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Reproductive Biology of Syntopic Blackside Dace and Southern Redbelly Dace in Two Kentucky Streams

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DEDICATION

This is dedicated to my mother who has always encouraged me to pursue my interests. She bought me my first aquarium when I was a child and I have been fascinated with fish ever since.

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ABSTRACT

When a rare endemic species and an ecologically similar, but more cosmopolitan species share habitat, understanding how they interact with each other is important for conservation efforts. This is especially true if both species rely on the same spawning habitat, which could potentially lead to competitive displacement or hybridization. It is known that the federally threatened Blackside Dace and common Southern Redbelly Dace are ecologically similar species, occur syntopically in Kentucky in the upper Cumberland River basin, and utilize some of the same host species for nest sites; however, it is unclear if the two fish species are spawning site competitors. This study examined reproductive interactions between these two species in the wild by 1) comparing spawning activities of both species in a stream where they are syntopic to those of Blackside Dace in a stream without Southern Redbelly Dace (i.e., spawning mode, habitat, co-occurrence in spawning aggregation), 2) discerning if spawning seasons of the two species differ temporally (based on stream temperature), and identifying differences in spawning and non-spawning microhabitats where the two species do and do not co-occur, 3) developing an ethogram of reproductive behaviors of the focal species and quantifying behaviors relevant to the question of reproductive competition between the two dace species from video recordings. All 8 spawning events observed in the Blackside Dace only stream and all 11 spawning events observed in the syntopic stream were in association with Creek Chub nests in stream habitats characterized as runs. Where the two species were syntopic, all spawning observations contained both species. The first and last observations of spawning activity in the Blackside Dace only stream occurred on earlier dates, and at lower temperatures (April 26th at 12.1 °C, and May 7th at 15.3°C respectively), than the corresponding dates and temperatures where the two species were syntopic (May 8th at 15.2°C, and June 18th at 18.9°C respectively). Spawning seasons for both species completely overlapped in the syntopic stream. However, the maximum number of individuals observed in spawning aggregations peaked at an earlier date for Blackside Dace (May 8th) compared to Southern Redbelly Dace (June 12th and June 14th) in the syntopic stream. The Blackside

Dace only stream had significantly lower mean values for conductivity, water velocity, stream width, and canopy cover compared to spawning sites in the syntopic stream. Spawning sites in both streams had an absence of silt and lack of substrate embeddedness compared to non-spawning sites. Canopy cover was significantly greater at spawning sites in the syntopic stream compared to non-spawning sites. All behaviors from the ethogram occurred between and within species. Male-male (agonistic) behaviors and male-female (courtship) behaviors generally occurred at greater rates within species than between. Males of both species formed spawning clasps with females of both species, but successful spawning clasps usually occurred at higher rates with female Southern Redbelly Dace. Competition occurred between male Blackside Dace and Southern Redbelly Dace for spawning opportunities with females of both species, but there were no indications that these species compete for spawning sites in a way that displaced one species from nests. The results demonstrates how behavioral observations in conjunction with habitat analysis can inform management and conservation of imperiled species by revealing that when syntopic both of these species rely on the same spawning habitat, spawn in the same nest at the same time, and spawn with each other across species.

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CHAPTER 1

INTRODUCTION

The Blackside Dace (*Chrosomus cumberlandensis*) is a federally threatened fish species that co-occurs with a more common species, the Southern Redbelly Dace (*Chrosomus erythrogaster*), in some tributaries of the Cumberland River. The Blackside Dace has a range limited to drainages of the upper Cumberland Plateau in southeastern Kentucky and northeastern Tennessee (Starnes and Starnes 1981). Streams inhabited by the Blackside Dace are characterized as high gradient with riparian zones of hemlock and *Rhododendron* spp. (Starnes and Starnes 1981). Inhabited streams usually have a riffle to pool ratio of 60:40 or below, with little to no silt (Starnes and Starnes 1981). The diet of the Blackside Dace consists mostly of algae and occasionally very small crustaceans. It is thought that the dace once had a more extensive range, but habitat fragmentation due to anthropogenic disturbances led to extirpation of the species in much of its former distribution (Starnes and Starnes 1981).

The Southern Redbelly Dace has an extensive range, occurring in Lake Michigan and Lake Erie to the upland areas of the Mississippi River basin (Etnier and Starnes 1993). Settles and Hoyt (1976) studied the reproductive ecology of *C. erythrogaster* in a spring fed tributary of the Green River, Warren Co., Kentucky. The stream was characterized as having an average gradient of 4.73 m/km, a width of 0.9-3.7 m with undercut banks, and long pools 0.3-0.6 m deep separated by frequent riffles.

Southern Redbelly Dace are probably more recent (in geologic time) inhabitants of the upper Cumberland River drainage compared to Blackside Dace (Starnes and Starnes 1978). Early accounts report few cases of Blackside Dace and Southern Redbelly Dace occurring syntopically (Starnes and Starnes 1981). Analysis by Mattingly and Black (2013) of more recent distributional data suggests an increase in co-occurrence of the two species in the upper Cumberland River drainage. In records from 1982-1994, both species co-occurred in 43.3% of all identified Southern Redbelly Dace collections and

27.4% of all Blackside Dace collections; whereas in 2003-2005, both species co-occurred in 90.3% of Southern Redbelly Dace collections and 35.9% of Blackside Dace collections.

Reproductive Tactics

Blackside Dace spawn April to July (Starnes and Starnes 1981). Members of the genus *Chrosomus* are broadcast spawners (Starnes and Starnes 1981), i.e., they spawn by releasing their gametes upon the substrate and do not practice parental care (Johnston and Page 1992). Blackside Dace and relatives (e.g., Southern Redbelly Dace) are also nest associates i.e., fish that spawn in the nest of other species (Mattingly and Black, 2013). Creek Chubs (*Semotilus atromaculatus*) and Central Stonerollers (*Campostoma anomalum)* are nest-building species that are known to provide spawning sites for Blackside Dace (Cicerello and Laudermilk 1996; Mattingly and Black 2013). Blackside Dace may be able to spawn independent of other species (Starnes and Starnes 1981; Cicerello and Laudermilk 1996; Mattingly and Black 2013); but thus far no observations of independent spawning (in the wild) have been reported. Starnes and Starnes (1981) first described the breeding behavior of Blackside Dace in the nest of a Stoneroller. Since then, all observations of Blackside Dace spawning activities have been in the nests of Creek Chubs (Cicerello and Laudermilk, 1996; Mattingly and Black 2013); except for captive breeding efforts (Rakes et al. 1999, Rakes et al. 2013). Species that serve as nest-hosts can be considered keystone species and should be taken into account when writing recovery plans for threatened species like the Blackside Dace (Johnston 1999).

In the wild, Blackside Dace are thought to rely almost entirely on nest-building species for spawning sites (Cicerello and Laudermilk 1996). Mattingly and Black (2013) analyzed patterns of Blackside Dace co-occurrence with nest building species, and found that Creek Chubs were more likely than Stonerollers to be a reliable nest-host in the distributional range of the Blackside Dace. Creek Chubs are often found in a wide range of freshwater habitats excluding lakes and large rivers (Etnier and Starnes, 1993). More commonly, Creek Chubs inhabit the slow-flowing channel and pool areas (instead of riffles) in small headwater, and sometimes-intermittent streams [Bart (1989) *cited in* Ross (2002)].

Blackside Dace spawning sites are characterized by gravel substrate lacking silt (Starnes and Starnes, 1981; Mattingly and Black 2013) and embedded substrate (Mattingly and Black 2013). Starnes and Starnes (1981) reported that although males frequently chased and nipped (i.e., courted) females outside of the nest, females resisted spawning in those areas with silt. Rakes et al. (1999) proposed that the presence of sediment could asphyxiate Blackside Dace eggs, embryos, and larvae during the days of development before the offspring emerge from gravel crevices. Nest-associates are thought to benefit from the behavior of nest-building species that remove sediment from spawning sites (Starnes and Starnes, 1981; Mattingly and Black 2013). Anthropogenic disturbances including deforestation and mining may be responsible for sedimentation of Blackside Dace inhabited streams; and can leave the nest of host-species as the only available silt free substrate (Starnes and Starnes 1981; Mattingly and Black 2013).

Mattingly and Black (2013) observed at sites associated with and without logging disturbance that all Blackside Dace spawning activity was in association with Creek Chub nests. Twenty of the 25 spawning events observed took place in sites designated as having no known logging disturbance, two events took place in sites with slight logging disturbance, and two events took place in sites designated as having moderate logging disturbance. The researchers suggested that the non-embedded silt-free gravel-nests of Creek Chubs allowed the Blackside Dace to persist in areas of logging disturbance where the unmodified substrate was otherwise inadequate for spawning. Although Mattingly and Black (2013) did not observe independent spawning or alternative nest hosts (other than Creek Chubs) for Blackside Dace; they did not think such possibilities should be ignored.

Several species of North American cyprinids are known to be plastic in their reproductive behavior. Some nest builders are capable of more primitive spawning modes such as broadcasting; while other nest builders occasionally use the nest of other species for spawning (Johnston 1999). The Southern Redbelly Dace has been observed breeding in nest associate fashion [over the nest of Creek Chubs, Stonerollers, and Common or Striped Shiners (*Luxilus spp.)]* as well as independently over gravel riffles (Etnier and Starnes 1993).

Information in the literature about the reproductive habitat of the Southern Redbelly Dace in Kentucky is deficient. April to July is the reported spawning season for *C. erythrogaster* in western Kentucky (Settles and Hoyt 1976). The information available suggests that the Southern Redbelly Dace may have more flexibility with reproductive habitat parameters than the Blackside Dace. For example, Settles and Hoyt (1976) reported what they considered reproductive activity from wild Southern Redbelly Dace when water temperature was 21 C°. All reports of wild Blackside Dace spawning events occurred at temperatures below 20 C° (Starnes and Starnes, 1981; Mattingly and Black 2013) with mean values between 14.0 and 17.9C° (Mattingly and Black 2013). Rakes et al. (2013) found that captive Blackside Dace failed to spawn when temperatures exceeded 21 C°.

Additionally, there have been no published observations indicating if Southern Redbelly Dace spawn with other species or independently in the upper Cumberland River basin in Kentucky. In western Kentucky, Settles and Hoyt (1976) attributed a nest, above which they observed possible Southern Redbelly Dace spawning activity, to a Stoneroller. It is of value to know where Southern Redbelly Dace spawn relative to Blackside Dace in areas where they co-occur. Blackside Dace and Southern Redbelly Dace are ecologically similar species (Starnes and Starnes 1978), occur syntopically in Kentucky in the upper Cumberland River basin (Starnes and Starnes 1981; Cicerello and Laudermilk 1996; Mattingly and Black 2013), and utilize some of the same host species (Etnier and Starnes 1993); however, it is unclear if the two fish species are spawning site competitors.

When a rare endemic species and an ecologically similar, but more cosmopolitan species share habitat, understanding how they interact with each other is important for conservation efforts. This is especially true if both species rely on the same spawning habitat. The work outlined herein provides results from the first study focused on whether or not competition exists between Blackside Dace and Southern Redbelly Dace for spawning sites. Co-occurrence of these related species could potentially lead to displacement or hybridization. Therefore, an examination of interactions between these two species during reproductive activity is essential for the management and conservation of the Blackside Dace. The objectives of this study were to 1) compare spawning activities of both focal species in a stream where they are syntopic to spawning activities of Blackside Dace in a stream without Southern Redbelly Dace (i.e., spawning mode, habitat, co-occurrence in spawning aggregation), 2) determine if spawning seasons of the two species differ temporally (based on stream temperature), and identify differences in spawning and non-spawning microhabitats where the two species do and do not co-occur, 3) quantify behaviors relevant to the question of reproductive competition between the focal species, and qualitatively note the identities and behaviors of other fish species present at spawning sites.

CHAPTER 2

STUDY AREAS

Study areas were the portions of Barren Fork and Mill Creek used as spawning habitat by the focal species. Mill Creek and Barren Fork are streams located in northeastern and eastern McCreary County respectively. Both streams are within the Daniel Boone National Forest, and the Southwestern Appalachians (Level III) and Plateau Escarpment (Level IV) Ecoregions of Kentucky (Woods et al. 2002). The geography of this region is characterized in upland areas by Pennsylvanian sandstone and coal and in more lowland areas by Mississippian carbonates (Woods et al. 2002). Both streams are within the Upper Cumberland River Basin.

Mill Creek is a direct tributary of the Cumberland River with its confluence northeast of its headwaters and below Cumberland Falls. Mill Creek is a 1st order stream, along the study area, and flows through Daniel Boone National Forest as well as privately owned lands. In addition to a number of small unnamed intermittent tributaries that flow into Mill Creek, Jim Blue Fork confluences with Mill Creek above the study area, and Sulfur Creek confluences with Mill Creek well below the study area. The area surrounding Mill Creek is mostly forested with a few undeveloped trails and unforested residential land beyond the immediate riparian zone. The riparian contains areas of open and closed canopy cover provided by trees including Eastern Hemlock, Maple species, American Holly and Rhododendron. There are isolated pockets of deep fine substrate within the stream where tree cover is more open and the vegetation along the stream bank consists of large amounts of grasses and weedy species. Below the study area is a long stretch of stream dominated by bedrock substrate. In the study area, there are large woody debris and the stream substrate consists primarily of boulders, cobble, pebble and gravel (excluding the aforementioned pockets of fine substrate). Evidence of recreational activity include a temporary stack of rocks across the stream, along with a fire pit and small amounts of litter in the surrounding forest.

Barren Fork is a 3rd order stream that flows northeast to its confluence with Indian Creek. Indian Creek flows directly into the Cumberland River above the falls. A number of tributaries flow into Barren Fork including Railroad Fork, above the study area, and Pigeon Roost Branch, below the study area. The area surrounding Barren Fork contains forested hiking and horse trails, as well as unimproved 4-wheel drive and gravel roads. The riparian includes Magnolia, Oak, and Maple species, in addition to Eastern Hemlock and Rhododendron. The tree canopy is primarily closed in the upstream portion of the study area and more open in the downstream portion. The stream contains ample large woody debris and undercut banks. The substrate in the study area consists of cobble, pebble, and gravel that in many locations is overwhelmingly dominated by sand and often covered in silt and leaf litter. For a detailed description of Barren Fork see Roghair et al. (2001).

CHAPTER 3

MATERIALS AND METHODS

Spawning Activity

Study sites were surveyed weekly from April through late June 2014 for signs of spawning activity. A two person field crew slowly walked the length of each study site and noted any spawning activity (aggregations of nuptial males) by Blackside Dace or Southern Redbelly Dace. When spawning activity was detected, it was video recorded for 20 min. Spawning activities were video recorded using a Hero3 video camera in a submersible Blackout Housing (GoPro, Inc., San Mateo, CA). The camera was mounted onto a 4x4" ceramic tile that resembled the color of the stream substrate. From the stream edge, a telescoping pole retrieval tool was used to place and retrieve the camera near the spawning site.

The spawning mode utilized by each species was determined by the presence or absence of a host nest below the spawning aggregation. The spawning mode was recorded as "independent spawning" if spawning occurred outside of the nest of a host. If spawning occurred within the nest of a host it was recorded as "nest association" and the host species was visually identified. Alternatively, if the host fish was not present, the host species was determined by the physical characteristics of the nest, with ridge-mound nests assigned to Creek Chubs.

The type of spawning habitat used by each spawning aggregation was determined in the stream with and the stream without Southern Redbelly Dace. Type of spawning habitat was defined by the habitat type (riffle, run or pool) occupied by the spawning aggregation. Habitat type was recorded as a riffle if it had shallow water that was flowing while lacking a smooth surface. Runs were classified as having a detectable flow and smooth surface water. Pools were identified as those locations lacking flow but having smooth surface water.

Whether or not both focal species were present in the same spawning aggregation was determined visually for each observation in the field, recorded on data sheets, and verified using video recordings.

Temporal Separation of Spawning Seasons and Physiochemical Measurements at Spawning and Non-spawning Microhabitats

Water temperature was recorded throughout the spawning season using Hobo Pendant Temp/Light Data Logger 8K (Onset Computer Corporation, Bourne, MA). Data loggers were placed before spawning commenced and set to record temperature in degrees Celsius every fifteen minutes. Data loggers were located at the lowest and highest points in the streams where the study species were observed during visual surveys. Dates of spawning events were recorded in the field during observations. Overlap of dates when each species was present in aggregations over spawning sites were compared. Water temperatures during spawning events were compared using visual interpretations of graphs to see if Blackside Dace stop spawning as water temperatures approach 21 C°, while Southern Redbelly Dace continue to spawn until some other threshold above 21 C° is reached.

After video recording spawning activity, microhabitat conditions for spawning sites were assessed and recorded on field data sheets. Quantitative variables measured at spawning microhabitats included conductivity (µmhos), temperature (◦C), wetted channel width (m), water depth (cm) and water velocity (cm/s). Temperature and conductivity were measured using a Multi-Parameter PCTestr 35 (OAKTON Instruments, Vernon Hills, Il). Wetted channel width was measured using a meter tape, and water depth was measured using a meter stick. Water velocity was measured using the float method and a velocity adjustment coefficient of 0.85 (Bain 1999). Both flow and depth were measured immediately above and below the spawning sites and averaged (this was to avoid disturbing the spawning site).

Estimations of substrate characteristics were made visually using 10-cm radius microhabitats in the center of each spawning site similar to Mattingly and Black (2013).

Substrate variables assessed at each microhabitat included dominant and sub-dominant substrate (fines, gravel, pebble, cobble, boulder, bedrock) using a modified Wentworth classification (Bain 1999), and level of embeddedness, which is the percentage of substrate particles as large or larger than gravel (≥2mm) that are covered in fines, i.e., silt and sand (Bain 1999). Attributes of the riparian zone recorded included percent canopy cover using a GRS densitometer (Geographical Resource Solutions, Arcata, CA) and identification of genera or species of trees providing cover. Similar to Mattingly and Black (2013), percent canopy cover was placed into one of five ordinal categories: 0 = 0%, 1 = 1—25%, 2 = 26—50%, 3 = 51—75%, 4 = 76—100%.

Like spawning microhabitats, non-spawning microhabitats were locations 10-cm in radius, where substrate and canopy cover data consisted of visual estimates and identifications using the same methods described above. Unlike spawning microhabitats, water depth and velocity were measured at non-spawning microhabitats rather than above and below. Microhabitat variables were assessed at 2 non-spawning locations: 1 upstream and 1 downstream from each spawning site. Each non-spawning microhabitat was located 1m away from the spawning site in a randomly selected direction. The first non-spawning site was determined while facing upstream. A randomly generated number was used to select a direction between 1—180 degrees from the nest. The direction directly upstream from the nest represented 90 degrees, the direction immediately to the left represented 0 degrees, and the direction immediately to the right represented 180 degrees. The second non-spawning site was selected using the same method, but the 90 degree direction was instead directly downstream.

Reproductive Competition.

A published ethogram was not found for any *Chrosomus* species, so an ethogram was developed through a literature review and ad libitum observations (Martin and Bateson 2007). From the relevant literature, descriptions of behaviors associated with spawning were compiled for Blackside Dace, Southern Redbelly Dace, other members of the genus *Chrosomus*, and a selection of broadcast spawning North American members of the family Cyprinidae. Ad libitum observations were made to identify behaviors (from the compiled list of spawning behaviors of related species) present in the Blackside Dace or Southern Redbelly Dace. Development of the ethogram was as follows: 1) Blackside Dace and Southern Redbelly Dace behaviors mentioned in the reviewed literature were added to the ethogram, 2) behaviors described in the literature for other *Chrosomus* species as well as those from a selection of additional broadcast spawning North American cyprinids, that were also observed during ad libitum observations of the focal species were added to the ethogram, 3) behaviors observed during ad libitum observations that were not identified in the previous steps were given descriptions and added to the ethogram. In the event that a behavioral description from the literature of the focal species did not agree with those observed during ad libitum observations, a more accurate description and/or name for the behavior was selected from the additional literature sources used in step 2, or given a new name and description if no accurate description was found in the reviewed literature. After the ethogram was developed, behaviors most relevant to the question of reproductive competition were identified based on the potential of the behavior to lead to promotion or prevention of successful spawning, i.e. courtship and agonistic behaviors.

Behaviors in the ethogram were scored from video recordings. Video recordings were also used to verify field observations including the identity and number of individuals of the focal species, identity of the host species, and identities of other fish species present at the spawning site. In addition, videos were used to determine if other species (non-focal species) were spawning in the nest, and to note any additional observations of interest.

The sampling rule used was behavioral sampling (Martin and Bateson 2007) also known as sampling all occurrences of some behaviors (Altman 1974). In addition, scan sampling at regular intervals (20 seconds) was used to determine the average number of male and female Blackside Dace and Southern Redbelly Dace in each aggregation (Martin and Bateson 2007). The recording rule used was continuous recording (Martin and Bateson 2007). Sometimes fish left the spawning site as the camera was set in position. Therefore, the scoring of videos began when focal species first occupied the spawning site and continued for 15min. The sample space encompassed interactions (i.e. selected behaviors identified from the ethogram as most relevant to the question of reproductive competition) among and between species and sexes over the spawning site.

The subjects in this study were groups (i.e. spawning aggregations) and not individual fish. Therefore, the sample size (n) for this study equaled the number of spawning aggregations. Of the 8 spawning aggregations recorded in Mill Creek, the video quality of 6 were deemed acceptable for analysis. Of the 11 spawning aggregations in Barren Fork, behaviors were quantified for 10, because the number of individuals in the aggregation of 1 video were too numerous to accurately score behaviors. The excluded videos included 2 spawning events on May 7th for Mill Creek, and 1 on June 12th for Barren Fork.

Behaviors were scored using the event logging software, The Observer XT (Noldus Information Technology Inc., Leesburg, VA). Each time a behavior listed in the ethogram was observed, it was logged as a new event. Data taken when an event occurred included the behavior itself (e.g. nudge), the species and sex that initiated (i.e. the sender), and the species and sex of the receiver. The number of individuals in a spawning aggregation varied from observation to observation, so behavioral rates were calculated as an average per individual of the sender's sex and species to make values comparable across aggregations. I.e., Interactions between individual fish (e.g. male-to-male nudges), were summed by the sender's species and sex then divided by the total number of individuals of the sender's sex and species present in the aggregation to give a single value of the behavior for the observation (e.g. average number of male-to-male nudges by Southern Redbelly Dace toward Blackside Dace).

When a particular sex of a species was absent from the spawning site, this was recorded as time-out for that sex-species combination. Time contingent measurements, such as rate, were calculated using time-in (i.e. time in which the relevant actors were present in the spawning site). Time-out was noted for each species and sex, and was not used in behavior rate calculations. Behavioral rates (per minute) were calculated from time-in for the actors (e.g. male Blackside Dace & male Southern Redbelly Dace interactions were calculated from the time when both species were present in the spawning site simultaneously for ≥ 60 ± 5 seconds).

Statistical Analyses

Two sample t-tests were used to determine if weather patterns during the study were similar to historic climate data. Monthly mean temperatures and precipitation during January-June for 2014 (year of the study) were compared to the same months in 2011-2013. Also mean monthly temperature from April-June (the spawning season) 2014 were compared to the same months in 2011-2013.

Microhabitat comparisons between Blackside Dace spawning sites, Southern Redbelly Dace spawning sites, and sites where both species spawn were compared using two samples t-tests. Mean values for microhabitat values at spawning sites were compared to those of associated non-spawning sites within both streams using dependent samples t-tests.

Descriptive statistic were used to summarize quantitative behavioral observations. The quantitative response variables were rates of behaviors. The categorical explanatory variables were species and sex. Comparisons were made within and between streams. Within-observer reliability was determined with a Pearson correlation coefficient. Three videos of spawning aggregations from Mill Creek and three from Barren Fork were randomly selected for analysis for a total of six videos. The observer recorded the rate of all eight behaviors (from the ethogram) performed by male dace during 1.5-minute observations. This was repeated twice for each of the six videos, once during the regular collection of data and then again at the end of the study. In two videos (one from each stream) few behaviors occurred or did not occur at all. These two observations were excluded from calculations of the Pearson correlation coefficients. Rates were represented as occurrences per minute of the behavior category for all males in the aggregation.

CHAPTER 4

RESULTS

Spawning Activity

All Blackside Dace and Southern Redbelly Dace spawning activities were in nest association, within the nests of Creek Chubs, for the 8 observations in Mill Creek and the 11 observations in Barren Fork. There were no observations of either species spawning independent of Creek Chub nests. Blackside Dace utilized the same spawning habitat in absence (i.e., Mill Creek) and presence (i.e., Barren Fork) of Southern Redbelly Dace. All spawning sites were in runs. Neither species was observed spawning in pools or riffles. Both species were present in every spawning aggregation observed in Barren Fork (see next section for number of individuals in spawning aggregations).

Temporal Separation of Spawning Seasons

The first and last observations of spawning activity by Blackside Dace in Mill Creek occurred on earlier dates, and at lower temperatures, than the respective dates and temperatures for Blackside Dace or Southern Redbelly Dace in Barren Fork. Spawning activity in Mill Creek was first detected on April 26th when mean water temperature was 12.1 °C (max = 16.8, min = 9.2; Figure 1)[1], and last detected on May 7th when mean water temperature was 15.3°C (max = 19.9, min = 12.8). Spawning activity in Barren Fork was first detected on May 8th when mean water temperature was 15.2°C (max = 17.3, min = 13), and last detected on June 18th when mean water temperature was 18.9°C (max = 20.7, min = 17.6).

There was no detection of a point in time in which one species began or completely ceased spawning before the other in Barren Fork. However, the maximum number of individuals observed in spawning aggregations peaked earlier for Blackside Dace compared to Southern Redbelly Dace (Figure 2).

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[1] Tables are located in Appendix A and Figures are located in Appendix B.

There was not a significant difference between monthly mean temperatures during January-June for 2014 compared to the same months in 2011-2013 ($t = 0.402$, $df = 22$, $p = 0.692$), nor for April-June (the spawning season) 2014 compared to the same months in 2011-2013 ($t = 0.166$, $df = 10$, $p = 0.871$). Likewise, there was not a significant difference between monthly mean precipitation during January-June for 2014 compared to the same months in 2011-2013 ($t = 1.219$, $df = 22$, $p = 0.236$), nor for April-June (the spawning season) 2014 compared to the same months in 2011-2013 ($t = 0.718$, $df = 10$, $p = 0.489$).

Physiochemical Measurements at Spawning and Non-spawning Microhabitats

The mean values for conductivity, water velocity, stream width, and canopy cover were all significantly lower for spawning sites with only Blackside Dace (i.e. Mill Creek) than for spawning sites with both Blackside Dace and Southern Redbelly Dace (i.e. Barren Fork) ($t \geq 2.210$, $df = 17$, $p \leq 0.041$, Table 1). Mill Creek spawning site canopy cover values ranged from 0-100%. Barren Fork spawning site canopy cover values were between 74-100%. There were no Southern Redbelly Dace only spawning sites in Barren Fork. Canopy cover over spawning sites was provided by a total of 5 tree taxa with one unique to Mill Creek spawning sites and another to Barren Fork spawning sites (Table 2).

In Mill Creek, dominant substrate size was smaller at spawning habitats than at downstream non-spawning sites ($t = 2.966$, $df = 7$, $p = 0.021$, Table 3). Barren Fork had significantly larger dominant substrate size at spawning sites ($t \geq 3.684$, $df = 10$, $p \leq 0.004$, Table 4). Substrates at spawning microhabitats were significantly less embedded in Mill Creek ($t \geq 7.937$, $df = 7$, $p < 0.001$, Table 3) and Barren Fork ($t \geq 6.421$, $df = 10$, $p < 0.001$, Table 4). Canopy cover at downstream non-spawning sites was significantly different from spawning sites in Barren Fork ($t = 2.324$, $df = 10$, $p = 0.042$, Table 4).

Comparison of Reproductive Behaviors

All behaviors from the ethogram occurred between and among species (Table 5). Male-male (agonistic) behaviors included nudges and pushes, as well as blocks of males from females. Male-female (courtship) behaviors included nudges, pushes, vertical alignments, and lateral displays, in addition to attempted spawning clasps and actual formation of spawning clasps with females. For most behaviors (seven out of eight) the within-observer reliability or Pearson correlation coefficient (*r*) was ≥ +0.96 (n=4; df = 2; Table 6), and for all behaviors (*r*) was ≥ +0.89 (n=4; df = 2; Table 6). Two additional fish taxa other than *Chrosomus* were observed at spawning sites in Mill Creek compared to three in Barren Fork (Table 7). There were no definitive observations of spawning by these other species.

Male Behaviors Toward Males

Generally, male Blackside Dace nudged males of their own species at higher rates in Mill Creek than in Barren Fork, where in the majority of cases, they nudged males of both species at similar rates (Figure 3 and Figure 4). Male Blackside Dace in Mill Creek and male Southern Redbelly Dace in Barren Fork nudged males of their own species at rates ≥2.280 per male per minute in all observations. The greatest nudge rates by males toward males were observed between Southern Redbelly Dace in Barren Fork.

In the observation on May 8th, males of both species were present in the nest in similar numbers (Figure 3), but they nudged males of their own species at higher rates than they did males of the other species. Male Southern Redbelly Dace nudged each other at a rate of 2.280 nudges per male per minute, but nudged Blackside Dace at 0.769 nudges per male per minute. In the same observation, male Blackside Dace nudged males of their own species at 1.979 nudges per male per minute, but nudged Southern Redbelly Dace at 0.922 nudges per male per minute.

Male Blackside Dace in Mill Creek pushed each other at rates that were often higher than those observed between male Blackside Dace in Barren Fork (Figure 5 and Figure 6). A notable exception to this was a single observation of male Blackside Dace in Barren Fork with a push rate of 2.976 pushes per male per minute, a push rate greater than all other observations. Male Blackside Dace and male Southern Redbelly Dace pushed males of their own species at rates with comparable ranges, which included values higher than those observed between species.

Block rates by male Blackside Dace, of males from female Blackside Dace, were generally the greatest values across all scenarios, especially in Mill Creek (Figures 7, Figure 8). A notable exception to this, was a single observation of male Southern Redbelly Dace blocking other male Southern Redbelly Dace from female Blackside Dace at 2.951 blocks per male per minute, a greater rate than all other observations.

Male Southern Redbelly Dace blocked males of their own species from female Southern Redbelly Dace at ≥0.405 blocks per male per minute, and these rates were greater than all other block rates of males from female Southern Redbelly Dace (Figure 9, and Figure 10). Only one instance of a male Blackside Dace blocking another male Blackside Dace from a female Southern Redbelly Dace was observed.

Male Behaviors Toward Females

There was notable overlap in the ranges of nudge rates by males toward females, with the highest nudge rates by males of each species directed toward females of their own species (Figure 11 and Figure 12). Male Blackside Dace in Mill Creek and male Southern Redbelly Dace in Barren Fork nudged females of their own species at ≥1.290 nudges per male per minute in all observations. There was a single observation in which male Southern Redbelly Dace nudged female Blackside Dace at 6.224 nudges per male per minute, a rate higher than all nudge rates by Blackside Dace toward female Blackside Dace.

Male Blackside Dace push rates toward female Blackside Dace were generally greater in Mill Creek than in Barren Fork (Figure 13 and Figure 14). Push rates by males of both species were almost always greater towards females of their own species compared to females of the other species. An exception to this was a single observation in which male Southern Redbelly Dace pushed female Blackside Dace at 1.073 pushes per male per minute, a rate greater than all other push rates toward females.

Males generally pursued females of their own species more often than females of the other species (Figure 15 and Figure 16). An exception to this was a single observation in which male Southern Redbelly Dace pursued female Blackside Dace at 1.932 pursuits per male per minute, a greater rate than all others observed.

There was considerable overlap in the range of vertical alignment rates by male Blackside Dace toward female Blackside Dace in both streams, but rates were often greater in Barren Fork (Figure 17 and 18). All alignments within species were ≥0.227 vertical alignments per male per minute, and were generally higher than rates between species. A notable exception to this was a single observation in which male Southern Redbelly Dace aligned with female Blackside Dace at 1.073 vertical alignments per male per minute, a rate greater than all observations of vertical alignment rates toward females of their own species.

The range of values for lateral display rates by Blackside Dace males toward females of their own species in both streams overlapped, with more values ≥1.65 lateral displays per male per minute in Barren Fork than in Mill Creek. In both streams, lateral display rates by Blackside Dace within species were generally greater than other lateral displays rates, with the exception of outliers, which contributed to much of the overlap between the ranges of all lateral display rates by males toward females (Figure 19 and Figure 20).

Clasp attempts by male Blackside Dace were observed in all Mill Creek observations and only 3 of Barren Fork observations, but when the behavior did occur, clasp attempt rates were higher in Barren Fork (Figure 21 and Figure 22). Across both streams, clasp attempt rates in the majority cases were ≤0.059 per male per minute. Males of both species in Barren Fork attempted to clasp with females of both species. The highest clasp attempt rates by males in Barren Fork were most often within species rather than between. An exception to this was 0.644 clasp attempts per male per minute by Southern Redbelly Dace toward female Blackside Dace, a rate greater than all other clasp attempt rates.

In Barren Fork, 1 spawning clasp between Blackside Dace was observed compared to a cumulative total of 6 in Mill Creek. Although more spawning clasps between Blackside Dace occurred in more observations from Mill Creek, the rates (per male per minute) were lower than the single observation from Barren Fork (Figure 23 and Figure 24). In Barren Fork, a cumulative total of fourteen male-female Southern Redbelly Dace clasps were observed compared to 3 by male Blackside Dace with female Southern Redbelly Dace. In general, the greatest rates of spawning clasp formations for males of both species were with female Southern Redbelly Dace. An exception to this was a single observation in which the spawning clasp rate by male Southern Redbelly Dace toward female Blackside Dace was 0.429 per male per minute, and greater than all other observations. A cumulative total of 4 spawning clasps by male Southern Redbelly Dace toward female Blackside Dace were observed in in Barren Fork.

CHAPTER 5

DISCUSSION

All spawning events by Blackside Dace and Southern Redbelly Dace were in association with Creek Chub nests. The extent to which both of these species rely on Creek Chub nests is uncertain, but the current study adds to observations by Mattingly and Black (2013) that support the prediction by Cicerello and Laudermilk (1996) that Blackside Dace spawn primarily in the nests of Creek Chubs. Mattingly and Black (2013) described Creek Chub nests as a “small island,” which can provide adequate spawning substrate for Blackside Dace in streams with otherwise silty and embedded substrates. In a stream such as Barren Fork, where much of the substrate outside of the nest is dominated by silt and sand, it is likely that Creek Chub nests are the only suitable spawning substrate available for both dace species.

Blackside Dace and Southern Redbelly Dace spawning sites were located in stream habitats categorized as runs. Although a few Creek Chub nests were observed in habitats where water velocity was negligible and the substrate seemed to be similar to other nests, the dace were not observed spawning in these pool locations. Riffles are another stream habitat that contained less sand and silt than other areas, but no spawning was detected in these locations. Although habitat conditions associated with Blackside Dace spawning microhabitats seem to be restricted to those selected by Creek Chubs (Mattingly and Black 2013), dace may have a preference within the range of available Creek Chub nests.

In Barren Fork, all spawning observations contained both species of dace. This could be because of similar response to cues such as those from heterospecific milt (Rakes et al. 2013), similar constraints on spawning habitat requirements, or both dace species could be attracted to the similar spawning behavior and nuptial coloration of the other dace species.

Spawning likely occurred between the dates of observation and may have extended before the start and after then end of the observational range of the present study for both streams. The dates of spawning events fall within the spawning season of April-June reported for Southern Redbelly Dace in Kentucky by Settles and Holt (1978) and for Blackside Dace by Starnes and Starnes (1981). Although the range of dates within the spawning seasons appear to overlap, the two species may have different peak spawning periods. Blackside Dace in Mill Creek ceased spawning earlier than populations in Barren Fork, while Blackside Dace in Barren Fork were more abundant in spawning aggregations at earlier dates, and Southern Redbelly Dace were more abundant in aggregations at a later period. Numbers of both species were low in late-June, with Southern Redbelly Dace numbers dropping dramatically within a week. The temperatures of the nest sites during observations fall within the range reported by other researchers in field studies (Mattingly and Black 2013) and captive propagation (Rakes et al. 2013) of Blackside Dace. The last observation of spawning in Barren Fork occurred when mean temperature was 18.9°C, and the maximum temperature of 20.7°C was similar to that of 21°C reported by Settles and Hoyt (1978) for a single observation of potential Southern Redbelly Dace spawning activity in Western Kentucky.

All spawning observations in Barren Fork contained both species, therefore the comparison of spawning sites with only Blackside Dace to those in which both species are present is also a general comparison between spawning microhabitats of the two streams. Conductivity was much higher in Barren Fork. Roghair et al. (2001) noted several old mines along Barren Fork and its tributaries, which may explain, in part, the higher conductivity measurements. Barren Fork is a larger stream compared to Mill Creek and that is the most likely explanation for the difference in water velocity and stream width at spawning microhabitats. The riparian zone along the length of stream in which spawning occurred on Barren Fork contained more large trees such as *Acer* and *Tsuga,* and trees with broad leaves such as *Magnolia*, which together provided 74-100% canopy cover above spawning sites. Compared to Barren Fork, the riparian along Mill Creek where spawning occurred often contained fewer large trees (e.g., *Acer* and *Tsuga* provided 20-45% canopy cover above spawning sites), more small trees overhanging the spawning sites (e.g., *Ilex opaca* and *Rhododendron* provided 95-100% canopy cover), and even a site without any canopy cover. Trash was commonly sighted along Barren Fork, and isolated areas contained reddish/orange colored water and substrate (see Roghair et al. 2001).

The absence of silt and the degree of substrate embeddedness were important differences between spawning and non-spawning sites in this study, and as previously reported by Mattingly and Black (2013). Dominant substrate size was smaller at Mill Creek spawning sites compared to downstream non-spawning sites, reflecting the presence of more pebbles, less cobble, and no boulders at spawning sites. Silt commonly dominated the substrate at non-spawning sites in Barren Fork sometimes completely covering the rocky substrate beneath in a thick layer. Roghair et al. (2001) noted hiking and horse trails as sources of sediment in Barren Fork.

Clinostomus, a genus notably absent from diving and electrofishing surveys by Roghair et al. (2001), was observed chasing and nudging almost all species in Barren Fork spawning sites. *Etheostoma* were observed probing the nest with their snout, which may indicate predation of eggs within the spawning site. No other fish species were observed interacting with *Etheostoma*, although they occasional chased and nudged each other.

Reproductive Behaviors

The following discussion focuses primarily on how observed behaviors relate to the idea of competition between Blackside Dace and Southern Redbelly Dace. The sample size was small, so an attempt was made to limit comparisons to general trends in the similarities and differences of behaviors based on visual interpretation of boxplots and histograms.

Johnston and Page (1992) indicated that behavioral observations might reveal territorial and clasping behavior in broadcast spawners. There were no indications that Blackside Dace and Southern Redbelly Dace competed for spawning sites in a way that displaced one species from nests, but males of both species were observed forming spawning clasps with females of both species on multiple occasions. Spawning clasps were consistent with the description provided by Johnston and Page (1992), that "A clasp occurs when a male places one pectoral fin under a female and curves his body close to hers, usually placing his caudal peduncle over hers." In this study, the formation of spawning clasps represented the best indicator of successful spawning. Gametes were not seen, likely because they are too small to be observed on video, and no attempt was made to confirm fertilization of eggs, so the success of each spawning clasp is uncertain. The interpretation of the results provided here is that males are competing within and between species for spawning opportunities with females of both species. Within mixed aggregations, both species were often observed closer to, and interacting with, members of their own species, but all recorded behavioral categories were observed to occur between species, often at lower rates than within species. Although the dace did not appear to compete for spawning sites, males did temporarily fight for and defend a small area immediately around a female from approaching males in a manner similar to that described by Hubbs and Walker (1942) for *Notropis longirostris*. Access to the space around a female gave males the best opportunity to form a spawning clasp when females were ready to spawn. Much of this competition between males to spawn with females can be seen in male-male interactions (i.e., pushing, nudging, and blocking).

Male Blackside Dace interacted with each other at generally higher rates in Mill Creek compared to Barren Fork. In Mill Creek, all Male Blackside Dace behaviors were directed toward one species, whereas in Barren Fork their total number of behaviors were divided between two species, which likely influenced the result of generally lower behavior rates toward males of their own species compared to Mill Creek. In interactions between species in Barren Fork, there were usually fewer male Blackside Dace for the larger number of male Southern Redbelly Dace to interact with. This difference in numbers probably influenced why the between species male-male behavior rates, per individual, were lower for behaviors initiated by male Southern Redbelly Dace and higher for behaviors initiated by male Blackside Dace.

Male-male nudges contained higher rates compared to other male-male interactions. This is likely because males nudged each other more often than pushed, even outside of the presence of females, and blocks only occurred in the presence of females. Nudges had a range of intensity from simply touching another male with their snout to ramming the other individual. The more intense nudges usually came from larger males.

In Barren Fork, the ranges of Blackside Dace nudge rates toward males of both species were similar, even though they were often outnumbered by Southern Redbelly Dace. Almost all within-species, male-male nudge rates by Southern Redbelly Dace were higher than the rates of nudges they initiated between species. In the observation on May 8th, when the numbers of males of both species in the spawning aggregation were similar, the nudge rates toward males within both species were more than double the respective rates between species. The observations mentioned above, seem to imply that both species focus the majority of their nudging on members of their own species.

Similar to nudge rates, male Blackside Dace generally pushed one another and blocked each other from female Blackside Dace more in Mill Creek, with their total number of behaviors in Barren Fork being distributed between members of both species. In addition to blocking males from female Blackside Dace, the total number of blocks performed by male Blackside Dace in Barren Fork also contained blocks of males from female Southern Redbelly Dace. This may explain why the block rates performed by male Blackside Dace toward female Blackside Dace differed between streams.

In general, male Southern Redbelly Dace blocked each other at rates similar to Mill Creek Blackside Dace. The majority of blocks of males from female Southern Redbelly Dace came from male Southern Redbelly Dace blocking males of their own species. Likewise, male Southern Redbelly Dace blocked each other from female Blackside Dace at higher rates than they blocked male Blackside Dace from female Blackside Dace. The highest rate of male Southern Redbelly Dace blocking each other from approaching females, involved a female Blackside Dace, and occurred on June 18th when there was only one Blackside Dace of each sex in the nest. The behaviors per minute for interactions between the single male and female Blackside Dace, on this date, were not calculated because they were only in the nest together for 36.2 s. Likewise, the only female Southern Redbelly Dace in the observation was present for 16.3 s, and therefore behaviors per minute involving her also could not be calculated.

In the observation mentioned above, the behaviors by male Southern Redbelly Dace toward female Blackside Dace, are also the overall highest rate values for pushes, pursuits, clasp attempts, and spawning clasps by any males toward any females, and the highest rates for Southern Redbelly Dace lateral displays and nudges toward female Blackside Dace. This was the last day that spawning behaviors were detected during this study and suggest increased rates of spawning behaviors by Southern Redbelly Dace toward the end of the spawning season. Excluding the observation mentioned above, males of both species generally directed the majority of their behaviors toward females of their own species.

Even though there were two species of females in Barren Fork toward which behaviors were directed, in many behavioral categories (i.e., nudges, lateral displays, vertical alignments, clasp attempts, and formation of spawning clasps) male Blackside Dace rates toward female Blackside Dace were generally greater in Barren Fork than in Mill Creek. This could be the result of a low sample size, or competition from male Southern Redbelly Dace. Another possibility is that in Mill Creek, where there were more Blackside Dace and the male to female ratios in the nests were higher, individual males interacted with each other at higher rates and with females at lower rates, because only a limited number of males could interact directly with the few females at any given time and they had to compete more for those opportunities. Whereas in Barren Fork, where there were fewer Blackside Dace and the male to female ratios in the nests were lower, slightly higher rates of interactions by males toward females were observed in many behavioral categories, possibly because males spent less time competing with each other and more time interacting with females, even when there were a large number of Southern Redbelly Dace, since most male-female behaviors seem to be primarily directed within species.

Nudges had the highest rates of any behaviors directed toward females. Often multiple nudges occurred during single pursuits and vertical alignments with females. Pushes and pursuits of females did not follow the same trend as other male-female behaviors, as there were somewhat higher rates in Mill Creek. Like male-male behaviors in Barren Fork, the total number of behaviors directed by males toward females were distributed between females of both species, but this may not explain why male-female Blackside Dace push and pursuit rates in Barren Fork are lower than those observed in Mill Creek, since the rates of those behaviors toward female Southern Redbelly Dace were much lower than toward female Blackside Dace.

Vertical aligning with the female may give the male a better view and position to temporarily defend the space around a female from other males. If the current reproductive status of females can be detected by males through cues from hormonal pheromones, as reviewed for fishes by Stacey and Sorensen (2009), the vertical alignment could potentially give males a chance to receive such cues from the female, since the male is often oriented with his snout below the vent of the female. Although males generally performed vertical alignments with females of their own species at higher rates, males vertically aligned with females of either species in almost all observations, with the exception of male Blackside Dace toward female Southern Redbelly Dace in two observations.

Lateral displays usually occurred when a single male was interacting with a female, and were often interrupted by the approach of other male dace. Male Blackside Dace lateral display rates toward female Blackside Dace were more consistently performed at rates ≥1.00 per male per minute, compared to other male-female lateral display rates, which may indicate more persistent courting between Blackside Dace.

When clasp attempts occurred, the rates of male Blackside Dace in Barren Fork toward females of both species were higher than those by male Blackside Dace in Mill Creek, but only one clasp was observed in Barren Fork compared to six in Mill Creek.

Males of both species attempted spawning clasps with females of both species, but rates of successful spawning clasps usually occurred at higher rates with female Southern Redbelly Dace.

A generalization of the typical sequence of events that describe the interactions between male and female dace, despite observed variation in such interactions within this study, is a follows: male behaviors toward females began with the female either already in the nest, entering the nest alone, or entering the nest while being pursued by the male(s). In the first or second situation, the male would approach the female with either a vertical alignment or a lateral display. The male would often persist near the female after one of these behaviors. A male interacting with a female would often gain attention from other males who would then approach the female. Usually one of two things would occur next; 1) The initial male would attempt to block other males from approaching the female, or 2) the approaching male would attempt to block the initial male from continuing to interact with the female. After the initial block there was usually a series of nudges and pushes between males and toward the female as each male jockeyed for a position next to the female with additional blocks. The female usually attempted to flee while both males pursued the female and continue to compete for dominance. The sequence may end in one male continuing to interact with the female while others give up, or the interaction can continue as males pursue the female within or outside of the nest.

In rare cases, during the initial lateral display toward the female, the male would attempt to form a spawning clasp with the female by aligning with her so that their flanks were in contact, placing his pectoral fin under her belly. When the male failed, which was frequently the case, the male would not extend his caudal peduncle over the female, and the female would push the male and swim away. In several cases, spawning clasp attempts were disrupted by other males, who would nudge and push the female and the first male, with one occasionally gaining a position along the other side of the female or in-between the female and the first male. Sometimes this behavior would lead to a successful spawning clasp by the second male. In very few instances, the initial lateral display would be followed by the first male forming a successful spawning clasp with the female, often proceeded by the male shaking its head. A similar head shaking behavior was describe by Reighard (1920) for *Catostomus commersonii*. Spawning clasp occurred while in contact with the substrate as well as while a few centimeters above the substrate. Spawning clasps were brief (≤1s). In most cases, after a spawning clasp was completed, the female swam away while the males, often several at a time, oriented head down in the substrate wiggling side-to-side in unison, presumably attempting to eat the eggs just released.

Although it is unknown if fertilization occurs between Blackside Dace and Southern Redbelly Dace, or if the offspring of such mating are viable, in three observations, male dace that were assigned to the Southern Redbelly Dace species in the behavior analysis appeared to contain intermediate phenotypes of Southern Redbelly Dace and Blackside Dace. The males were nuptial individuals as large as the other dace and had two black horizontal lines (like Southern Redbelly Dace) that then merged or nearly merged toward the caudal peduncle. It is unknown how common such individuals are in the population. It is also unknown if these individuals are hybrids or represent phenotypic variation within the population of Southern Redbelly Dace inhabiting the Upper Cumberland River Basin. Roghair et al (2001), observed potential Blackside Dace x Southern Redbelly Dace hybrids during their survey of Indian Creek and its tributaries, which include Barren Fork. Hybridization has been documented between Blackside Dace x Creek Chubs (Eisenhour and Piller 1997). Southern Redbelly Dace have been documented as producing hybrid offspring with many fishes including Creek Chubs (Grady and Cashner 1988), Central Stonerollers (Grady and Cashner 1988), *Clinostomus elongatus* (Trautman 1957), *Clinostomus funduloides* (Trautman 1957), *Notropis cornutus* (Greenfield et al. 1973), and *Notropis pilsbryi* (Robison and Miller 1972). The observation of spawning clasps between Blackside Dace and Southern Redbelly Dace suggests they are potentially hybridizing where they are syntopic.

Competition between male Blackside Dace and Southern Redbelly Dace for spawning opportunities with females of both species may help explain how interactions between these closely related species contribute to Blackside Dace decline. Reproductive success is imperative for threatened species to maintain a healthy population size. This study illustrates why, as detailed by Shumway (1999) the observation and quantification of reproductive behaviors of aquatic organisms is an important tool for species management and conservation.

In summary, this study provides the first detailed quantification of behaviors related to spawning Blackside Dace and Southern Redbelly Dace, as well as the complex competition that occurs between males of both species for spawning opportunities. Although both species were observed spawning in Barren Fork, in streams where habitat conditions are degraded, Southern Redbelly Dace may prove more resilient than Blackside Dace, and may become the dominant dace species. Also because these two species spawn in the same nest at the same time and with each other across species, hybridization of such syntopic populations is a risk of which those involved with managing the Blackside Dace should be aware.

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APPENDIX A:

Tables

Table 1. Independent samples t-test to determine if mean values for habitat variables differed between spawning habitats in Mill Creek and Barren Fork, McCreary County, KY, 2014. The t-values are reported as absolute values. Bold text indicates statistically significant results.

Habitat Variable	Mill Creek spawning microhabitats		Barren Fork spawning microhabitats		Independent samples t-test		
	Mean values	Standard deviation	Mean values	Standard deviation	t	df	p
Conductivity (µmhos)	55.5	6.302	80.7	8.063	7.348	17	**0.000**
Nest temperature (°C)	16.1	1.732	16.9	1.402	1.096	17	0.288
Water depth (cm)	13.53	7.461	11.98	6.415	0.487	17	0.632
Water velocity (cm/s)	6.91	4.368	14.53	8.961	2.210	17	**0.041**
Stream width (m)	3.1	0.729	5.3	1.383	4.187	17	**0.001**
Canopy cover index	2.5	1.690	3.8	0.405	2.515	17	**0.022**
Dominant substrate index	3.1	0.354	3.5	0.522	1.541	17	0.142
Subdominant substrate index	2.1	0.354	2.6	0.820	1.353	17	0.194
Embeddedness index	0.0	0.000	0.0	0.000	n/a	n/a	n/a

Table 2. Taxa list and number of occurrences of trees providing canopy cover over spawning sites used by *Chrosomus* species in 8 locations in Mill Creek and 11 locations in Barren Fork, McCreary County, KY, 2014.

	Observed number of occurances	
Genus/Species	Mill Creek	Barren Fork
Acer	2	6
Ilex opaca	3	0
Magnolia	0	3
Rhododendron	2	3
Tsuga canadensis	3	4

Table 3. Paired samples t-test to determine if values differed between spawning and non-spawning habitats in Mill Creek, McCreary County, KY, 2014. Spawning habitats were compared to non-spawning habitats upstream and also to non-spawning habitats downstream. The t-values are reported as absolute values. Bold text indicates statistically significant results.

Habitat Variable	Spawning microhabitat: Mean values	Spawning microhabitat: Standard deviation	Upstream non-spawning microhabitats: Mean values	Upstream non-spawning microhabitats: Standard deviation	Downstream non-spawning microhabitats: Mean values	Downstream non-spawning microhabitats: Standard deviation	Paired t-tests: Spawning site and upstream non-spawning site: t	df	p	Paired t-tests: Spawning site and downstream non-spawning site: t	df	p
Water depth (cm)	13.53	7.461	18.94	12.275	10.25	2.591	2.252	7	0.059	1.547	7	0.166
Water velocity (cm/s)	6.91	4.368	9.03	14.624	9.03	6.979	0.400	7	0.701	1.188	7	0.274
Dominant substrate index	3.1	0.354	3.8	0.886	4.0	0.535	1.930	7	0.095	2.966	7	**0.021**
Subdominant substrate index	2.1	0.354	1.3	1.389	1.9	1.246	1.594	7	0.155	0.475	7	0.649
Embeddedness index	0.0	0.000	3.0	1.069	2.4	0.744	7.937	7	n/a	9.029	7	n/a
Canopy cover index	2.5	1.690	3.0	1.604	2.1	1.808	1.323	7	0.227	0.753	7	0.476

Table 4. Paired samples t-test to determine if values differed between spawning and non-spawning habitats in Barren Fork, McCreary County, KY, 2014. Spawning habitats were compared to non-spawning habitat upstream and to non-spawning habitats downstream. The t-values are reported as absolute values.

Habitat Variable	Spawning microhabitat: Mean values	Spawning microhabitat: Standard deviation	Upstream non-spawning microhabitats: Mean values	Upstream non-spawning microhabitats: Standard deviation	Downstream non-spawning microhabitats: Mean values	Downstream non-spawning microhabitats: Standard deviation	Paired t-tests, Spawning site and upstream non-spawning site: t	df	p	Paired t-tests, Spawning site and downstream non-spawning site: t	df	p
Water depth (cm)	11.98	6.415	34.45	78.414	9.35	6.455	0.964	10	0.358	2.015	10	0.072
Water velocity (cm/s)	14.53	8.961	10.66	12.471	24.19	25.575	0.969	10	0.356	1.672	10	0.126
Dominant substrate index	3.5	0.522	0.9	1.375	1.7	1.489	5.864	10	n/a	3.684	10	**0.004**
Subdominant substrate index	2.6	0.820	2.1	1.221	1.9	1.136	1.336	10	0.211	1.750	10	0.111
Embeddedness index	0.0	0.000	3.6	0.934	2.6	1.362	12.587	10	n/a	6.421	10	n/a
Canopy cover index	3.8	0.405	3.3	1.191	3.0	1.414	1.604	10	0.140	2.324	10	**0.042**

Table 5. Ethogram of behaviors relevant to reproductive competition in Blackside Dace and Southern Redbelly Dace. When possible definitions were constructed from literature sources that described behaviors for Blackside Dace or Southern Redbelly Dace. Some definitions were constructed from sources that described behaviors in other cyprinid fishes. When an adequate definition was not found in the relevant literature, a behavioral category and definition was created that best describe the behavior. The source of definition for such behaviors is marked as ad libitum observations, but is not intended to imply that these are the first observations or description of these type of behaviors in cyprinid fishes.

Behavior Category	Definition	Source of definition	Species referenced in source
Nudge	A fish makes contact with another by touching its snout to the others body.	Platania and Altenbach (1998)	Six cyprinid species
Push	A fish aligns laterally with another. It then makes contact with the other fish by thrusting its flank into the other fish with an undulation of its body from the anterior to posterior all in a single motion. Often follows a lateral display.	Ad libitum observations	*Chrosomus cumberlandensis* & *Chrosomus erythrogaster*
Block	A male positions himself between another male and the female that both males are attempting to interact with.	Ad libitum observations	*Chrosomus cumberlandensis* & *Chrosomus erythrogaster*
Pursue	One fish follows another for distance equal to at least one body length of the pursuer.	Starnes and Starnes (1981)	*Chrosomus cumberlandensis*
		Smith (1980)	*Chrosomus erythrogaster*
Vertical alignment	A male positions itself below and posterior to the female, and usually maintains his head under the belly, tail or vent of the female.	Ad libitum observations	*Chrosomus cumberlandensis* & *Chrosomus erythrogaster*
	This is similar to a description of "a male generally approached a female from below."	Platania and Altenbach (1998)	*Hybognathus placitus*
Lateral display	A fish aligns with another in a horizontal plane with all median fins erect.	Ad libitum observations	*Chrosomus cumberlandensis* & *Chrosomus erythrogaster*
Attempted clasp	Male places pectoral fin under female but does not curl tail over female's tail.	Ad libitum observations	*Chrosomus cumberlandensis* & *Chrosomus erythrogaster*
Spawning clasp	A male aligns laterally with a female so that their flanks are touching. The male situates his pectoral fin under her belly and curls his caudle peduncle over hers while bend his body towards the female.	Stasiak (1978)	*Chrosomus. neogaeus*
		Johnston and Page (1992)	n/a

Table 6. Within-observer reliability for logging male dace reproductive behaviors expressed as Pearson's correlation coefficient (*r*). Behavior rates were recorded during 1.5-minute observations. This was repeated twice for each of four videos, once during the regular collection of data and then again at the end of the study. Two videos were of Blackside Dace spawning aggregations in Mill Creek = Mill, and 2 videos were of spawning aggregations containing Blackside Dace and Southern Redbelly Dace in Barren Fork = Barr. Rates represent occurrences per minute of the behavior category for all males in the aggregation.

		Rate per minute				Pearson correlation coefficient (r)	Coefficient of determination (r^2)
Behavior	Occasion	Mill 1	Mill 2	Barr 1	Barr 2		
	First	20.00	75.33	17.33	14.67		
Lateral display	Second	21.33	88.67	15.33	15.33	1.00	1.00
	First	8.67	48.67	14.67	12.67		
Vertical alignment	Second	20.00	49.33	26.67	18.00	0.98	0.96
	First	12.00	30.67	10.00	18.00		
Block	Second	14.67	38.67	12.00	16.00	0.97	0.93
	First	132.00	488.67	372.67	216.67		
Nudge	Second	124.00	556.00	304.00	227.33	0.96	0.92
	First	12.67	163.33	29.33	12.67		
Push	Second	23.33	212.00	45.33	29.33	1.00	1.00
	First	9.33	22.67	18.00	12.00		
Pursue	Second	15.33	28.00	19.33	10.67	0.89	0.78
	First	0.00	0.00	0.00	0.67		
Clasp spawning	Second	0.00	0.00	0.00	0.67	1.00	1.00
	First	0.00	0.00	2.00	0.00		
Clasp attempt	Second	0.00	0.00	2.67	0.00	1.00	1.00

Table 7. Taxa list, number of occurrences, and primary activities of fishes present in spawning sites used by *Chrosomus* species in 6 locations in Mill Creek and 10 locations in Barren Fork, McCreary County, KY, 2014.

Genus/Species	Observed number of occurances		Activities performed while in the spawning site
	Mill Creek	Barren Fork	
Clinostomus	0	2	Chasing and nudging *Clinostomus*
			Occasionally chasing and nudging *Semotilus*
			Occasionally nudging *Chrosomus*
Etheostoma	1	8	Probing nest (probably eating eggs)
			Occasionally chasing and nudging *Etheostoma*
Sematilus atromaculatum	4	5	Digging
			Chasing, nudging and lateral displaying toward *Semotilus*
			Occasionally chasing, nudging, and pushing *Chrosomus*
			Occasionally chasing, nudging and lateral displaying toward *Clinostomus*
			Occasionally Probing nest (possibly eating eggs)
			Possible spawning event (view obstructed)

APPENDIX B:

Figures

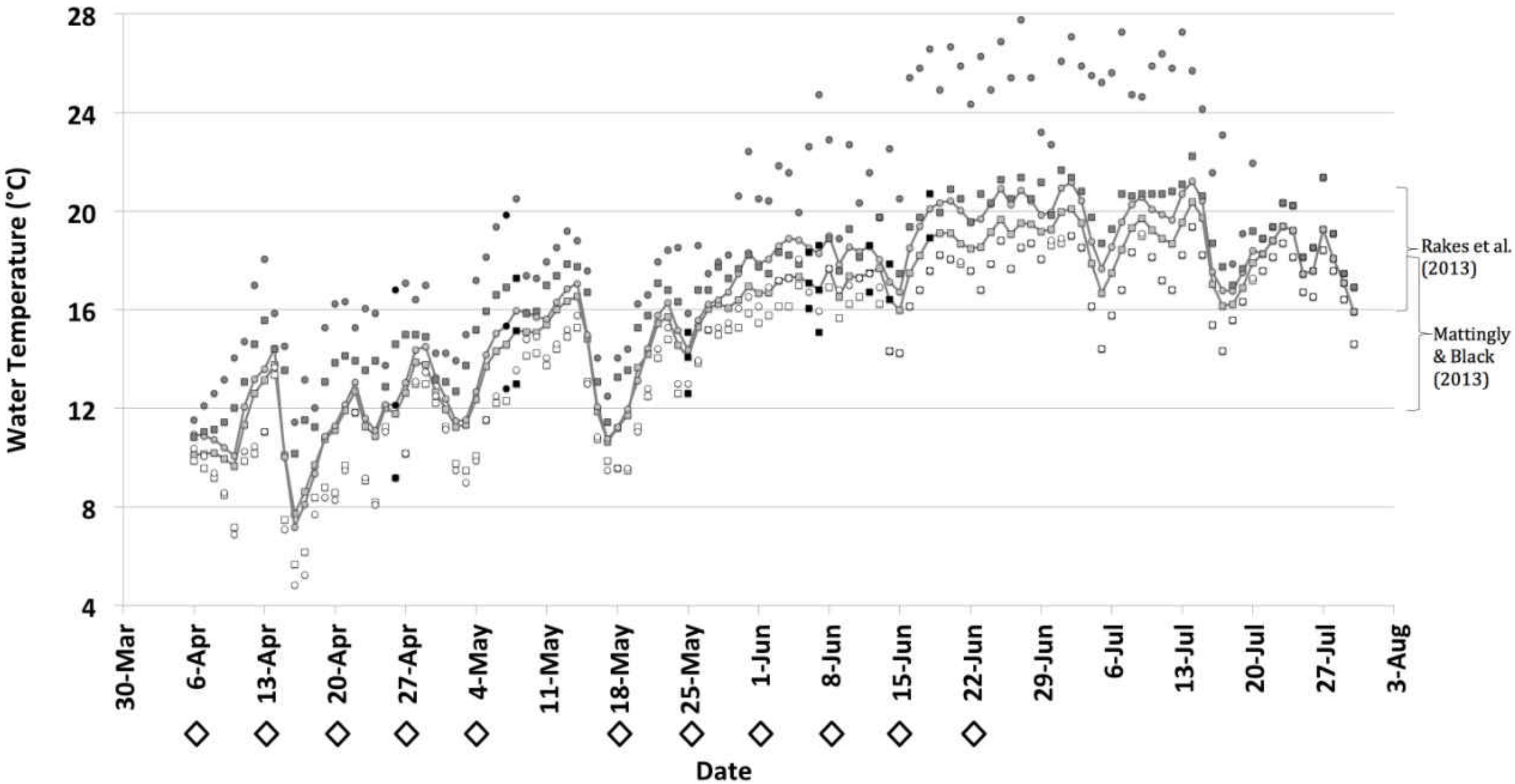

Figure 1. Water temperature for Mill Creek and Barren Fork, McCreary County, KY, 2004. Circles represent temperature data from Mill Creek, and squares are temperature data from Barren Fork. Black shapes show maximum (top), mean (middle), and minimum (bottom) temperature on dates when spawning activity was observed. Black circles denote Blackside Dace only spawning aggregations in Mill Creek, whereas squares are spawning aggregations containing both Blackside Dace and Southern Redbelly Dace in Barren Fork. Dark gray markers represent maximum daily temperatures, light gray markers connected by a line represent mean daily temperatures, and white data markers with black outline represent minimum daily temperatures. Diamonds below dates represent weeks when field observations were made. The citations on the right side of the figure represent observed temperature ranges for Blackside Dace spawning activity in past studies.

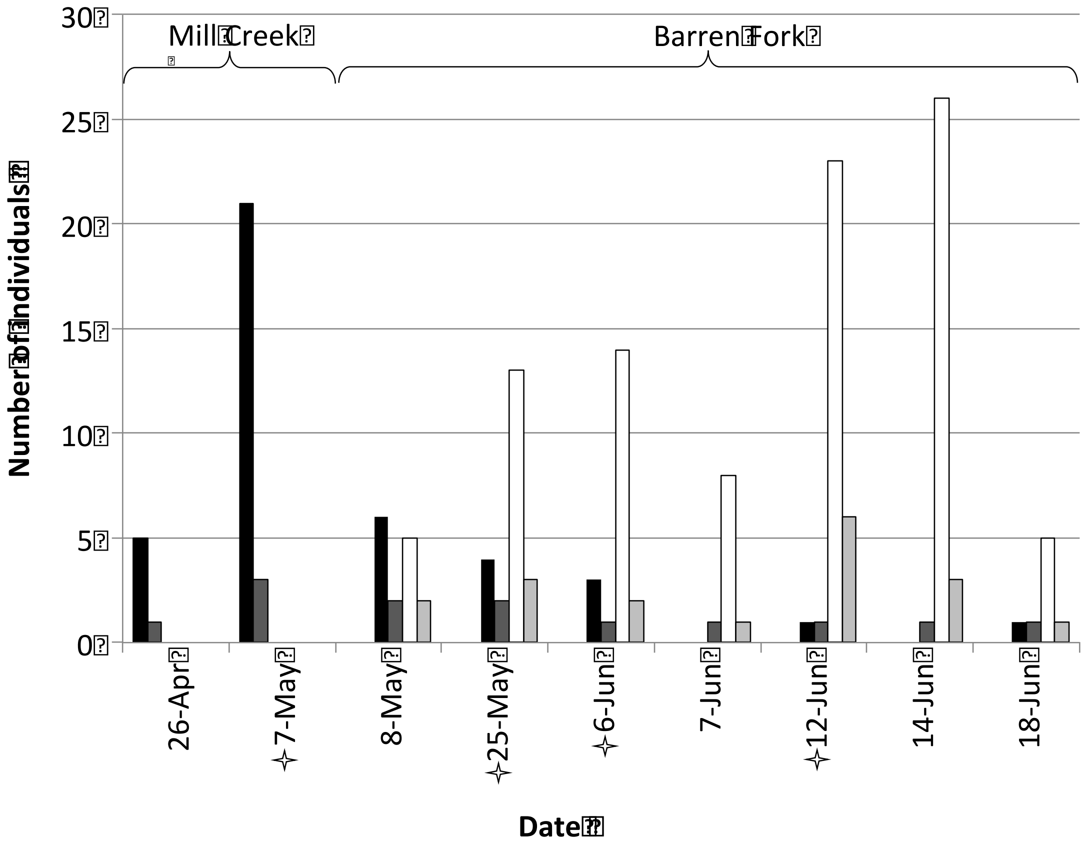

Figure 2. Number of individuals in spawning aggregations in Mill Creek and Barren Fork, McCreary County, KY 2004. Black bars = male Blackside Dace, dark gray bars = female Blackside Dace, white bars = male Southern Redbelly Dace, and light gray bars = female Southern Redbelly Dace. Dates with a star next to them represent the average of two or more spawning aggregations with values rounded up to the nearest whole number. The total number of male Blackside Dace on May 7th (n=6) averaged 20.8 (s=13); on May 25th (n=3) averaged 3.7 (s=0.6); on June 6th (n=2) averaged 2.5 (s=0.7); on June 12th (n=2) averaged 0.5 (s=0.7). The total number of female Blackside Dace on May 7th (n=6) averaged 3 (s=1); on May 25th (n=3) averaged 2 (s=0); on June 6th (n=2) averaged 0.5 (s=0.7); on June 12th (n=2) averaged 1 (s=0). The total number of male Southern Redbelly Dace on May 25th (n=3) averaged 12.3 (s=1.2); on June 6th (n=2) averaged 13.5 (s=2.1); on June 12th (n=2) averaged 22.5 (s=14.9). The total number of female Southern Redbelly Dace on May 25th (n=3) averaged 2.7 (s=1.2); on June 6th (n=2) averaged 1.5 (s=0.7); on June 12th (n=2) averaged 5.5 (s=2.1).

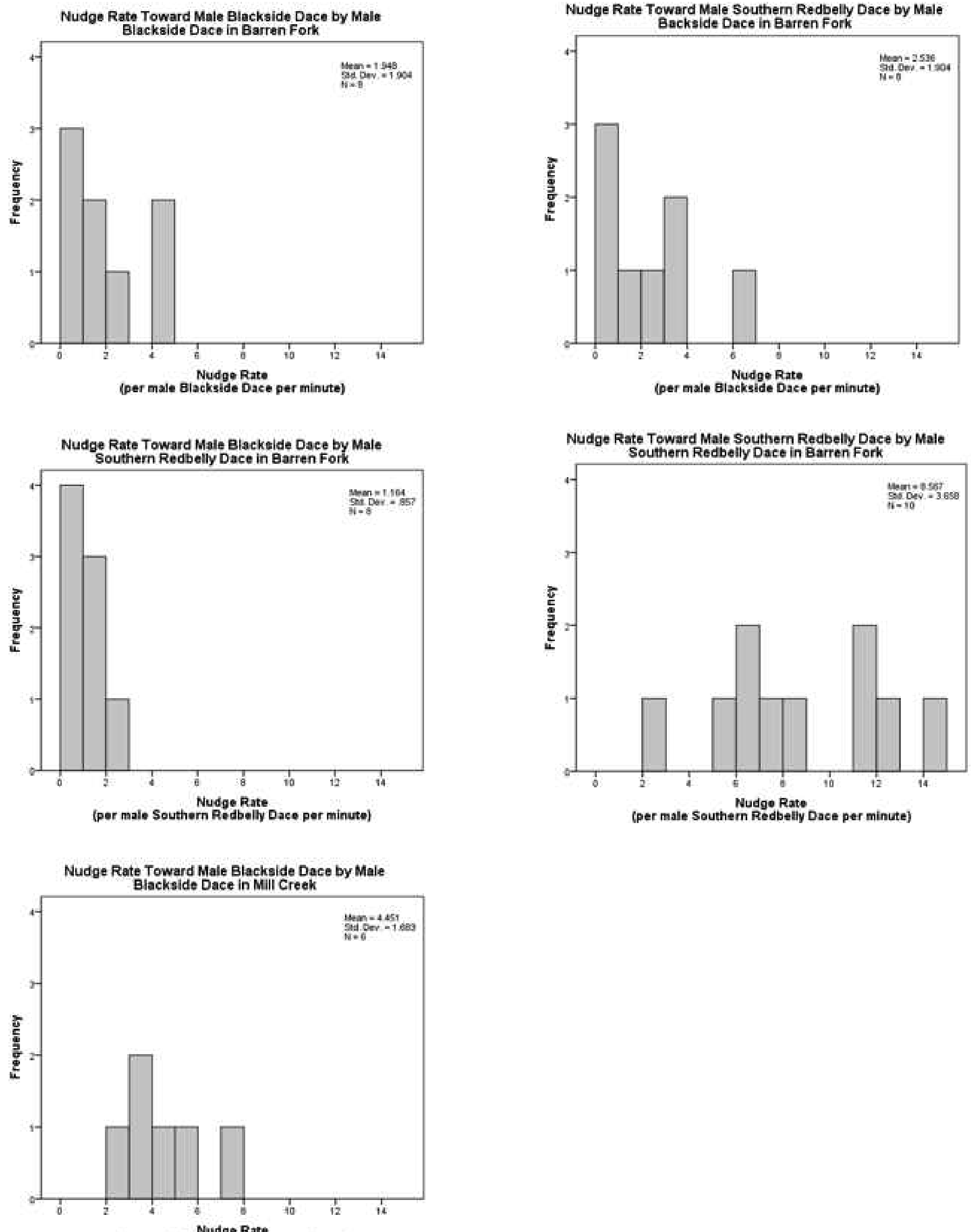

Figure 3. Male-male nudge rate histograms. Male Blackside Dace and male Southern Redbelly Dace behavior related to spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Nudge rates represent the average number of nudges performed per minute and per individual of the species doing the nudging. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

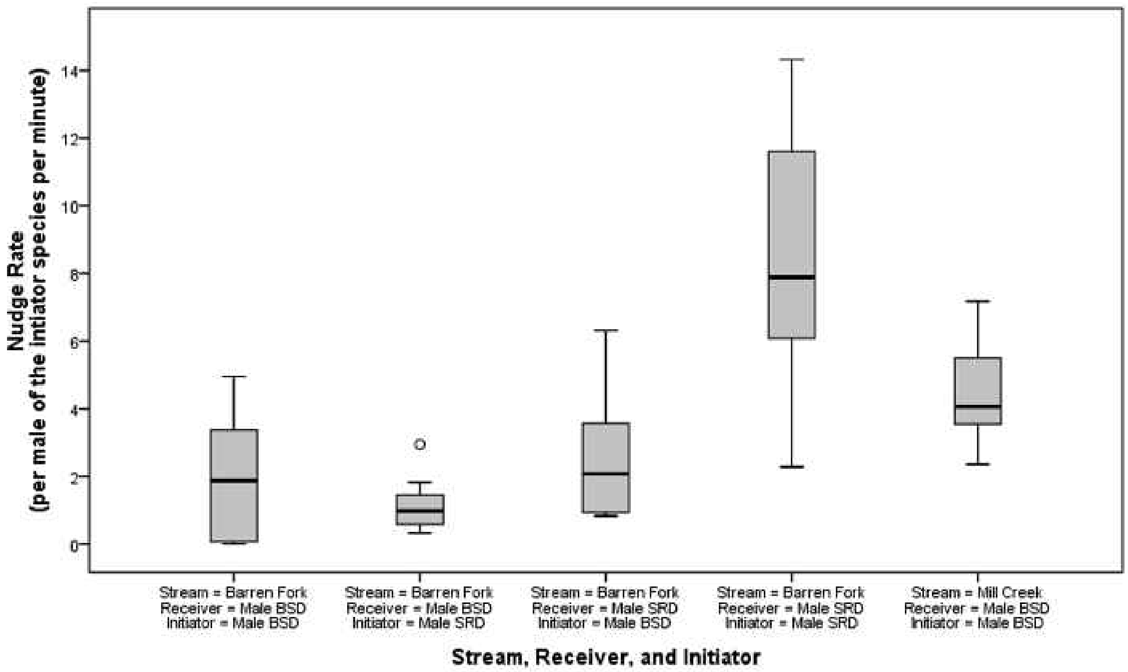

Figure 4. Male-male nudge rate boxplot. Male Blackside Dace and male Southern Redbelly Dace behavior related to spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Nudge rates represent the average number of nudges performed per minute and per individual of the species doing the nudging. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more extreme than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle.

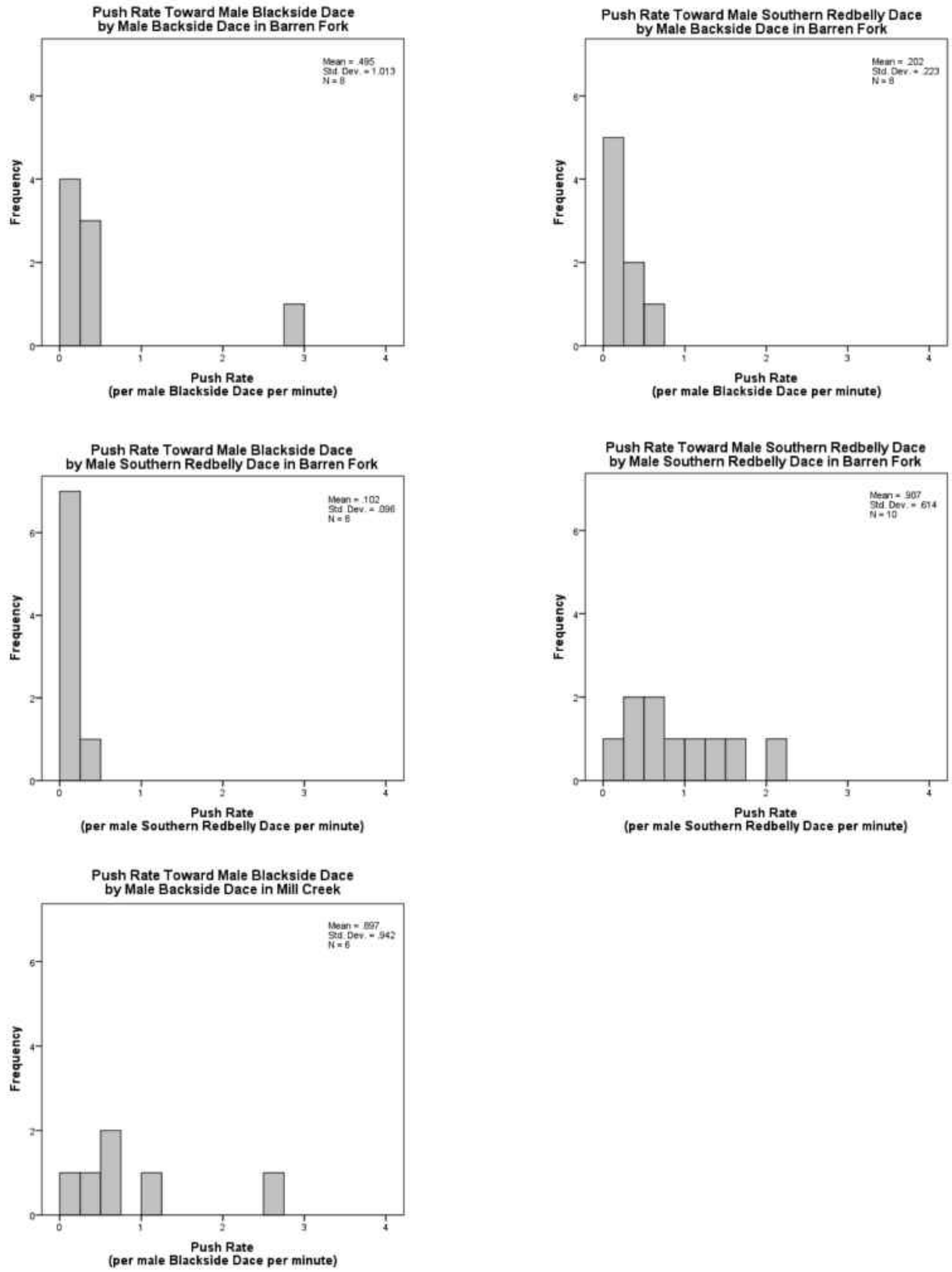

Figure 5. Male-male push rate histograms. Male Blackside Dace and male Southern Redbelly Dace behavior related to spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Push rates represent the average number of pushes performed per minute and per individual of the species doing the pushing. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

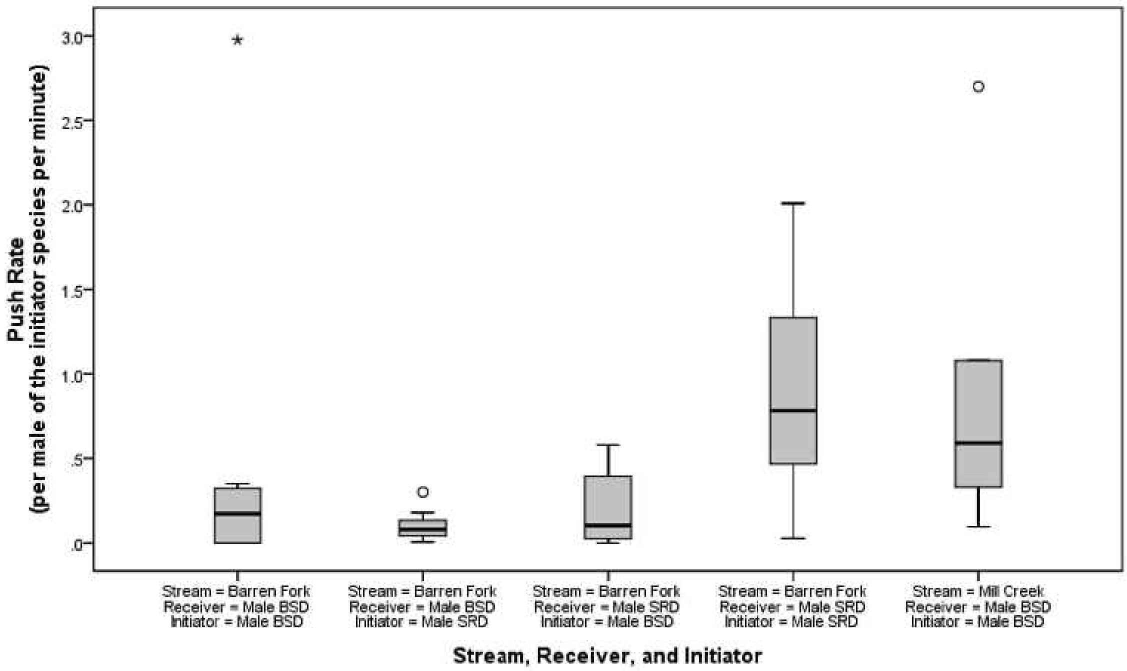

Figure 6. Male-male push rate boxplot. Male Blackside Dace and male Southern Redbelly Dace behavior related to spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Push rates represent the average number of pushes performed per minute and per individual of the species doing the pushing. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

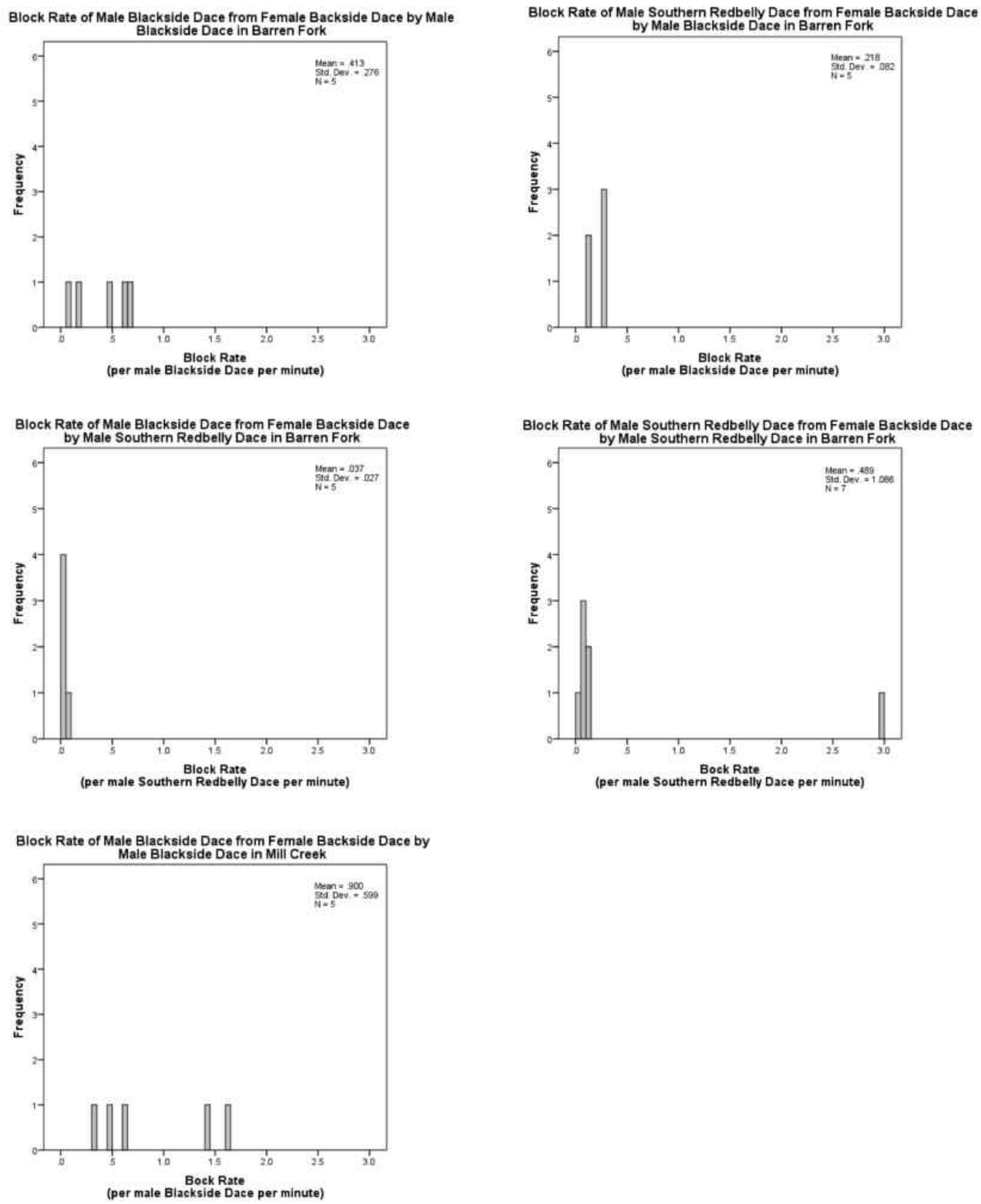

Figure 7. Block rate histograms (block male from female Blackside Dace). Rate histograms for the behavior of male dace blocking another male dace from approaching a female Blackside Dace in Barren Fork and Mill Creek, McCreary County, KY, 2014. Block rates represent the average number of blocks performed per minute and per individual of the species doing the blocking. The sample size (*N*) designates the number of observations where the actors (the male initiator and the male and female receivers indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

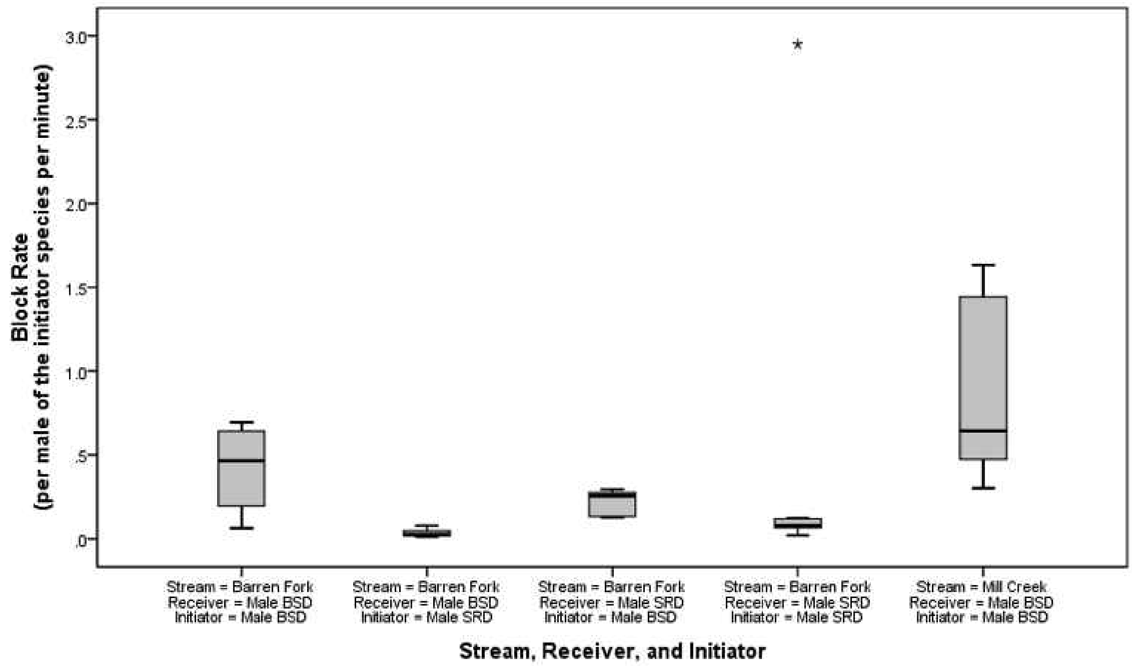

Figure 8. Block rate boxplot (block male from female Blackside Dace). Boxplot for the behavior rate of male dace blocking another male dace from approaching a female Blackside Dace in Barren Fork and Mill Creek, McCreary County, KY, 2014. Block rates represent the average number of blocks performed per minute and per individual of the species doing the blocking. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

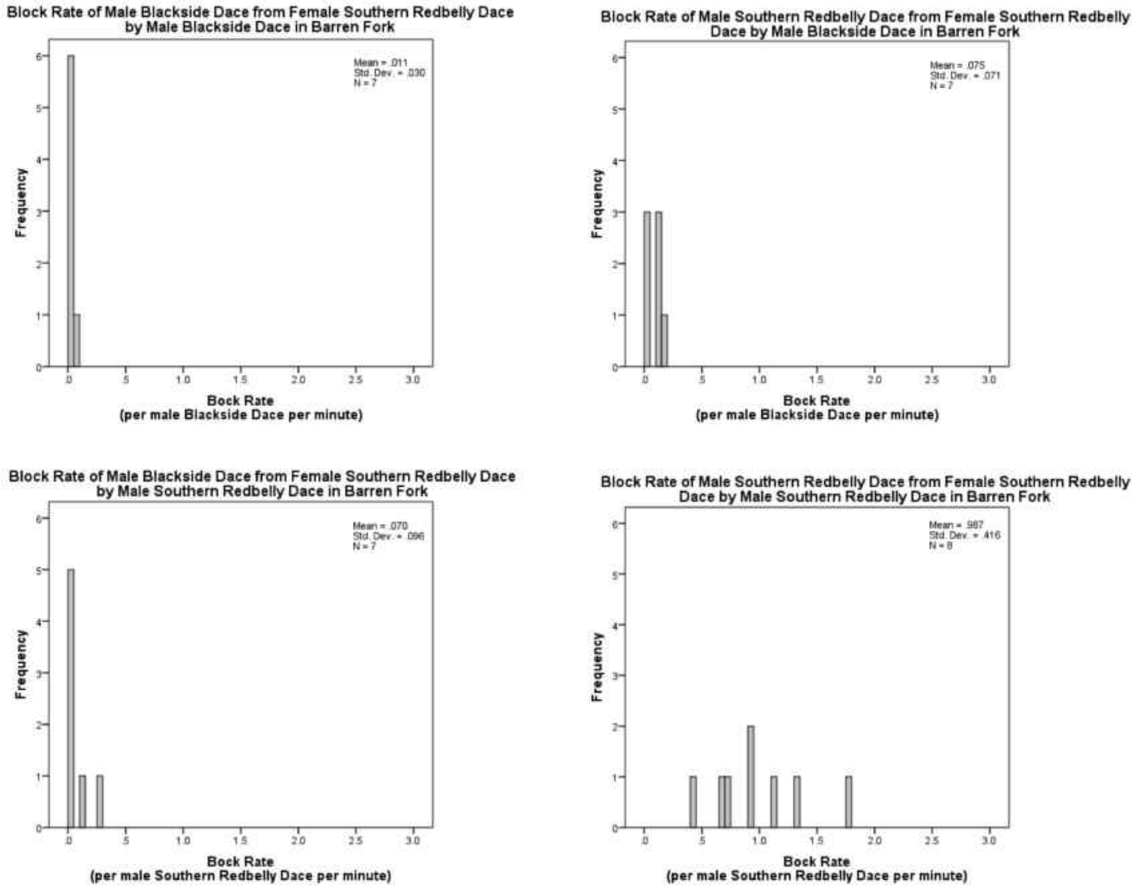

Figure 9. Block rate histograms (block male from female Southern Redbelly Dace). Rate histograms for the behavior of male dace blocking another male dace from approaching a female Southern Redbelly Dace in Barren Fork and Mill Creek, McCreary County, KY, 2014. Block rates represent the average number of blocks performed per minute and per individual of the species doing the blocking. The sample size (*N*) designates the number of observations where the actors (the male initiator and the male and female receivers indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

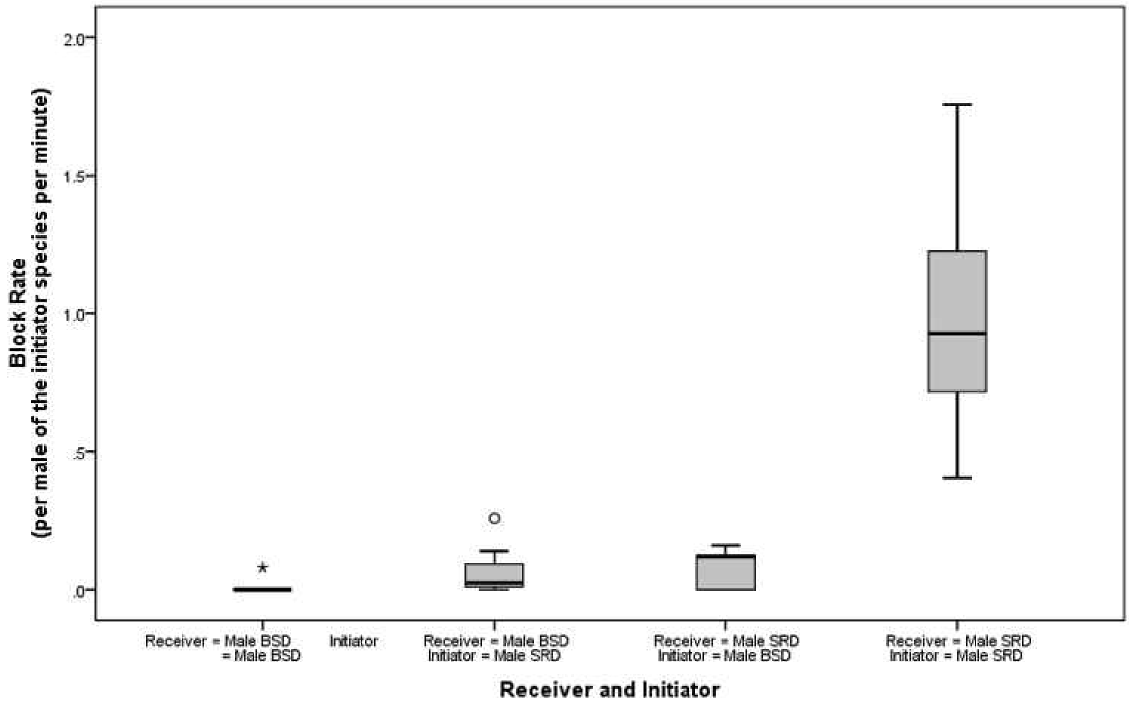

Figure 10. Block rate boxplot (block male from female Southern Redbelly Dace). Boxplot for the behavior rate of male dace blocking another male dace from approaching a female Southern Redbelly Dace in Barren Fork and Mill Creek, McCreary County, KY, 2014. Block rates represent the average number of blocks performed per minute and per individual of the species doing the blocking. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

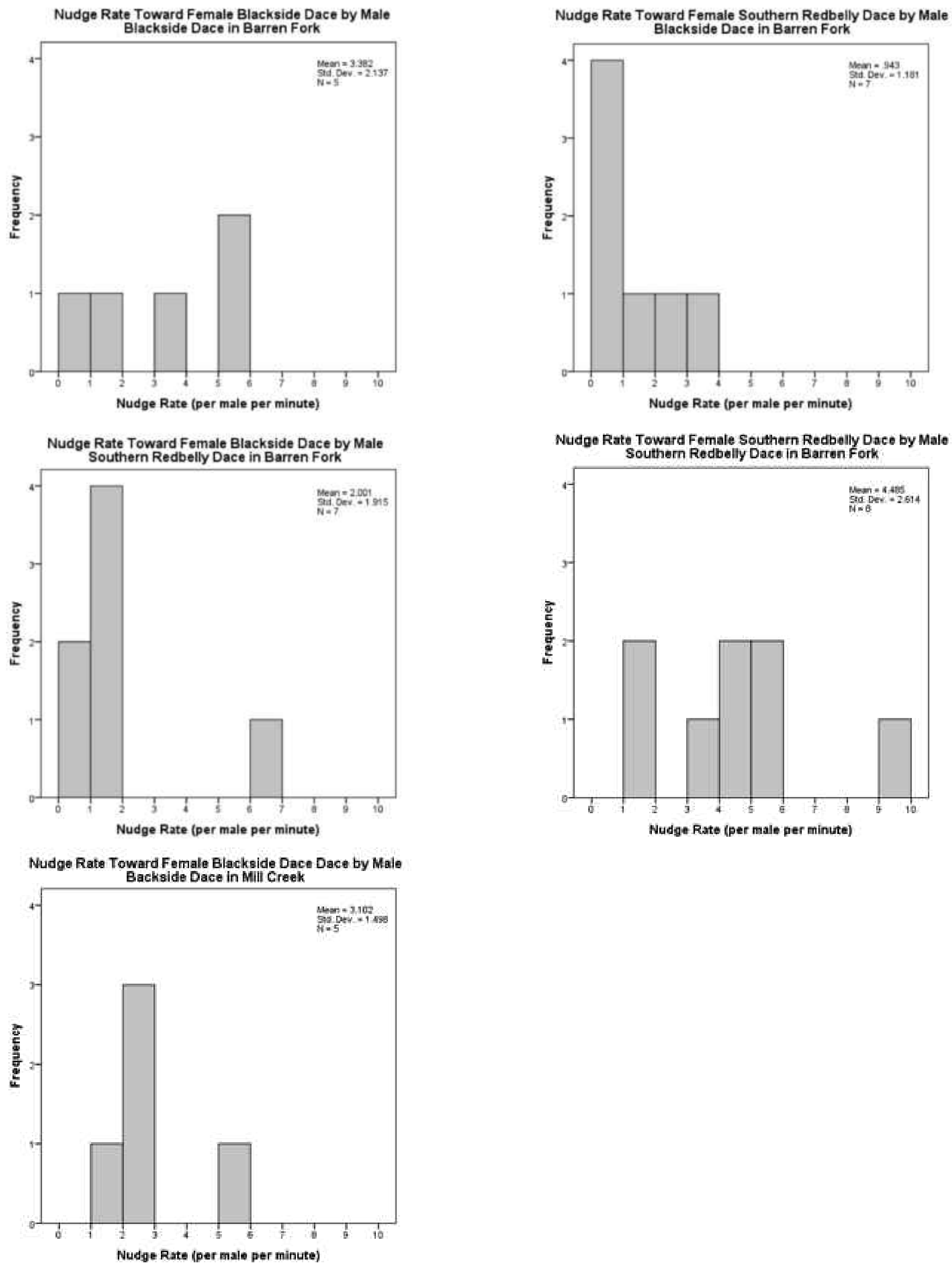

Figure 11. Male-female nudge rate histograms. Rate histograms for the behavior of male Blackside Dace and male Southern Redbelly Dace nudging females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Nudge rates represent the average number of nudges performed per minute and per individual of the species doing the nudging. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

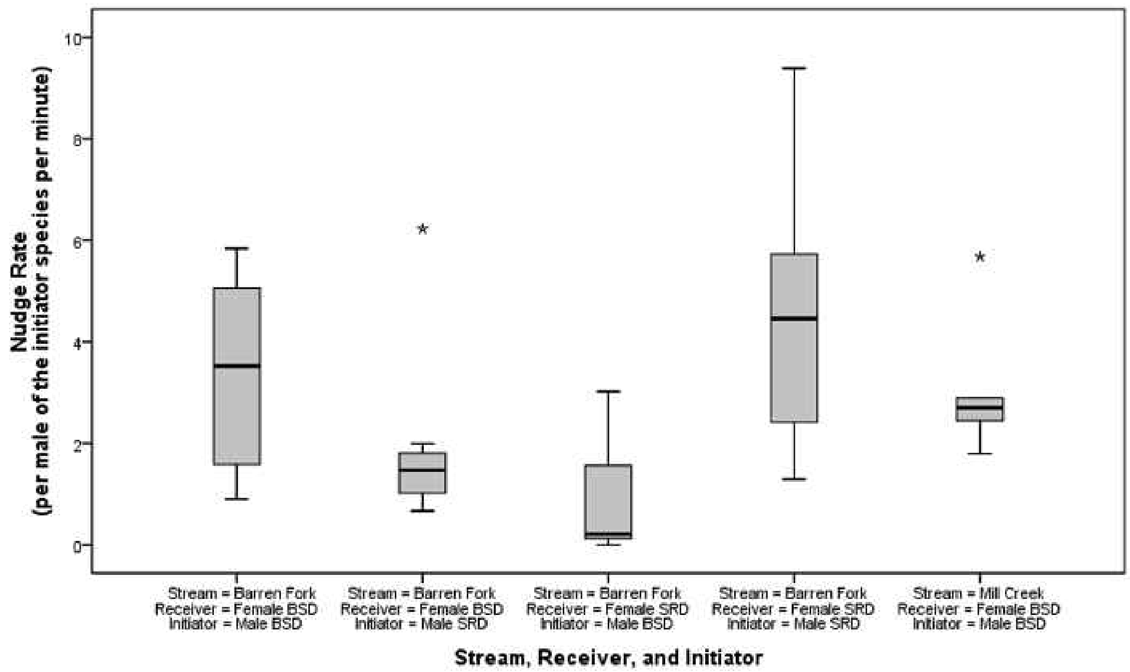

Figure 12. Male-female nudge rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace nudging females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Nudge rates represent the average number of nudges performed per minute and per individual of the species doing the nudging. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

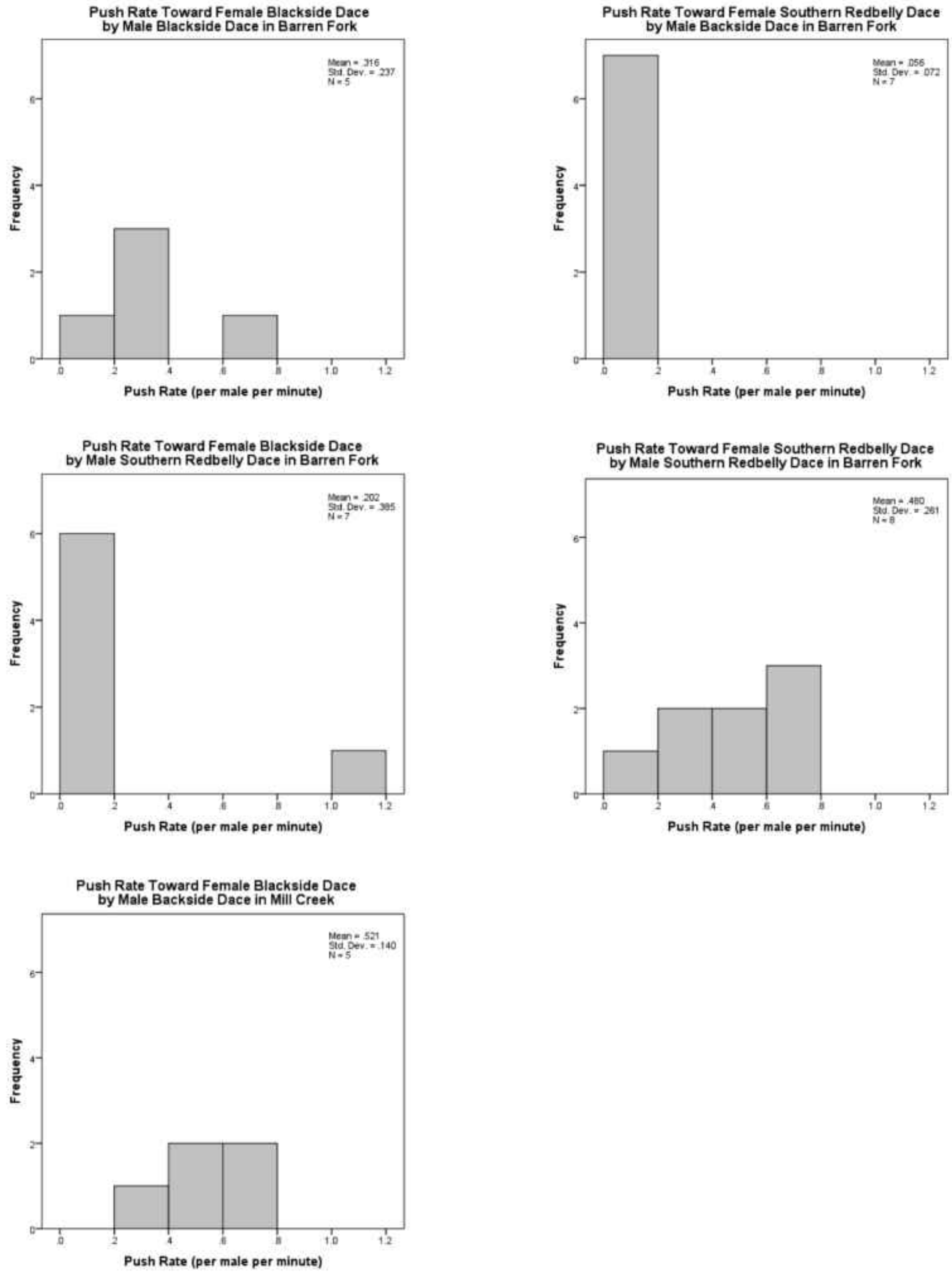

Figure 13. Male-female push rate histograms. Rate histograms for the behavior of male Blackside Dace and male Southern Redbelly Dace pushing females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Push rates represent the average number of pushes performed per minute and per individual of the species doing the pushing. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

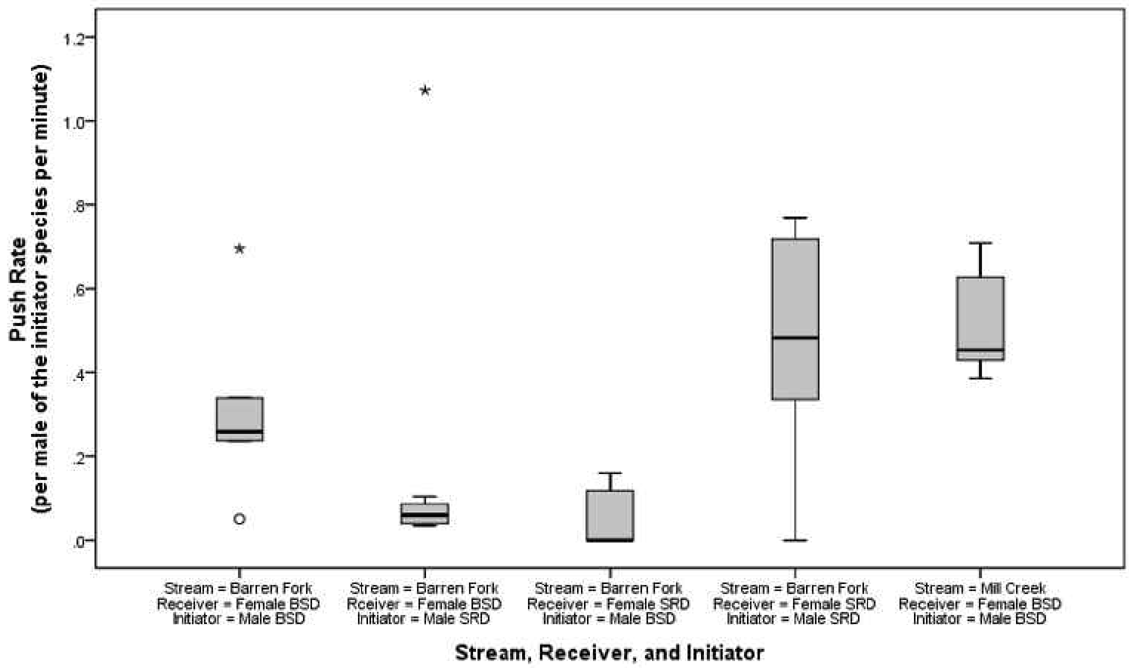

Figure 14. Male-female push rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace pushing females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Push rates represent the average number of pushes performed per minute and per individual of the species doing the pushing. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

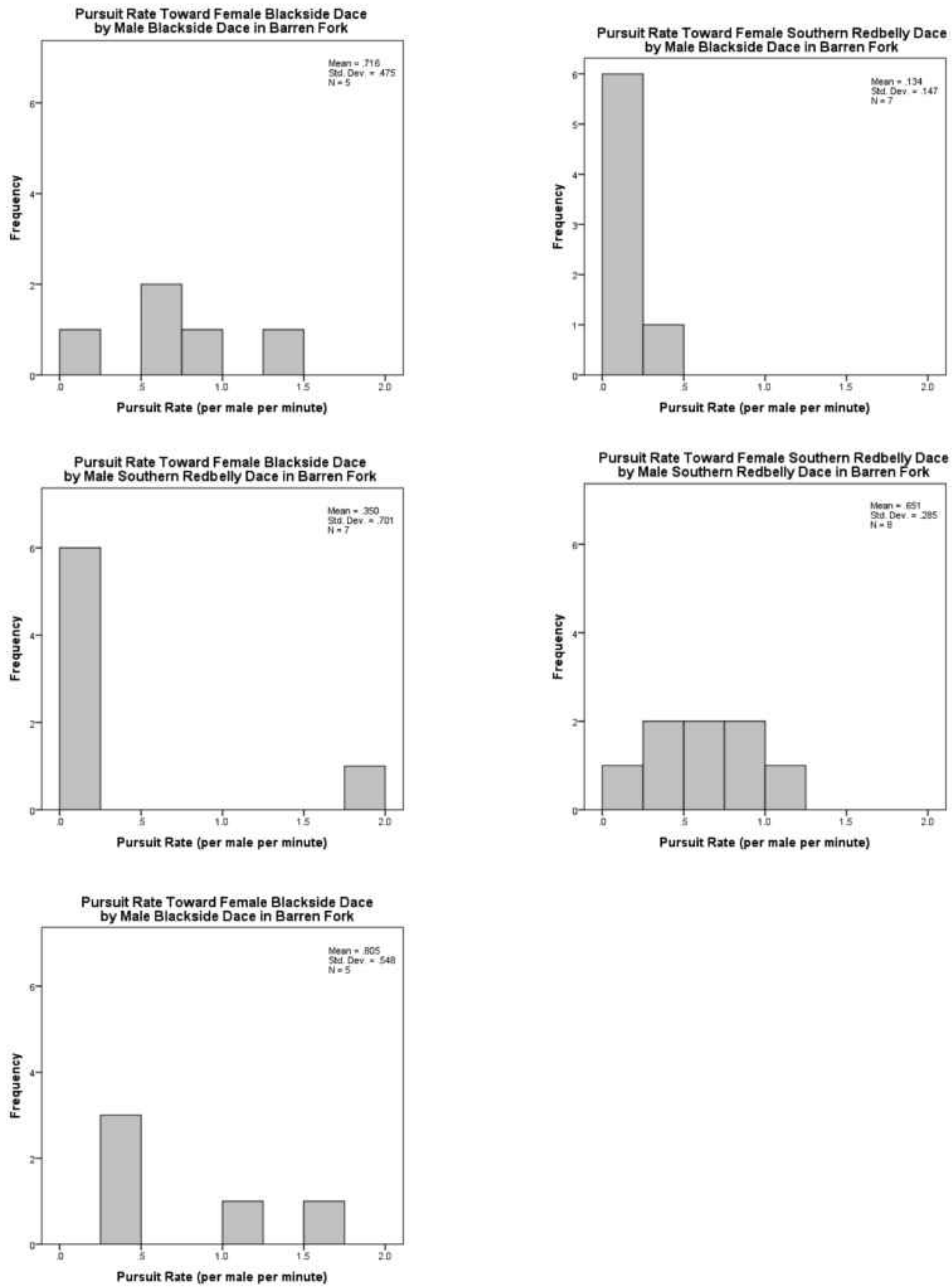

Figure 15. Pursuit rate histograms. Rate histograms for the behavior of male Blackside Dace and male Southern Redbelly Dace pursuing females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Pursuit rates represent the average number of pursuits performed per minute and per individual of the species doing the pursuing. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

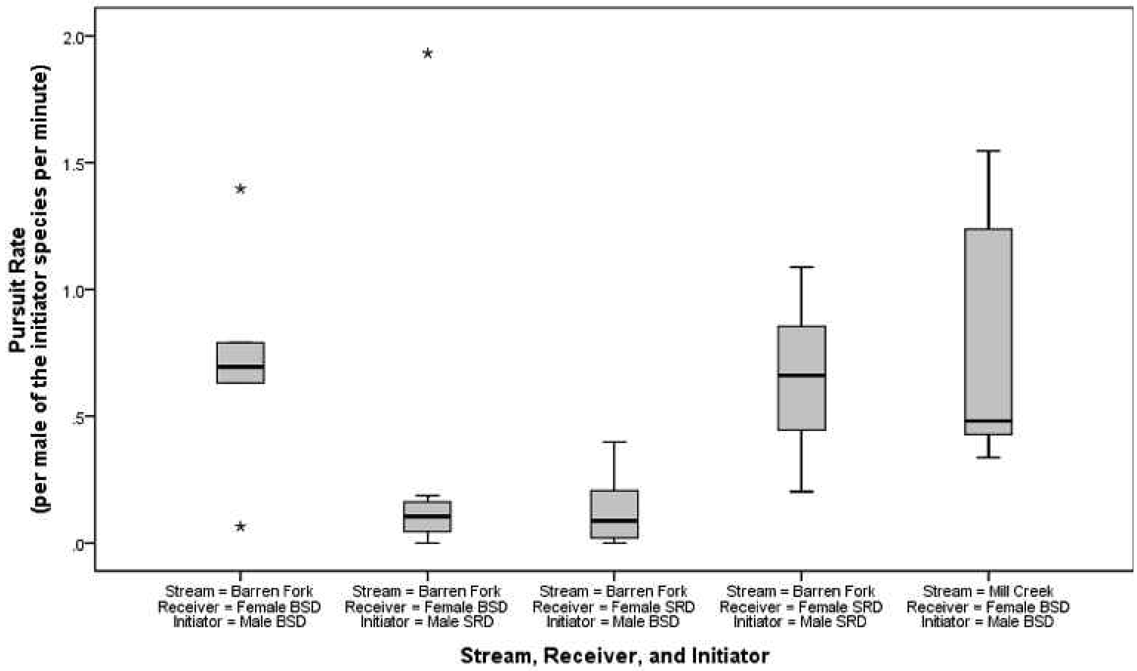

Figure 16. Pursuit rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace pursuing females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Pursuit rates represent the average number of pursuits performed per minute and per individual of the species doing the pursuing. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

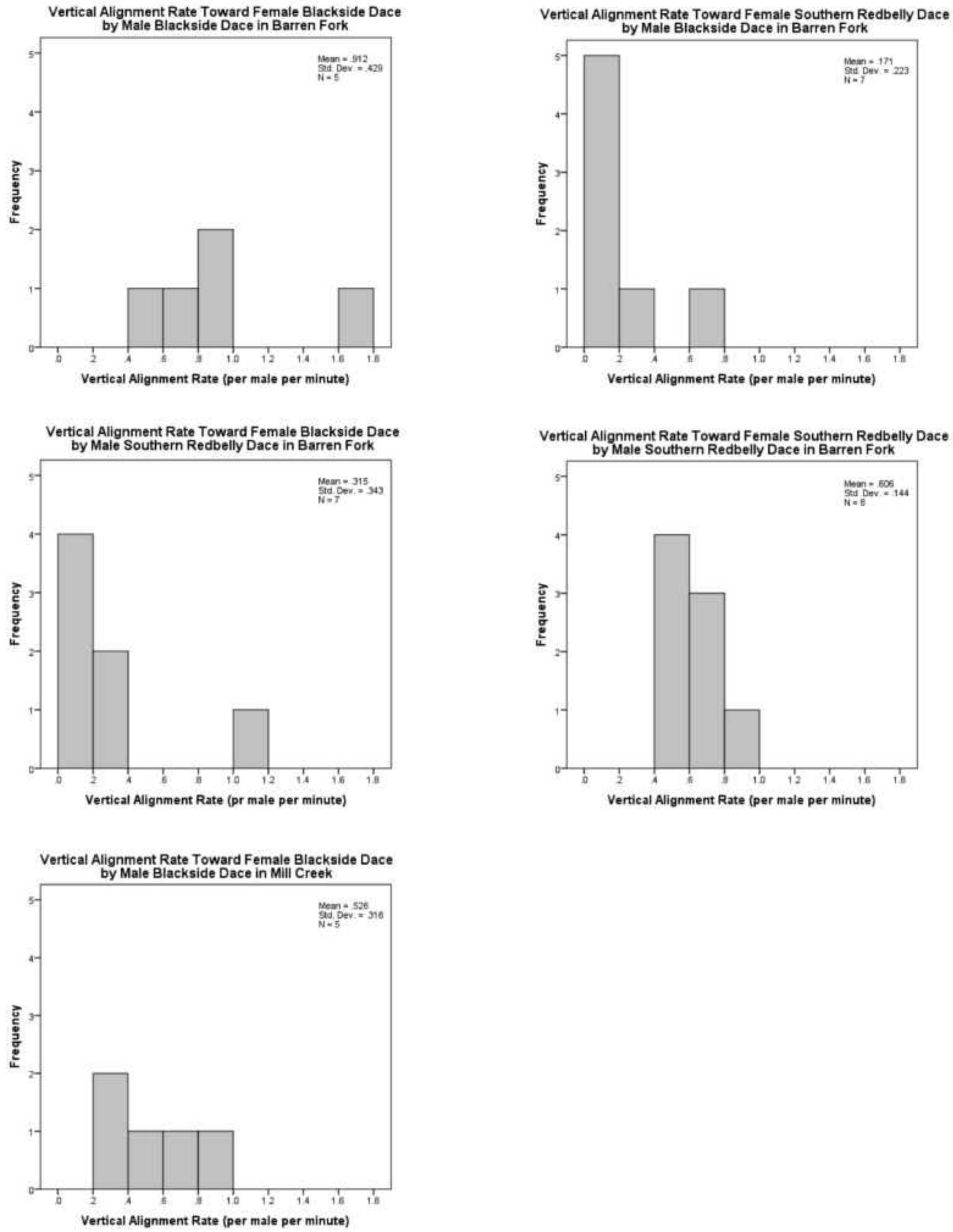

Figure 17. Vertical alignment rate histograms. Rate histograms for the behavior of male Blackside Dace and male Southern Redbelly Dace vertically aligning with females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Vertical alignment rates represent the average number of vertical alignments performed per minute and per individual of the species doing the aligning. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

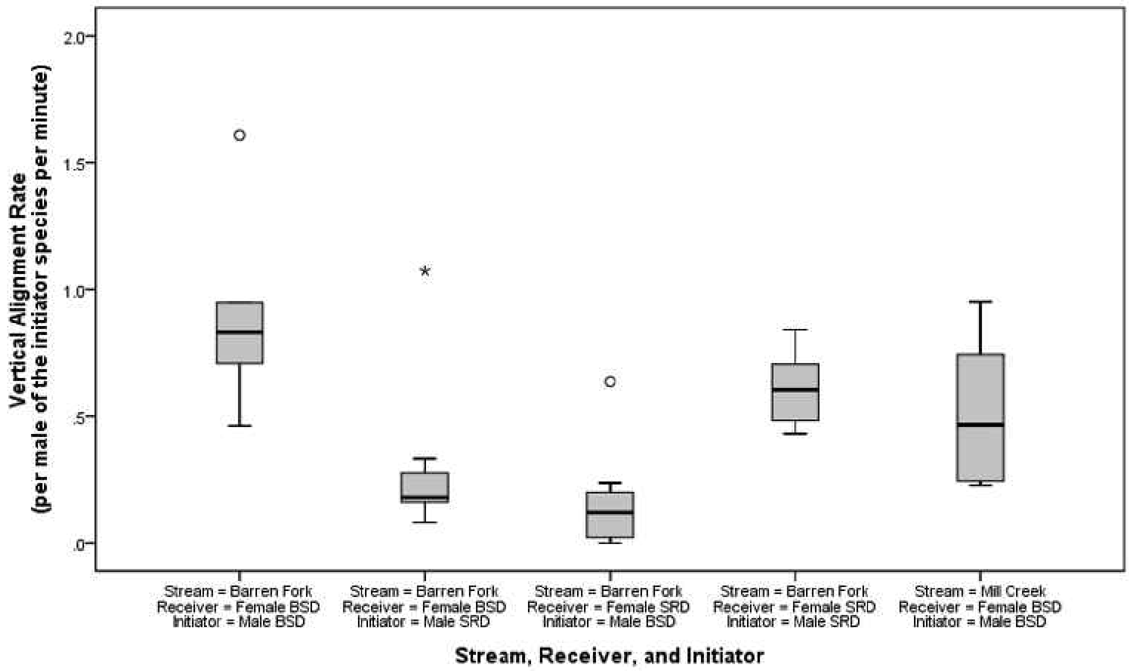

Figure 18. Vertical alignment rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace vertical aligning with females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Vertical alignment rates represent the average number of vertical alignments performed per minute and per individual of the species doing the aligning. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

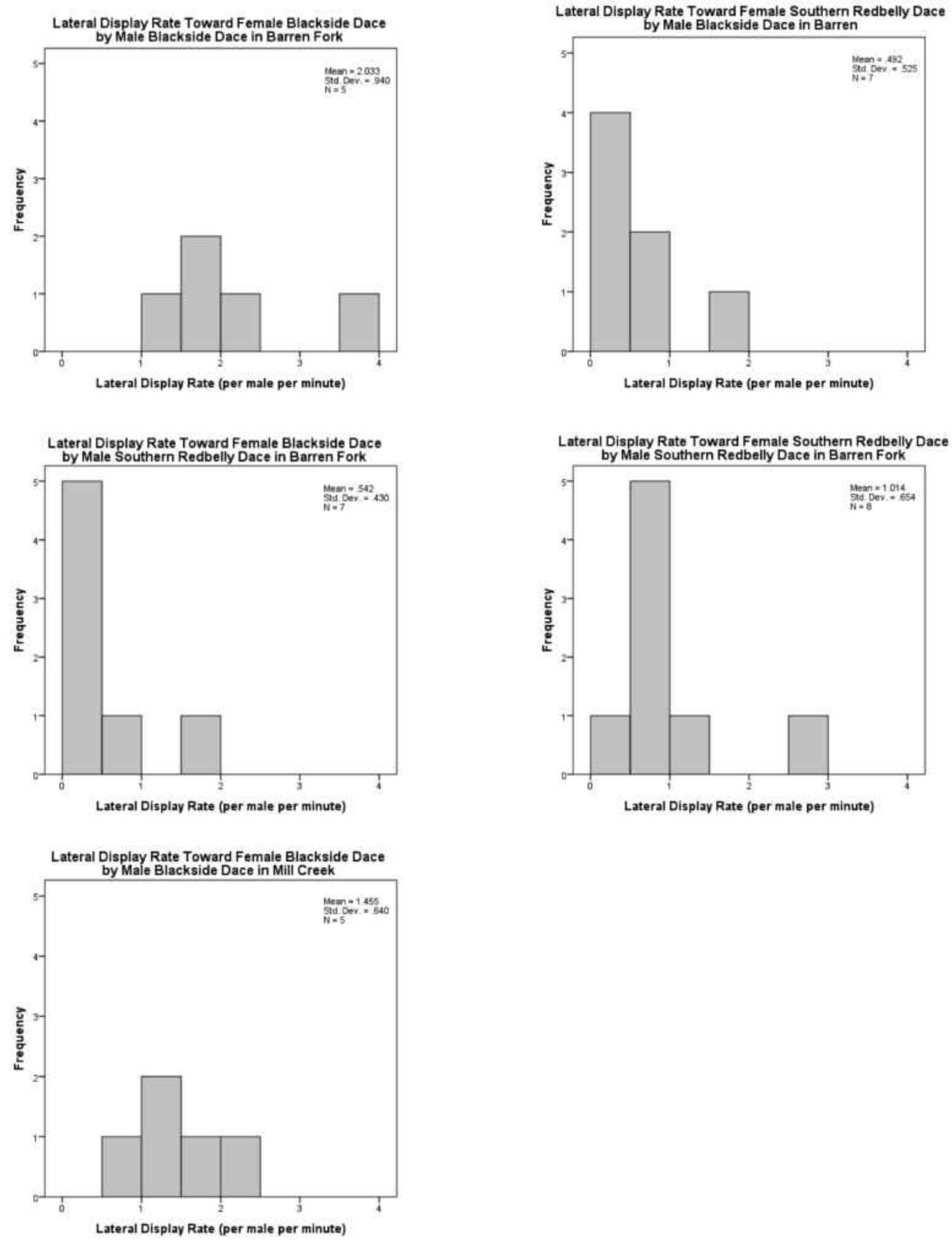

Figure 19. Lateral display rate histograms. Rate histograms for the behavior of male Blackside Dace and male Southern Redbelly Dace lateral displaying toward females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Lateral display rates represent the average number of lateral displays performed per minute and per individual of the species doing the displaying. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

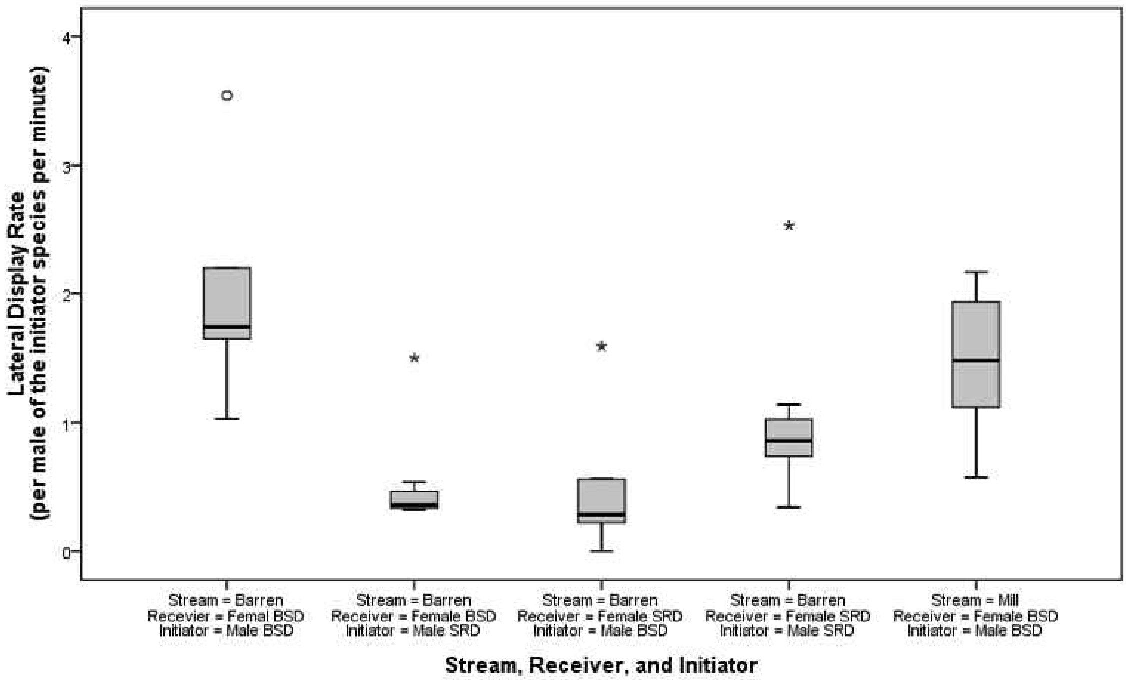

Figure 20. Lateral display rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace lateral displaying toward females during spawning activity in Barren Fork and Mill Creek, McCreary County, KY, 2014. Lateral display rates represent the average number of lateral displays performed per minute and per individual of the species doing the displaying. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

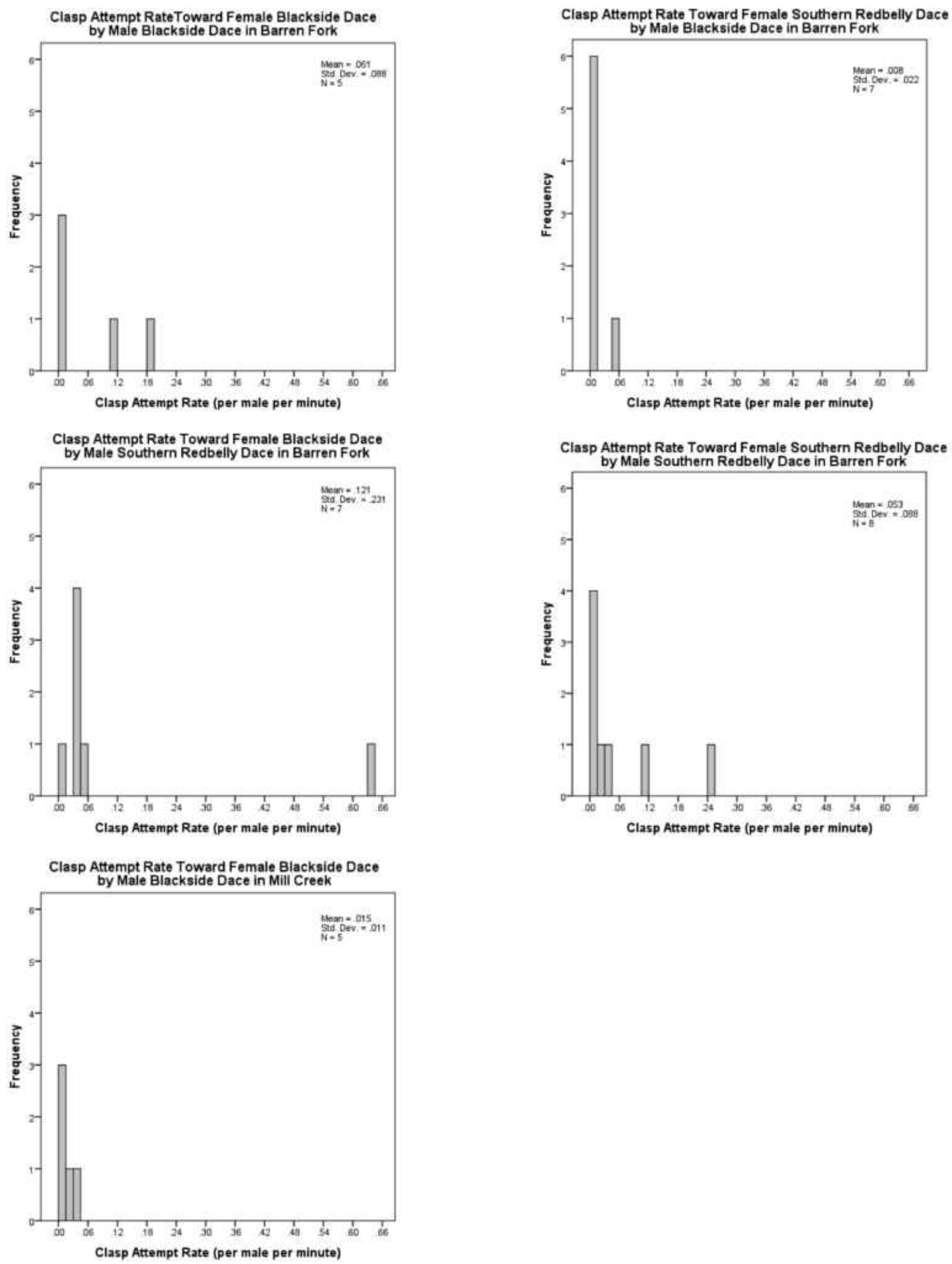

Figure 21. Clasp attempt rate histograms. Rate histograms for male Blackside Dace and male Southern Redbelly Dace attempting (but failing) to form a spawning clasp with females in Barren Fork and Mill Creek, McCreary County, KY, 2014. Clasp attempt rates represent the average number of clasp attempts performed per minute and per individual of the species attempting to form clasp with the female. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

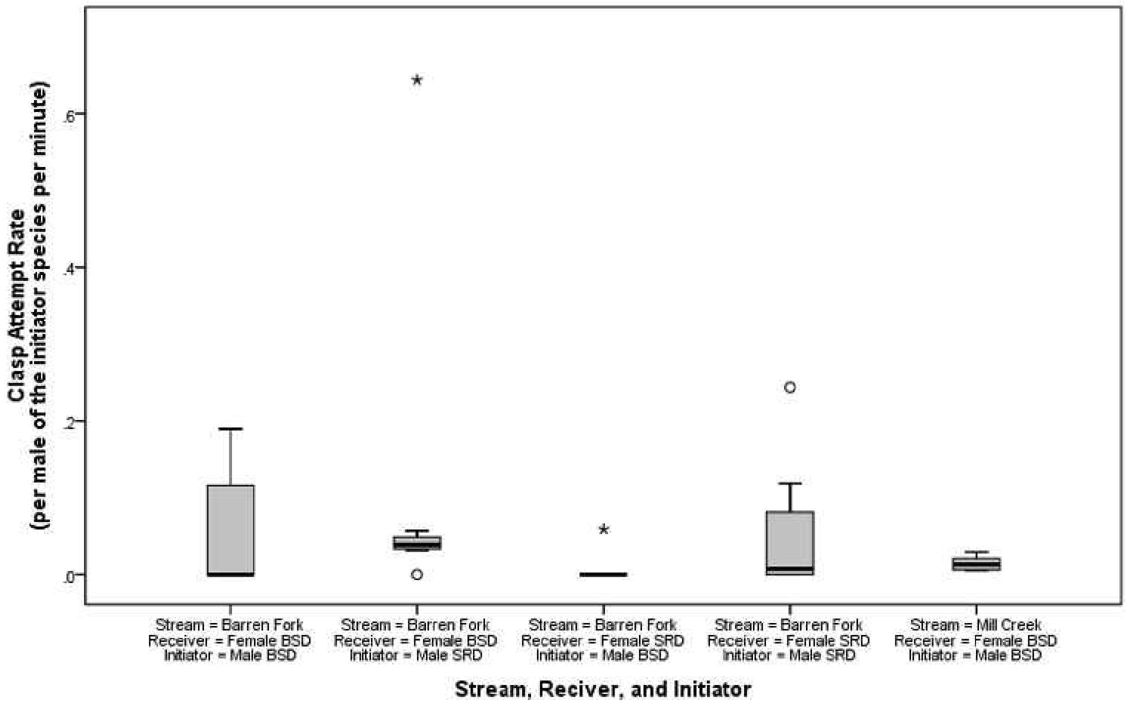

Figure 22. Clasp attempt rate boxplot. Boxplot for the behavior rate of male Blackside Dace and male Southern Redbelly Dace attempting (but failing) to form a spawning clasp with females in Barren Fork and Mill Creek, McCreary County, KY, 2014. Clasp attempt rates represent the average number of clasp attempts performed per minute and per individual of the species attempting to form clasp with the female. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.

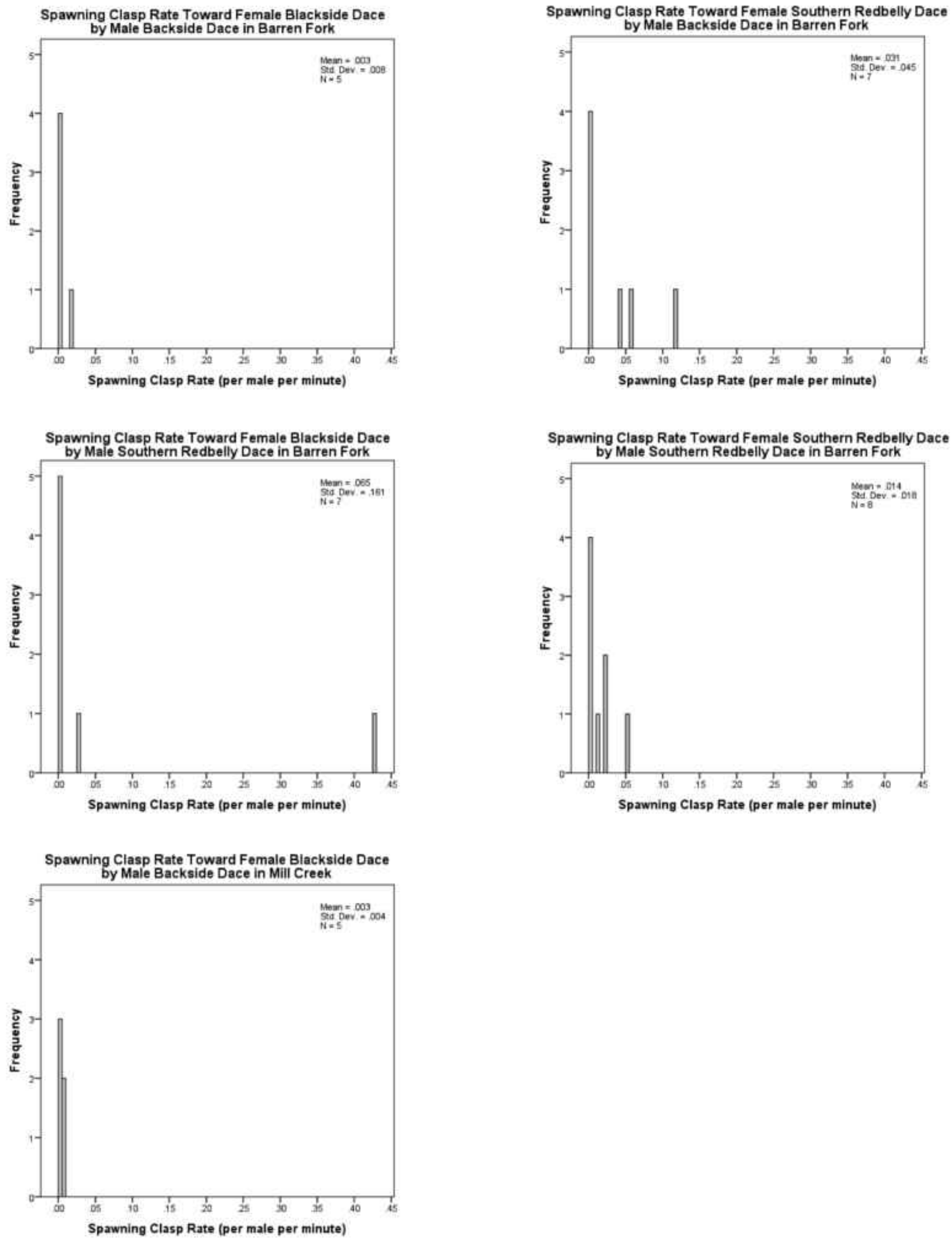

Figure 23. Spawning clasp rate histograms. Rate histograms for male Blackside Dace and male Southern Redbelly Dace forming a spawning clasp with females in Barren Fork and Mill Creek, McCreary County, KY, 2014. Spawning clasp rates represent the average number of spawning clasps performed per minute and per individual of the species forming a clasp with the female. The sample size (*N*) designates the number of observations where the actors (the initiator and receiver indicated by each histogram title) in the interaction were simultaneously present in a spawning location for > 60 ± 5 seconds.

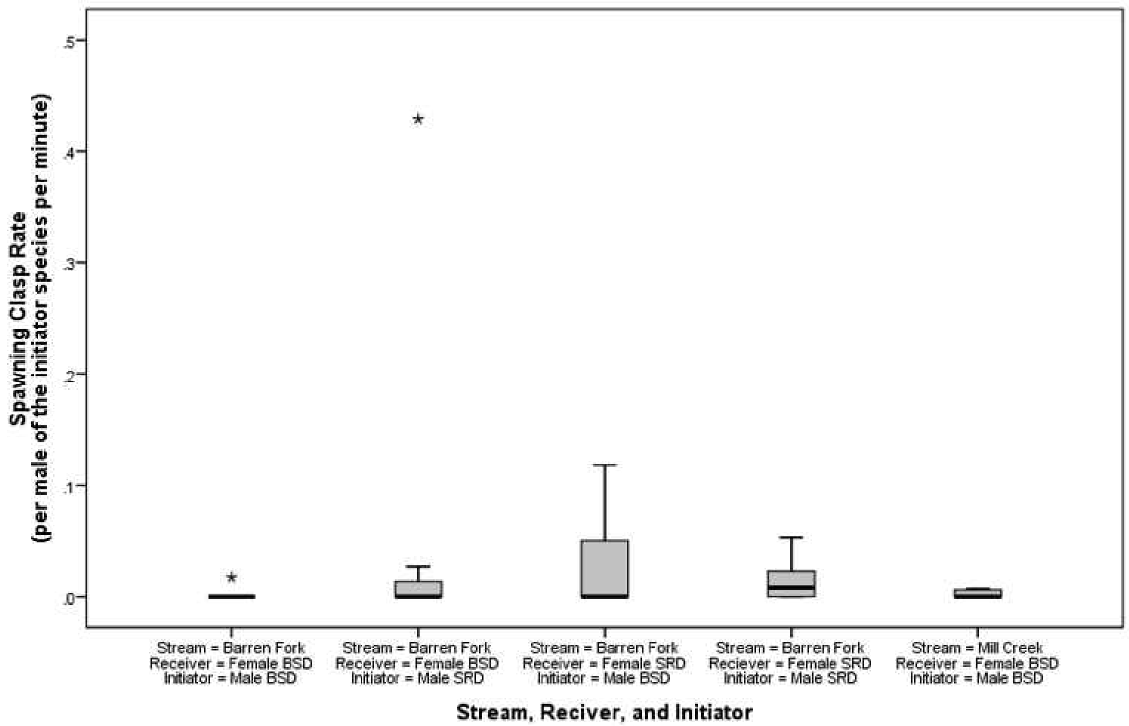

Figure 24 Spawning clasp rate boxplot. Boxplot for behavior rate of male Blackside Dace and male Southern Redbelly Dace forming a spawning clasp with females in Barren Fork and Mill Creek, McCreary County, KY, 2014. Spawning clasp rates represent the average number of spawning clasps performed per minute and per individual of the species forming a clasp with the female. Male BSD = male Blackside Dace, and Male SRD = male Southern Redbelly Dace. Data points that are more than Q1 – 1.5*IQR or Q3 + 1.5*IQR are outliers and marked with a circle. Data points that are more than Q1 – 3*IQR or Q3 + 3*IQR are extreme outliers and marked with a star.