MATING BEHAVIOR AND THE EFFECTS OF TURBIDITY ON

PREFERENCES FOR SIZE IN THE FOUNTAIN DARTER,

ETHEOSTOMA FONTICOLA

by

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A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Population and Conservation Biology
May 2014

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everlasting encouragement and motivation. Finally, I thank my incredible fiancé, Nathaniel Snell, for his unwavering encouragement and support of me these past few years and all of the sacrifices he has made to make my graduate education possible. Without his insistence in my moving across the country and his persistent belief in me, I would not be where I am today.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Rising turbidity levels from anthropogenic stressors impact visual communication systems in aquatic communities and may affect the behavior of individual animals, including altering foraging and anti-predator behavior as well as mating preferences. The fountain darter, *Etheostoma fonticola*, is federally endangered making it an especially important species for examining the effects of turbidity on social behavior. *Etheostoma fonticola* is endemic to only two clear, spring-fed rivers in central Texas that are currently facing a number of anthropogenic threats that may potentially influence turbidity levels. We tested male and female association preferences and the effects of low (~40NTU) turbidity on association preferences in *E. fonticola*. We found that association preferences for larger size were not present in adult male or female *E. fonticola*. However, low levels of turbidity did significantly decrease female strength of preference for larger males. Similarly, male and female *E. fonticola* spent less time associating with individuals of the opposite sex when their vision was reduced by simulated low levels of turbidity than when vision was not reduced. These results suggest that rising levels of turbidity may have significant consequences on the mating behavior of the federally endangered *E. fonticola*.

While association preferences are frequently used as a proxy for mate choice in fishes, excluding potentially important influences of intrasexual selection may provide misleading results when examining mating preferences. We examined whether female *E.*

fonticola prefer to mate with larger males. We also examined whether larger and smaller male *E. fonticola* exhibit differences in agonistic behaviors and consequently, mating success. We found that larger males exhibited higher rates of aggressive behaviors and smaller males in turn exhibited more defensive behaviors. However, differences between larger and smaller males in male-male interactions were not correlated with differences in spawning success. These results suggest that male size influences dominance relationships in *E. fonticola* but not mating success.

Sexual selection, communication, and turbidity

Communication

Animal communication involves potentially complex interactions between signalers and receivers that vary in the modalities of signaling and signal components (reviewed in Johnstone 1997). Communication at the most basic level involves a direct interaction between a signaler and a receiver. In mate choice, communication is important for mate recognition as well as mate quality recognition. Mate recognition involves signals that aid in the identification and assessment of appropriate mates through species recognition or provide information about genetic compatibility. Assessing mate quality consists of receivers interpreting signals that communicate information about the quality of the sender such as high fecundity (Pfenning 1998).

The main signal modalities are acoustic, visual, tactile, and chemical (Bradbury and Vehrencamp 1998). Signals can be transmitted alone, or may consist of several different modalities. Multi-modal cues can provide greater accuracy in the detection of signals or communicate information about various attributes of an individual. Several hypotheses have been proposed to explain the evolution of signaling using multiple modalities. The 'multiple-message' hypothesis states that different components of an individual's quality can be conveyed through multiple signal modalities (Møller and Pomiankowski 1993). Similarly, the 'backup signal' hypothesis states that signalers may use multiple modalities to ensure receivers are able to accurately assess a single attribute of the signaler (Møller and Pomiankowski 1993). Which modality is used and whether

signals are presented singularly or in conjunction with other signals may vary based on the context and the environment in which the communication is taking place.

The environment in which communication occurs may obscure interpretation and affect what modality is utilized (Endler 1993). Back-up signals may improve the accuracy of a receiver's interpretation, and may also function to compensate for impairment of primary signals. In aquatic environments, low-light conditions or suspended sediment (turbidity) influence the efficacy of communication, which results in greater reliance on multi-modal cues (Ward and Mehner 2010). For example, in the three-spined stickleback (*Gasterosteus aculeatus*), females spend more time associating with males when presented with visual rather than olfactory cues in a dichotomous choice test in clear water but reverse their relative cue reliance in turbid water (Heuschele et al. 2009). Heuschele et al. (2009) argue that females vary their sensory reliance based on which cues are more easily detected.

<u>Mate Choice</u>

Sexual selection is the differential reproductive success of individuals as a result of their ability to attract and obtain mates (Darwin 1871). In many species, males develop exaggerated characteristics such as larger body size, color patterns, and ornamental displays and females exhibit mate preferences. Both intersexual (usually female mate choice) and intrasexual (male-male competition) selection are important components of sexual selection (Darwin 1871) which can reinforce the evolution of these sexually dimorphic traits (Andersson and Iwasa 1996). For example, females in many species prefer larger males because larger males may be superior competitors and or more

2

attractive (Ryan and Keddy-Hector 1992). Preferences for larger individuals may proximally arise due to the increase in visual stimulation of larger body size (Rowland 1989).

Typically, studies on intersexual selection focus on female mate preferences due to differences in the level of parental investment leading females to be more discriminating than males (Trivers 1972). However, male mate choice does occur, especially in instances of significant size-related variation in female fecundity (Johnson 1982) or when males invest more in reproduction and parental care (Rosenqvist 1990). Males prefer larger females in several species of fish (Downhower and Brown 1981; Loiselle 1982; Berglund et al. 1986; Sargent et al. 1986; Côte and Hunte 1989; Rowland 1989; Rosenqvist 1990; Nuttall and Keenleyside 1993; Gabor 1999). Males may prefer larger females because larger females produce more eggs, allowing males to obtain greater fertilization success (Lawrence 1986; Sargent et al. 1986; Rowland 1989). However, in other species of fish such as mosquitofish, *Gambusia holbrooki* (McPeek 1992), chum salmon, *Onchorhynchus keta* (Schroder 1981), and orangethroat darters, *Etheostoma spectabile* (Pyron 1996), males do not exhibit preferences for larger females. A lack of male preference for larger females may be due to low variation in female quality, high costs attributed to searching for females, a male-biased sex ratio, or because males are unable to assess female quality (Pyron 1996).

Mate choice studies typically measure mating preferences through dichotomous choice trials with association time as a measure of mating preference. Association preferences in dichotomous choice trials are a reliable proxy of mate choice in several species of freshwater fish (Brooks and Endler 2001; Aspbury and Basolo 2002;

Cummings and Mollaghan 2006; Lehoten and Lindstrom 2008; Jeswiet et al. 2011).

However, association preferences for larger conspecifics have been shown for same sex

individuals as well, suggesting that association preferences may not necessarily arise

through sexual selective pressures but possibly through pre-existing biases, predation

pressures, or shoaling behavior (Gabor 1999; Aspbury and Basolo 2002). Alternatively,

males may prefer to associate with males that they assess as more attractive (larger) to the

opposite sex than themselves (Dugatkin and Sargent 1994).

A second complication of dichotomous choice studies is that the design

intentionally excludes the potential for male-male interactions. Competitive interactions

between males have been thoroughly studied across taxa (review in Andersson 1994). In

many species, specific morphological traits such as weaponry are important in

determining the competitive success of males and therefore affect their mating success

(Parker 1974). Additionally, exaggerated traits may increase a male's competitive ability

without being used in direct contests (Davies and Halliday 1978). Weapons and elaborate

coloration provide signals to potential competitors, allowing males to evaluate each other

and in some cases, avoid the energetic costs and potential injuries that may result from

direct conflicts (Maynard Smith and Parker 1976; Maynard Smith and Brown 1986). In

species that do not exhibit elaborate secondary sexual characteristics such as color

patterns or ornaments, direct competitive interactions between males may be the primary

determinant of mating success and larger body size may contribute to increased success

in competitive interactions (Farr 1989). For example in the mosquitofish (*Gambusia*

holbrooki), larger males dominate in male-male aggressive interactions, which translates

into greater access to females, and increased mating advantages for larger males (McPeek

4

1992). While controlling for male-male competition is important for investigating female mate preferences, exploring the interactions between mechanisms of sexual selection is necessary for understanding the evolution of male secondary sexual traits and female preferences for them (Hunt et al. 2009).

<u>Turbidity</u>

In aquatic environments, low-light conditions and turbidity (suspended sediment) can greatly reduce the accuracy of communication signals. The degradation of the quality and efficacy of visual signals can impair an animal's ability to perform behaviors such as foraging (Vogel and Beauchamp 1999), recognizing predators (Ferrari et al. 2010), and mate selection (Seehausen et al. 1997). Reduced visibility due to a rise in turbidity can influence the use of visual cues for mate choice and therefore impact the evolution of secondary sexual characteristics (Engström-Öst and Candolin 2007). When vision is impaired, the ability to receive visual signals to evaluate a potential mate is impaired as well the ability to exhibit preferences, leading to weaker sexual selection on traits that are used in mate choice (Candolin and Heuschele 2008). Consequently, when the environment is turbid, fish may compensate for impaired visual cues by increasing reliance on chemical cues. For example, fathead minnows (*Pimephales promelas*) that are exposed to chemical alarm cues in trials where vision is impaired by turbidity exhibit an increased response (rapid dashes) to a predator than when vision is not impaired (Hartman and Abrahams 2000). This indicates that some aquatic prey species utilize available stimuli to compensate for impairment of the modality they primarily rely on for communication. Conversely, in systems which normally have very low turbidity levels,

aquatic species may be less adapted to utilizing backup cues. For example, when vision is reduced or completely blocked due to simulated turbidity, male broad-nosed pipefish (*Syngnathus typhle*) do not show association preferences for larger females as they do in full vision regardless of whether olfactory cues are present or not (Sundin et al. 2010). Therefore, changes in turbidity levels may affect aquatic species differently depending on their ability, or lack there of, to compensate for impaired vision by relying on alternate cue modalities.

Study System & Species

The fountain darter, *Etheostoma fonticola*, is a small federally endangered (IUCN Red list 2013) fish that is endemic to the spring-fed headwaters of the San Marcos and Comal Rivers in Hays County, Texas, USA (Schenck and Whiteside 1976). These rivers originate from outflows of the Edwards Aquifer that create unique aquatic ecosystems with constant water temperatures, moderate flow, and support many endemic species, including seven that are threatened or endangered (USFWS 1996). Both the San Marcos and Comal Rivers are currently facing several anthropogenic threats including pollution, introduced species, increased groundwater withdrawal, and recreational use. Many of these threats are likely to cause rising levels of turbidity, especially during increased periods of drought experienced by Central Texas caused by global climate change. In the Edwards Aquifer system in particular, climate change is anticipated to cause increased water shortages (Loaiciga et al. 2000). Normal turbidity levels in the San Marcos River range from 0.26-5.76 nephelometric turbidity units (NTU) at the headwaters and become increasingly turbid (up to 13 NTU) downriver (Groeger et al. 1997; Saunders et al. 2001).

However, turbidity levels can become significantly elevated, up to 47 NTU during drought events and periods of heavy recreational use (T. Hardy unpublished data). Recent studies on *E. fonticola* show that low levels of turbidity have significant impacts on their anti-predator response (Swanbrow Becker and Gabor 2012) and foraging behavior (Swanbrow Becker 2012).

Etheostoma fonticola is the smallest species of darter reaching a maximum size of 35.5mm standard length (SL) in the wild (Page and Burr 1979) although individuals in the laboratory are observed up to 42.4mm SL (personal observation). *Etheostoma fonticola* prefer habitats with constant water temperatures, moderate flow, and vegetated substrate (Schenck and Whiteside 1976; Linam et al. 1993). *Etheostoma fonticola* are found within a small range, about 11 km total of stream habitat within the San Marcos and Comal Rivers (Schenck and Whiteside 1976), and exhibit high site fidelity, only moving an average of 10 ± 17 m ($\pm$ 1 SD) annually (Dammeyer et al. 2013). Their restricted range coupled with limited dispersal tendencies are particularly concerning in light of the combination of anthropogenic and extreme weather events that are implicated in the 1956 extirpation of *E. fonticola* from the Comal River (Schenck and Whiteside 1976). The potential reduction in spring flow caused by drought and increased water withdrawal from the Edwards Aquifer, threatens the survival of these darters (Bonner and McDonald 2005). Additionally, during summer months both the San Marcos and Comal Rivers are subject to intense recreational use. Wild caught *E. fonticola* are maintained by US Fish and Wildlife at the San Marcos Aquatic Resource Center in San Marcos, Texas in the event that reintroduction to the San Marcos and Comal Rivers is required.

However, habitat fragmentation and degradation along with high site fidelity are predicted to make recolonization efforts difficult (Dammeyer et al. 2013).

<center>Mate Choice in Darters</center>

Darters in the genus *Etheostoma* typically exhibit sexually dimorphic secondary sexual characteristics, particularly nuptial color patterns in males (Mendelson et al. 2007). Females in this genus exhibit mate preferences based on visual cues (Gumm et al. 2011). Sexual selection is hypothesized to be important in *Etheostoma* due to the prevalence of conspicuous color patterns and ornaments in males of most of the species. However, mate preference and intraspecific competition has not been thoroughly studied in darters (Fuller 2003).

Research has produced contrasting results on female preference for male size in *Etheostoma.* In tessellated darters, *Etheostoma olmstedi*, females prefer larger males as indicated by the propensity of females to lay eggs in nests occupied by larger males over smaller males (Stiver and Alonzo 2010). However, female *E. spectabile* show no preference for larger or smaller males in simultaneous choice tests in field or laboratory conditions (Pyron 1995). Female rainbow darters, *Etheostoma caeruleum*, from two different populations that differ significantly in size, show no association preferences but perform more nose digs (pre-mating behavior in which the female submerges the ventral half of her body into the substrate) in front of males from the population with larger males (Fuller 2003). Therefore, studies need to be conducted on each species in the genus *Etheostoma* to determine whether females show mate preferences based on male size.

<center>8</center>

There are very few studies on male mating preferences in *Etheostoma*. Some *Etheostoma* species exhibit different forms of paternal care and nest defense suggesting that males may mate discriminately as a result of increased reproductive investment. In one example of a territorial nesting species in this genus, the blackfin darter, *Etheostoma nigripinne*, males exhibit individual preferences for one female over the other in a dichotomous choice test although these preferences are not correlated with female size or species (O'Rourke and Mendelson 2010), suggesting that males in this species have the ability to evaluate potential mates and exhibit mate preferences. However, male *E. spectabile* do not show a preference to spawn with larger over smaller females (Pyron 1996). Other species in the genus *Etheostoma* may exhibit male mate preferences but these preferences may vary between species as has been shown for female mate choice and thus both male and female mate choice need further exploration.

Male – Male Interactions in Darters

Males in many species of *Etheostoma* exhibit striking species-specific nuptial color patterns, which are important for conspecific recognition, female preferences, and male-male competition (Mendelson et al. 2007; Gumm 2011; Martin and Mendelson 2013). However, in some *Etheostoma* species, such as *E. fonticola*, males are comparably drab and exhibit muted or cryptic coloration. In these species, non-color based visual stimuli such as larger body size may be important for female preferences as well as aggressive interactions among males.

There are a limited number of studies on mating systems in the genus *Etheostoma* and fewer still that examine the effects of male-male interactions on mating success. In *E.*

caeruleum, some male interactions such as guarding females, influence their spawning success, while other behaviors such as attacks and chases, are not correlated with spawning success (Fuller 2003). Similarly in *E. spectabile*, male guarding is correlated with spawning success while male aggressive behaviors are not (Pyron 1995). Interestingly, both Pyron (1995) and Fuller (2003) show that female preference is not associated with male spawning success, but the ability of the male to guard the female from competing males predicts spawning success. Therefore, association preferences may be a reliable indicator of mate choice but there may be additional interactions that influence mating success. Further studies that investigate the influences on mate choice and mating success in each species of *Etheostoma* are necessary to illuminate the complexities of mating behavior in this system.

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CHAPTER II

Effects of turbidity on association preferences for size in the fountain darter,

***Etheostoma fonticola*[1]**

Abstract

Anthropogenic activities such as aquatic recreation, and increased groundwater
withdrawal pose serious threats to aquatic ecosystems. Rising levels of turbidity as a
result of these threats have serious consequences for aquatic organisms as turbidity
degrades visual communication. Here we tested the impact of simulated turbidity on
association preferences in the federally endangered fountain darter, *Etheostoma fonticola*,
which is endemic to the clear spring-fed headwaters of two rivers in central Texas. We
examined whether male and female *E. fonticola* exhibit preferences for larger over
smaller individuals of both sexes across two vision levels; clear and reduced visibility
(simulated turbidity). We found that neither female nor male *E. fonticola* exhibit
preferences for larger over smaller individuals of the opposite sex or the same sex.
However, reduced visibility (~40NTU) decreased the total amount of time both female
and male *E. fonticola* spent associating with the opposite sex. A reduction in the amount
of time spent associating with the opposite sex may reflect a reduction in the time spent
evaluating potential mates, thereby weakening sexual selection for traits important for
mate choice. These results indicate that compromised visibility hampers association

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behavior in *E. fonticola,* which may be of concern for the conservation and management of this endangered species.

<u>Introduction</u>

Turbidity degrades visual signals and impairs the quality and efficacy of visual information by narrowing the visible light spectrum and reducing light intensity (Seehausen et al. 1997), greatly reducing signal propagation in aquatic environments (Heuschele et al. 2009). Turbidity impacts behaviors such as foraging (Vogel and Beauchamp 1999) and predator recognition (Ferrari et al. 2010). In turbid conditions, mating behavior may also be impacted as the ability to evaluate potential mates is impaired as well as the ability to exercise mate choice (Candolin and Heuschele 2008).

Turbidity affects mating behavior through weakening mate choice preferences, which in turn relaxes sexual selection (Seehausen et al. 1997; Järvenpää and Lindström 2004; Sundin et al. 2010). Additionally, turbidity alters the amount of time males and females spend associating with potential mates (Heubel and Schlupp 2006; Sundin et al. 2010). By reducing the amount of time the choosier sex spends evaluating mates, turbidity may lead to increased courtship activity as this may be required to attract equivalent attention to what would be received in non-turbid conditions (Engström-Öst and Candolin 2007).

Body size variation in both males and females is common across taxa and typically larger size has been sexually selected for by both male and female mate choice (reviewed in Andersson 1994). Larger males may experience increased mating success due to their superior competitive abilities over smaller males and larger female body size

is frequently correlated with higher reproductive success (Ryan and Keddy-Hector 1992). Alternatively, preferences for larger individuals may proximally arise due to the increase in visual stimulation affected by increased body size (Rowland 1989). Individuals may also exhibit preferences for larger individuals of the same sex, suggesting that preferences for larger size may not necessarily arise through sexual selective pressures but pre-existing biases, predation pressures, or shoaling behavior (Gabor 1999; Aspbury and Basolo 2002).

We propose to examine the effects of simulated turbidity on mate choice in fountain darters, *Etheostoma fonticola*. *Etheostoma fonticola*, is a federally endangered fish (IUCN Red list 2013) that is endemic to the spring-fed headwaters of the San Marcos and Comal Rivers (Schenck and Whiteside 1976). This species is adapted to habitats characterized by clear water, with normal turbidity levels ranging from 0.26-5.76 nephelometric turbidity units (NTUs) in the headwaters of the San Marcos River (Groeger et al. 1997; Saunders et al. 2001) and 0.81-4.48 NTUs in the headwaters of the Comal River (Hubbs 2001). Rising turbidity levels may potentially affect association preferences and may impair mate choice. Darters rely on visual cues to evaluate mates (Gumm et al. 2011), and *E. fonticola* are unable to compensate for impaired vision by relying on chemical cues (Swanbrow Becker and Gabor 2012). Additionally, turbidity even at very low levels (8.7 and 23.2 NTUs) significantly impacts the foraging behavior of *E. fonticola,* reducing the time to initiate foraging as well as prey consumption (Swanbrow Becker 2012).

While darters in the genus *Etheostoma* typically exhibit sexually dimorphic secondary sexual characteristics, particularly nuptial color patterns in males (Mendelson

et al. 2007), male *E. fonticola* have more cryptic coloration consisting of only two brightly colored bands on their dorsal fin. Given their lack of coloration we chose to focus on mate preference for size. Some studies on species of *Etheostoma* have found evidence for female preference for size (Fuller 2003; Stiver and Alonzo 2010), while other studies have not (Pyron 1995). There are few studies on male mating preferences in *Etheostoma*. Some *Etheostoma* species exhibit different forms of paternal care and nest defense suggesting that males may mate discriminately as a result of increased reproductive investment. Therefore, examining mate choice in both males and females in *Etheostoma* species may provide greater insight into mating behavior in this genus.

Here we tested the hypothesis that male and female *E. fonticola* exhibit association preferences for larger size that are based on mate preferences and that these preferences will be affected when their vision is reduced by simulated turbidity. We determined whether male and female *E. fonticola* prefer larger over smaller individuals of the opposite sex. We predicted that females would prefer to associate with larger males and that males would exhibit a preference to associate with larger females. In this species, female fecundity is correlated with body size (Schenck and Whiteside 1977) therefore males may prefer to mate with larger females who have more mature ova. In addition to examining male and female size preferences for the opposite sex, we also determined whether *E. fonticola* exhibit preferences for size in the same sex. Examining preferences for size in the same sex helps to elucidate whether association preferences are based on mate choice (Gabor 1999). If males and females base association preferences on mate choice, it is less likely that males and females will exhibit a preference for size in the same sex.

19

Simultaneously, we determined how simulated turbidity affects female and male preferences to associate with other *E. fonticola*. We predicted that the strength of association preferences would be lower in simulated turbidity than in clear water. In addition, we predicted that male and female *E. fonticola* would spend less time associating with stimulus individuals overall when vision is reduced by simulated turbidity. Alternatively, male and female *E. fonticola* may spend more time associating with others in turbid conditions due to the reduction of visual information available and the potential for increased predation risk.

Methods

We conducted trials in the laboratory using hatchery-reared, first generation adult *E. fonticola*. We housed and maintained test fish in holding tanks with recirculating well water (22 °C) at the San Marcos Aquatic Resource Center, in San Marcos, Texas. Each tank held approximately 60 individuals with an equal number of males and females in each holding tank. We fed test fish a diet of black worms (*Lumbriculus variegatus*) once daily. We conducted trials between July 2013 and February 2014. *Etheostoma fonticola* spawn year round with two annual spawning peaks, one in late summer and one in late winter to early spring (Schenck and Whiteside 1977).

We examined the association preferences of adult *E. fonticola* (N=40 females, N=40 males) in two treatments; (1) male and (2) female stimulus individuals across two vision levels: (a) clear and (b) turbid (simulated turbidity). We used semi-transparent window tinting to simulate the reduced vision caused by low levels of turbidity following Sundin et al. (2010) and Swanbrow Becker and Gabor (2012). Traditionally, turbidity has

been experimentally manipulated using some type of bentonite. However, these materials may produce chemicals or toxins that may interfere with olfactory cues or impact behavior. We used window tinting to simulate turbidity to control for these effects. We affixed tinting that allowed for 50% visible light transmission (VLT) to clear plastic dividers. We approximated the turbidity range of the semi-transparent tinting to NTU by comparing the light absorbency to that of known NTU standards across the visible light spectrum (400-790 nm) using a spectrometer. The 50% VLT tinting corresponded with a turbidity range of 25-55 NTU, hereafter referred to as 40 NTU. The clear vision level dividers with no affixed tinting allowed for approximately 100% VLT, equivalent to zero NTU.

Our experimental set-up consisted of two 57 liter aquaria containing a fine gravel substrate and 10cm of water (changed after each trial). We covered each tank on three sides with opaque black plastic and the front facing side with one-way tinting to minimize disturbance and allow for observation. We divided tanks into three compartments, two at either end for stimulus individuals, and one in the center for the focal fish. We separated the compartments by plexiglass dividers, which we placed 10cm from either edge of the tank. Dividers were held in place using silicone guide rails and were not attached to the testing tank, allowing dividers to be removed and replaced with the alternate vision level between trials. We visually divided the center compartment of the tank into three zones by lines marking the outside of the tank into a no preference, neutral zone flanked by two 5cm association zones for each stimulus fish. The clear vision level (a) consisted of two clear dividers with holes drilled in them to allow water and chemical cues to flow through. The turbid vision level (b) consisted of two clear

dividers with 50% VLT window tinting affixed to one side and holes drilled through both the plexiglass and the tinting to allow water and chemical cues to flow through.

For each treatment, we haphazardly chose a small and a large stimulus individual of the same sex from stock tanks, measured their standard length (SL), and placed each individual into one of the side compartments at either end of the test tank. We randomized the side in which the small and large stimulus individuals were placed prior to each trial. Stimulus individuals had a minimum of 5mm difference in SL (± 1 SD from the laboratory population mean of 35.5mm SL). We placed the focal fish in the neutral zone in a acclimation chamber, a clear 2 liter plastic bottle with the bottom removed and holes drilled in the sides, to allow the focal fish exposure to both visual and chemical cues from both stimulus individuals prior to each trial. We allowed the focal fish to acclimate for a minimum of 40 minutes before we removed the acclimation chamber and began the trial. We observed the focal fish for 15 minutes, during which we recorded the amount of time spent within each association zone via separate stopwatches. After 15 minutes we again placed the focal fish inside of the acclimation chamber, removed the stimulus individuals and placed them into the opposite side compartments, replaced the dividers with the dividers for the remaining vision level, and repeated the trial protocol. We measured and recorded the SL of the focal fish after the final trial. We randomized the order of vision level prior to each trial. We tested each focal fish in a total of two trials, one for each vision level, with one stimulus sex (male or female) only.

We examined the effects of simulated turbidity and stimulus size on association preferences for large and small stimulus individuals using Wilcoxon Signed Rank tests. We used repeated measures ANOVAs to analyze the total amount of time spent

associating with stimulus individuals and strength of preference (SOP) for larger stimulus

individuals using vision level and vision level order as our model effects. We calculated

strength of preference (SOP) for large as the amount of time spent associating with large

individuals divided by the total amount of time spent associating with both stimulus

individuals. We conducted repeated measures ANOVAs on each response variable (SOP

and total association time) separately and for each combination of stimulus sex treatment

(male or female) and vision level (clear or turbid) for male and female focal fish

separately. We excluded from analysis any focal individuals that did not associate with

either stimulus individual. We carried out all analyses using JMP 11 software (SAS

Institute Inc., SAS Campus Drive, Building S, Cary, NC, 27513, USA).

<u>Results</u>

Females of *E. fonticola* did not show a significant preference for female size in

either the clear (z=-31, *P*=0.26) or the turbid (z=-21, *P* =0.30) vision levels (Figure 1a).

Similarly, females did not exhibit a significant preference for male size (clear: z=-37, *P*

=0.15; turbid: z=-19.5, *P* =0.19; Figure 1b). Male *E. fonticola* did not exhibit preferences

for female size (clear: z=-37.5, *P* =0.11; turbid: z=-5, *P* =0.73; Figure 2a) or male size

(clear: z=-42.5, *P* =0.09; turbid: z=-12.5, *P* =0.41; Figure 2b).

Females of *E. fonticola* SOP for larger female stimulus individuals was

significantly affected by vision level (Table 1). Female SOP for larger females was lower

when vision was reduced by simulated turbidity than when vision was clear. Female SOP

for larger male stimulus individuals was not significantly affected by vision level (Table

1). However, vision level had a significant effect on the total time females spent

associating with male stimulus individuals (Table 2). When vision was reduced by simulated turbidity, females spent less time associating with male stimulus individuals. Vision level did not have a significant effect on male SOP for larger stimulus individuals, regardless of sex (Table 3). The total amount of time males spent associating with stimulus individuals was significantly affected by vision level when males were tested with female stimulus individuals but not male stimulus individuals (Table 4). Males spent less time associating with female stimulus individuals when vision was reduced by simulated turbidity. For all analyses there was no significant effect of vision level order or the interaction between vision level and vision level order (Tables 1-4).
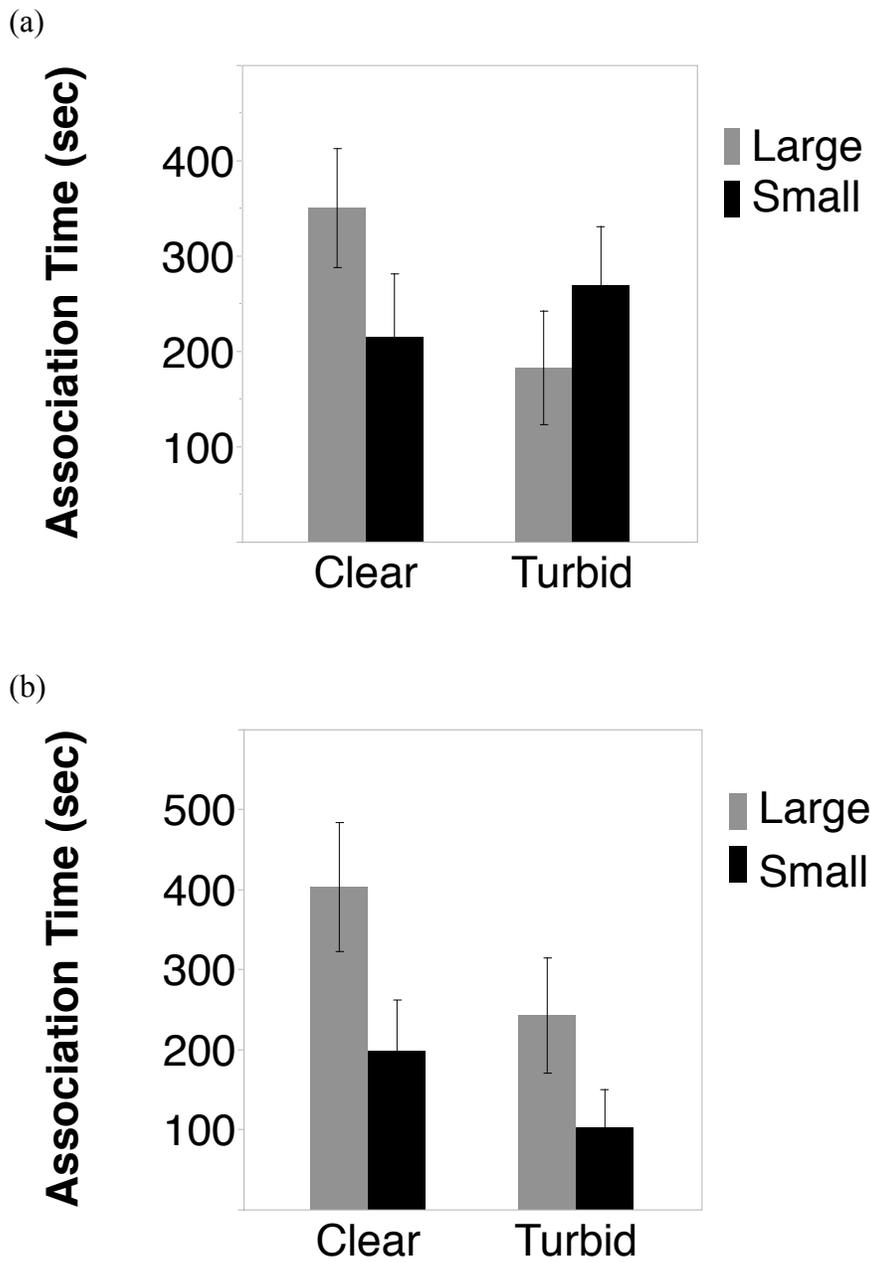
Fig. 1

Mean ± SE association time for female *Etheostoma fonticola* tested with (a) female or (b) male stimulus individuals across the clear and turbid (~40 NTU) vision levels

(a)

(b)
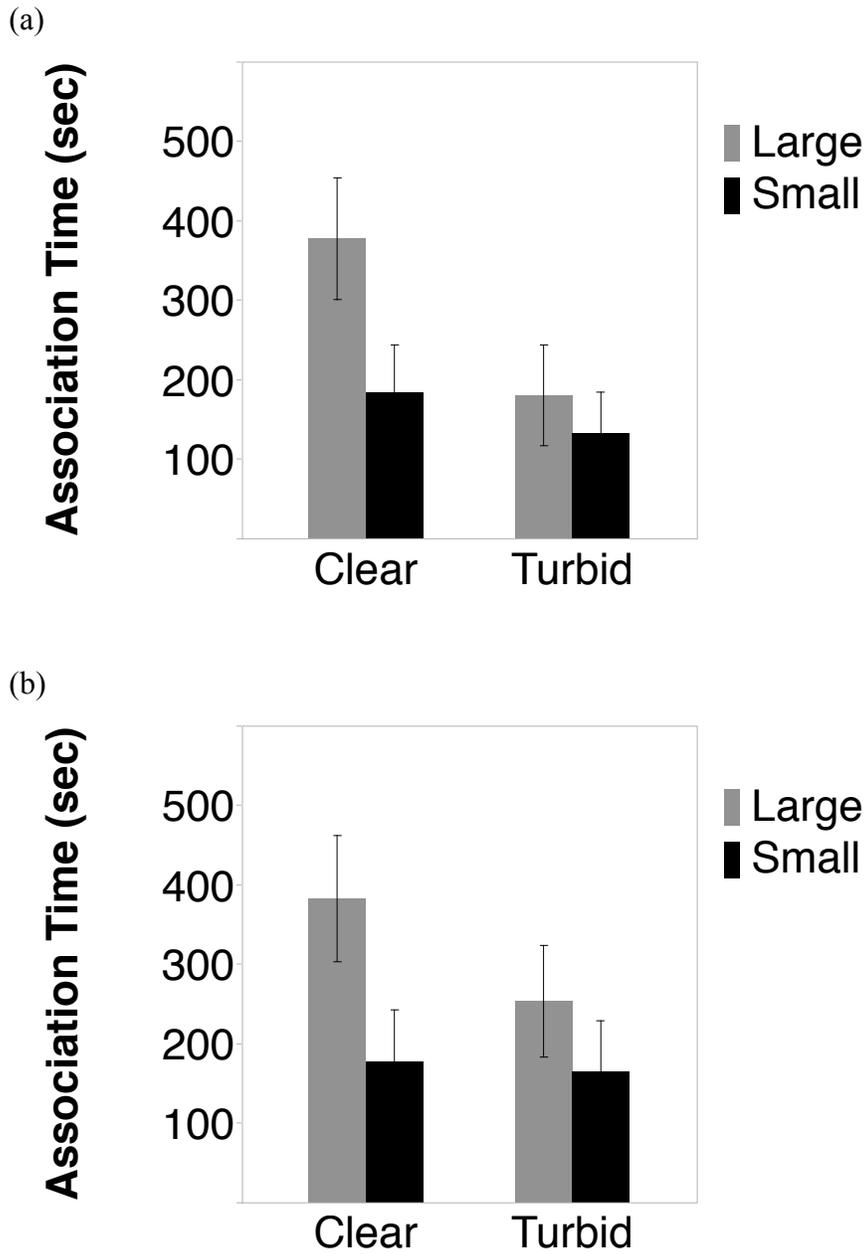
Fig. 2

Mean ± SE association time for male *Etheostoma fonticola* tested with (a) female or (b) male stimulus individuals across the clear and turbid (~40 NTU) vision levels

26

Table 1

Results from repeated measures ANOVAs for female SOP for larger stimulus individuals

	df	Male stimulus		Female stimulus	
		F	*P*	F	*P*
Vision Level	1	0.78	0.39	13.17	0.002
Vision Level Order	1	0.05	0.82	1.04	0.32
Vision Level * Vision Level Order	1	0.75	0.40	0.04	0.85
Error	18				

Table 2

Results from repeated measures ANOVAs for the total time female focal fish spent associating with stimulus individuals

	df	Male stimulus		Female stimulus	
		F	*P*	F	*P*
Vision Level	1	8.55	0.009	2.86	0.11
Vision Level Order	1	0.40	0.54	4.01	0.06
Vision Level * Vision Level Order	1	0.001	0.97	0.41	0.53
Error	18				

Table 3

Results from repeated measures ANOVAs for male SOP for larger stimulus individuals

		Female stimulus		Male stimulus	
	df	F	*P*	F	*P*
Vision Level	1	0.17	0.18	1.92	0.18
Vision Level Order	1	2.18	0.16	0.60	0.45
Vision Level * Vision Level Order	1	1.17	0.69	0.12	0.73
Error	18				

Table 4

Results from repeated measures ANOVAs for the total time male focal fish spent associating with stimulus individuals

		Female stimulus		Male stimulus	
	df	F	*P*	F	*P*
Vision Level	1	7.47	0.01	1.59	0.22
Vision Level Order	1	3.82	0.07	0.41	0.53
Vision Level * Vision Level Order	1	0.008	0.93	1.11	0.31
Error	18				

<u>Discussion</u>

In several species of fish, turbidity has been found to reduce time spent associating with the opposite sex (Engström-Öst and Candolin 2007; Sundin et al. 2010). Similarly, we found that females spent less time overall associating with male stimulus individuals in the turbid vision level as compared to the clear vision level. These results suggest that while females do not exhibit association preferences for male size, they spend less time associating with males when vision is reduced, which decreases the amount of time they are able to spend evaluating potential mates. Additionally, males spent less time overall associating with female stimulus individuals in the turbid vision level as compared to the clear vision level. Our results are consistent with studies in other fish that found turbidity reduced total association time (Engström-Öst and Candolin 2007; Sundin et al. 2010). Reducing the amount of time spent associating with and evaluating mates may require an increase in the amount of time and energy expended on courtship to compensate and elicit comparable attention (Sundin et al. 2010). Alternatively, if courtship behavior does not increase to make up for reduced association times in turbid conditions or the increase does not change female choice, mate choice preferences may not be maintained, which in turn relaxes sexual selection on traits that are used in mate choice (Seehausen et al. 1997; Järvenpää and Lindström 2004; Sundin et al. 2010).

Research on mate preference has produced contrasting results on female preference for male size in *Etheostoma* (Fuller 2003). As has been found in some darter species (Pyron 1995; Fuller 2003), female *E. fonticola* do not exhibit an association preference for male size. This lack of preference may be due to females not being the

choosier sex in this species. However, males also did not exhibit a preference for female size. Our results suggest that larger body size may not indicate mate quality in *E. fonticola*. Alternatively, the experimental design did not allow us to accurately assess mate choice.

We also examined intrasexual association preferences. Interestingly, female strength of preference for larger females was significantly affected by reduced vision. Specifically, female strength of preference for larger females was higher in the clear vision level than in the turbid vision level. While the amount of time females spent associating with larger and smaller females was not significantly different, the proportion of time they associated with larger females out of the total time they spent associating with female stimuli was significantly different between vision levels. However, male strength of preference for larger males was not significantly higher in the clear vision level. These results suggest that in *E. fonticola*, turbidity affects female association behavior for both female and male stimulus individuals

It is clear that turbidity has some impact on association behavior in *E. fonticola*, which may have consequences for mating behavior in this species. While body size may not be important for *E. fonticola* in terms of mate preferences, conspecific association preferences, or even conspecific recognition, there may be other visual signals that are important for communicating this information in this species. If turbidity has significant effects on female association preferences for a trait that is not necessarily used in mate choice, then there may be greater impacts on traits that are important for mate choice and sexual selection. Additionally, conspecific association can be driven by mechanisms other than sexual selection such as foraging behaviors and predation pressure. Therefore,

the effects of turbidity on association behaviors may impact other social behaviors important for the fitness and survival of *E. fonticola*.

The San Marcos and Comal Rivers are currently facing several anthropogenic threats including pollution, increased groundwater withdrawal, and recreational use. Many of these threats are likely to cause rising levels of turbidity, especially during increased periods of drought experienced by Central Texas. In the Edwards Aquifer system in particular, climate change is anticipated to cause increased water shortages (Loaiciga et al. 2000). The potential reduction in spring flow caused by drought and increased water withdrawal from the Edwards Aquifer, threatens the survival of these darters (Bonner and McDonald 2005). Additionally, recreational use of the San Marcos and Comal Rivers may be a significant source of fluctuating turbidity levels in *E. fonticola*'s limited habitat. Low levels of turbidity have significant effects on *E. fonticola* anti-predator behavior (Swanbrow Becker and Gabor 2012) and foraging behavior (Swanbrow Becker 2012), and here we have shown the impact on association preferences. The turbidity level simulated in this study, 40 NTU, is consistent with levels that are found in *E. fonticola's* natural range during peaks in recreational activity (T. Hardy unpublished data). Our results combined with prior studies (Swanbrow Becker and Gabor 2012; Swanbrow Becker 2012) provide a multi-faceted argument for how visual impairment caused by turbidity has consequences on behaviors that are important for the survival and mating of this endangered species. Rising levels of turbidity may be especially detrimental to the behavior of species that are not adapted to environments that are typically characterized by high turbidity (Bonner and Wilde 2002). Therefore, the implications of this study and others are not limited to *E. fonticola* or the San Marcos and

Comal Rivers, but may be relevant to other historically clear freshwater systems and species that are adapted to low turbidity levels. As environmental changes and anthropogenic stressors associated with elevated turbidity continue to threaten aquatic ecosystems, our findings may be informative for the conservation and management of threatened and endangered species in the San Marcos and Comal Rivers or other similar systems.

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CHAPTER III

Mating behavior and male – male interactions in the fountain darter,

Etheostoma fonticola

Abstract

Association preferences are frequently used as a proxy for mate choice in fishes. However, excluding potentially important influences of intrasexual selection may provide misleading results when examining mate choice. Association preferences in male and female *E. fonticola* do not reveal a size preference for same sex or opposite sex individuals. However, male-male interactions may influence mating behavior in *E. fonticola* and larger and smaller males may experience variation in mating success regardless of female preferences for size. We examined whether larger and smaller male *E. fonticola* exhibit differences in agonistic behaviors and mating success. We found that larger males exhibited higher rates of aggressive behaviors and smaller males in turn exhibited more defensive behaviors. However, differences between larger and smaller males in male-male interactions were not translated into differences in spawning success. These results suggest that male size influences dominance relationships in *E. fonticola* but not mating success. The lack of sexual selection in this species, and possibly the minimal sexual dimorphism may be an outcome of many components in their breeding system including a year round breeding season, females lay eggs individually, eggs are fertilized individually by males, and close to a 50/50 OSR.

Introduction

Sexual selection is the differential reproductive success of individuals as a result
of their ability to attract and obtain mates (Darwin 1871). Many studies focus on how
mating preferences influence male mating success (review in Andersson 1994), and
typically measure female mating preferences through dichotomous choice trials using
association time as a proxy for mate choice. In some species of freshwater fish,
association preferences exhibited in dichotomous choice trials are a reliable estimate of
mate choice (Brooks and Endler 2001; Aspbury and Basolo 2002; Cummings and
Mollaghan 2006; Lehoten and Lindstrom 2008; Jeswiet et al. 2011). However,
association preferences may not necessarily reflect mating preferences as they may arise
through other social behaviors or in response to predation risk (Gabor 1999).
Additionally, dichotomous choice designs deliberately exclude the potential for
interactions between individuals. Removing the influence of male-male interactions is
useful for investigating the presence of female mate choice and which male traits are
under selection (Hunt et al. 2009). However, both male-male competition and female
mate choice are important mechanisms of sexual selection and the interactions between
these selection pressures drive the evolution of sexually selected traits. There may also be
instances in which female association preferences accurately reflect female mate choice
but do not correlate with male mating success due to processes that override female
choice such as competition between males (Andersson 1994), alternative male mating
strategies (Henson and Warner 1997), and post-copulatory mate choice (Eberhard 1996).

Female preference for larger body size is common in many species and may arise
due to larger individuals possessing superior competitive abilities, genetics, or

37

reproductive success (Ryan and Keddy-Hector 1992). Alternatively, preferences for larger individuals may proximally arise due to the increase in visual stimulation affected by increased body size (Rowland 1989). Male-male competition can also influence the evolution of larger body size (review in Andersson 1994). In direct contests between males, larger males are generally better able to dominate male competitors, and males that employ alternative mating strategies or tactics are typically smaller males (Basolo 2004). Larger body size may confer or be an outcome of superior competitive abilities but may also function as a signal to potential competitors, allowing males to evaluate each other and in some cases avoid the energetic costs and potential injuries that may result from direct conflicts (Maynard Smith and Parker 1976; Maynard Smith and Brown 1986).

Darters in the genus *Etheostoma* typically exhibit sexually dimorphic secondary sexual characteristics, particularly nuptial color patterns in males (Mendelson et al. 2007), which are important for conspecific recognition, female preferences, and male-male competition (Mendelson et al. 2007; Gumm 2011; Martin and Mendelson 2013). However, some *Etheostoma* species exhibit comparably muted coloration, suggesting that alternative visual stimuli, such as larger body size, may play a role in female preferences as well as aggressive interactions among males. Research has produced contrasting results on female preference for male size in *Etheostoma*, with some species exhibiting preferences for size (Fuller 2003; Stiver and Alonzo 2010), while others do not (Pyron 1995). In species that do not exhibit elaborate secondary sexual characteristics, such as ornate coloration, direct competitive interactions between males may be the primary determinant of mating success and larger body size may contribute to increased success

in competitive interactions (Farr 1989). In some species of *Etheostoma*, female preference is not associated with male spawning success, but the ability of the male to guard the female from competing males predicts spawning success (Pyron 1995; Fuller 2003). Therefore, association preferences may be a reliable indicator of mate choice but there may be additional interactions that influence mating success.

The fountain darter, *Etheostoma fonticola*, is a small, endangered fish that is endemic to the spring-fed headwaters of the San Marcos and Comal Rivers in Hays County, Texas, USA (Schenck and Whiteside 1976). Previous work revealed that male and female *E. fonticola* do not show association preferences for size in the same sex or the opposite sex (DeColo et al. chapter 2). *Etheostoma fonticola* may base mate choice on other modalities not permitted in the prior design or on phenotypic characteristics other than body size. Here we determined whether female *E. fonticola* exhibit mating preferences based on male size and whether mating success is influenced by male-male interactions. We predicted that female *E. fonticola* would prefer to mate with larger over smaller males. Alternatively, females may not show a preference for male size and may base mate choice on other male traits not tested here. Additionally, female mate choice may be obscured by male-male interactions, resulting in an apparent lack of female preference. We predicted that interactions between males would influence the mating success of larger and smaller males. Particularly, we predicted that differences in aggressive behaviors would be translated into variation in mating success between different sized males.

Methods

Darters in the genus *Etheostoma*, do not exhibit complex courtship behavior (Winn 1958). Typically a mating event is characterized by a male following a female into an area suitable for egg laying, the male will assume a mounted position on top of the female, and both male and female will vibrate their bodies rapidly, fertilizing and attaching a single egg to the surface of an aquatic plant (Winn 1958). *Etheostoma fonticola* spawn year round with two annual spawning peaks, one in late summer and one in late winter to early spring (Schenck and Whiteside 1977). *Etheostoma fonticola* preferentially spawn on *Rhizoclonium sp.* and *Ludwiga repens,* native vegetation in their habitat (Phillips et al. 2011). *Etheostoma fonticola* will spawn readily in laboratory conditions without manipulation or acclimation and have been observed to spawn within hours of being placed in novel testing environments (Phillips et al. 2011).

We examined the mating preferences of adult female *E. fonticola* (N=20) in a free spawning setup. We conducted trials in the laboratory using hatchery-reared, first generation adult *Etheostoma fonticola*. We housed and maintained test fish in holding tanks with recirculating well water (22 ^{o}C) at the San Marcos Aquatic Resource Center in San Marcos, Texas. Each tank held approximately 60 individuals. We separated males and females two weeks prior to testing into separate holding tanks. We fed test fish a diet of black worms (*Lumbriculus variegatus*), once daily. Holding tanks contained four 16cm long sections of 5cm PVC pipe cut in half, which provide shelter as well as a surface on which eggs may be attached. We conducted trials in March 2014.

Our experimental set-up consisted of two 75 liter aquaria containing fine gravel substrate and 15cm of water (changed after each trial). We covered each tank on three

sides with black plastic to minimize disturbance and left the front facing side clear to allow for video recording. We divided each testing tank into three equal sections during acclimation periods using two clear plastic dividers with holes drilled in them to allow all test fish access to visual and olfactory cues. We placed one plastic aquatic plant in the center of the back side of the testing aquaria. *Etheostoma fonticola* will deposit eggs on artificial plants that resemble natural vegetation types (Phillips et al. 2011). For each trial, we haphazardly chose one large and one small male stimulus individual from the male holding tank, measured their standard length (SL), and placed them into the sections on opposite ends of the testing tank. Stimulus males differed in SL by at least 5mm (± 1 SD from the laboratory population mean of 35.5mm SL). Subsequently, we haphazardly chose one focal female from the female holding tank, measured her SL, and placed her into the center section of the testing tank. We allowed all test fish to acclimate to the testing aquaria for a minimum of 15 hours. We recorded all trials via digital cameras (Canon HD Vixia HFR series). We began video recording immediately prior to removing the dividers and continued recording for 5 hours. We placed all individuals in a holding tank for tested fish after video recording was completed. No individuals were used in more than one trial.

We analyzed each 5 hour video and recorded the behaviors (as defined in Table 5) of both the small and large males and the female fish. We recorded the number of times each behavior was exhibited by males towards the other male, males towards the female, and the female towards each male. We analyzed the differences between large and small males for each combination of behavior and recipient using Mann-Whitney U tests as our data did not conform to assumptions required for parametric tests. We carried out all

analyses using JMP 11 software (SAS Institute Inc., SAS Campus Drive, Building S, Cary, NC, 27513, USA).

Results

There was no significant difference between larger and smaller males in the number of times they move towards or approached other males (Table 6). However, larger males were significantly more likely than smaller males to perform aggressive behaviors towards other males such as attempt solicitations, follow, chase, and attack (Table 6; Fig.3). Conversely, smaller males were significantly more likely than larger males to perform defensive behaviors towards other males such as move away (Table 6; Fig. 4a) and hide (Table 6; Fig. 4b).

There was no significant difference between the behaviors of larger and smaller males towards females (Table 7), except larger males were more likely than smaller males to follow females (Table 7; Fig. 5). There was no significant difference in the number of times females moved towards or followed larger and smaller males (Table 8). Females approached larger males significantly more often than they approached smaller males (Table 8; Fig. 6a). However, females also moved away from larger males significantly more often than from smaller males (Table 8; Fig. 6b). In total there were very few successful mating attempts. Only 8 males had successful mating attempts out of the 32 males that attempted to mate.

Table 5

Description of behaviors of *Etheostoma fonticola* recorded for large male, small male, and female test fish

Behavior	Description
Move Towards	Actor fish moves towards receiver fish within no more than one half of the length of the tank.
Move Away	Actor fish moves away from receiver fish within no more than one half of the length of the tank.
Approach	Actor fish moves within one body length of receiver fish.
Follow	Actor fish moves towards the receiver fish several times along the same path at a normal pace.
Chase	Actor fish moves towards the receiver fish several times along the same path at a rapid pace.
Attack	Actor fish darts rapidly toward receiver fish with dorsal fin raised, deliberately making contact with body of receiver fish.
Hide	Actor fish buries all or part of body into gravel substrate and remains motionless for several seconds.
Unsuccessful Solicitation	Actor fish mounts all or the majority of body on top of receiver fish and initiates body oscillations.
Successful Solicitation	Actor fish mounts all or the majority of body on top of receiver fish and initiates body oscillations, female reciprocates body oscillations, and pair moves horizontally upward together.
Total Mating Attempts	The combination of unsuccessful and successful solicitations.

Table 6

Results from Mann-Whitney *U* tests for differences between the behavior of large and small males towards males. Bold values indicate a significant difference (*P*<0.05)

Behavior	df	Mean ± SE large	Mean ± SE small	Z	*P*
Move Towards	19	58.85 ± 10.6	50.85 ± 10.6	-27.5	0.32
Move Away	19	55.65 ± 29.5	152.9 ± 29.5	86	**0.0006**
Approach	19	102.05 ± 19.6	64.9 ± 19.6	-48	0.07
Follow	19	10.1 ± 3.8	3.85 ± 3.8	-34	**0.053**
Chase	19	26.7 ± 8.9	1.4 ± 8.9	-60	**<0.0001**
Attack	19	21.2 ± 4.6	5.8 ± 4.6	-85.5	**<0.0001**
Hide	19	0.75 ± 0.4	2.1 ± 0.4	50.5	**0.002**
Unsuccessful Solicitation	19	7.7 ± 4.3	3.45 ± 4.3	-55.5	**0.006**

Table 7

Results from Mann-Whitney *U* tests for differences between the behavior of large and small males towards females. Bold values indicate a significant difference (*P*<0.05)

| Behavior	df	Mean ± SE large	Mean ± SE small	Z	*P*
Move Towards	19	78.7 ± 11.1	66.05 ± 11.1	-41	0.13
Move Away	19	46.95 ± 9.9	38.75 ± 9.9	-30.5	0.27
Approach	19	151.4 ± 30.8	88.15 ± 30.8	-48	0.08
Follow	19	24.6 ± 5.3	12.65 ± 5.3	-45.5	**0.047**
Chase	19	6.8 ± 3.5	1.25 ± 3.5	-12.5	0.23
Unsuccessful Solicitation	19	15.9 ± 4.3	9.55 ± 4.3	-33	0.12
Successful Solicitation	19	5.65 ± 2.9	2.95 ± 2.9	-1.5	0.84
Total Mating Attempts	19	21.55 ± 6.1	12.5 ± 6.1	-34	0.11
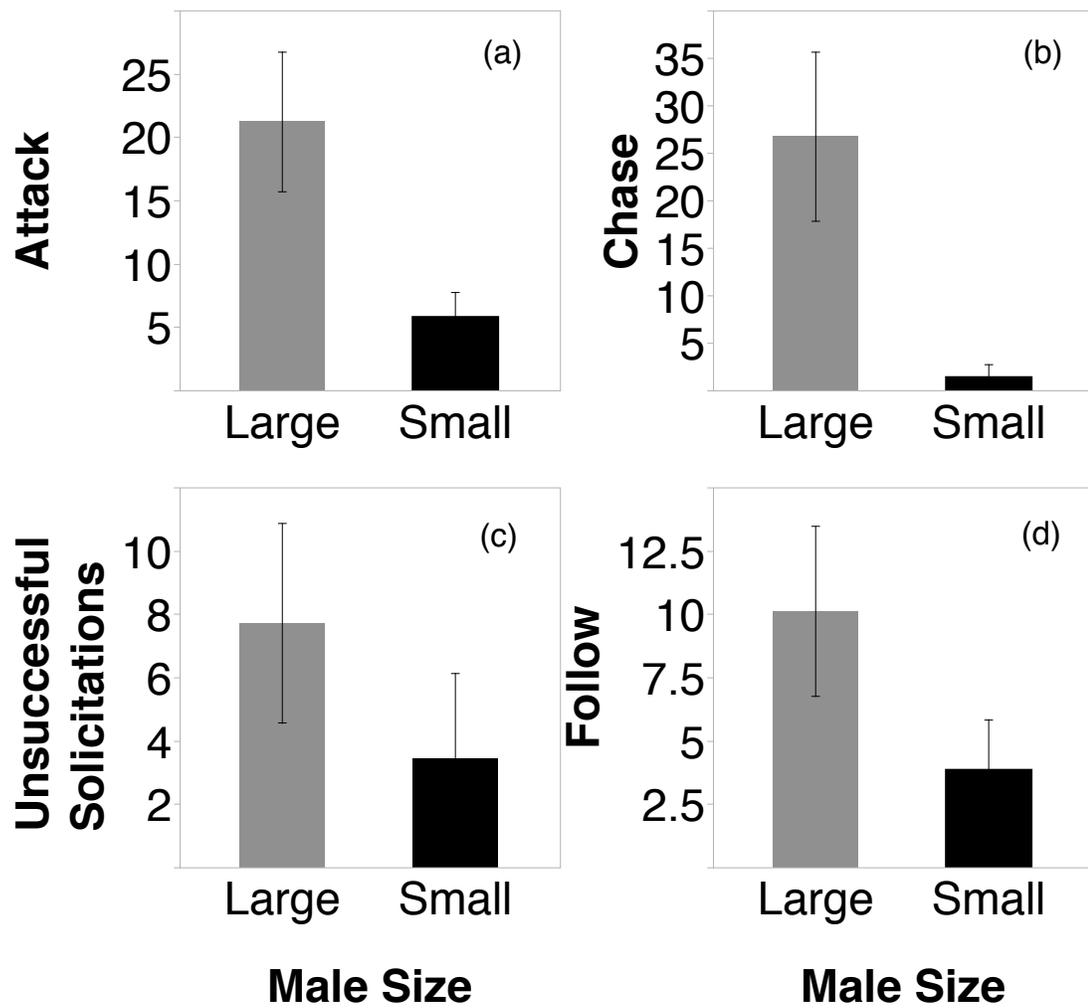
Fig. 3

Differences between large and small male *Etheostoma fonticola* in aggressive behaviors towards other males in mean ± SE (a) attacks, (b) chases, (c) unsuccessful solicitations, and (d) follow
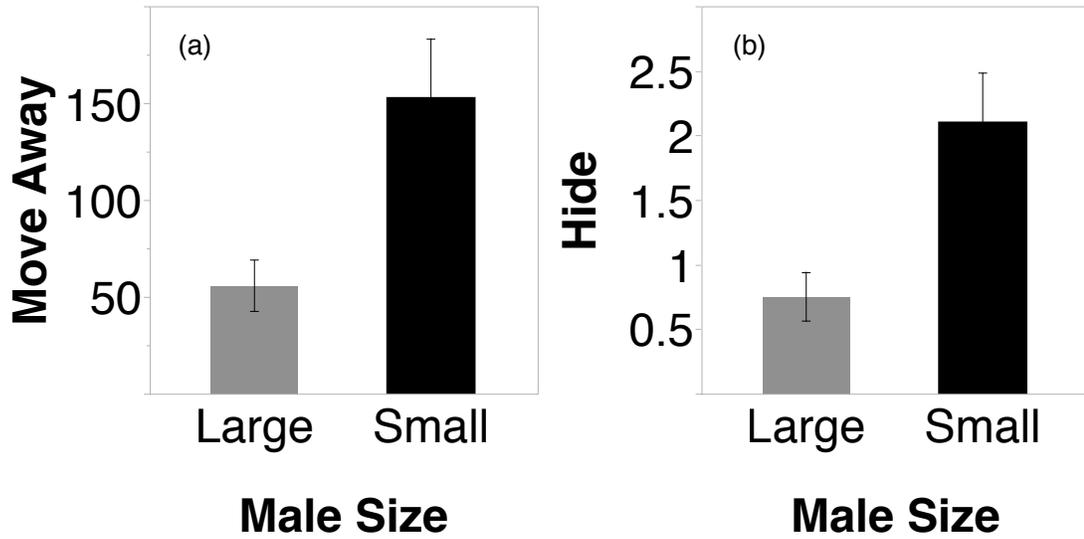
Fig. 4

Differences between large and small male *Etheostoma fonticola* in defensive behaviors towards other males in mean ± SE (a) move away and (b) hide
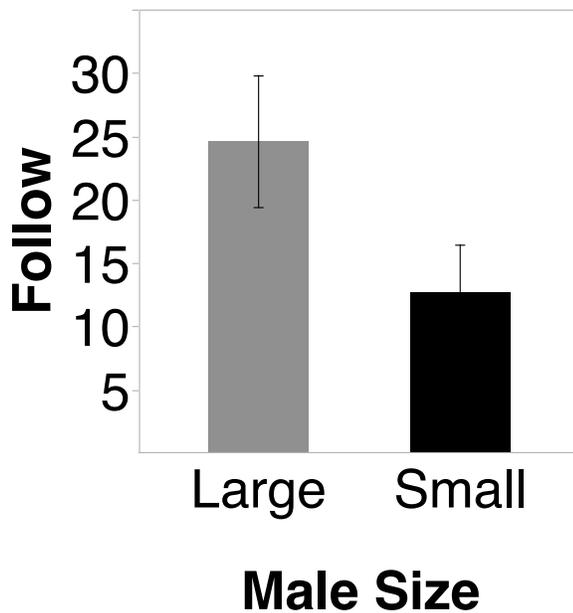

Fig. 5

Differences between large and small male *Etheostoma fonticola* in mean ± SE number of times observed following the female

Table 8

Results from Mann-Whitney *U* tests for differences in behaviors of females towards large and small males. Bold values indicate a significant difference (*P*<0.05)

| Behavior	df	Mean ± SE large	Mean ± SE small	Z	*P*
Move Towards	19	47.95 ± 2.9	43.55 ± 2.9	41.5	0.07
Move Away	19	189.9 ± 40.6	107.15 ± 40.6	51	**0.058**
Approach	19	44.6 ± 4.9	31.75 ± 4.9	67	**0.0103**
Follow	19	1.75 ± 0.7	1.4 ± 0.7	4.5	0.8
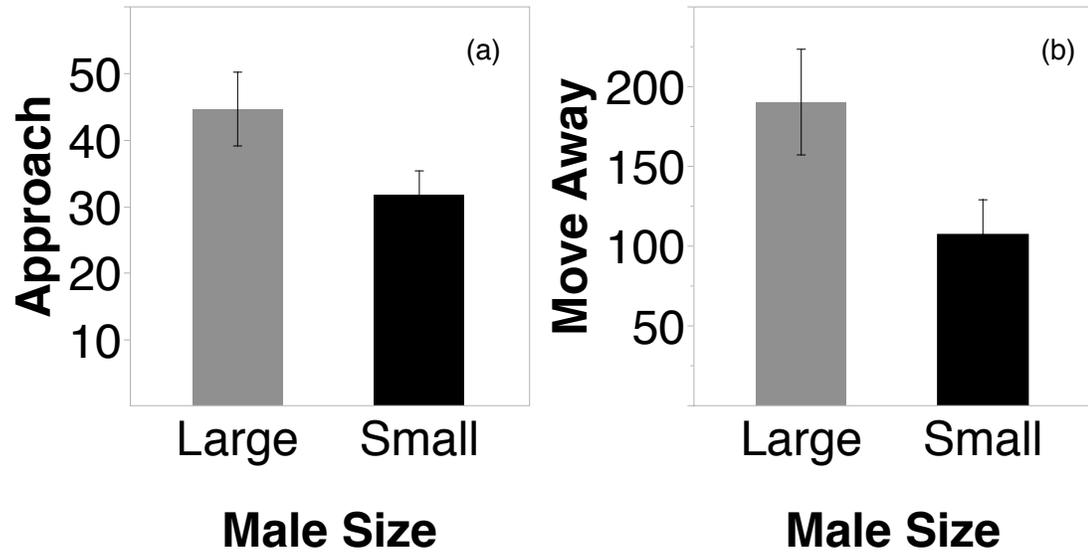
Fig. 6

Differences in female *Etheostoma fonticola* behaviors towards large and small males in mean ± SE (a) number of times approach and (b) number of times moved away

Consistent with our previous work examining association preferences in *E. fonticola* (DeColo et al. chapter 2), we found no support for female mate choice based on male size. Female mating preferences in *E. fonticola* had not been tested previous to this study, and may be based on other male characteristics such as banding patterns on the body or coloration on the dorsal fin. Alternatively, female mate preferences may be based on other visual stimuli or other cues not directly evaluated here. Additionally, females may not be the choosier sex in this species.

In contrast to the lack of association preferences for larger males previously found in *E. fonticola* (DeColo et al. chapter 2), we found that females approach larger males more than smaller males. Females may be evaluating some characteristic other than size that larger males possess, but this behavior does not translate into a mating advantage for those males because either females are not choosing to mate with those males, or male-male interactions may be interfering with the ability of females to exercise their preferences. Additionally, females moved away from larger males more than smaller males. This may be due to the increased number of times females approached larger males. Females were not preferentially mating with those males so it may result in them also moving away from them more frequently. There was no difference in the behaviors of larger and smaller males towards females, except that larger males follow females more than smaller males do. This increased following behavior may have contributed to the propensity of females to move away from larger males more than smaller males.

Males and females were separated two weeks prior to testing which may not be a sufficient amount of time to incentivize females to mate in *E. fonticola*. Out of the 40

male stimulus individuals, 32 males attempted to mate with the female, although only a small number (8) successfully mated with the female. There was only one trial in which neither male solicited any mating attempts. Females may be choosing to not mate with the males that are attempting to mate with them or they may simply be choosing to not mate at all. Alternatively, our experimental design may have provided a habitat that females did not find suitable to spawn in, although previous studies have found that *E. fonticola* will readily spawn in laboratory conditions on artificial plants and spawning mops (Brandt et al. 1993; Simon et al. 1995; Bonner et al. 1998; Phillips et al. 2011).

While there was no difference in the mating behaviors exhibited by large and small male *E. fonticola,* males differed significantly in their interactions with other males. We found that larger males exhibited more aggressive behaviors. Larger males follow, chase, and attack the other male more often than smaller males. Larger males are also more likely than smaller males to solicit mating attempts towards the other male. To our knowledge, this behavior has not been described as an aggressive behavior before in *Etheostoma*. Winn (1958) found that in several *Etheostoma* species males will mount other males and were even observed to initiate body vibrations. However, Winn (1958) suggested that this behavior is due to the absence of sex recognition in males in these species. Even though *E. fonticola* lack the extreme sexual dimorphism in coloration that is characteristic of other *Etheostoma* species, there are many differences in the behaviors male *E. fonticola* direct towards males and females such as being more aggressive towards other males than females. These findings suggest that at least male *E. fonticola* are capable of sex recognition. We propose that a male mounting a competing male is an aggressive behavior for establishing dominance relationships.

In other species of *Etheostoma,* male behaviors such as guarding females is correlated with male spawning success while aggressive behaviors such as attacks and chases are not correlated with spawning success (Pyron 1995; Fuller 2003). Male guarding behavior was not directly tested in this study nor were males observed guarding females in any trials. The absence of guarding behavior in *E. fonticola* may explain why larger males do not experience increased mating success although they exhibit more dominant behaviors than smaller males. Alternatively, our experimental design may not have provided the appropriate conditions for males to exhibit guarding behavior such as a more strongly male biased sex ratio.

Consideration of several life history traits and environmental factors may offer some insight into the mating behavior of *E. fonticola* and may provide insight into the lack of female mating preferences for male size. Ecological factors provide a framework for shaping a mating system and the intensity of sexual selection within that system (Emlen and Oring 1977). For instance, there is no parental care exhibited in *E. fonticola*, eggs are attached to the surface of a plant and subsequently abandoned. Female preferences for a variety of male traits have been found in several species of *Etheostoma* (Grant and Colgan 1983; Knapp and Sargent 1989; Strange 2001; Porter 2002; Fuller 2003; Stiver and Alonzo 2010; O'Rourke and Mendelson 2014). However, parental care is exhibited in all of these species with the exception of *E. caeruleum* (Fuller 2003). In another species that does not exhibit parental care, *E. spectabile*, female preferences for male size were not found (Pyron 1995). Additionally, *E. fonticola*'s native habitat is characterized by a temporally and spatially uniform distribution of native vegetation types that *E. fonticola* are primarily associated with (Schenck and Whiteside 1976; Linam

et al. 1993) and that are preferentially used by *E. fonticola* for spawning (Phillips et al. 2011). The large supply and predictable availability of spawning sites would suggest there is little need for male *E. fonticola* to defend resources or territories in order to gain access to mates. It is not known whether there is high variation between male *E. fonticola* in terms of mate quality. Our results suggest that there is variation between large and small males in their aggressive behaviors towards other males, but this does not influence their relative mating success. Female *E. fonticola* exhibit high variation between individuals in the number of mature ova they contain (Schenck and Whiteside 1977) as well as high variation between and within individuals in the number of eggs they release per day and the duration of days that eggs are released (Brandt 1993). This suggests that males may be able to increase their reproductive success by preferentially mating with females with higher reproductive output. However, *E. fonticola* release a single egg per mating event, restricting the potential for males to increase their fertilization success by mating discriminately. Finally, given the reproductive biology of *E. fonticola*, and survival in the lab, we assume that the operational sex ratio is 50/50 in this species. Considering the combination of these behavioral, biological, and environmental elements, it is not surprising that we found no evidence for female preferences for male size in *E. fonticola*.

In conclusion, our data do not support the hypothesis that male body size influences female mating preferences in *E. fonticola*. However, larger and smaller males differ in their behavior towards other males. It is important to note that we only tested pairs of males that differed in SL by 5mm and did not test pairs of males that were size matched, or any other variation of size combinations. We found that interactions between

larger and smaller males result in significant differences in aggressive and defensive behaviors between the two sizes. However, when the size difference between competing males is decreased, males may increase agonistic interactions in order to establish dominance relationships or they may be able to assess that their competitive abilities are more evenly matched and subsequently reduce direct contests. For example in the mosquitofish, *Gambusia holbrooki*, when there is less size differentiation between male competitors, the frequency and intensity of aggressive interactions decreases (McPeek 1992). Examining male-male interactions across a spectrum of size differentiation would provide insight into male dominance relationships in *E. fonticola*.

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