1,128 fish captured) through the intentional recapture electrofishing. Within the 4 streams, 1–12% of these captured fish carried tags (mean, 8%; SE, 2%).

The RFID dissection stations were effective—data from the RFID detection stations, in conjunction with the original tagging location, identified 94% of all observed passages; whereas the electrofishing recapture data only identified 6% of observed passages. However, the RFID stations, often crippled by poor performance, had limitations. For example, the stations detected 24% of observed passing fish on only 1 antenna (rather than both). These fish passed undetected through or around one of the two antennas and we were only able to infer that the fish had passed a study reach by also consulting the electrofishing location data (both original tagging and recapture locations).

Movement and passage

We detected 118 fish passing the study reaches 246 times (Fig. 4). In all streams, fish passed at similar rates both upstream and downstream. The RFID stations did not always operate both antennas continuously, but fish passages scaled to RFID station operating time (which varied among streams) followed a similar pattern as data presented here.

When we pooled the 3 species, passage rates appeared dependent on “stream” (four study streams; $\chi^2 = 166$, $df = 3$, $P < 0.01$). We also observed this pattern

Table 4. The percentage of fish (by species) that were detected by the RFID station on each the 4 streams.

Stream	Species			Mean
	Creek Chub	Highland Stoneroller	Longear Sunfish	
Bear Creek	12%	5%	4%	11%
Crystal Prong	10%	18%	4%	12%
Long Creek	8%	11%	10%	10%
Little Missouri River	24%	16%	14%	20%
Mean	14%	14%	9%	13%

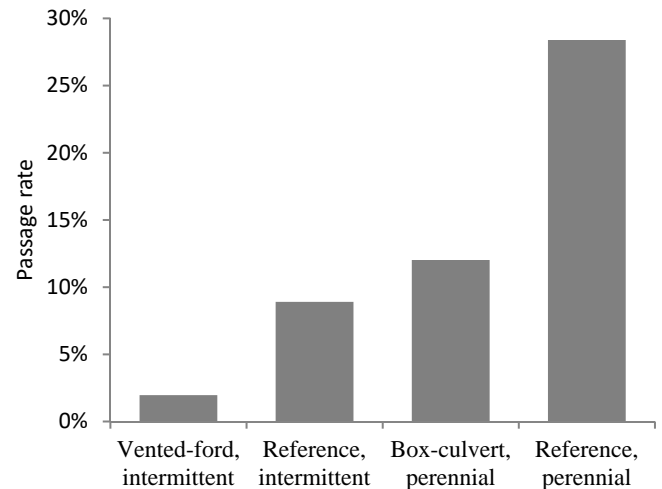


Figure 4. Fish passage rate (proportion of detected passages to RFID-tagged fish [that did not pass] within each stream).

of significant stream-effects when we analyzed the 2 most abundant species, Creek Chub and Highland Stoneroller, separately ($\chi^2 = 230$, $df = 3$, $P < 0.01$ and $\chi^2 = 9.62$, $df = 3$, $P < 0.05$, respectively). However, this analysis was inappropriate for Longear Sunfish because of the low frequency of detected passages.

When we grouped the streams into pairs (based on hydrologic regime) and pooled the 3 species, passage rate was significantly greater in the reference reaches than the road crossings for both pairs of streams (perennial: $\chi^2 = 31.7$, $df = 1$, $P < 0.01$ and intermittent: $\chi^2 = 20.3$, $df = 1$, $P < 0.01$). Likewise, when we grouped the streams into pairs (based on crossing type) and pooled the 3 species, passage rates in perennial streams were greater than in intermittent streams (culverted streams: $\chi^2 = 34.4$, $df = 1$, $P < 0.01$ and reference streams: $\chi^2 = 56.4$, $df = 1$, $P < 0.01$).

Log-linear modeling indicated significant three-way interaction among the design variables, stream and species, with respect to passage rate as a response variable ($G^2 = 1080$, $df = 17$, $P < 0.01$). Each of the associated two-way interactions were also significant. Passage rates differed among the 3 species for both road crossings ($\chi^2 = 25.5$, $df = 2$, $P < 0.01$) and reference reaches ($\chi^2 = 38.3$, $df = 2$, $P < 0.01$). Furthermore, the pairwise comparisons indicated a significant trend across species with respect to passing the reference reaches; with Creek Chub passing at higher rates, followed by Highland Stoneroller, and finally Longear Sunfish (each $\chi^2 > 10$, $df = 1$, and $P < 0.01$).

For Highland Stonerollers and Longear Sunfish, the lengths of fish passing versus not passing were similar, but passing Creek Chub were significantly longer than

those not passing (126 mm versus 109 mm, respectively; ANOVA, $F = 42.5$, $df = 1$, $P < 0.01$). Four individuals (2 Creek Chub and 2 Highland Stonerollers) moved as far as 950 m from their original capture locations to pass a study reach, but the average movement was approximately 262 m (SE, 22.3 m); thus, we considered the 2-km study zone sufficiently large to avoid biased assessment of fish movements.

Hydrology and passage

Water levels, as measured by the water level recorders from July 2012–April 2013, fluctuated between 0.07 and 1.35 m (mean, 0.47 m; SE, 0.002 m) near the vented ford on intermittent Bear Creek and ranged from 0.08 to 1.01 m (mean, 0.18 m; SE, 0.001 m) near the box culvert on perennial Long Creek. At Long Creek, water depth always exceeded 3 cm and we never observed swim zone velocities >0.5 m/s within the culvert.

Alternatively, the vented ford on intermittent Bear Creek presented hydraulic challenges for fish passage. The culvert pipes were clogged—thus, fish passage was restricted to movement over the top of the large concrete slab or to adjacent portions of the floodplain. At low water levels (less than 0.24 m on the staff gauge), water did not pass over the road crossing. As water levels rose above 0.45 m, water flowed over the concrete roadway and down the steep concrete slope on the downstream side of the road prism, but never exceeded 3 cm depth and velocities ranged from 2.3–2.8 m/s. At higher flows (water levels ≥ 0.69 m), the downstream slope supported a potential swim-zone with depths ≥ 3 cm. However, during these conditions, water velocities of 2.5–4.5 m/s exceeded typical swimming speeds of warmwater fishes (Leavy and Bonner 2009).

We categorized water levels on the culverted streams as either “low” and “high” to assess passage rates based upon water levels. For the vented ford on intermittent Bear Creek, water levels <0.7 m did not produce the 3-cm potential swim zone. Therefore, we assigned passages when water levels were <0.7 m to the low category and >0.7 m to the high category. Because the perennial streams supported a swim zone at all measured conditions, we did not identify a clear hydraulic cutoff for water levels and arbitrarily categorized water levels as low or high relative to 0.6 m, which mimicked the categories assigned to the intermittent streams and distinguished baseflow levels from less frequent high water events.

Fish passed study reaches in the 3 streams with passable “swim zones” predominantly when water levels were low (corresponding with baseflow

hydrologic conditions) and only passed study reaches 7–17% of the time during high water levels (Table 5). Alternatively, 25% of fish passages for the vented ford on intermittent Bear Creek were at high water levels. This high-water passage rate was significantly greater than the rates for the other 3 streams (when the 3 dominant species were pooled for sufficient sample size in a log-linear analysis with flow regime (i.e., perennial vs. intermittent) and crossing presence (i.e., road crossing vs. reference reach) as design variables; $G^2 = 4.62$, $df = 1$, $P < 0.05$). We hypothesize that fish opportunistically utilized higher water levels to pass the hydraulically-challenging vented ford on Bear Creek.

We utilized all available location data (i.e., RFID station data *and* electrofishing data) to determine if fish passed the study reach because the RFID stations alone sometimes failed to detect fish passage. However, despite our best intentions, these data had often recorded a fish’s location prior to the actual moment of passage, sometimes even months before the fish was detected on the far side of the study reach; therefore, we could not always ascertain the precise moment for a particular passage. Consequently, we conservatively analyzed the relationship between water level and passage based on the highest water level occurring between the 2 detection events. We acknowledge that this presents a potential bias, whereby it appears that fish passed during high water levels.

Discussion

RFID detection stations

The RFID stations improved the probability of re-encountering tagged fish over traditional methods such as electrofishing mark and recapture (similar to benefits observed by Roghair *et al.* 2014). Additionally, except when damaged, the RFID stations detected passage during high flow conditions when electrofishing was unsafe or less efficient and they enabled us to link fish passage to flow fluctuations in natural stream cross-sections. Alternatively, electrofishing sampling allowed us to detect fish in more locations, ameliorating the inherent limitation of a fixed-location RFID station.

Our approach could not accurately assess detection (or missed detection) rates for the RFID stations as others have reported for more controlled settings such as fishways (Axel *et al.* 2005; Aymes and Rives 2009). The RFID stations (without help from the electrofishing detection data) missed 24% of the total observed passages. Thus, we acknowledge that the reported counts of fish passages were biased low. We designed the RFID stations to provide uniform and efficient fish

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Table 5. The number of fish passages when water levels were “low” and “high”. The vented ford on Bear Creek did not sustain a hydraulically-favorable “swim zone” at low water levels; whereas, both perennial streams supported a hydraulically-favorable swim zone at all water levels measured.

Species	Number of passages		Proportion of high water passages
	Low water levels	High water levels	
Bear Creek- vented ford, intermittent			
Creek Chub	9	2	0.18
Highland Stoneroller	0	0	N/A
Longear Sunfish	0	1	1.00
Total	9	3	Mean 0.25
Crystal Prong- reference, intermittent			
Creek Chub	17	5	0.23
Highland Stoneroller	21	1	0.05
Longear Sunfish	3	0	0.00
Total	41	6	Mean 0.13
Long Creek- box culvert, perennial			
Creek Chub	4	2	0.33
Highland Stoneroller	21	3	0.13
Longear Sunfish	19	4	0.17
Total	44	9	Mean 0.17
Little Missouri River- reference, perennial			
Creek Chub	98	9	0.08
Highland Stoneroller	23	0	0.00
Longear Sunfish	4	0	0.00
Total	125	9	Mean 0.07

detection, but unavoidable intra-station and inter-station variations limited the validity of such comparisons (also noted by Aymes and Rives 2009). Station downtime led to missed detections, which contributed to fewer observed passages. These design limitations probably contributed to the observed interactions among species and streams (with respect to the frequency of detected passage). Future studies seeking more rigorous analysis could address this limitation by installing additional RFID stations to achieve greater replication.

We explored the technological limits of the RFID detection equipment by building large antennas located substantial distances from the reader without rigid, in-stream structures. Antenna efficiency, already limited by the read range of 12-mm HDX, RFID tags, declined when we added a second antenna to the multiplex reader and as each antenna increased in size and distance from the reader. To mitigate the limited antenna efficiency associated with the simple pass-through loop designs, we developed a figure-eight crossover design, which produced multi-fold improvements in antenna efficiency and largely eliminated tag detection gaps within the antenna plane. However, the crossover design, with its vertical columns, was more prone to damage during high flow events and was more complex to build and repair. Fortunately, an experienced a two-

person team could rebuild and tune this type of antenna in less than 3 hours using inexpensive and widely available materials.

We initially designed larger antennas that encompassed more of the floodplain above bankfull to maximize antenna cross-section (and hence *path efficiency*) and detect fish during higher stormflow events. We later reduced antenna size to achieve consistently higher *antenna efficiency* during average flow conditions. These small antennas were also less vulnerable to high flow damage. An antenna installed in a pool increased detection probability because fish often resided in the pool for extended periods. However, to accommodate the pool depth, these antennas were large and had reduced antenna efficiency. Runs and riffles permitted squat antennas with excellent antenna efficiency, but the high stream velocity and associated debris damaged antennas.

Norman *et al.* (2009) called for longer-term studies (months to years rather than weeks) to evaluate the impact of hydrologic variability (i.e., fluctuations in stream discharge) on fish passage at semi-permeable road crossings. We sought to measure the water level at the moment of fish passage to investigate passability at various hydrologic conditions. However, our conclusions were limited by the long spans of time

between detection events, which were exacerbated by equipment downtime. Future improvements to RFID technology will likely yield smaller tags, improved antenna read range and efficiency, more resilient antenna designs, and more stable electrical operating systems. Pass-over antenna designs, such as those designed by Connolly *et al.* (2008), may someday offer the same level of detection performance as more vulnerable pass-through designs. By placing multiple antennas on each stream (i.e., more than just 1 upstream and 1 downstream of the study reach e.g., Connolly *et al.* 2008) researchers can improve the spatial accuracy of fish movement studies and minimize missed detections. We believe RFID detection stations can help evaluate and prioritize the removal of the worst passage barriers in large stream networks as called for by Kemp and O'Hanley (2010).

Fish detection and passage

The community of fishes within the 4 streams varied in both assemblage and abundance—as evidenced by the variable tagging rates of the 3 species across streams (Table 2)—and we detected fish at different rates across species and streams (Table 4). To enable comparisons, we attempted to control for differences by focusing on only three common species and normalizing detection and passage rates to tagging rates. However, we acknowledge that ecological and experimental variability impacted our study.

Alternatively, the higher detection rates may not be experimental variability but may indicate higher movement rates (hence greater likelihood that the RFID station would detect these individuals). Specifically, species that were more likely to move (e.g., Creek Chub and Highland Stoneroller) or streams that may have allowed more movement (e.g., Little Missouri River) resulted in higher detection rates.

Even when accounting for differences in numbers of fish tagged and RFID station operating time, Creek Chub and Highland Stoneroller passed reference reaches more often than road crossings. This conclusion is consistent with results of other studies of fish passing road crossings in warmwater streams (Benton *et al.* 2008; Bouska and Paukert 2009). Data for Longear Sunfish across treatment type was inconclusive due to low numbers of observed passages.

Passage was higher on the perennial streams, likely because the hydrologic discontinuities of the intermittent streams, most pronounced during the dry summer months (see Girondo 2011 for related details), cumulatively reduced movement. In other words, stream dryness converted the intermittent streams into a series

of isolated pools that reduced long-distance fish movement and passage across the study reaches.

Passage rates were lowest at the vented ford on intermittent Bear Creek where the clogged culvert pipes prevented water and fish passage *through* the road crossing. Furthermore, the stream's summer intermittency appeared to exacerbate passage problems by causing discontinuous surface flow that prevented water and fish from passing *over* the road crossing—a common condition among road crossings in this ecoregion. Consistent with these observations, Schanke *et al.* (2017) concluded that culverts and stream intermittency in this and other nearby streams contributed to reduced gene flow among Longear Sunfish and Highland Stoneroller subpopulations. When water did flow over the crossing at moderate water levels, the steep downstream slope of the structure produced a sheet of water with high velocity and insufficient depth for most fish species to pass (especially upstream). Thus, we conclude that fish opportunistically crossed this barrier when water rose near or above bankfull and flowed over and around the road crossing, creating low-velocity swim zones. Helfrich *et al.* (1999) and Norman *et al.* (2009) have also observed fish opportunistically crossing otherwise impermeable barriers during high flows. Our results should be interpreted cautiously due to the inconsistent performance of the RFID detection stations and small sample sizes, but we document a trend of reduced movement that may concern conservationists and resource managers.

At the full range of water levels observed, the box culvert on perennial Long Creek sustained hydraulic conditions (i.e., adequate swimming depth and low velocities) that appeared favorable for fish passage. Indeed, fish passed this box culvert at higher rates than they passed the vented ford on intermittent Bear Creek. While the confounding factor of hydrologic regime (intermittent versus perennial) and lack of replication preclude strong inference, this observation is corroborated by an independent analysis of gene flow patterns (Schanke *et al.* 2017) and supports previous observations that box culverts facilitate more passage than other types of road crossings that restrict flow (Warren and Pardew 1998; Standage and Gagen 2007; Norman *et al.* 2009).

The RFID stations on reference streams detected higher passage rates of Highland Stonerollers than Longear Sunfish, which is consistent with their respective swimming abilities (inferred through swimming velocity) as reported by Leavy and Bonner (2009) and with gene flow studies in these headwater

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streams (Schanke *et al.* 2017). Leavy and Bonner (2009) reported that the Creek Chub's swimming ability in the laboratory was poor among Cyprinidae; however, our results support the possibility that Creek Chub have a greater tendency to move in natural settings. On average, Creek Chub that did not pass our study reaches were likely two-year-old fish; whereas those that did pass were likely three-year-old fish (based on Gunning and Lewis's [1956] study of length at age in Illinois). Because even the two-year-old Creek Chub were likely sexually mature (Schemske 1974) and thus motivated to move frequently, we attribute the higher passage rates of the larger Creek Chub to their more powerful swimming capabilities.

Fish traveling on the culverted streams encountered not only the road crossing with the RFID station, but additional road crossings, which may have compounded the challenges of long-distance movements. Conclusions about long-distance movement and passage at a particular crossing must be considered in the context of the overall stream system because the benefit of any given "fish-friendly" road crossing is likely diminished by other barriers along the stream continuum (Helfrich *et al.* 1999; Zydlewski *et al.* 2006; Cote *et al.* 2009; Kashiwagi and Miranda 2009; Ryles 2012).

Despite ongoing research (Park *et al.* 2008; Bouska and Paukert 2009; Schanke *et al.* 2017), there are still many unknowns regarding the ecological effects of stream fragmentation and the degree of road crossing permeability necessary to maintain genetic diversity and viability of non-migratory fish populations over longer time scales. This presents a challenge to resource managers who wish to maintain access to remote areas while preserving natural fish movements. Flow-constricting road crossings are ubiquitous in many headwater streams, where they were commonly designed to intentionally restrict and intensify cross-sectional stream flow to ensure that strong flows would clear debris and substrate from the road surface. Thus, additional effort is needed to identify and improve aging road crossing designs, such as vented fords, which restrict flow, alter stream hydraulics, and impair fish passage (also see Warren and Pardew 1998; Bouska and Paukert 2009).

In this study, we applied RFID technology in a novel way to investigate road crossings as barriers to individual fish movements. Concurrently, Schanke *et al.* (2017) reported patterns of DNA microsatellite variability in 2 fish species in the same stream systems and documented evidence of longer-term, population-level, impacts of road crossings. A combination of

research methods, such as genetic analyses at the population level, hydraulic evaluation of road crossings, and observation of individual fish movements may help answer the question—how much passage is enough?

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