Effect of substrate size on sympatric sand darter benthic habitat preferences

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ABSTRACT
The western sand darter, Ammocrypta clara, and the eastern sand darter, A. pellucida, are sand-dwelling fishes that have undergone range-wide population declines, presumably owing to habitat loss. Habitat use studies have been conducted for the eastern sand darter, but literature on the western sand darter remains sparse. To evaluate substrate selection and preference, western and eastern sand darters were collected from the Elk River, West Virginia, one of the few remaining rivers where both species occur sympatrically. In the laboratory, individuals were given the choice to bury into five equally available and randomly positioned substrates ranging from fine sand to granule gravel (0.12–4.0 mm). The western sand darter selected for coarse and medium sand, while the eastern sand darter was more of a generalist selecting for fine, medium, and coarse sand. Substrate selection was significantly different (p = 0.02) between species in the same environment, where the western sand darter preferred coarser substrate more often compared to the eastern sand darter. Habitat degradation is often a limiting factor for many species of rare freshwater fish, and results from this study suggest that western and eastern sand darters may respond differently to variations in benthic substrate composition.

KEYWORDS
Ammocrypta; benthic habitat; sand; siltation; substrate

Introduction
The western sand darter Ammocrypta clara and the eastern sand darter A. pellucida are the only sympatric species of the genus Ammocrypta (Near et al. 2000), and the Ohio River drainage is the sole region where both distributions are known to overlap (Cincotta & Welsh 2010). Over the years, the number of rivers where each species occurs has declined, presumably in response to degradation of physical stream habitat or water quality (Lachner 1956; Kuehne & Barbour 1983). Historically, the two species co-occurred in the Wabash (IN), Green (KY), Cumberland (KY), Kentucky (KY), Big Sandy (KY, WV), and Kanawha (WV) river systems (Williams 1975; Cincotta & Welsh 2010). However, the western sand darter is presumed to be extirpated from the Licking River in the Kentucky River drainage, the Big Sandy River drainage, and portions of the Wabash River drainage (Williams 1975; Burr & Warren 1986; Simon 2006). Several researchers have suggested that siltation of sand habitats has contributed to declines and local extirpations of sand darter populations, and as a result, the western and eastern sand darters have a threatened or critical status throughout most of
their ranges (Holm & Mandrak 1996; Warren et al. 2000; Adams & Burr 2004; Grandmaison et al. 2004; Driver & Adams 2013).

Western and eastern sand darters are psammophiles, or organisms that occupy and thrive in sandy environments and possess a combination of specialized traits to flourish in a habitat of predominantly sand (Schaefer et al. 2005; Zuanon et al. 2006; Carvalho et al. 2014). Both species are slender, elongate, slightly translucent with cryptic pigmentation, and are known for burying in sand substrates (Williams 1975). Consequently, siltation of the stream bed may affect the ability of sand darters to bury into sand habitat (Daniels 1989; Holm & Mandrak 1996; Facey 1998). Sand darters are habitat specialists and thus sensitive to habitat alterations, which is why both species are often valued as indicators of ecosystem integrity (Grandmaison et al. 2004; Drake et al. 2008). Habitat use and sand grain size preference is well documented for the eastern sand darter (Daniels 1993; Facey & O’Brien 2004; Drake et al. 2008; O’Brien & Facey 2008; Tessler et al. 2012; Dextrase et al. 2014), but literature pertaining to the western sand darter is sparse, and little information is available from areas where the two species are sympatric.

Western and eastern sand darters inhabit medium to large rivers with a moderate current, loose sand, and gravel substrates, and spend the majority of the time buried just below the surface of sandy streambeds (Williams 1975; Daniels 1989). Daniels (1993) regarded the eastern sand darter as one of the most habitat selective fishes of all freshwater species preferring areas of greater than 90% sand. In the field and laboratory setting, eastern sand darters exclusively associated with sand substrates, while water velocity, depth, and distance from the bank had little to no effect on the distribution of individuals (Daniels 1993). Laboratory studies that investigated eastern sand darter substrate use reported that individuals generally used medium (0.25–0.5 mm) sized particles (Daniels 1993; O’Brien & Facey 2008). Furthermore, field surveys revealed that the species was commonly detected in sand comprised of fine to medium (0.12–0.5 mm) particles, while few individuals were detected in areas where the particle size was larger than 1.0 mm (Facey & O’Brien 2004; Tessler et al. 2012; Dextrase et al. 2014). Pflieger (1971) noted that in Missouri the western sand darter avoided strong currents, inhabiting shallow backwater areas, as well as quiet margins of a drainage canal at depths up to 1.5 m. Simon et al. (1992) observed the western sand darter spawning and described the habitat as an area on the downstream side of an island with a slow current over coarse sand.

Although sand grain size appears to be an integral life history component of the western sand darter, little information is available on substrate size preference for this species. Additionally, little research exists documenting habitat selection behavior where both the western and eastern sand darters are sympatric. This study sought to examine western and eastern sand darter benthic habitat preferences in laboratory aquaria to (1) determine substrate size selection for the western sand darter, (2) evaluate substrate selection in an environment where the two species are sympatric, and (3) assess if substrate selection differs significantly between the two species.

**Methods**

**Fish collection and aquaria setup**

A total of 20 western sand darters and 20 eastern sand darters were collected from the lower Elk River during October 2015 and transported to the laboratory in an aerated cooler (Figure 1). The laboratory trials took place over six weeks from October to early December 2015. The western and eastern sand darters mean total length was 52.7 mm (SD 2.30 mm) and 56.9 mm (3.24 mm), respectively. For collection, a straight 1.5 × 3 m seine with 3 mm mesh was used in wadable areas of the river, including upstream and downstream parallel seine hauls and perpendicular hauls pulled into shore (O’Brien & Facey 2008; Driver & Adams 2013). Sampling for the sand darters occurred during the fall because the Elk River experiences lower flows (i.e. <350 cfs) during this time. In the laboratory, fish were placed initially in a 473 L (183 cm × 46 cm × 56 cm) glass aquaria and allowed to acclimate prior to the start of the substrate selection trials. Water conditions within each tank were
maintained by a sequence pump (2.6 L/min) recirculating water from a 379 L sump to the aquaria. Water quality was controlled with carbon filters, bio balls, and freshwater substitutions. The room temperature within the laboratory during late fall remained between 15 and 17 °C. Photoperiod was maintained with wide spectrum fluorescent plant bulbs and an electric timer (12 h light, 12 h dark). The fish were fed frozen bloodworms (chironomids) every other day throughout the experiment. On a day with a substrate trial, the fish were fed after the experiment.

**Experimental design**

The experimental design was similar to other aquaria-based fish burying behavior and habitat selection studies (Daniels 1989, 1993; Smith et al. 2011). The sand darters were allowed to acclimate in the laboratory aquaria for at least seven days prior to the start of the experiment. After the acclimation period, a subset of individuals were placed into the experimental aquarium sections. The purpose was to assess western sand darter habitat selection with and without the presence of the eastern sand darter. Two 473 L (183 cm × 46 cm × 56 cm) glass aquaria were divided in half by a mesh barrier, thus creating a total of four aquarium sections of equal size. For the first experiment, six western sand darters were placed in one aquarium section, and another six western sand darters were placed in a second aquarium section. For the second experiment, three western sand darters and three eastern sand darters were placed in the third aquarium section, and another three western sand darters and three eastern sand darters were placed in the fourth aquarium section. The same individuals were used in each section throughout the 15 trials.

Each aquarium section contained five plastic containers (24.9 × 15.7 × 5.3 cm) filled with approximately 5 cm of substrate: fine sand (0.125–0.25 mm), medium sand (0.25–0.5 mm), coarse sand (0.5–1.0 mm), very coarse sand (1.0–2.0 mm), and granule gravel (2.0–4.0 mm) (Wentworth 1922). Sand for this study was collected primarily from the Elk River (90%), as well as a later addition of aquaria sand (CaribSea Super Naturals Aquarium sand; 10%) to supplement the finer sand that was lost in suspension between trials. The sand for the substrate trials was sifted using a Gilson
20.3 cm (8 in) sieve Shaker (115 V/60 Hz) with US Standard brass sieves. Finer substrate was not used since particles smaller than fine sand may not remain settled due to fish activity and current from the water filter (O’Brien & Facey 2008).

At the start of each trial, fish were released into an aquarium section and given a choice of five equally available and randomly positioned substrate types. The random placement and the equal proportion of substrate types in a controlled environment allowed us to evaluate benthic habitat selection and preference (e.g. Garshelis 2000). A total of 15 trials were conducted with each trial lasting 48 h. At the end of each trial, the substrate containers were capped and transferred individually to a separate container, where the darters were gently removed from the sand, identified to species, and enumerated. We were concerned that an ideal-free distribution effect (i.e. Fretwell & Lucas 1969) could prevent six individuals in an aquarium section from using the same substrate container. A pilot study, however, found that 10 individual fish of both species would readily bury in an individual substrate container. In the Elk River, both species are syntopic and have been detected within the same sandbar (Cincotta & Welsh 2010). It is also possible that another density-dependent effect occurred, where an individual was attracted to a substrate container because it was being used by one or more individuals. Our study design, however, did not allow for measurement of this type of effect, and no known literature exists suggesting this type of social attraction. Overall, our sample size of fish was low because of difficulty of detecting the western sand darter and the conservation status associated with the western sand darter (WVDNR 2015).

Data analysis

To evaluate resource selection, the log-likelihood ratio test was used to determine if sand darter substrate selection was in proportion to its availability (i.e. random or nonrandom) within the experimental aquaria (Manly et al. 2002). Data for the western sand darter only aquaria and the combined species aquaria were pooled over the two tanks for each substrate size class and trial. Selection ratios were then calculated following Manly et al. (2002) to evaluate substrate size preference. This method assumes that there is no unique identification of individuals, the proportion of resource categories are known, and a random sample of used resources is taken. The selection ratio for a given group is the proportion of used units in a category over the proportion of available categories (Manly et al. 2002). Selection ratio values greater than 1 indicate selection, and values less than 1 indicate avoidance (Manly et al. 2002). Bonferroni 95% confidence intervals were calculated for each selection ratio and were considered statistically significant when the interval did not contain the value of 1 (Manly et al. 2002). Furthermore, pairwise comparisons with Bonferroni 95% confidence intervals were generated to assess differences between grain size preferences. The substrate category granule gravel was removed from all statistical analyses because neither species utilized this habitat type.

A multinomial logistic regression model was used to compare if substrate size selection differed significantly between the two species of sand darters (Hosmer & Lemeshow 2000). Following Smith et al. (2011), the response variable was substrate type and the explanatory variables were species and trial. Deviance statistics were used to determine if substrate selection significantly differed between the western and eastern sand darters. Furthermore, odds ratios were assessed to evaluate the effect of species on substrate selection. Odds are the ratio between the probability of using or not using a substrate type. The category ‘fine sand’ was designated as our reference category for the odds ratio. The reference category is an arbitrary designation and should be one which makes the subsequent inference the simplest or the most meaningful (Rogers & White 2007). Wald confidence intervals were estimated for the odds ratios to further examine significant differences between substrate size selections. Wald’s confidence intervals that did not contain the value of 1 were statistically significant (Hosmer & Lemeshow 2000). The selection ratios, Bonferroni 95% confidence intervals, and pairwise comparisons were calculated using statistical software R (version 3.2.3), and the multi-logit model, odds ratios, and Wald’s confidence intervals were generated using SAS (version 9.4).
Results

Western sand darter habitat use

The log-likelihood ratio revealed that substrate selection was not in proportion to its availability \((p = 0.04)\), indicating that sand grain size selection in the aquaria was nonrandom. Across the 15 trials, there were 174 instances of western sand darters found buried in the substrate, while there were 6 instances in which individuals were found above the surface of the sand. Western sand darters primarily buried in coarse (33%) and medium sand (28%), followed by fine (20%) and very coarse (19%) sand. No individuals were detected in granule gravel. Western sand darters selected for coarse \((\hat{w}_c = 1.31)\) and medium \((\hat{w}_m = 1.10)\) sand, and selected against fine \((\hat{w}_f = 0.81)\) and very coarse \((\hat{w}_v = 0.78)\) sand (Table 1). However, the Bonferroni 95% confidence intervals did not indicate significant selection for or against a specific substrate category (Table 1). The substrate selection ratios were further examined with a pairwise comparison with Bonferroni 95% confidence intervals, which revealed that coarse sand was selected with a higher probability compared to very coarse sand \((p = 0.05)\) and coarse sand was selected over fine sand but not significantly \((p = 0.08)\) (Table 2). Although the results were not all statistically significant, they suggest that western sand darters exhibited a tendency to select for sand habitat ranging in size from medium to coarse grains (0.25–1.0 mm) (Figure 2).

Sympatric microhabitat use

In the combined species aquaria, western and eastern sand darters each displayed nonrandom substrate selection \((p < 0.01)\). Across the 15 trials, western sand darters were found buried 88 times and eastern sand darters were found buried 84 times. Western sand darters were detected above the substrate just two times, and eastern sand darters were detected above the substrate six times. Western sand darters primarily buried in coarse (39%) and medium (32%) sand, while eastern sand darters primarily buried in medium (34%) and fine (33%) sand. To examine selection or avoidance, selections ratios were calculated for each species. Western sand darters significantly selected for coarse sand, while significantly selecting against fine and very coarse sand (Table 1). Eastern sand darters selected for fine, medium, and coarse sand, while significantly selecting against very coarse sand (Table 1). The pairwise comparisons revealed that western sand darters had a significantly higher probability of selecting coarse sand over fine and very coarse sand, as well as medium sand over very coarse sand (Table 2). In contrast, the eastern sand darter had a significantly higher probability of selecting fine, medium, and coarse sand over very coarse sand (Table 2).

| Table 1. Substrate selection ratio estimates and the Bonferroni confidence intervals for the western sand darter only aquaria and combined species aquaria. Selection ratio values > 1 indicate selection, while values < 1 indicate avoidance. An asterisk * indicates significant selection or avoidance. |
|---------------------------------|--------|-------|--------|
| Substrate type                  | Selection ratio | Lower CI | Upper CI |
| Western sand darter (single species aquaria) |                |        |        |
| Fine sand                       | 0.81   | 0.532 | 1.078  |
| Medium sand                     | 1.10   | 0.798 | 1.408  |
| Coarse sand                     | 1.31   | 0.992 | 1.628  |
| Very coarse sand                | 0.78   | 0.513 | 1.051  |
| Western sand darter (sympatric aquaria) |                |        |        |
| Fine sand                       | 0.64   | 0.287 | 0.985* |
| Medium sand                     | 1.27   | 0.827 | 1.719  |
| Coarse sand                     | 1.55   | 1.079 | 2.011* |
| Very coarse sand                | 0.55   | 0.218 | 0.872* |
| Eastern sand darter (sympatric aquaria) |                |        |        |
| Fine sand                       | 1.33   | 0.872 | 1.794  |
| Medium sand                     | 1.38   | 0.915 | 1.847  |
| Coarse sand                     | 1.05   | 0.618 | 1.478  |
| Very coarse sand                | 0.24   | 0.007 | 0.469* |
Comparing the selection ratios, western and eastern sand darters each demonstrated a strong preference for medium sand. However, eastern sand darters selected for fine sand ($\hat{w}_i = 1.33$), while western sand darters selected against fine sand ($\hat{w}_i = 0.64$; Figure 2). The multinomial logistic regression demonstrated that substrate size selection differed significantly between the sand darter species for at least one substrate category ($p = 0.02$). The effect of sand darter species on substrate selection was further evaluated using odds ratios and Wald confidence intervals, with the reference category fine sand. The odds ratios demonstrated a significant effect for species on coarse (odds ratio 3.26, CI [1.31, 8.13]) and very coarse (odds ratio 5.25, CI [1.50, 18.40]) sand. No species effect was found for medium sand (odds ratio 1.97, CI [0.81, 4.83]). Thus, western sand darters compared to eastern sand darters are 5.25 times more likely to select very coarse sand over fine sand and 3.26 times more likely to select coarse sand over fine sand. Overall, the western sand darter had a higher probability of selecting medium, coarse, and very coarse sand over fine sand compared to the eastern sand darter.

### Discussion

Our aquaria-based study represents the first evaluation of sand grain size selection and preference for the western sand darter and the first investigation of substrate selection and preference in a region where the two species of sand darters are sympatric. The western and eastern sand darters are habitat specialists, occupying areas that consist of predominantly sand (Simon et al. 1992; Daniels 1993). Western sand darters preferred coarse to medium sand, while the eastern sand darters substrate preference mirrored previous studies, with the species preferring fine to medium sand grains and coarse sand to a lesser extent (e.g. Daniels 1993; Facey & O’Brien 2004). In general, western sand darters selected for a narrower range of substrate sizes compared to the eastern sand darter, and habitat use overlap occurred most often in the medium sand category. This study was contingent upon five designated substrate types that were similar to previous sand darter habitat use studies (Daniels 1993; O’Brien & Facey 2008) and represented a range of benthic habitats both species may encounter (Welsh & Perry 1998; Facey & O’Brien 2004; Tessler et al. 2012). Habitat use in the field could vary compared to the aquaria-based study, but our results demonstrated that each sand

| Substrate comparison | Lower CI | Upper CI | $p$ Value |
|----------------------|---------|---------|-----------|
| Western sand darter (single species aquaria) |         |         |           |
| CS vs. FS | $-0.039$ | $1.032$ | $0.080$ |
| CS vs. MS | $-0.349$ | $0.722$ | $1.000$ |
| FS vs. MS | $-0.846$ | $0.226$ | $0.710$ |
| CS vs. VCS | $-0.002$ | $1.069$ | $0.051$ |
| FS vs. VCS | $-0.498$ | $0.573$ | $1.000$ |
| MS vs. VCS | $-0.188$ | $0.883$ | $0.489$ |
| Western sand darter (sympatric aquaria) |         |         |           |
| CS vs. FS | $0.263$ | $1.496$ | $0.002^*$ |
| CS vs. MS | $0.275$ | $0.892$ | $1.000$ |
| FS vs. MS | $-0.504$ | $0.012$ | $0.058$ |
| CS vs. VCS | $0.978$ | $1.594$ | $<0.01^*$ |
| FS vs. VCS | $0.098$ | $0.714$ | $1.000$ |
| MS vs. VCS | $0.702$ | $1.318$ | $0.017^*$ |
| Eastern sand darter (sympatric aquaria) |         |         |           |
| CS vs. FS | $-0.948$ | $0.565$ | $1.000$ |
| CS vs. MS | $-1.043$ | $0.470$ | $1.000$ |
| FS vs. MS | $-0.852$ | $0.661$ | $1.000$ |
| CS vs. VCS | $0.135$ | $1.647$ | $0.013^*$ |
| FS vs. VCS | $0.226$ | $1.838$ | $0.001^*$ |
| MS vs. VCS | $0.421$ | $1.934$ | $<0.01^*$ |
Figure 2. Western sand darter only experiment (A), western sand darters from the combined aquarium (B), eastern sand darters from the combined aquaria (C), and combined selection results (D). Values above the dashed line at 1 indicate selection and values below indicate avoidance. Bonferroni 95% confidence intervals that do not contain the value of 1 are statistically significant.
darter species exhibited nonrandom selection of substrate types, indicating that a certain benthic habitat was preferred compared to the other available sizes.

The western sand darter was recently discovered in the Elk River, where it was previously misidentified as the eastern sand darter (Cincotta & Welsh 2010). After the discovery, eastern sand darter museum specimens collected from 1986 to 2006 were reexamined, and a total of 17 western sand darters were documented in the lower 36 rkm of the Elk River, and all co-occurred with the eastern sand darter. Therefore, the two species are sympatric within the lower 36 rkm of the Elk River, whereas the eastern sand darter can be detected up to 135 rkm from the mouth (Figure 1; Welsh & Perry 1998; Cincotta & Welsh 2010). The sand darters differences in substrate selection may influence the western sand darter’s limited range in the Elk River. The restricted range of the western sand darter compared to the eastern sand darter in the Elk River is of conservation concern, since the Elk River is the only known location in West Virginia where the western sand darter persists, and represents the southeastern extent for both species (Cincotta & Welsh 2010).

Habitat availability below the Sutton Dam (lower 190 rkm) is potentially limited for the western sand darter compared to its more habitat generalist sister species. As a result, the quality of a certain sand habitat likely varies depending upon its position in the river, which is ultimately controlled by fluvial geomorphology, as well as soil development and vegetation (e.g. Vannote et al. 1980; Jackson et al. 2001; Wang et al. 2003). Given that western sand darters are restricted to the lower 36 rkm of the Elk River, this implies that this section of the river has more suitable habitat for the western sand darter (i.e. higher proportions of medium to coarse sand). Thus, larger more contiguous sand patches are presumably more available in the lower reaches of the Elk River, where additional sediment is added to the system from contributing tributaries. Furthermore, the maintenance of these sandy depositional areas is likewise influenced by the presence of the Sutton Dam, which impounds a large 6 km² reservoir. The dam alters natural flow regimes and changes scouring and depositional patterns (e.g. Baxter 1977; Power et al. 1996; Poff & Hart 2002).

The aquaria-based experiment provided information on habitat use of two species of sand-dwelling darters, data that can be challenging to obtain in the field because of their burying behavior. Preference for larger substrate sizes may indicate that western sand darters are more sensitive to fine sediment deposition compared to eastern sand darters. Habitat selection study results indicate where a species is likely to find a set of conditions within their physiological tolerance (Rice 2005). Thus, the siltation of the sand darters preferred habitat is potentially more limiting for the western sand darter and may be a contributing factor to the western sand darters sporadic distribution in the Elk River and the Ohio River drainage. For instance, the eastern sand darter persists in the Licking (Kentucky), Tug (Kentucky, West Virginia), and Wabash (Indiana) rivers, whereas the western sand darter is presumed to be extirpated (Burr & Warren 1986; Simon 2006). Likewise, in the Wabash River, the eastern sand darter has been reported as increasing in distribution and abundance, while the western sand darter remains undetected or extirpated from the main channel (Simon 2006). Furthermore, in Indiana, the western sand darter is a species of special concern, while the eastern sand darter was delisted following a statewide survey of the species (INDNR 2004). Similar research could be conducted in other rivers that contain western sand darters, which would further document sand grain size preference across the range of this species.

The types of rivers that western and eastern sand darters occupy (i.e. moderately large with a low gradient) are often located in landscapes that attract urban development, industrialization, and agriculture. Therefore, these species face potential impacts from land use activities that increase the amount of siltation in the watershed; however, with improved land use practices, and other efforts to minimize impacts to watersheds, it is possible to protect and enhance sand darter populations (Grandmaison et al. 2004; COSEWIC 2009; Tessler et al. 2012). There are several places where the eastern sand darter was absent for more than 50 years and have since recolonized improved reaches of rivers (Tessler et al. 2012; Hopkins & Zimmerman 2014). Thus, understanding habitat use preferences can aid in the recovery of both species. Further information gained from sand darter
habitat use studies may provide insight into the health and overall quality of an aquatic ecosystem, especially in large river systems that are impacted by urban development and intensive agriculture.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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