FOUNTAIN DARTER MOVEMENT UNDER LOW-FLOW CONDITIONS IN THE COMAL SPRINGS / RIVER ECOSYSTEM

FINAL REPORT

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EXECUTIVE SUMMARY

A vital component of the Edwards Aquifer Habitat Conservation Plan (HCP) is the development of an ecological model to predict responses of the covered species to various flow regimes. Although development of the model is well underway, additional ecological data on the covered species is necessary to parameterize this model. This report describes studies conducted to examine fountain darter *Etheostoma fonticola* movement under deteriorating habitat conditions caused by low-flow scenarios.

Initial study plans included a field component examining movement of wild fountain darters in the Comal Springs / River ecosystem as well as a manipulative pond investigation in the event that low-flow conditions were not encountered in the wild. However, since extended low-flow conditions presented themselves in the Comal system during 2014 and complications were encountered in the experimental pond; all resources were diverted to the field study to maximize the information gained from the project.

Previous research conducted in the Old Channel of the Comal River has shown that fountain darters move little in quality habitat under a stable flow regime (Dammeyer et al. 2013). Specifically, fountain darters moved an average of 10 meters (m) over the course of the year, with a maximum movement of 95 m in 26 days. However, should habitat conditions begin to deteriorate; movement could potentially increase as fountain darters search for more suitable conditions. To investigate this, over 2,000 individual fountain darters were captured from the headwaters of the Comal River, injected with fluorescent visual implant elastomer (VIE) marks under their skin, and released during a low-flow period in spring and summer 2014. A variety of methods were used to relocate the tagged fountain darters and thus monitor movement and habitat utilization.

Over the course of the study, total system discharge at Comal Springs declined drastically, reaching levels that had not been experienced in over 20 years. During late August and early September, spring flow within the study area was essentially zero (<1 cfs), although some groundwater infiltration was noted in certain areas along the river bottom. Aquatic vegetation, which is the key fountain darter habitat component within the study reach, became covered in filamentous algae and eventually disappeared completely. Water temperatures, which typically fluctuate between 23°C and 26°C over the course of a year peaked at over 30°C, with two straight weeks over 26°C. Extremely low discharge conditions, coupled with extensive habitat decline, provided the study team with a very favorable situation to observe movement of wild fountain darters in a stressed environment.

A total of 149 fountain darters were relocated during the study. In general, despite the low-flow conditions observed, fountain darters were relatively sedentary, moving an average of 20.9 m (median = 17.9 m) from their release point over the course of the study. However, two fountain darters, which were tagged in Blieders Creek, made relatively long movements of approximately 130 m toward areas of increased spring influence in the Upper Spring Run. These represent the longest recorded movements ever documented for wild fountain darters. Despite these two relatively long movements from Blieders Creek to the Upper Spring Run, no fountain darters were documented moving downstream of the Upper Spring Run into the spring-influenced

habitat that was available near Spring Island. The distance to this habitat (>250 meters), along with observations made by divers suggesting that much of the wetted area between became comparatively warm and stagnant, may have presented a barrier to fountain darter movement.

Average distance moved (20.9 m) and maximum distance moved (131 m) in this study was slightly greater than that documented under stable habitat conditions by Dammeyer et al. (2013) (10 m and 95 m, respectively). This may suggest slightly increased movement as fountain darters searched for more suitable habitat. However, this may also be an artifact of a more expansive study area.

This study provided interesting insight into fountain darter movement, habitat selection, and potential population dynamics under low-flow, no-vegetation conditions. When aquatic vegetation disappeared in July and early August, and water temperatures increased, rather than moving, fountain darters adjusted their habitat utilization to that available within the local area. They were observed using interstitial spaces in gravel and cobble substrates as concealment, and were occasionally seen occupying open silt flats during this time period. These changes in habitat utilization could result in decreased prey availability and increased susceptibility to predation. As a result, an eventual decline in fountain darters would be anticipated should these conditions persist. It will be important to closely examine the HCP biological monitoring program data at the conclusion of this year's sampling to evaluate if a concurrent decline in fountain darter abundance occurred in the Upper Spring Run in late summer 2014.

HCP Ecological Model Parameterization

Results of this study show that even under extreme low-flow conditions, long-distance movement of fountain darters was rare. This has direct implications to ecological model parameterization. Currently, a decline in habitat within the ecological model results in a concomitant decline in the number of fountain darters occupying that habitat. At present, there is no movement factor incorporated. This study suggests that movement/emigration of fountain darters from disappearing vegetation/habitat is not likely to completely counteract a projected population decline, particularly if additional habitat is more than approximately 20 m away. At maximum, fountain darters were observed moving over 100 m. However, this is based on the maximum distance moved by only a few fountain darters. Perhaps a more appropriate cutoff to represent movement potential in the HCP ecological model would be the median distance moved during extreme low-flow conditions (17.9 meters).

In addition to providing input to the ecological model on movement potential under low-flow scenarios, this study also provided data on fountain darter population size within the study reach. These estimates may be useful in HCP ecological model calibration or validation within this reach. Finally, changes in habitat utilization that could result in decreased prey availability and increased susceptibility to predation should be considered during ecological model parameterization. Although the report puts forward parameters for consideration in model parameterization, it is emphasized that specific use of any of the 2014 applied research will be determined by the HCP ecosystem modeling team with guidance from the HCP Science Committee.

Recommendations for Future Applied Research

Should low-flow conditions continue or rebound in the first 6 months of 2015, it is recommended that follow-up relocation surveys in the Upper Spring Run reach be conducted. These surveys would test the following two hypotheses: (1) a complete loss of marked individuals would occur during extended drought, or (2) higher relocation percentages would accompany a rebound in total system discharge and subsequent anticipated habitat improvements in the spring. Additionally, determining population sizes in other study reaches in the Comal and San Marcos rivers would likely be beneficial in ecological model calibration or validation in those reaches.

Acknowledgments

The project team would like to acknowledge the U.S. Fish and Wildlife Service (USFWS) San Marcos Aquatic Resource Center (SMARC) scientists and staff. In particular, we thank Dr. Ken Ostrand and Dr. Tom Brandt for their guidance, assistance and cooperation. Dr. Ostrand was integral in all marking activities and his dedication to this project is very much appreciated.

1.0 INTRODUCTION

Section 6.3.4 of the Edwards Aquifer Habitat Conservation Plan (HCP) outlines applied research activities focusing heavily on the fountain darter *Etheostoma fonticola* and the Comal Springs riffle beetle *Heterelmis comalensis* (EARIP 2011). Additional ecological data on these species is needed to populate an ecological model, which is under development and serves as a crucial component to meet HCP goals and inform management decisions in coming years. To provide input to the ecological model, the initial round of applied research activities in 2013 focused on addressing several key questions regarding physical habitat and food source responses relative to the fountain darter under low-flow conditions. Specifically, applied research studies conducted in 2013 were aimed at determining the low-flow-induced abiotic conditions, which would result in impacts to aquatic vegetation (fountain darter habitat) and amphipod populations (fountain darter food source) (BIO-WEST 2013). Such habitat deterioration parameters can be incorporated into the ecological model, thus resulting in impacts to the fountain darter population as habitat conditions deteriorate. However, this assumes that degradation of habitat results in a similar degradation of the fountain darter population and does not account for the ability of fountain darters to move away from deteriorating habitat to more suitable areas. Therefore, to build upon the 2013 studies, a key question for 2014 applied research related to how fountain darter movement may be influenced by changes in habitat under low-flow-induced conditions. This report describes applied research conducted in 2014 relating to movement of fountain darters under such conditions.

One previous study, Dammeyer et al. (2013), has examined movement of wild fountain darters within the Old Channel of the Comal River. Their results show that fountain darters are not highly mobile, moving an average of 10 meters (m) within a year, and up to a maximum of 95 m. Fountain darters move among habitats more frequently than other darters, most often towards low-growing vegetation such as bryophytes or filamentous algae and most often in an upstream direction. However, the Dammeyer et al. (2013) study was conducted in the Old Channel of the Comal River, which typically exhibits a stable hydrograph and consistent habitat conditions. By contrast, the goal of this study was to further investigate movement relative to changes in habitat and temperature caused by low-flow regimes.

To accomplish this, a two-part study design was developed involving a field movement study in the Upper Spring Run reach of the Comal River as well as a manipulative pond study in an experimental pond at the U.S. Fish and Wildlife Service (USFWS) San Marcos Aquatic Resource Center (SMARC). Prior to initiation of the study, an extensive literature review was conducted relating to movement of freshwater fishes under varying hydrologic regimes, particularly fountain darters and other similar species. This literature review is summarized in Section 2. Section 3 provides information on the study design, describes the methods used, and presents challenges observed during implementation of these studies. Results are provided in Section 4, followed by conclusions and recommendations in Section 5. Finally, Section 6 lists the references cited throughout the document.

2.0 LITERATURE REVIEW

Movement of freshwater stream fish depends on an array of physical and environmental factors (Jackson et al. 2001). The restricted-movement paradigm predicts that small-bodied, resident fishes are relatively sedentary, moving <50 m under normal hydrological conditions (Gerking 1959, Gowan et al. 1994). *Etheostoma*, a very speciose genus composed of the smallest species of darters, conform to the predictions of the restricted-movement paradigm, being highly sedentary with 80 to 97% of individuals remaining within habitat patch of initial observation (Boschung and Nieland 1986, Labbe and Fausch 2000, Mundahl and Ingersoll 1983, Roberts and Angermeier 2007b). Among a few mobile individuals, mean distance moved is <200 m (Mundahl and Ingersoll 1983, Roberts and Angermeier 2007a). Movement among highly sedentary darters coincides with non-reproductive seasons (Mundahl and Ingersoll 1983, Scalet 1973), shifting habitat preferences as the darters grow (Labbe and Fausch 2000), and declining habitat quality (Mundahl and Ingersoll 1983, Roberts and Angermeier 2007b). Among swift-water darters, a 5% area loss of riffle habitats (i.e., shallow water habitats) because of summertime dewatering prompted fantail darters (*E. flabellare*) to move away from riffles (Roberts and Angermeier 2007b). Movement is also associated with density dependent mechanisms. Darter movement from patches has been shown to increase as resources became limited (Mundahl and Ingersoll 1983).

Fountain darters, like other darters, appear highly sedentary, moving on average 10 m within a year and up to 95 m within 26 days under a stable hydrograph (Dammeyer et al. 2013). When movement occurs, fountain darters move among habitats more frequently (51%) than other darters (3 to 20%; Mundahl and Ingersoll 1983; Labbe and Fausch 2000), most often towards low-growing vegetation, upstream, and during the winter and spring-summer seasons. Determining how and why fountain darters disperse throughout the Comal River system could be vital to the conservation of this species. Dammeyer et al. (2013) have offered insight into this fundamental question under a stable hydrograph; however, the goal of this study is to further investigate movement relative to changes in habitat and temperature caused by low-flow regimes. A wealth of information on aquatic vegetation preference by the fountain darter is available through long-term biological monitoring on both the Comal and San Marcos systems (BIO-WEST 2014a, BIO-WEST 2014b).

A mark-and-relocate study was conducted to determine how movement of fountain darters is affected by habitat and temperature changes under low-flow conditions. Fountain darter mark-and-relocate techniques utilized methods previously developed for darters and other small-bodied fishes, with visual implant elastomer (VIE) as the marking material. Although recapture success rate varies among movement studies (9–37%) (Belica and Rahel 2008, Dammeyer et al. 2013, Labbe and Fausch 2000, Roberts and Angermeier 2007b, Schaefer et al. 2003, Skyfield and Grossman 2008), VIE marking has been thoroughly tested (Belica and Rahel 2008, Holt et al. 2013, Labbe and Fausch 2000, Phillips and Fries 2009, Roberts and Angermeier 2004, Weston and Johnson 2008) and shows a high rate of retention (79–100%) accompanied with high survivorship (85–100%). Additionally, laboratory studies using darters (Phillips and Fries 2009, Roberts and Angermeier 2004) found VIE advantageous compared with other marking mediums, such as acrylic paint or photonic dye. Both visual (re-sight) and physical (dip net, recapture) methods were used for relocating fountain darters due to their habitat affinity (i.e., benthic fish occupying areas of dense vegetation) (Alexander and Phillips 2012, Linam et al. 1993), characteristics of the study reach, and successes/suggestions of previous studies (Belica and Rahel 2008, Dammeyer et al. 2013, Holt et al. 2013, Jordan et al. 2008, Labbe and Fausch 2000, Skyfield and Grossman 2008).

3.0 DESIGN, METHODS, AND IMPLEMENTATION

3.1 Field Movement Study

3.1.1 Study Area

The Upper Spring Run reach of the Comal River near the Blieders Creek confluence (Figure 1) provided a well-suited area for the field movement study for two primary reasons. First, due to the elevation of springs in this reach, this area is the first to be impacted by springflow reductions as overall system discharge declines. Previous monitoring conducted in this reach as part of the HCP biological monitoring program has documented deterioration of habitat within this reach under low-flow conditions, as well as a corresponding decline in fountain darter abundance. Second, Blieders Creek merges with the river near the head of this reach. This intermittent creek is dependent upon local runoff and has a different temperature regime than the Upper Spring Run reach (Figure 2). Water temperatures in the middle and upper portions of Blieders Creek get much higher during the summer and much lower during the winter when compared to the spring-fed Comal River. Fountain darters are known to use the lower portions of the creek, although use of the area is expected to be seasonal, as water temperatures allow. Therefore, even if high-quality habitat conditions persisted in the Upper Spring Run reach over the course of the field movement study, habitat conditions in Blieders Creek were known to deteriorate each summer. This provided the study team an opportunity to observe movement of fountain darters in low springflow conditions. To keep track of water temperature conditions throughout the project area over the course of the study, three stationary water temperature monitors (HOBO tidbit V2) were placed at key locations and set to collect water temperature hourly (Figure 1).

3.1.2 Marking

Fountain darters were marked with fluorescent VIE tags using products and materials commercially available from Northwest Marine Technology, Inc. (www.nmt.us). Visual implant elastomer tags consist of a two-part silicone based material that is mixed immediately before use, injected under the skin as a liquid, and soon cures to a pliable solid. Visual implant elastomer marking has been thoroughly tested and has shown a high rate of retention (79–100%) accompanied with high survivorship (85–100%) on small fishes and darters, including the fountain darter (Belica and Rahel 2008, Holt et al. 2013, Labbe and Fausch 2000, Phillips and Fries 2009, Roberts and Angermeier 2004, Weston and Johnson 2008). Laboratory studies using darters found VIE advantageous compared with other marking mediums such as acrylic paint or photonic dye (Phillips and Fries 2009, Roberts and Angermeier 2004).

Figure 1. Map of Upper Spring Run and Blieders Creek study area.

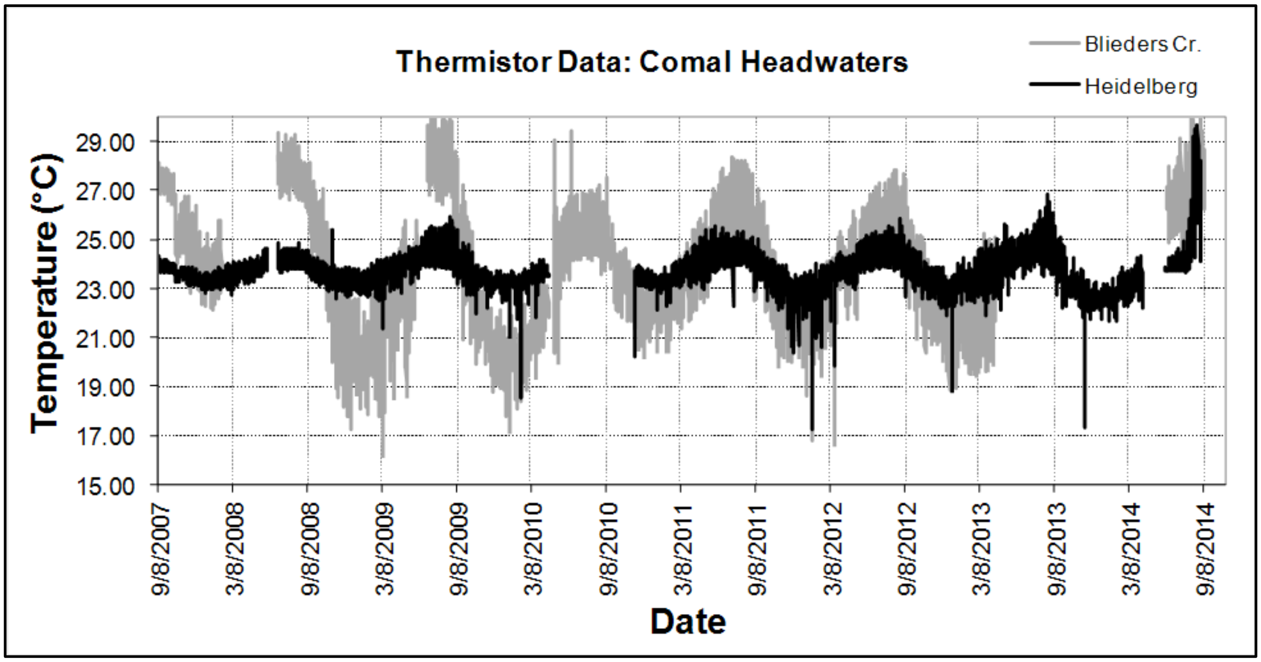

Figure 2. **Water temperature data from Blieders Creek and the Upper Spring Run reach (Heidelberg).**

To test mark retention and survivorship and provide marking practice prior to initiation of the field study, 35 adult fountain darters were marked at the SMARC on March 19, 2014. These fish were “extra” F1 hatchery stock scheduled to be euthanized if not used for another purpose. They were captured from their tank with a small aquarium net, injected with fluorescent blue VIE marks approximately 2–3 millimeters (mm) in length along the left side of the dorsal fin (Figure 3), immediately placed back into their tank of origin, and monitored for several weeks after tagging. These fish exhibited 100% survivorship and tag retention 1 month after marking, and were kept alive to be used later in the manipulative pond experiment.

From March 24 to August 7, 2,212 individual fountain darters were marked within four study locations (Table 1). Fountain darters were captured from two Upper Spring Run and two Blieders Creek (BC) sampling areas using dip nets and cohort marked according to area of initial capture (Figure 4). Darters from the upstream Upper Spring Run site (USR1) were marked with yellow fluorescent VIE on the right side of and adjacent to their dorsal fin, while fountain darters from the downstream Upper Spring Run site were marked with yellow fluorescent VIE on the left side of their dorsal fin. Upstream BC (BC2) were marked with red VIE on their left side, and downstream BC (BC1) fountain darters were marked with red on their right side (Figure 5).

Captured fountain darters were held in floating containers in the river that allowed water exchange and had shade covers until marking (Figure 6). During marking, fountain darters were removed from the floating container using an aquarium net, injected with a VIE mark, and quickly placed back into a separate container containing fresh river water. Insulated and aerated bait buckets were used as post-marking containers to reduce stress and mortality on hot days (Figure 7). To reduce handling stress, fountain darters were not individually measured. However, fountain darters less than approximately 20 mm total length were released without marking.

Figure 3. Visual implant elastomer -marked fountain darters from the initial trial run at the SMARC on March 19, 2014.

Table 1. Marking dates, marks used, and number of darters marked in each sampling area.

Location	Marking Dates	Mark	Total Number
USR1	Mar. 24, Apr. 18	Yellow / Right Dorsal	185
USR2	Mar. 24, Apr. 18, May 30, Aug. 7	Yellow / Left Dorsal	1,810
BC1	Mar. 25, Apr. 18	Red / Right Dorsal	154
BC2	Mar. 28, Apr. 18	Red / Left Dorsal	63
Total			2,212

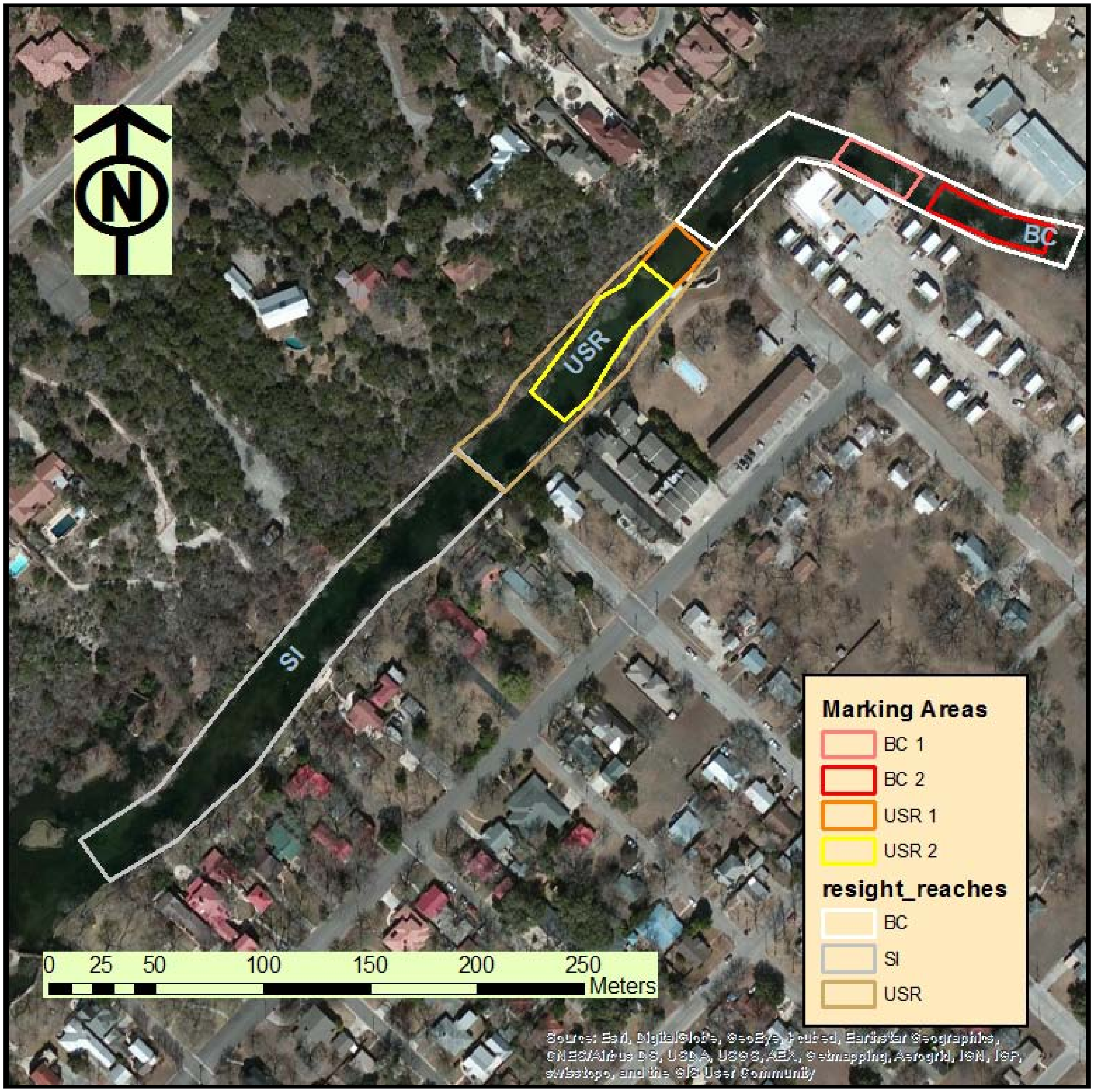

Figure 4. **Areas where fountain darters were collected and marked (Marking Areas) and areas where visual searches were conducted (resight_reaches).**

Figure 5. Examples of visual implant elastomer (VIE) marked fountain darters.

Figure 6. Floating container constructed to retain darters in river water during marking events.

Figure 7. BIO-WEST and USFWS personnel marking fountain darters at USR1.

After marking, fountain darters were held briefly to observe for mortality, they were released at designated release points within each marking area. Observed mortality rates prior to release averaged 2.5% (range: 0–14%) and consisted mainly of fountain darters in the smaller size classes. The number of fountain darters marked during an event was determined either by the maximal number that could be captured (Blieders Creek) or the number that could be marked by the end of day (Upper Spring Run).

Fountain darters that were recaptured from previous marking events during a new marking event were held in the aforementioned containers *in situ* for the duration of the event to prevent re-counting. For these relocations, the same data were recorded as during relocation surveys described below.

3.1.3 Relocating

Relocating marked fountain darters was conducted using three separate methods: recapturing them with dip nets (Figure 8), daytime SCUBA/snorkel visual surveys, and nighttime SCUBA/snorkel visual surveys with the aid of specially designed ultra-violet underwater flashlights (VI light; Northwest Marine Technology, Inc.) (Figure 9). The VI lights radiate a deep purple light that causes the tags to fluoresce, increasing visibility substantially when used in the dark or shade. Initially (April–June), daytime dip net and visual surveys were used to collect


Figure 8. Dip netting for marked fountain darters in the Upper Spring Run reach.

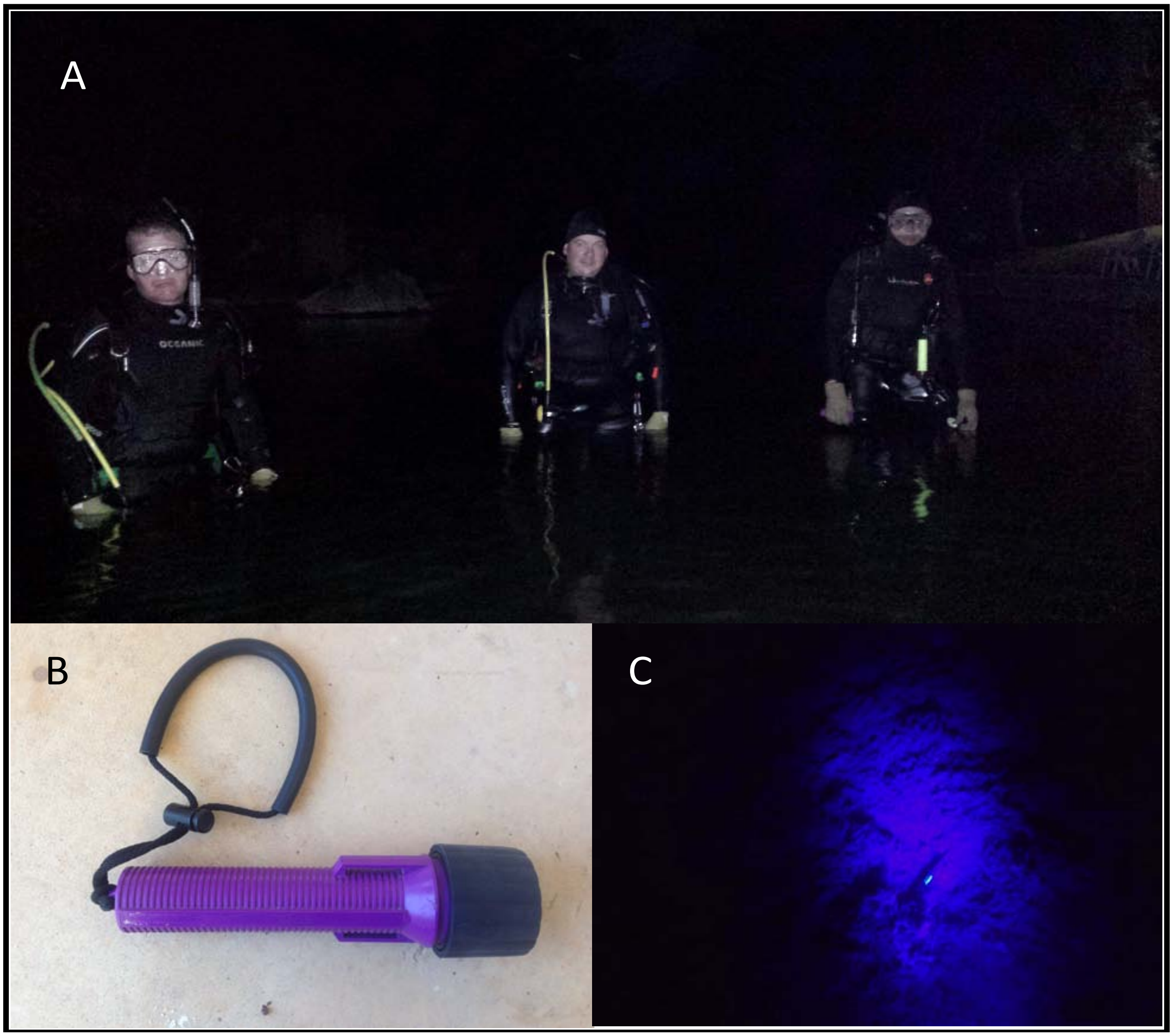

Figure 9. **BIO-WEST divers preparing for night relocation surveys (A) using VI lights (B) to relocate fountain darters with fluorescent visual implant elastomer (VIE) tags (C).**

most relocation data. However, preliminary data from relocation events in the Upper Spring Run using each technique showed that catch-per-unit-effort (CPUE) was highest using night visual surveys due to the substantial increase in visibility of the fluorescent tags (Table 2). Therefore, night SCUBA/snorkel visual surveys were used to relocate darters for the remainder of the study period (July–September).

Night visual surveys were conducted using either SCUBA or snorkel equipment according to the reach being surveyed and water level conditions at the time of the event. The study area was split into three different survey areas delineated by feasible access points (Figure 4). Each survey area could be covered thoroughly in a single night dive which typically lasted approximately 2–3 hours. Two to five observers swam through the chosen survey area parallel to one another in an upstream direction using VI lights to scan the substrate for fluorescent tags. Large rocks, aquatic

Table 2. Catch-per-unit-effort (CPUE) from initial relocation surveys using three different methods.

Date	Relocation Method	Total Person-Hours Effort	Number of Relocations	CPUE (darters/person-hr)
5/30/2014	Day Dipnet	25	4	0.16
6/24/2014	Day SCUBA/snorkel	10	4	0.40
7/1/2014	Night SCUBA/snorkel	9.75	18	1.85

vegetation, and algal mats were often moved to search for fountain darters hiding underneath or around these structures. In addition to VI lights, which provide little illumination, standard dive lights were also carried by each diver to help orient themselves in the dark underwater environment. Waterproof tank lights were strapped to each diver's back so that divers could keep track of each other's positions. An attempt was made by the divers to move through the study area at approximately the same rate, thus reducing potential overlap and ensuring that unique portions of the survey area were observed by each diver. An additional biologist accompanied divers in a kayak to record data and assist. Each time a marked fountain darter was relocated, a GPS waypoint was collected using a Garmin eTrex 30 handheld GPS. Additionally, time, mark description (color, location on fish), and notes on habitat (vegetation and/or substrate) were also recorded. During visual surveys, the number of unmarked fountain darters observed by each diver was also recorded. Standard water quality parameters (temperature, pH, conductivity, and dissolved oxygen [DO]) were recorded at the onset of each marking event using a HydroTech water-quality sonde. The number of observers and the time spent searching was recorded for each relocation event and CPUE was calculated as darters/person-hour.

3.1.4 Habitat Analysis

A variety of statistical analyses were used to explore relationships between fountain darter movement data and various habitat and discharge variables. Due to low sample size in other populations, only data from fountain darters marked in the USR1 and USR2 were subjected to statistical analysis. Analysis of Variance (ANOVA) was used to investigate association of recaptures (scaled by total number marked at the time of recapture) with habitat type. Linear regression was used to investigate relationships between the same response variable and weekly average temperature (from HOBO logger), DO (empirical from sampling event), and system discharge (based on U.S. Geological Survey [USGS] gauge #08169000). System discharge and weekly average temperature values were used in analyses as they were found to have significant ($p<0.001$), near-perfect (>0.90) Pearson's correlation with empirical data, but were more complete. For the same set of observations, the estimated distance of each relocation from the release point was calculated using ArcMap 10.2.2 (ESRI, Redlands, CA). These data were analyzed using linear regression to incorporate the variables study days (days since beginning of study), weekly average temperature, and discharge. Data in all statistical analyses were visually examined for departures from model assumptions in R version 3.0.3 (R Development Core Team 2008) using residual and quantile plots.

3.2 Manipulative Pond Study

For the manipulative pond study, it was proposed to use an experimental pond at the USFWS San Marcos Aquatic Resource Center (SMARC) to conduct a series of experiments investigating movement of fountain darters. The initial experiments were designed to investigate the use of vegetated vs. non-vegetated habitat patches by fountain darters. Follow-up experiments would then examine movement of fountain darters once vegetated habitat patches were removed and pond levels were altered. One hundred thirteen experimental fountain darters were given fluorescent VIE (Northwest Marine Technology, Shaw Island, WA) marks adjacent to the dorsal fin to enhance observation during experiments and housed in a holding tank at SMARC.

Vegetated patches consisted of specially designed Mobile Underwater Plant Propagation Trays (MUPPTS) planted with *Ludwigia repens* (Figure 10). Vegetation was established in the pond in mid-April, approximately one month prior to the planned beginning of experiments. This was done to allow for colonization of the vegetated patches by invertebrates providing a food source for the experimental fountain darters representative of a natural system. To provide an initial population of invertebrates, amphipods were stocked from a nearby SMARC raceway on several occasions in April and May. Vegetated patches were arranged interspersed with equally-sized non-vegetated patches. The MUPPTS and pots as well as non-vegetated areas were covered with pea gravel to prevent fountain darters from using the structure of the MUPPTS as habitat (Figure 11). A system of dam boards was constructed at the pond drain to allow manipulation of water levels to facilitate experiments involving manipulation of the draw down rate of the pond and its effect on movement of the fountain darters among habitats. The inflow plumbing to the pond was modified to allow for manipulation of inflow rates. Finally, two HOBO tidbit V2 temperature loggers were placed in the pond to record water temperature approximately 2 inches above the pond bottom spaced equally from the shallow (inflow) end to deep (outflow) end of the pond.

In late May, as the experimental pond setup neared completion, temperature loggers documented large diel swings in water temperature with afternoon water temperatures exceeding 29°C. With hot summer conditions expected in the coming months, it was determined that water temperatures similar to the natural environment of the fountain darter could not be maintained at the current inflow rate and pond level. Therefore, extensive experimentation was conducted to examine the effect of various flow rates and pond water levels on water temperature within the pond. Over a series of preliminary experiments in May and June, pond inflow rates were adjusted from approximately 9 gallons/minute (gpm) to over 80 gpm, and pond levels were lowered by removing dam boards to reduce overall retention time in the pond. After each successive change in flow rate and/or water level, temperature dataloggers were used to monitor water temperatures in the pond. Even at the lowest possible water level amenable to experiments and the highest flow rate tested, water temperatures in the pond during mid-June pushed or exceeded 30°C with diel swings of 8–10 degrees. Therefore, in an attempt to further reduce retention time in the pond, a dividing wall was built with sandbags on June 25 (Figure 12). This wall cut the surface area of the pond approximately in half. However, even under this reduced surface area and a high flow rate, water temperatures in the pond still exhibited a large diel swing with maximum temperatures exceeding 30°C (Table 3). Therefore, in early July the decision was made to abandon the pond study and focus efforts on the field movement study. This decision was aided by the fact that discharge conditions in the Upper Spring Run reach and for the Comal System as a whole were approaching levels not observed in over 20 years. As such, the natural system was providing the perfect laboratory and efforts were thus expanded in the field to take advantage of these rarely seen conditions.

Figure 10. **MUPPTS being planted with *Ludwigia repens* in an experimental pond at the SMARC.**

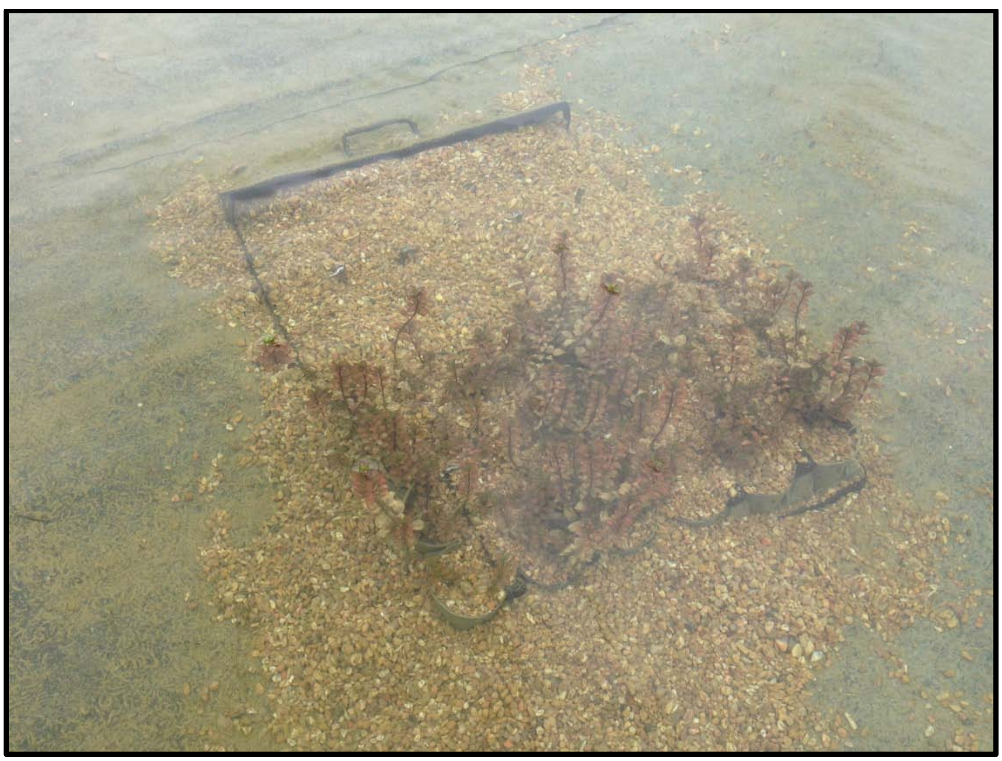

Figure 11. **Pea gravel being applied to mask MUPPT structure and homogenize available habitat patches for experimental trials.**

Figure 12. Sandbag wall being constructed to reduce the area of the experimental pond, thereby increasing water exchange rates to facilitate improved water temperature control.

Table 3. Summary statistics of water temperature recorded from two different locations in the experimental pond during various temperature manipulation trials.

Date	Shallow End Water Temperature (°C)			Deep End Water Temperature (°C)		
	Max	Min	Avg.	Max	Min	Avg.
May 09 – May 23	29.27	20.24	24.46	28.94	20.22	24.46
May 23 – May 30	29.27	20.25	24.46	28.94	20.22	24.46
May 30 – Jun 09	30.98	23.57	26.43	31.18	23.62	26.51
June 09 – June 13	31.89	22.94	26.74	32.30	22.92	26.66
June 13 – June 16	31.43	23.71	26.53	31.08	23.67	26.23
June 16 – June 19	29.67	23.30	25.55	29.46	23.30	25.50
June 19 – June 25	32.67	22.68	26.57	32.87	22.30	26.47
June 25 – June 30	31.38	20.08	25.60	31.48	20.08	25.25
June 30 – July 03	28.94	23.11	25.00	30.17	23.06	25.41

4.0 Results

4.1 Field Movement Study

4.1.1 Habitat Conditions Observed

Extreme low-flow conditions occurred at Comal Springs over the course of the study period (Figure 13). In fact, daily mean discharge dipped as low as 65 cubic feet per second (cfs) at the end of August. This represented the lowest daily mean discharge observed in over 20 years. Additionally, total system discharge remained below 100 cfs for 43 straight days from early August through mid-September. These conditions resulted in cessation of spring flow from many spring areas in the Upper Spring Run. As a result, Upper Spring Run discharge approached zero (<1 cfs) during late-August. During this time, water temperatures within the Upper Spring Run and Blieders Creek climbed considerably with daily average temperature approaching 30°C (Figure 14). However, even under these conditions, some spring flow/groundwater influence was still evident along the bottom in certain areas.

Aquatic vegetation represents the main fountain darter habitat component within the Upper Spring Run study reach and consists mainly of bryophytes with occasional summer blooms of filamentous algae. Blieders Creek typically contains large mats of muskgrass (*Chara* sp.), along with *Hygrophila polysperma*, filamentous algae, and *Nuphar* sp. Although coverage of aquatic vegetation within Blieders Creek remained relatively stable, coverage of aquatic vegetation within the Upper Spring Run reach varied considerably over the course of the year due to low springflow conditions. Data collected by BIO-WEST as part of the ongoing HCP biological monitoring shows that in early April, bryophytes covered approximately 34% of the Upper Spring Run study area (Figure 15). This had dropped to less than 1% coverage of bryophytes by August (Figure 16).

Low-flows and resulting deterioration of aquatic vegetation and water quality conditions in the study area provided the study team with a favorable situation for observing fountain darter movement patterns under extreme low-flow and unstable habitat conditions. Data on the number of fountain darters relocated, and their movement patterns, are described in the following sections.

4.1.2 Relocation Efficiency

In total, 149 fountain darters were relocated over the course of the study. The majority of these (136) were located during 22 separate relocation events between April 18 and September 10, 2014 (Table 4). Thirteen additional fountain darters were relocated incidentally during sampling for other applied research studies or during HCP biological monitoring activities during the study period. Given the total number of fountain darters marked (2,212), if each relocation is considered an independent observation, this results in an overall relocation rate of 6.7%. Although slightly lower, this is comparable to the recapture percentage (8.7%) observed in the previous fountain darter marking study conducted in the Old Channel Reach of the Comal River (Dammeyer et al. 2013). It is not surprising that the relocation rate was slightly lower in this study, given that the Upper Spring Run is a much more expansive aquatic environment than the Old Channel.

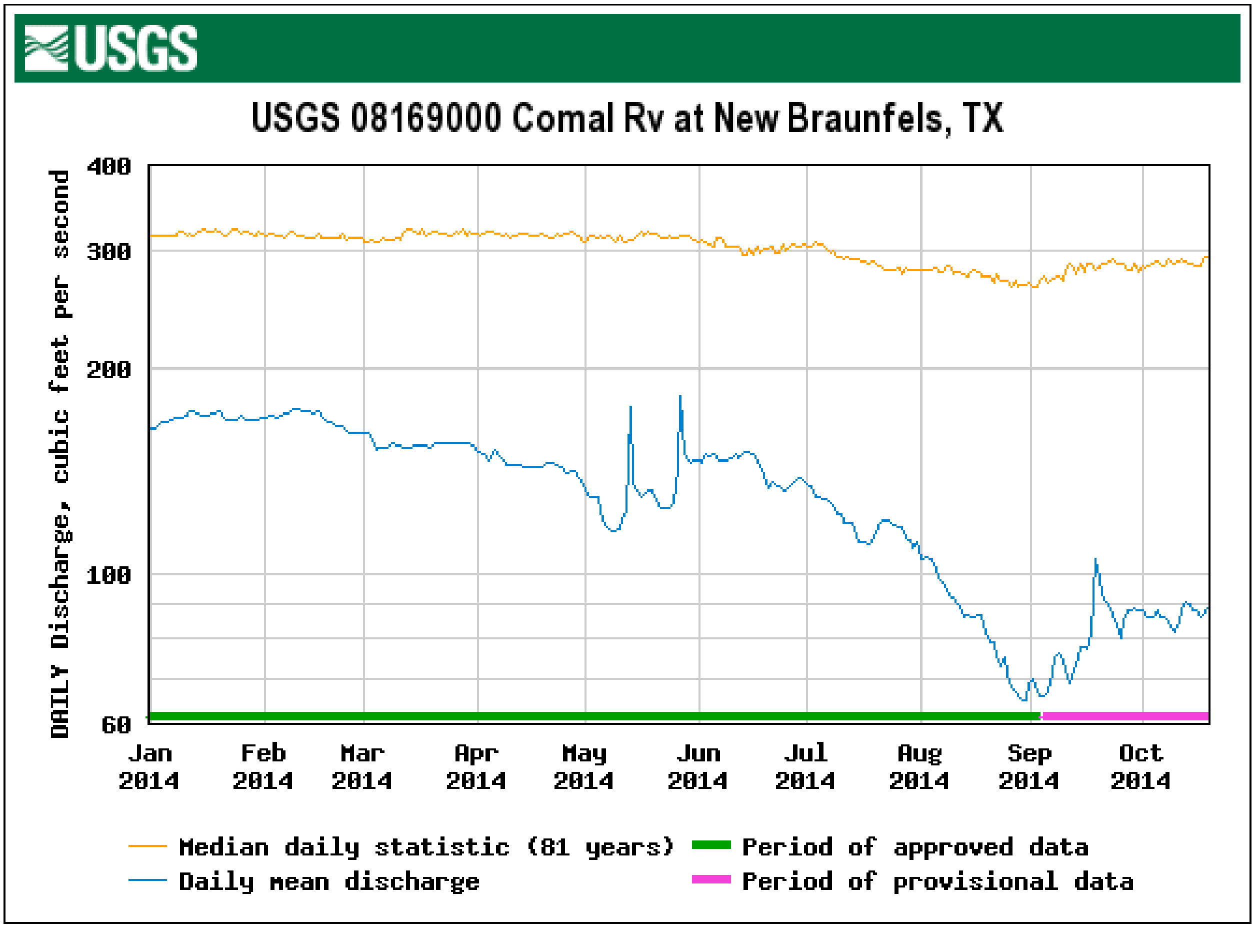

Figure 13. **Mean daily discharge (cfs) from the USGS gauge (#08169000) on the Comal River at New Braunfels, Texas from January 1–October 19, 2014.**

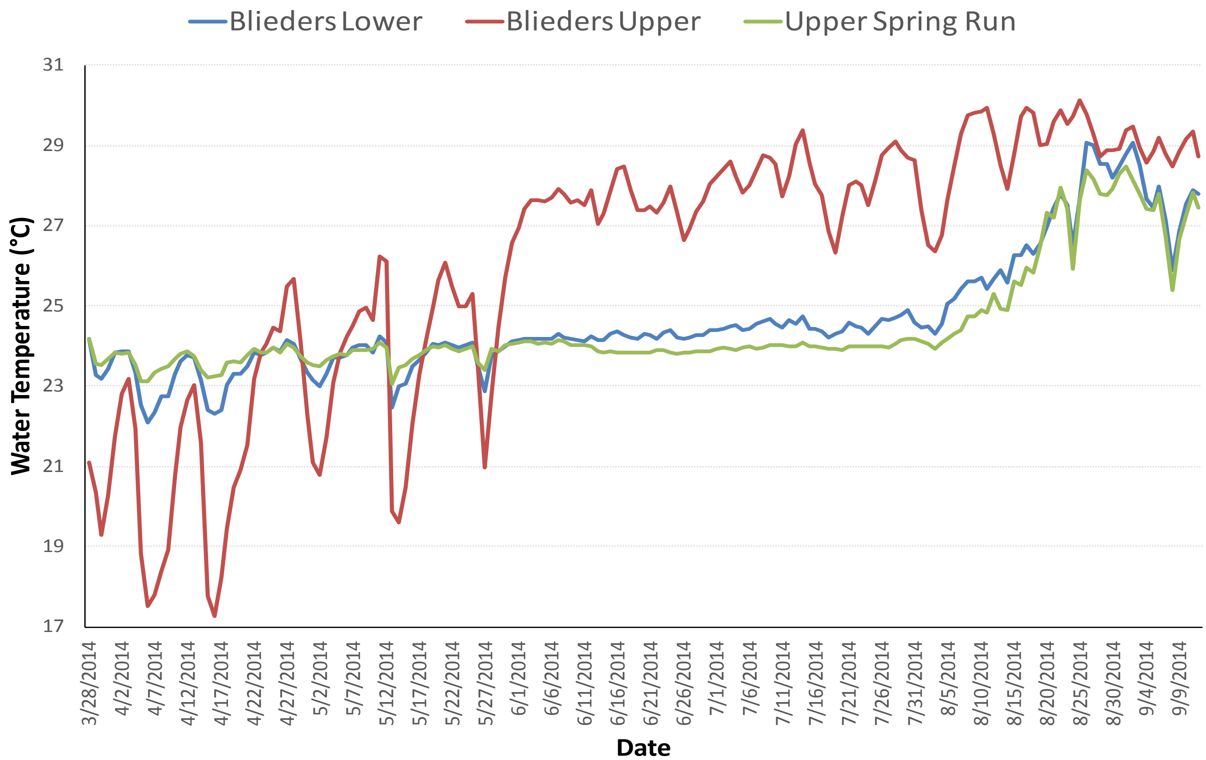

Figure 14. Daily average water temperature taken from three locations within the study area.

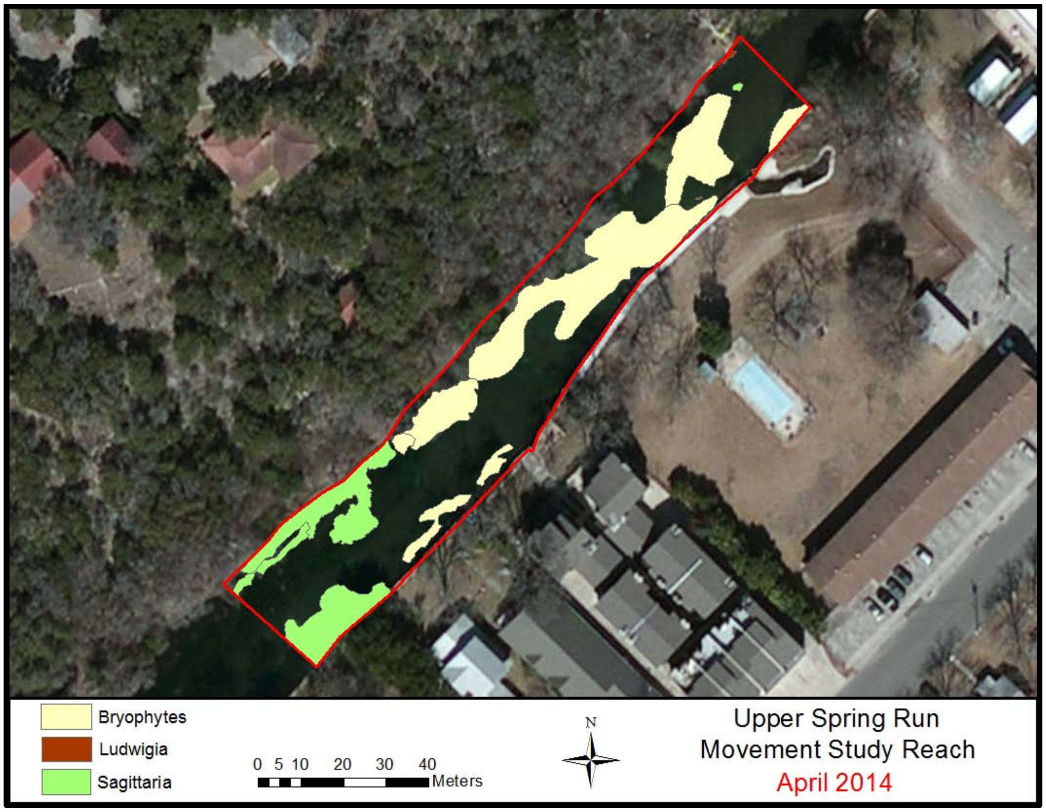

Figure 15. **Aquatic vegetation present in the Upper Spring Run reach during April 2014.**

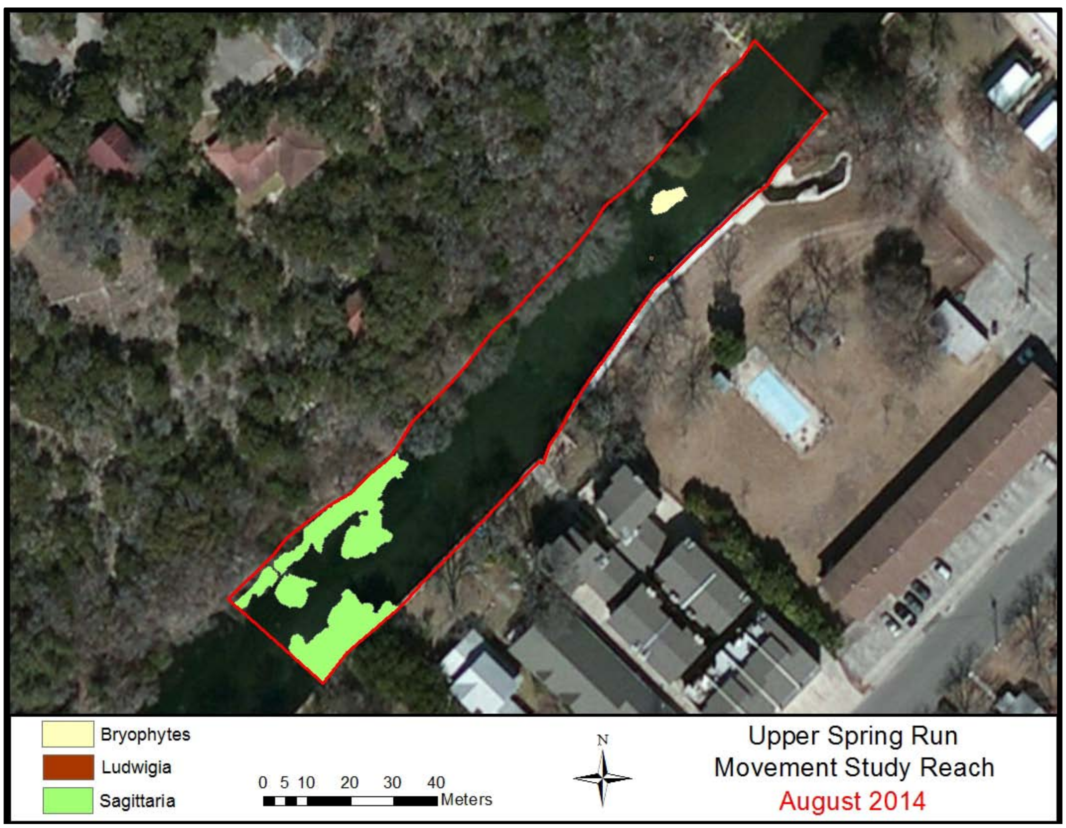

Figure 16. **Aquatic vegetation present in the Upper Spring Run reach during August 2014.**

Table 4. Date and details of fountain darter relocation events conducted from April through September 2014.

Date	Survey Area	Method	Number of Observers	Time Spent Searching (Hours)	Effort (Person-Hours)	Number of Relocations	Total Number Marked on Date of Survey	CPUE (Darters/person-hr)
4/18/2014	BC	Day Dipnet	4	1.00	4.00	2	386	0.50
4/18/2014	USR	Day Dipnet	4	3.00	12.00	2	386	0.17
4/24/2014	USR	Day Dipnet	4	1.25	5.00	4	757	0.80
5/16/2014	USR	Day Dipnet	4	4.50	18.00	8	757	0.44
5/30/2014	USR	Day Dipnet	5	5.00	25.00	4	757	0.16
6/23/2014	SI	Day Visual	4	2.00	8.00	0	1,348	0.00
6/24/2014	USR	Day Visual	4	2.50	10.00	4	1,348	0.40
6/26/2014	BC	Day Visual	3	2.00	6.00	0	1,348	0.00
7/1/2014	USR	Night Visual	3	3.25	9.75	18	1,348	1.85
7/9/2014	SI	Night Visual	3	2.75	8.25	0	1,348	0.00
7/10/2014	BC	Night Visual	3	2.50	7.50	1	1,348	0.13
7/23/2014	BC	Night Visual	3	2.25	6.75	0	1,348	0.00
7/24/2014	USR	Night Visual	3	2.25	6.75	17	1,348	2.52
7/29/2014	SI	Night Visual	4	1.50	6.00	0	1,348	0.00
7/30/2014	USR	Night Visual	3	1.75	5.25	9	1,348	1.71
8/12/2014	SI	Night Visual	4	1.50	6.00	0	2,212	0.00
8/13/2014	USR	Night Visual	4	1.75	7.00	42	2,212	6.00
8/26/2014	USR	Night Visual	4	2.00	8.00	17	2,212	2.13
8/28/2014	SI	Night Visual	4	1.75	7.00	0	2,212	0.00
9/9/2014	USR	Night Visual	4	1.50	6.00	8	2,212	1.33
9/10/2014	BC	Night Visual	2	1.50	3.00	0	2,212	0.00
9/10/2014	SI	Night Visual	3	1.75	5.25	0	2,212	0.00
Total				**49.25**	**180.50**	**136**	**2,212**	
Average CPUE								**0.82**

Also, it should be noted that this 6.7% estimate is slightly misleading since fountain darters were marked repeatedly through the course of the study, and many relocations took place with fewer total fountain darters marked. Regardless, low relocation rates are to be expected given the small size of the fountain darter (maximum length <2 inches) and its preference for complex benthic habitats.

Overall average CPUE was 0.8 fountain darters per person-hour (range: 0.0–6.0) and was highly dependent on the area surveyed, the total number of fountain darters marked, and the relocation technique used (Table 4). If only data from night visual surveys is analyzed since it was the most effective relocation technique, then CPUE within the Upper Spring Run survey area averaged 2.6 fountain darters/person-hour (range: 1.3–6.0). Data from night visual surveys show an average CPUE of 0.04 and 0.00 in the Blieders Creek and Spring Island survey areas, respectively. Although no fountain darters were marked in the Spring Island survey area, it was repeatedly surveyed to document any potential emigration from the Upper Spring Run Survey area as discharge declined. Two-hundred and seventeen fountain darters were marked within Blieders Creek (both sample areas combined). However, dense macrophyte beds within the creek made visual relocation of fountain darters difficult in this area, perhaps leading to the reduced CPUE.

4.1.3 Movement Patterns

In general, relocation data showed that fountain darters moved little from their initial area of capture (Figure 17). In fact, 84% of darter relocations were within the initial area of capture. The overall average distance moved was 20.9 m (median = 17.9 m) and ranged from a minimum of less than one meter to over 130 m (Table 5). This 130 m movement was a fountain darter that was tagged in BC1 during March or April and moved to the middle of USR2 by August 7. This exceeds the maximum movement found by Dammeyer et al. (2013) and, therefore, represents the longest movement ever recorded for a wild fountain darter. Additionally, one other fountain darter marked in Blieders Creek moved over 128 m. This red-marked darter was spotted in the upper portion of USR2 on April 22, 2014, during fish community sampling as part of the HCP biological monitoring. However, it was not determined whether this fish was tagged on the left or right side. Therefore, it was assumed this fish moved from BC1, which would be the closest site in Blieders Creek.

If the two long movements out of Blieders Creek are removed from analysis, then average distance moved at a given marking location varied between 14.0 and 21.0 m, with maximum movements ranging from 18.8 to 63.3 m. This represents slightly higher average movement than that reported by Dammeyer et al. (2013), who reported an average movement of 10 m in the Old Channel. Larger average movement in the Upper Spring Run during low-flow periods may represent fountain darters moving more in search of better physical habitat and/or feeding opportunities. Fountain darters in stable habitats within the Old Channel may have to move less to obtain the necessary resources. However, more movement within the Upper Spring Run may just be an artifact of a less confined study area.

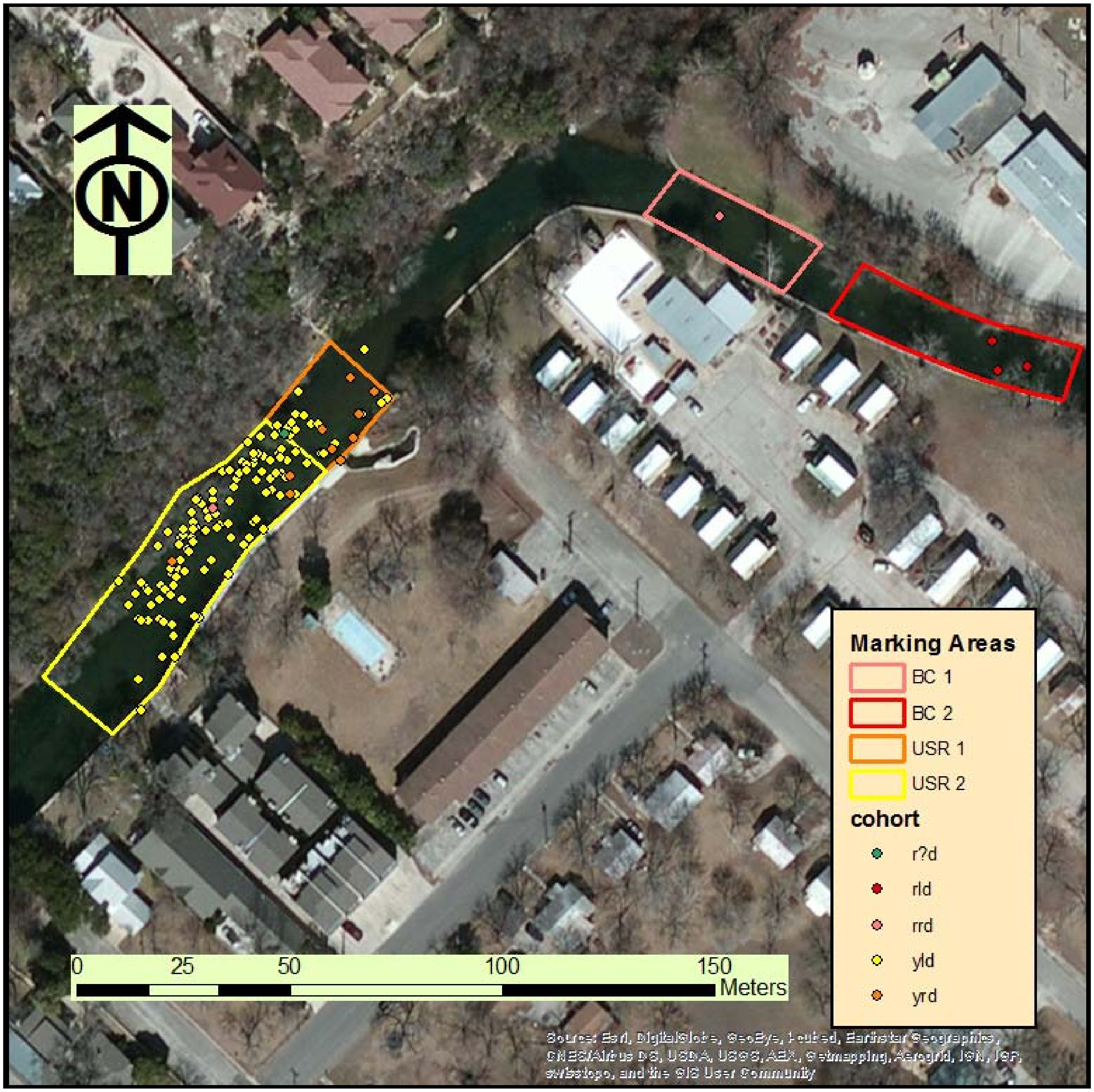

Figure 17. Fountain darters relocated over the course of the study period. Relocation points are represented by the same color as the area in which fountain darters were originally marked.

Table 5. **Number of relocations, number of emigrations, and summary statistics for distance moved for each marking area.**

Marking Area	Number of Relocations	Number of Emigrations	Distance Moved (meters) Avg	Max	Min
BC1	2	1	74.5	131.1	17.9
BC2	3	0	14.0	18.8	11.3
BC?	1	-		≥128.6	
USR1	10	3	21.0	63.3	3.7
USR2	133	20	19.4	52.8	0.5
Overall	149	24	20.9	131.1	0.5

Despite over 27 person-hours of effort, no movements were observed from USR2 downstream into the Spring Island survey area. During extreme low-flow conditions in late August, more-thermally-stable vegetated habitat still occurred downstream at Spring Island, yet no fountain darters were observed moving in that direction. This may have been a result of lower quality habitat in the upstream portion of the Spring Island survey area. During late August surveys, divers noticed cooler spring-fed water in the downstream portion of the Spring Island survey area, followed by warmer more-stagnant conditions in the upstream portion of the Spring Island survey area. This middle area of warmer water may have prevented fountain darters from traveling in a downstream direction toward Spring Island. Instead, fountain darters remained within the Upper Spring Run survey area.

No temporal patterns in movement or location were observed. With discharge declining in early August, and relocations declining, a large marking event was conducted to boost relocation numbers and assess if movement under such conditions was different than during better conditions in spring. However, despite the conditions observed, relocations were continually found near the area of capture.

4.1.4 Habitat Analysis

No clear patterns were evident between relocation data and the various habitat variables recorded. ANOVA results did not show any relationship (p=0.201) between habitat type (bryophyte, algae, leaf litter/detritus, open substrate or under substrate) and the relative percentage of marked fountain darters detected (detections/# marked x 100). Linear regression of discharge, weekly mean temperature and DO on relative percentage of marked fountain darters detected showed no significant relationships or interactions among variables (adjusted r-squared -0.044, F=0.8799 on 7 and 13 df, p=0.5476). Using distance from release point as a response variable rather than percent capture, linear regression was conducted against the following variables: study days (days since inception of study), weekly average temperature, DO, and USGS discharge values. This analysis also found no significant relationships (adjusted r-squared 0.0177, F=1.555 on 4 and 119 df, p=0.1908) to exist in the data.

4.1.5 Population Estimates

Although this study was not explicitly designed for such purpose, the pooled relocation data from USR1 and USR2 was used to generate an abundance estimate for the Upper Spring Run study reach. Estimates of abundance and accompanying confidence intervals were produced by both Schnabel and Schmumacher-Eschmeyer methods using methods implemented in the R package “fishmethods” in R version 3.0.3 (R Development Core Team, 2008). As both estimation methods make some assumptions that the population is “closed” to immigration and emigration during the period data were collected, estimates were made using only the last three sampling occasions (August 13 and 26, and September 9) to generate a data set where the assumptions are most likely met.

The Schnabel method estimated the fountain darter population of the Upper Spring Run to be 21,692 with a 95% confidence interval of 18,099 to 27,064 individuals. Estimates from the Shumacher-Eschmeyer method were 16,138 individuals with a 95% confidence interval of 9,083–72,269.

5.0 Summary and Conclusions

In summary, habitat conditions observed in the study area during summer 2014 provided a very favorable scenario to observe fountain darter movement under low-flow-induced unstable habitat conditions. Bryophytes, which provide high-quality fountain darter habitat based on drop net density estimates from biomonitoring data (BIO-WEST 2014a) and are common in the study area during normal flow conditions, completely deteriorated from the study reach by mid-summer. Initially, bryophytes were overtaken by filamentous algae, which had bloomed during the intense sunlight and low-flow conditions. Such algal blooms are common in the Upper Spring Run reach under low-flow summertime conditions. However, by late August, as flows continued to drop, even the filamentous algae had disappeared and the reach contained essentially no aquatic vegetation. Drop net density data from the biomonitoring program suggest that areas containing no vegetation harbor few fountain darters. Additionally, the fairly stable water temperature typical in this reach increased substantially beginning in early August. Water temperatures, which typically fluctuate between 23 and 26°C in a normal year, peaked at over 30°C and remained above 26°C for over two straight weeks. Despite these conditions, relocation data showed that fountain darters remained in the Upper Spring Run area. No fountain darters were observed moving downstream toward vegetated spring-influenced habitats near Spring Island. Instead, fountain darters seemed to congregate around the few areas in the study reach that still had some groundwater influence. Although total discharge in this area was approaching zero, divers conducting visual surveys noted that some springflow was still trickling from a few areas along the river bottom. Noticeable stratification had developed in many areas with cooler spring-water along the river bottom and much warmer water in the upper two-thirds of the water column. These were the areas in which most relocations occurred during late August and early September when discharge was the lowest.

Although fountain darters marked in the Upper Spring Run were not observed to make large movements, two longer movements were observed from fountain darters that were marked in Blieders Creek. Blieders Creek is not spring-fed, and temperatures in the creek fluctuate much more than in the Upper Spring Run. Fountain darters had been previously documented in the lower and middle portion of the creek on occasion, although use of the area was considered to be seasonal since water temperatures often exceed 30°C in the middle portion of the creek during summer months. Water temperature data collected as part of this study showed that temperatures in the upper portion of the creek were consistently above 27°C in early June. However, temperatures in the lower portion of the creek mirrored those in the Upper Spring Run and remained below 26°C until early August. Although only 6 relocations were made based on 217 fountain darters marked in Blieders Creek, two of those relocations represented fountain darters making large movements (> 125 m) from the creek downstream into the Upper Spring Run. These two movements represent the longest documented movements of wild fountain darters and document seasonal use of Blieders Creek.

When combined, relocation data from the Upper Spring Run and Blieders Creek show that even under extreme low-flow conditions (< 1 cfs), fountain darters are rather sedentary, moving on average 20.9 m (median = 17.9 m) from their release location. At maximum, fountain darters moved up to 131 m, although movements of this magnitude were rare (≈1% of all movements). This has direct implications to ecological model development. Currently, a decline in habitat within the ecological model results in a concomitant decline in fountain darter populations occupying that habitat, with no movement factor currently incorporated. This study suggests that movement/emigration of fountain darters from the area is not likely to counteract this projected population decline, even under extreme low-flow conditions, and particularly if additional habitat is more than approximately 20 m away.

In addition to providing input to the ecological model on movement potential under low-flow scenarios, this study also provided data on fountain darter population size within the study reach. Population size estimations calculated from mark recapture data from two different techniques ranged from approximately 16,000 to approximately 22,000 individuals within the Upper Spring Run study area. These estimates and associated error seem reasonable based on previous experience and HCP biological monitoring abundance estimates. In particular, population estimate data may be useful in HCP ecological model calibration or validation within this reach. Similar studies may be useful in the future for determining population size and model evaluation within other reaches.

Finally, this study provided interesting insight into fountain darter habitat selection and potential population dynamics under extreme low-flow, no-vegetation conditions. When aquatic vegetation disappeared from the Upper Spring Run study area in July and early August and water temperatures increased, fountain darters did not move out of the area looking for more suitable habitat. Instead, they were often observed hiding under gravel and cobble substrate in areas where springflow maintained adequate interstitial spaces for concealment. They were also observed using open silt substrate at times. These changes in habitat utilization could result in decreased prey availability and increased susceptibility to predation. In this case, an eventual decline in fountain darters would be anticipated should these conditions persist. An alternative interpretation is that the lack of movement from the study area may suggest that habitat within the area was still adequate for maintaining the fountain darter population. It will be important to closely examine the HCP biological monitoring program data at the conclusion of this year's sampling to evaluate if a concurrent decline in fountain darter abundance occurred in the Upper Spring Run in late summer 2014.

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