SHORTER CONTRIBUTIONS

Disentangling Female Mate Choice and Male Competition in the Rainbow Darter, *Etheostoma caeruleum*

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Sexual selection via female choice is assumed to be common in darters despite few studies demonstrating the phenomenon. This study examines the breeding behaviors of the rainbow darter, *Etheostoma caeruleum*, and addresses the following four questions: Do females exhibit mating preferences? Which behavioral variables are good measures of female mating preferences? Which male behaviors are used in the context of mating? How do female choice and male competition affect mating success? In dichotomous choice tests, where females could choose between males from two different populations, females performed a precopulatory behavior (no-sedigs) significantly more often in front of males from Prairieville Creek than they did in front of males from Seven Mile Creek. However, no pattern of choice was detected when preference was measured as the amount of time spent associated with each male. When the animals were allowed to interact freely, males competed aggressively over the female by chasing and attacking each other. Group spawns occurred where two males mated simultaneously with the female. Male spawning success was positively correlated with male guarding ability (the ability to prevent a competing male from coming between himself and the female). However, there were no significant correlations between overt aggression (chases and attacks) and male spawning success or between female preference (measured in dichotomous choice tests) and male spawning success. Male interactions, in the form of guarding, appear to play a larger role in determining male mating success than does female choice.

SEXUAL selection, defined as differential reproduction owing to variation in the ability to obtain mates, is thought to be common in freshwater fishes, particularly those possessing sexually dimorphic color patterns (Farr, 1989; Ryan and Keddy-Hector, 1992; Seehausen and van Alphen, 1998). Recent studies have focused on the role of mating preferences in determining male mating success in fishes (reviews in Andersson, 1994; Dugatkin and FitzGerald, 1997; although see Evans and Norris, 1996). Many of these studies measure female choice using dichotomous choice tests in which the time spent in association with a male is the measure of female preference. There are two problems associated with such studies. First, it is unclear whether such measures of choice indicate a mating preference or simply a tendency to aggregate. Second, these studies intentionally exclude male/male competition so as not to obscure patterns of preference. Although understandable, this leaves open the question of whether female preferences can be realized in the face of male/male competition.

Darters (Teleostei, Percidae) are a species-rich group of North American freshwater fishes (162 species; Nelson, 1994). Sexual selection is assumed to be important in the evolution of darters (Page, 1983; Page and Swofford, 1984; Porterfield, 1999). Males of most darter species bear conspicuous color patterns or presumably expensive ornaments such as egg-mimics. The roles of mate choice and competition in darter mating systems are poorly understood, and few studies have identified specific behaviors that provide clues to the mode of sexual selection in these fishes. The subgenus *Catonotus*, in which males of several species possess egg-mimic structures, has been most thoroughly studied. Knapp and Sargent (1989) demonstrated that male *Etheostoma flabellare* with egg-mimic structures on the first dorsal fin are more likely to spawn than males that have had their egg-mimics removed. Similarly, females of four species (*Etheostoma oophylax, Etheostoma neopterum, Etheostoma pseu-*

dovulatum, and *Etheostoma squamiceps*) spawned in the nests of males with intact egg mimics as opposed to males whose egg-mimics had been removed (Strange, 2001). In *E. virgatum,* Porter et al. (2002) found a positive correlation between the number of females spawning with a male and the number of egg mimics (spots on dorsal fins) possessed by the male. Thus, egg-mimics are an important determinant of mating success in male *Catonotus* individuals, however, whether selection is based on female choice or male competition is unclear. In the subgenus *Boleosoma,* Grant and Colgan (1983) demonstrated that male *Etheostoma nigrum* that actively guard their nests are visited more often by females and have more eggs in their nests. Finally, in the subgenus *Oligocephalus,* Pyron (1995) found no evidence for female mate choice in *E. spectabile* where preference was measured as the amount of time spent associated with a male. In addition, Pyron (1995) found no evidence that male secondary sexual traits are involved in male competition.

Here I examine the breeding behaviors in the rainbow darter, *Etheostoma caeruleum* (subgenus *Oligocephalus*) and address the following four questions: (1) Do females exhibit mating preferences? (2) Which behavioral variables are good measures of female mating preference? (3) Which male behaviors are used in the context of mating? (4) How do female choice and male interactions affect male mating success?

MATERIALS AND METHODS

Etheostoma caeruleum is a small bottom-dwelling fish that inhabits shallow riffles in swift streams and gravel areas in clear lakes (Page, 1983). Adult males exhibit brilliant blue and red color patterns, whereas females and juveniles are cryptic (Page, 1983; Page and Burr, 1991). The mating system is promiscuous. During the breeding season, males maintain moving territories on riffles (Winn 1958b). Breeding males remain on their territories except for brief periods when they leave to forage (Winn 1958b). Outside of mating, females remain off of the territories and forage (pers obs). When a female is ready to spawn, she moves to the riffle and is immediately followed and defended by a male. The male attempts to keep competing males away by chasing and attacking them. The female solicits spawnings from the male by performing nosedigs, a behavior in which the female digs her head into the gravel, quivers, and then moves down and forward into the gravel so that her ventral half is buried in the substrate. The male then assumes a position on

top of the female, and the two fish vibrate rapidly during which time eggs and sperm are released (Winn, 1958a,b). Occasionally, nearby males dash in and release their sperm next to the pair of spawning fish (pers obs). There is no parental care, and eggs are left buried in the substrate (Page, 1985).

Etheostoma caeruleum individuals were collected with a kicknet between March and May 1995 at Seven Mile Creek, Kalamazoo County, and at Prairieville Creek, Barry County, Michigan, USA. These streams are two separate branches of the Kalamazoo River drainage (MI). Animals were returned to Kellogg Biological Station where females and males were kept in separate 40-liter aquaria containing a gravel substrate. Water was filtered with carbon filters. Other than this filtration, there was no water current in the aquaria. Animals were fed daily with live tubifex worms and chironomids. All fish were returned to their native populations at the end of the experiment.

Mate choice trials.—Females from both populations were given a choice between Seven Mile and Prairieville Creek males. Fish from these two populations were used because they appeared to differ in their color pattern and body size. Males from Seven Mile Creek appeared darker with more large patches of blue. Prairieville Creek males appeared lighter with more contrast between red and blue hues (pers. obs.). Seven Mile Creek males were larger than Prairieville Creek males (Seven Mile Creek: mean = 52.174 ± 1.589 mm SE, Prairieville Creek: mean = 45.731 ± 0.835 mm SE, $t = 3.710$, df = 47, $P = 0.001$). Similarly, Seven Mile Creek females were larger than Prairieville Creek females (Seven Mile Creek: mean = 50.000 ± 1.113 mm SE, Prairieville Creek: mean = 44.158 ± 1.018 mm SE, $t = 3.210$, df = 24, $P = 0.004$).

Three sequential mate choice trials were conducted on each group of fish. A group consisted of one Prairieville Creek male, one Seven Mile Creek male, and one female from one of the populations. In all three trials a mixture of fluorescent and incandescent spotlights provided lighting. Aquaria contained gravel substrate. There was no water filtration in these aquaria as fresh water was replaced between trials. Because of a paucity of animals, two males from Seven Mile Creek were reused. One male was used in two replicates—one replicate in which spawning occurred and another in which spawning did not occur. Another male was used in three replicates, and no spawning occurred in any of these replicates (see Appendix 1). Correction of the analysis for the reuse of these two
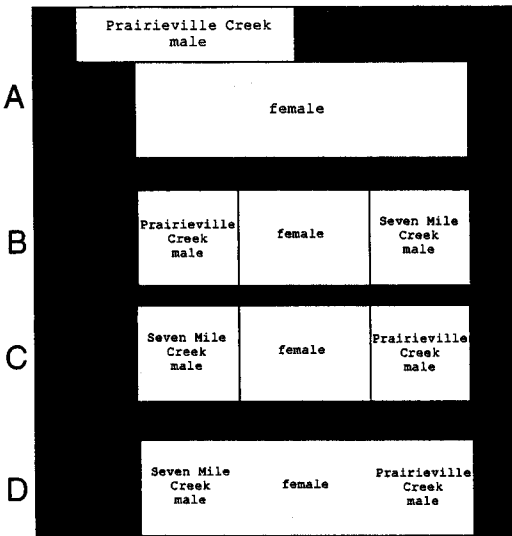
Fig. 1. Mate choice aquaria set-up. (A) Trial 1. The female has visual access to both males. (B) Trial 2. The female has both visual and olfactory access to both males. (C) Trial 3. Identical aquarium set-up as in trial 2 only the positions of the males have been reversed. (D) 1.5-h observation period. Fish are allowed to interact freely for 1.5 h. For the purpose of demonstration the Prairieville Creek male is initially placed in the left aquarium, but the position of males was randomized across replicates.

males did not alter the results qualitatively. No males from Prairieville Creek were reused, and no females from either population were reused. The night prior to experimentation, the standard lengths of the animals were measured, and animals were placed in the aquaria for trial 1 (Fig. 1A). Males were placed randomly in one of the two 20-liter aquaria. Pieces of cardboard were placed between all the aquaria to prevent visual access prior to the experiment.

In trial 1, the female was given visual access to the two males. The piece of cardboard preventing visual access between the males remained in place. Animals were allowed to interact for 30 min. In trial 2, the female's 40-liter aquarium was divided into thirds with Plexiglas® barriers creating three separate compartments (Fig. 1B); these barriers were not waterproof and allowed olfactory cues to pass. The female was placed in the central compartment, and males were transferred from the 20-liter aquaria to the end compartments. Males retained their relative positions from trial 1. Animals were allowed to interact for 30 min. In trial 3, the position of the males was switched to control for any side biases that females may have (Fig. 1C). Again, the fish were allowed to interact for 30 min. These trials were performed sequentially,

and, hence, were completed in 90 min. No additional acclimatization was allowed for trials 2 and 3.

Immediately following the three mate choice trials, the fish were allowed to freely interact in the 40-liter aquarium with no partitions for 1.5 h. If no spawning took place during the 1.5-h observation period, then the animals were left in the aquarium for 24 h. Reproductive behavior was recorded from videotape, and aquaria were searched for the presence of eggs. Each replicate was categorized as either having spawned (spawning occurred during the 1.5-h observation or during the subsequent 24 h) or not spawned (no spawning took place during either the 1.5-h observation period or during the subsequent 24 h).

In all three choice trials, behaviors were recorded with a video camera. Tapes were used to assess the number of nosedigs females performed in front of each male and the amount of time females spent in front of each male. A female was recorded as spending time with a male if she was located on that male's side of the aquarium. A female was recorded as performing a nosedig in front of a male if she performed a nosedig on that male's side of the aquarium. The number of nosedigs performed by the female was summed from all three trials yielding total number of nosedigs. The total number of nosedigs was compared between replicates where spawnings did and did not take place to determine whether this behavior is associated with mating condition.

In addition, nosedig and time scores were calculated for each trial. Nosedig scores were calculated as the proportion of nosedigs performed per trial to the Prairieville Creek male (number of nosedigs performed to Prairieville Creek male divided by the total number of nosedigs performed in that trial). Similarly, time scores were calculated as the proportion of time each female spent in front of the Prairieville Creek male. This measure of preference is similar to that used by Pyron (1995) where females in the field were designated as spending time with a focal male if she was within 5 cm of his cubicle. Finally, overall nosedig and time scores were calculated as the proportion of nosedigs and time spent with each male pooled over the three trials. Scores above 0.5 indicate that females spent more time near the Prairieville Creek male, and scores below 0.5 indicate that females spent more time near the Seven Mile Creek male.

The preferences of the females from the two populations were pooled providing no significant differences existed. A Kolmogorov-Smirnov

Lilliefors test was used to discern whether the variables were normally distributed, and either a parametric one-sample t-test or a nonparametric Wilcoxon Signed Ranks test (depending on whether the assumptions of normality were met) was used to test whether female preferences differed statistically from 0.5, a null expectation of no choice.

Spawning dynamics.—During the 1.5-h observation period following the three mate choice trials, the number of attacks, chases, and guards performed by each male were recorded from direct observation. Guarding behavior occurred when one male was closer to the female and successfully prevented a competing male from coming between him and the female. From these data, the total number of attacks, chases, and guards were calculated as well as scores for each variable (e.g., proportion of the behaviors performed by the Prairieville Creek male, an attack score greater than 0.5 indicates that Prairieville Creek males performed more attacks than Seven Mile Creek males). The number of times each male was closer to the female when she performed a nosedig and the number of times each male spawned with the female were also recorded. From these data, I calculated the spawning score (proportion of spawns with Prairieville male) and the male proximity nosedig score (proportion of time Prairieville male was closer to the female when she performed a nosedig). In addition, the number of group spawnings where the two males simultaneously spawned with the female and the number of spawnings in which each male mated as the dominant, guarding male and as the sneaker male were recorded. The dominant, primary male was defined as the male that initiated spawning with the female. The sneaker male was the male that released sperm next to a pair of fish that had already begun spawning. Male spawning success was defined as the number of spawns where a male spawned as the dominant, primary male.

Mann-Whitney U-tests and t-tests were performed on the total number of attacks, chases, and guards to determine whether these behaviors were performed primarily in the context of spawning. The correlation coefficients between spawning and attack, chase, guard, male proximity nosedig scores, and preference scores were calculated to determine which behaviors were most closely related to spawning success. All probability tests were two-tailed, and results were considered significant at $P < 0.05$. All analyses were performed with SAS v.8.
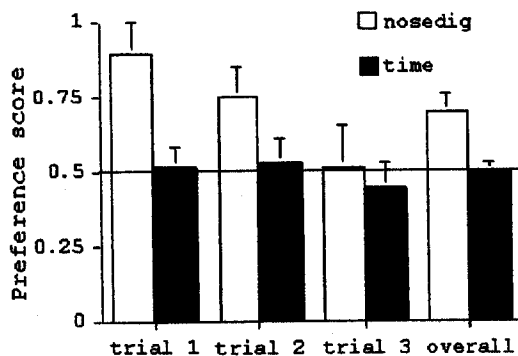

Fig. 2. Preference scores in trials 1, 2, 3, and the overall preference score using nosedigs and time as measures of preference. Data are shown for replicates where spawnings occurred within 24 h (i.e., during 1.5-h observation period or within subsequent day). Sample sizes are as follows: nosedig scores trial 1 $n = 4$, trial 2 $n = 10$, trial 3 $n = 10$, overall $n = 12$; time scores $n = 15$ in all cases. Means and standard errors are shown. Preference is detected when the preference scores differ from a null expectation of 0.5 denoted by the black line.

RESULTS

Female mate choice.—Of the 26 replicates, 11 contained spawns during the 1.5-h observation period, six contained spawns during the subsequent 24 h, and nine contained no spawns. To discern which variables were appropriate measures of female mating preferences, the behavior of females that spawned was compared with the behavior of females that did not spawn. Nosedigs were only performed in replicates where females went on to spawn within 24 h ($P = 0.0015$, $n = 26$).

When measured as nosedig scores, female mating preferences differed significantly from a null expectation of no choice (Fig. 2). Females performed more nosedigs to Prairieville Creek males in trials 1 and 2 (trial 1: Wilcoxon Signed Ranks test $Z = -1.890$, $n = 4$, $P = 0.059$, trial 2: One sample t-test $t = 2.632$, df = 9, $P = 0.027$). The overall nosedig score also differed significantly from the null expectation of 0.5 (One sample t-test $t = 3.487$, df = 11, $P = 0.005$). Nosedig scores did not differ significantly from 0.5 in trial 3 where the position of the males was switched ($t = 0.109$, df = 9, $P = 0.916$). Sample sizes were less than 17 because some females did not perform nosedigs in all of the trials. Female mating preferences were not detected with time scores (Fig. 2). Neither time scores nor the overall time score differed significantly from 0.5 in any of the trials ($P > 0.500$ in all cases).

In trial 3, reversing the position of the males

TABLE 1. SPEARMAN CORRELATION COEFFICIENTS BETWEEN SPAWNING SUCCESS AND MALE BEHAVIORS (IN **BOLD**) AND FEMALE PREFERENCES (IN NORMAL TYPE). Unadjusted probability values and sample sizes are shown.

Variable	r_s	P	n
Male proximity nose dig	0.915	0.0001*	11
guard score	0.880	0.0004*	11
attack score	0.368	0.323	9
preference—trial 3 (nose dig)	0.247	0.491	10
chase score	0.154	0.716	8
preference—trial 3 (time)	0.134	0.694	11
preference—trial 2 (nose dig)	−0.014	0.974	8
preference—trial 2 (time)	−0.084	0.805	11
preference—trial 1 (time)	−0.453	0.161	11
preference—trial 1 (nose dig)	−0.775	0.225	4

* Statistically significant after sequential Bonferonni correction.

had strong effects upon preference scores in replicates where spawns occurred. All preference scores were negatively correlated in trials 2 and 3 (nosedig scores: $r = -0.834$, $P = 0.010$, $n = 8$, time scores: $r = -0.537$, $P = 0.026$, $n = 17$). Conversely, in replicates where no spawns occurred, reversing the position of the males had a less dramatic effect on female mating preferences. There were no statistically significant relationships between preference scores in trials 2 and 3 (time scores: $r = -0.158$, $P = 0.685$, $n = 9$).

Spawning dynamics.—Males behaved more aggressively within the context of mating. Males performed more attacks, chases, and guards in replicates where spawns occurred within 24 h (Mann-Whitney, $n = 26$, df = 1, total attacks: $P = 0.0084$, total chases: $P = 0.0023$, total guards: $P = 0.0030$). As in the female choice experiment, females only performed nosedigs in replicates where spawns took place ($P = 0.0015$).

Group spawns occur when an alternate male dashes in and releases his sperm next to a spawning pair of fish. Group spawns occurred in five of the 11 replicates in which animals spawned during the 1.5-h observation period. In three of the five replicates, both males spawned as the dominant, guarding male at least once. In four of the five replicates, the smaller of the two males acted as the sneaker male in the majority of the group spawns. The absolute difference in body size between the two males was smaller in replicates where group spawns took place (Mann-Whitney, $P = 0.043$, $n = 11$, df = 1). More chases and guards also took place in replicates where group spawns took place (Mann-Whitney, $n = 11$, df = 1, total chases: $P = 0.043$, total guards: $P = 0.028$).

Aggression levels were higher in replicates where the two males were of similar size. The absolute size difference between the two males was inversely correlated with the total number of chases and attacks performed in replicates where spawns occurred during the 1.5-h observation period ($r_s = -0.721$, $P < 0.02$, $n = 11$; $r_s = -0.676$, $P < 0.05$, $n = 11$, respectively). Males from the two populations did not differ in their tendencies to perform attacks, chases, guards, group spawns, or in their ability to obtain spawns (Wilcoxon Signed Ranks Test, $P > 0.50$ in all cases). Chase and attack scores were positively correlated ($r_s = 0.638$, $P < 0.05$, $n = 12$). Chase and attack scores were not correlated with guard scores ($r_s = 0.181$, $n = 12$, $r_s = 0.156$, $P > 0.500$, $n = 13$).

The relationship between female mating preferences, aggressive male behaviors, and spawning success is unresolved. There were no significant correlations between female mating preferences as measured in trials 1–3 and male spawning score (Table 1). Similarly, none of the overall preference scores correlated with spawning or nosedig scores. Guard scores were highly correlated with spawning scores in the observation period (Table 1). Furthermore, male proximity nosedig scores and spawning scores were highly correlated indicating the male that was closer to the female when she performed a nosedig in the 1.5-h observation period was most likely to spawn with her. However, overt aggression was not strongly correlated with mating success. Neither attack scores nor chase scores were correlated with spawning scores.

DISCUSSION

Which behavioral variables are good measures of female mating preference?—Nosedig behaviors are the most sensitive measures of female mating preference. In *E. caeruleum*, female nosedig behavior provides an accurate measure of female

mating preferences because the motivation for this behavior is unambiguous. In nature, females only perform nosedigs prior to spawning (Winn, 1958a,b; pers. obs.). The results presented here support this finding, since nosedigs were only performed in replicates where spawns took place. The fact that 11 females spawned during the 1.5-h observation period suggests that females had ovulated while being held in the female stock tanks but had not yet dropped their eggs. Unfortunately, the exact duration of the postovulatory period is unknown. In many *Etheostoma* species, females exhibit some type of "head behavior" where females jab at the spawning substrate with their snouts directly before copulating (Bart, 1992; Walters, 1994; Porterfield, 1999). Therefore, these behaviors may provide convenient and representative measures of mating preference for many darters.

The amount of time spent with a given male does not appear to be an accurate measure of female choice. Measures of female mating preferences based on time assume that females are constantly choosing among males, a potentially erroneous assumption. In this study, the average time scores incorporate data from all three trials of all replicates where females spawned. If females were not actively choosing among males at all times, my conclusion of no female mating preferences based on time scores may be erroneous because mating preferences were inferred at times when no preferences existed.

Measures of female mating preferences based on time have worked well in fish species where the reproductive state of the female is easily assessed. In poeciliids, females can store sperm and directly fertilize their eggs when they ovulate. As a result, these females are typically receptive to males as virgins (when they have no sperm) and after giving birth (Kodric-Brown, 1985; Farr and Travis, 1986; Endler and Houde, 1995). In other species, researchers have demonstrated that mating preferences based on time measures correlate with mating success and are therefore accurate measures of mating preferences (Forsgren, 1992; Berglund, 1993). However, when conducting mate choice experiments on previously unstudied organisms, it is imperative to either use a biologically relevant variable (i.e., a variable associated with reproduction) or demonstrate those time measures are associated with some aspect of subsequent spawning behavior.

Examining female choice after the positions of the males had been reversed indicated the presence of side biases. Female mating preferences became linked to their original spatial location. Nosedig scores were negatively correlated between trials 2 and 3 (which differed only in the location of the two males) indicating that females did not follow the male they preferred in trial 2 to the other side of the aquarium in trial 3. This result cannot be accounted for by a preference for simply one side of the aquarium because Prairieville males were placed in the left and right aquaria at random in trial 1. What can account for this behavior? Females may never have to follow males in the wild. Typically, females enter an area and are themselves followed and defended. Females may conceivably choose among males by performing nosedigs at locations that they associate with specific males. Another possibility is that olfaction plays a role in mate choice. Although a given male may be moved to another compartment, the remaining scent may take some time to dissipate. The practice of reversing the position of males to control for side biases has been used predominantly in studies of poeciliids (e.g., Kodric-Brown, 1985) where males are very mobile and spawning is not restricted in location to a specific substrate. In those cases, it is reasonable to expect that females should follow preferred males.

What accounts for female mating preferences?—Females from both populations performed significantly more nosedigs in front of the Prairieville Creek males. Given that females of many fish species prefer larger males (Andersson, 1994; Ptacek and Travis, 1997; Rosenthal and Evans, 1998), this result is unexpected. Females did not prefer larger males, as Seven Mile Creek males were larger than Prairieville Creek males. Females must have relied on visual cues for mate choice because a preference was detected in trial 1 where there were no olfactory cues. Prairieville Creek males may have appeared more attractive because the experimental lighting conditions closely approximated lighting conditions of Prairieville Creek. The sensory environments of the two populations are different. Seven Mile Creek is deeper, contains more dissolved organic material, and has a darker substrate than Prairieville Creek (pers. obs.). Blues may be used more often in waters containing high levels of organic matter because this works to increase the perceived contrast with other color elements (Fuller, 2002). Different sensory environments may result in the evolution of different male color patterns, and these color patterns may be perceived as more attractive when signaling takes place in their own environment (Endler 1992, 1993).

Which male behaviors are used in the context of mating?—Males competed aggressively over spawning opportunities. Attacks, chases, and guards were performed primarily within the context of mating, and aggression levels were higher when males were of similar size and presumably competitive ability. Group spawns occurred predominantly in replicates where males were of similar size and neither was capable of completely dominating the other. The fact that males acting as sneakers in group spawns would also mate as dominant, guarding males indicates that these males may be making the best of a bad situation (Dunbar, 1982; Magnhagen, 1992, 1994). Alternative male mating strategies have been documented for other *Oligocephalus* species including *E. lepidum* and *E. spectabile* (Breder and Rosen, 1966; Robinson and Buchanan, 1992; Pyron, 1995) and for one species of the subgenus *Ozarka*, *E. cragini* (Distler, 1972).

How do female choice and male interactions affect male mating success?—Males that successfully guarded females had higher spawning success. However, overt aggression (attacks and chases) was not correlated with male spawning success. Furthermore, female mating preferences were not associated with male spawning success. Pyron (1995) obtained similar results in *E. spectabile*, a close relative of *E. caeruleum*. He found that neither female mating preferences nor overt male aggression was correlated with spawning success. However, male guarding ability was correlated with spawning success in *E. spectabile*. At one level, the data in the current study are somewhat limited because of low sample size. Larger sample sizes would undoubtedly increase the power to detect relationships between overt aggression and mating success and also between female preference and mating success. Endler (1985) has shown that low-selection differentials are most likely quite common and can produce dramatic changes in phenotypes over many generations. Hence, additional studies with higher sample sizes are vital to understanding sexual selection in darters. Still, this study shows that male guarding ability (ability to prevent other males from coming between himself and the female) and male ability to be closest to the female when she performs a no-sedig are strong predictors of male mating success.

Female mating preferences, presuming they exist, are most likely weak predictors of male mating success for two reasons. First, group spawning will hamper selection acting through female mating preferences even if dominant, guarding males are preferred (Jones et al.,

2002). In this study, group spawns occurred in five of 11 replicates. In another population, 80% of the observed spawns involved 2–5 males simultaneously spawning with the female (Gull Lake population; unpubl. data). Paternity analysis indicates that the expected fertilization success of the guarder and sneaker male is equivalent in-group spawns involving two males (Fuller, 1999). Females may have limited control over male paternity, particularly in areas of high male density.

The second reason female choice may be limited is because exerting preference is costly. Female *E. caeruleum* are fractional spawners and must mate multiple times. Fuller (1998a) estimates females mate an average of 39 times over the course of a breeding season. Costs may accrue through increases in female susceptibility to predation (Forsgren, 1992; Chivers et al., 1995; Fuller and Berglund, 1996) or through decreases in egg viability caused by prolonged mating activities. Survival costs to females accrue in a multiplicative fashion making costs of choice very high if females make a new choice for each spawning. In addition, females have a limited time frame over which they need to spawn their eggs (unpubl.). Females can retain unfertilized eggs for at least two days (perhaps longer), but egg viability decreases with time since ovulation. Excessive choosiness increases the time required to spawn the entire clutch of eggs, particularly in the face of male/male interactions (Fuller, 1998b). Decreases in egg viability with time since ovulation are quite common in external fertilizers (Bry, 1981; Vincent, 1994; De Gaudemar and Beall, 1998). Theoretical investigations suggest that fractional spawners should have decreased levels of preference in comparison to females that can spawn their entire clutch at once (unpubl.).

This is the second study to find no correlation between female preference and male mating success for a species from the subgenus *Oligocephalus*. In contrast, it seems likely that female mating preferences are common and are important determinants of male mating success in the subgenus *Catonotus*, although more definitive experiments are needed (Knapp and Sargent, 1989; Strange, 2001; Porter et al., 2002). In *Oligocephalus*, males guard and fight directly over females producing high levels of male competition and possibly making female choice costly (Fuller, 1998b). In comparison, males of *Catonotus* species compete over breeding sites and most likely have restricted movement. This may allow females to exercise preference with lower costs.

In conclusion, three main findings emerge

from this study. First, the observed pattern of female mating preferences depends critically on the variable used to assess preference. Preference for males from Prairieville Creek was detected when nosedigs were used as the measure of preference. However, no pattern of preference was found when preference was assessed with the amount of time spent near a given male. Nosedigs are only performed when females have ovulated and, hence, provide a useful control for female motivation (i.e., mate choice vs shoaling). The second finding to emerge from this study is that female preference and male aggression are weakly correlated with male mating success. Male guarding behaviors are the best predictors of male mating success. Finally, group spawning was common and was most likely to occur when males were similar in size and competitive ability.

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APPENDIX 1. RAW BEHAVIORAL DATA. Replicate, female population of origin, and female standard length (SL) are listed. The remaining columns list data for males from both populations (Prairiville Creek (P) data on top, Seven Mile Creek (S) data below). The amount of time the female spent in front of each male in trials 1–3 is listed as time 1, and time 2, and time 3. The number of nosedigs females performed in front of each male are listed as ND 1, ND 2, and ND 3. Attack, chase, and guard refer to the number of times each male attacked, chased, or guarded the female from the other male during the 1.5-hour observation period. ND refers to the number of times the female performed a nosedig while the male was closest in proximity during the 1.5-hour observation period. Spawn as dominant refers to the number of times each male spawned as the dominant male. Spawn as sneak refers to the number of times each male sneaked in and spawned next to a spawning pair. Whether the animals spawned within 24 hours is listed. One Seven Mile Creek male was used in two replicates (7, 10) and is marked in *bold italics*. One Seven Mile Creek male was used in three replicates (4, 11, 14) and is marked in **bold**. Another Seven Mile Creek male was used in two replicates (7, 10) and is marked in *bold italics*.

Rep	♀ origin	♀ SL	P/S	SL	Time 1	Time 2	Time 3	ND 1	ND 2	ND 3	Attack	Chase	Guard	ND	Spawn as Dominant	Spawn as Sneak	Spawn w/in 24 h
1	P	50	P	44	1010	500	1570	0	0	0	0	0	0	0	0	0	no
			S	58	790	1300	230	0	0	0	0	0	3	0	0	0	
2	S	52	P	46	1140	10	1800	0	0	0	3	2	5	0	0	0	yes
			S	56	660	1790	0	0	0	0	4	11	38	0	0	0	
3	S	51	P	50	1740	530	1350	31	17	27	3	1	4	4	2	7	yes
			S	52	60	1270	450	0	24	0	32	115	29	20	11	0	
4	S	51	P	40	1030	330	1270	0	0	0	0	1	0	0	0	0	no
			S	56	770	1470	530	0	0	0	0	1	0	0	0	0	
5	P	44	P	47	1330	240	380	9	0	1	0	5	5	0	0	1	yes
			S	45	470	1560	1420	0	0	7	1	0	49	18	2	0	
6	P	50	P	39	850	350	590	0	0	0	0	0	0	0	0	0	yes
			S	53	950	1450	1210	0	0	0	0	0	0	0	0	0	
7	P	40	P	42	1330	1470	130	0	35	2	0	0	0	4	0	0	yes
			S	*50*	470	330	1670	0	3	17	1	8	37	2	1	0	
8	S	49	P	46	320	1010	1730	0	10	17	18	29	5	6	6	0	yes
			S	45	1480	790	70	0	5	0	53	0	58	0	0	4	
9	P	51	P	50	0	830	1010	0	0	0	4	0	29	0	0	0	yes
			S	65	1800	970	790	0	0	0	1	0	69	0	0	0	
10	P	40	P	46	590	300	210	0	0	0	42	2	28	0	0	0	no
			S	*50*	1210	1500	1590	0	0	0	2	0	9	0	0	0	
11	P	49	P	46	70	540	70	0	0	0	1	0	2	0	0	0	no
			S	**56**	1730	1260	1730	0	0	0	0	0	0	0	0	0	
12	P	41	P	50	130	420	1450	0	0	0	0	0	0	0	0	0	no
			S	52	1670	1380	350	0	0	0	0	0	0	0	0	0	

APPENDIX 1. CONTINUED

Rep	♀ origin	♀ SL	SL P	SL S	Time 1 P	Time 1 S	Time 2 P	Time 2 S	Time 3 P	Time 3 S	ND 1 P	ND 1 S	ND 2 P	ND 2 S	ND 3 P	ND 3 S	Attack P	Attack S	Chase P	Chase S	Guard P	Guard S	ND P	ND S	Spawn as Dominant	Spawn as Sneak	Spawn w/in 24 h
13	P	42	50	65	890	910	1470	330	910	850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no
14	P	43	46	56	620	1180	270	1530	40	1760	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	no
15	P	37	40	54	730	1070	1690	110	670	1130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no
16	P	43	44	62	950	850	810	990	100	1700	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	yes
17	P	49	41	50	790	1010	230	1570	1640	160	7	0	2	0	13	0	1	0	0	0	2	3	13	6	6	0	yes
18	P	43	53	44	1600	200	1220	580	970	830	5	15	18	13	8	4	16	1	23	0	1	2	2	8	6	2	yes
19	P	40	46	44	1090	710	1760	40	1040	760	0	0	7	2	0	0	9	1	9	0	2	3	0	0	1	0	yes
20	P	38	55	38	1080	720	1790	10	290	1510	0	0	25	0	14	0	0	0	0	0	3	0	10	14	8	0	yes
21	P	43	52	56	1200	600	340	1460	1410	390	0	0	0	0	0	0	67	0	3	0	1	2	0	0	2	0	yes
22	P	47	46	43	180	1510	1660	140	0	1800	0	0	10	0	17	0	26	0	35	0	2	0	6	0	6	0	yes
23	P	49	42	56	780	1020	1190	610	320	1480	0	0	7	2	3	1	7	0	0	0	2	3	5	1	5	0	yes
24	S	48	41	44	550	1250	1450	350	640	1160	0	0	0	0	4	4	40	0	58	0	2	30	1	8	4	4	yes
25	S	54	43	62	20	1780	0	1800	1800	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	no
26	S	45	44	48	840	960	1280	520	150	1650	0	0	1	0	0	0	31	3	34	0	25	0	12	0	0	0	yes