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Head over heels: An examination of a possible mating signal in female swordtails, *Xiphophorus cortezi*

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Abstract

Females of many species display overt behavioural signs that provide information to males about their willingness to mate. We have observed female swordtails (*Xiphophorus cortezi*) performing “headstands” and/or “pecks” toward the substrate of their tank in the presence of males. To determine if this behaviour is an attempt at foraging, a mating signal, or a sign of aggression or stress, we exposed satiated individual female swordtails to either no fish, a female or a male fish and measured association time and frequency of headstand/pecking behaviours. Females did not perform these behaviours when there was no other fish in the tank. Although they spent equal amounts of time associating with either a male or female stimulus fish, they only performed headstands/pecks in the presence of males. Furthermore, in dichotomous choice tests with large and small males, females preferred to associate with large males and performed significantly more headstands/pecks in their presence. Finally, males were simultaneously exposed to videos of a free-swimming female and the same female intermittently pecking the bottom of the tank in order to examine their response to the signal. Larger males spent more time with and performed more courtship behaviours towards the pecking female, whereas smaller males spent more time associating with and courting the freely swimming female. These results suggest that headstands/pecks performed by female swordtails in the presence of males may be a signal of a female’s willingness to mate and that the size of a male affects his response to this signal.

Keywords

female mating signals; swordtails; *Xiphophorus cortezi*; sexual selection; female preference; male preference; headstands

Females of many species, particularly those for which sexual receptivity is cyclical, display overt behavioural signs that provide information to males about their willingness to mate (Beach 1976; Montgomerie & Thornhill 1989; Rowland et al. 1991; Ringo 1996; Rowland 2000; Semple & McComb 2000). Female mating signals can be beneficial for both females and males. For females, it can serve to attract males and incite male-male competition, increasing her choices and enhancing her likelihood of mating with the most desirable males (Farr 1989). For males, female mating signals allow them to focus their courtship behaviour and energy on cooperative or receptive females rather than on all females in a population

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(Beach 1976; Sumner et al. 1994). This would be particularly important if there is a reproductive cost associated with mating, such as, diminishing sperm reserves following copulation (Wedell et al. 2002), costly sperm production (Dewsbury 1982, Aspbury & Gabor 2004), or there is a refractory period between consecutive copulations during which sperm cannot be produced (Pilastro & Bisazza 1999). Furthermore, a signal of a female's willingness to mate would allow a male to synchronize his behaviour with a female's, improving his ability to orient and position himself properly in order to effectively deposit his sperm (Beach 1976). Sperm transfer in guppies has been shown to be more successful when a female cooperates with a male (Pilastro et al. 2002). Finally, ecological factors such as territorial defense and predation risk impose additional costs on courtship behaviour (insects: Sih 1988; fish: Magnhagen 1991, Candolin 1997; frogs: Ryan 1985) that would influence natural selection by favoring males that mate non-randomly with the limited number of receptive females in the population.

Though female mating signals can be displayed in a variety of forms, one of the more common overt behaviours reported in many species of fishes is a change in body posture in the presence of a male (Beach 1976; Rowland et al. 2002). For example, Tinbergen (1948) described a head-up body orientation performed by female threespine stickleback fish and hypothesized that it was a social releaser that elicited male courtship behaviour. Experimental studies have confirmed that the head-up posture in threespine sticklebacks first described by Tinbergen is a solicitation behaviour by females to elicit courtship by males (Bakker & Rowland 1995; Rowland 2000). During behavioural observations, we have observed female swordtails (*Xiphophorus cortezi*, Poeciliidae, Cyprinodontiformes) performing headstand behaviours in the presence of males during which their bodies are inclined toward the substrate in a 30–45° degree angle. These headstands are sometimes, but not always, accompanied by pecking behaviours toward the substrate of the tank. Head down displays by both males and females have been described anecdotally as threats of aggression in many species of fish including sticklebacks (Mori 1987; McLennan 1993; Losey & Sevenster 1995; McKinnon 1996; Bolyard & Rowland 2000), cichlids (Neil 1984; Walter & Trillmich 1994; Ochi 1996), hamlets (Lobel 1992), red and crimson sea bream (Kudoh & Yamaoka 2004), Atlantic sharpnose puffers (Sikkel 1990) and *Xiphophorus cortezi* (Moretz 2005). However, a head-down posture performed by males in front of females may also be part of the male courtship display in *Xiphophorus nezahualcoyotl* (Morris et al. in review) and Caribbean rosy razorfish (*Xyrichtys martinicensis*; Victor 1987). Interestingly, in Caribbean rosy razorfish, Victor (1987) observed that if this behaviour is not reciprocated by consenting behaviour in the female, the male leaves to court other females suggesting that the male waits for a behavioural sign that a female is willing to mate.

Only two studies have described a head-down posture performed by female fish in the context of reproductive behaviour. Female sunbleak (*Leucaspius delineatus*), for example, adopt a headstand position during both spawning and pseudospawning (Gozlan et al. 2003). In smallmouth bass (*Micropterus dolomieu*), an approach by a male elicits a head-down posture in receptive females (Ridgway et al. 1989). However, no experimental studies have been conducted to validate that this behaviour is a mating signal as opposed to a random behaviour or threat of aggression toward the approaching male. Though female mate choice has been extensively studied in *Xiphophorus*, to our knowledge the function of headstands/pecks performed by females has been neither described nor empirically examined.

Female poeciliids are live-bearing fishes that have a 30-day reproductive cycle during which new fertilizable ova are available for a limited amount of time (Constantz 1989). Though female poeciliids can store sperm for up to eight months (Constantz 1989), the sperm that is deposited most closely to ovulation is the most viable (Farr & Travis 1986). Because individual female poeciliids do not synchronize ovulation, Farr (1989) hypothesized that male poeciliids would

increase their reproductive success by being able to recognize fertile females, court exclusively these few receptive females, and prevent copulations by other males. Male mate choice would also increase a male's reproductive success because research suggests that sperm production by male poeciliids is costly (Aspbury & Gabor 2004). A recent study of *Xiphophorus nigrensis* suggests that females perform a gliding motion or glide response more often in the presence of preferred males suggesting that they advertise their willingness to mate (Cummings & Mollaghan 2006). A similar behaviour has been described in guppies (*Poecilia reticulata*, Houde 1997). However, the male response to these potential mate solicitation behaviors has not been explicitly tested. In order to definitively determine that a behaviour functions as a signal, the response of the receiver must also be evaluated. Therefore, in this study we examined not only the function of the headstand/peck behavior of *X. cortezi* females, but also the response of males to the behavior.

Xiphophorus is widely distributed throughout much of Central America (Rosen 1979). *Xiphophorus cortezi* has a large geographic distribution (Rauchenberger et. al 1990) and is found in many rivers and tributaries of the Rio Panuco basin that empties into the Gulf of Mexico near Tampico, Mexico. *X. cortezi* is often found sympatric with other *Xiphophorus* species (primarily *X. variatus* and *X. birchmanni*) although frequently not with other sworded *Xiphophorus* species (i.e. complex sword, per Basolo 1996). Mating behaviors in *Xiphophorus* are found in both males and females (for descriptions see Ryan & Causey 1989), with males frequently employing behaviours that display phenotypic traits used in mate choice decisions (e.g. figure eight swimming displays to assess vertical bars bilaterally, Morris 1998). Although some *Xiphophorus* species employ alternative male mating strategies (*X. nigrensis*, Ryan & Causey 1989; *X. multilineatus*, Morris & Ryan 1992) in which large males perform courtship displays whereas small males perform sneak-chase behaviors and forced copulations, there is no evidence in the literature that this mating strategy is present in *X. cortezi* males. There is continuous variation in *X. cortezi* male size (i.e. no distinct male size classes; Rauchenberger et. al 1990), and we have observed the full range of courtship displays in even the smallest *X. cortezi* males.

There were three main objectives of this study. First, we wanted to determine if headstands/pecks directed toward the bottom of the tank are mating signals in *X. cortezi*, rather than attempts at foraging or signs of aggression or stress. In the first experiment, we exposed food satiated individual female *X. cortezi* to either no fish, a female fish or a male fish and measured association time and frequency of headstands or pecks directed toward the bottom of the tank. We predicted that females would perform more headstands/pecks in the presence of males than in the presence of females or no stimulus. Second, we wanted to examine if headstands/pecks are performed more often in the presence of preferred males than non-preferred males. Food satiated females were given the choice between large and small males in dichotomous choice tests, and we measured association time as well as the frequency of headstands/pecks performed in each choice zone. We predicted that females would spend more time with large males and perform more headstands/pecks in their presence. Finally, we wanted to determine if males engage in more courtship behaviours or spend more time associating with a female performing headstand/pecking behaviours. This would indicate whether or not males perceive these behaviours as mating signals and respond differentially in the presence of a female performing headstands/pecks. Therefore, in the third experiment individual males were simultaneously exposed to videos of a freely swimming female and the same freely swimming female intermittently pecking the bottom of the tank. We predicted that males would spend more time with females performing headstands/pecks than with freely swimming females.

GENERAL METHODS

Experiments were conducted between October 2006 and May 2007. Adult male and female *X. cortezi* were collected in April 2006 from the San Pedro River near the town of San Martin (21°22'N, 98°40'W) in San Luis Potosí Province, Mexico. At this collection site, *X. cortezi* was the predominate *Xiphophorus* species, although there were moderate numbers of *X. variatus* and a limited number of *X. birchmanni*. Before experimentation began, fish were visually isolated and housed separately in 19L isolation tanks. All females were housed in isolation for a minimum of 4 months before being tested in the first and second experiment. Standard laboratory conditions included a daily diet of TetraMin flakes, 12 D:12 L cycle, constant air temperature of $22 \pm 1^\circ\text{C}$, and 40W Vitalite® (Durotest, Philadelphia) fluorescent lighting. All experiments were conducted between 0800 hours and 1200 hours. In each experiment, focal fish and those used as stimuli were fed until satiated in their resident tanks ten minutes before being placed in the experimental tank. Fish were considered satiated when they no longer fed and there were still flakes in their tank. Every third day of testing, experimental tanks were drained completely and gravel rinsed thoroughly before being refilled. A single observer (LT) collected all observational data in the first two experiments and a single observer collected all data in the third experiment to maintain consistent observations and classification of the headstand/peck behaviours.

EXPERIMENT 1

Methods

The experimental tank was a standard 208L choice tank ($120 \times 30 \times 40$ cm) divided into five equal zones (~24 cm) separated by clear solid partitions. The central three zones were visually delineated on the outside of the tank. For this experiment, only one end of the choice tank was used at a time. The 'choice' zone was the zone immediately adjacent to the location of the test stimulus, whereas the 'neutral' zone consisted of the remaining two central zones. Therefore, the neutral zone was twice the size of the choice zone.

The testing procedure consisted of placing a stimulus fish or no stimulus in the end zone and recording the number of headstands/pecks at the substrate performed as well as the association time of each female. Association time was the amount of time the female spent in the zone adjacent to the end zone during each 10-minute condition trial. A headstand was recorded when the body of the fish was inclined toward the substrate in a 30–45° degree angle. Pecks were recorded when the female adopted the headstand position and pecked toward the substrate. Each subsequent peck was recorded even if the female did not break the headstand position, though one single peck was most frequently performed. Nineteen females were tested under three treatments: male stimulus, female stimulus, and alone (no fish in end zone). At the beginning of each trial, the test females were placed in an opaque holding tube that was situated in the middle of the neutral zone. The holding tube was 10 cm in diameter, and its length was long enough extend out of the test tank water while resting on bottom (~50 cm). The width of the tube, as well as a twin fluorescent light fixture with Vitalite® bulbs (Durotest, Philadelphia) suspended approximately 30 cm above the water line, ensured that females were not dark-adapted during the acclimation periods. The total duration of each test was 45 minutes including 10 minutes for each condition and 5 minute acclimation periods at the beginning and between each stimuli condition. The females were exposed to each treatment consecutively, and the placement (i.e. two end zones) and presentation order of stimulus conditions were randomized.

Nineteen females were tested; however, the data from two females were eliminated from analysis because they displayed a side bias such that they spent the entire 10-minute trial in either the neutral zone or on the same side of the test tank across each of the three trials (i.e. regardless of the condition present). Therefore, the data from 17 individuals were analyzed

using a two-tailed repeated measures analysis of variance (ANOVA). All data met the assumptions of normality and the results were considered statistically significant at $p < 0.05$.

Results

In Experiment 1, there was a significant main effect of test stimulus on female association time ($F_{1,16} = 8.61$, $P = 0.01$; Fig. 1). Bonferroni adjusted pairwise comparisons revealed that the females spent equal amounts of association time with either a male or female stimulus ($P = 0.26$). There was also no significant difference in association time between no fish and females ($P = 0.17$). However, there was a significant difference in association time between the no fish condition and the male stimulus condition ($P = 0.02$). Because headstands without pecking at the substrate occurred at a relatively low frequency, we summed the number of headstands and the number of headstands accompanied by pecking towards the substrate (hereafter referred to as headstands/pecks). Females performed significantly more headstands/pecks when in the presence of a male than when in the presence of either no fish or a female ($F_{1,16} = 15.11$, $P < 0.001$; Fig. 1). In fact, females never performed headstands/pecks when either alone in the test tank or in the presence of a female.

EXPERIMENT 2

Methods

In experiment 2, we used the same experimental tank that was used in experiment 1. In this experiment, however, females were simultaneously presented with two stimuli, a large and a small male. Large males were classified as males > 40 mm SL ($X \pm SE = 44.16 \pm 0.72$) and males < 40 mm SL were considered small males ($X \pm SE = 35.18 \pm 0.29$). Nineteen females were randomly assigned to be tested with seven different male pairs. To minimize potential changes in reproductive state, each female was tested 5 days after she was tested in the first experiment. Females were placed in the opaque holding tube for a five-minute acclimation period after the males had been positioned in the end zones. The 15-minute trial began as soon as the tube was lifted out of the tank. We measured association time as the time the female spent in the two 'choice' zones immediately adjacent to the end zones housing the stimulus males. We also recorded the number of headstands/pecks. At the end of the first trial, the female was returned to the holding tube and the male's position switched to control for any side bias by the female. After the five-minute acclimation period, the second 15-minute observation trial began. On the third day, each female was tested again with this same protocol but using a different pair of stimulus males to ensure that females were not associating with a particular male but rather size classes of males. Assignment of female-male pairs and the placement of large and small males on the right or left side of the choice tank were randomized. We averaged the number of headstands/pecks performed by the test female and the total amount of time each female associated with the large and small males across test days. Two-tailed paired t-tests were used to compare the mean association time and the frequency of headstands/pecks the females performed with either large or small males. All data met the assumptions of normality, and the results were considered statistically significant at $p < 0.05$.

Results

Females spent significantly more time associating with large males than with small males ($t_{18} = 26.58$, $N = 20$, $P < 0.001$; Fig. 2a). Furthermore, females performed significantly more headstands/pecks ($t_{18} = 16.72$, $P = 0.001$; Fig. 2b) while in the choice zone associated with large males than in the choice zone associated with small males.

EXPERIMENT 3

Methods

Video Preparation—Three females were filmed in a 3L plastic observation container with a high definition video camcorder (Hitachi VM-H100LA). White construction paper was used to cover the sides and back of the container, providing a uniform backdrop for all videos. Two inches of natural color gravel lined the bottom of the container. A 24 inch fluorescent light fixture with a Vitalite® bulb (Durotest, Philadelphia) was placed 25 cm above the container to provide consistent lighting for the videos. Each female was first filmed swimming freely in the container. Afterwards, the female was removed from the container and the bottom of the container was baited with freeze dried *Artemia*. *Artemia* were used because of its small particle size and its coloring, which appeared inconspicuous against the brown gravel. Only small amounts of *Artemia* were used, most of which settled in between the gravel on the bottom of the tank. Each female was filmed again as she performed headstands/pecks towards the substrate. No food particles visible during the recordings of the three females performing the headstands/pecks behaviour.

All video clips were edited using iMovie (Apple Computer, Inc., Cupertino, CA, U.S.A.) on a MacBook Pro laptop. Raw footage was edited so that each trial contained a two-minute lead-in (backdrop only) and eight minutes of female behaviour. Female behavioural footage was looped in each of the trials to obtain eight minutes of video (minimum = 4, maximum = 8). Care was taken to maintain continuity between sequences. Female headstands/pecks were spliced into the free swimming video of that female such that a male saw the same female trial with or without headstands/pecks. Female headstands/pecks represented approximately 20% of the eight minute trial in each of the three female videos.

Methods—The experimental protocol consisted of testing a male's response ($N = 22$) to the simultaneous presentation of a single freely swimming female and the same freely swimming female intermittently performing headstands/pecks at opposite ends of a $50 \times 25 \times 30$ cm tank. The tank was visually delineated into three equal zones (~ 16.5 cm), two 'choice' zones and a central neutral zone. The back and sides of the tank were covered with brown paper with the exception of $16 \text{ W} \times 12 \text{ H}$ cm rectangular cutouts on each side to view the monitor display. Two seventeen inch Samsung syncmaster 740BX LCD monitors with a maximum resolution of 1280×1024 were used for video display. Dual Macmini computers connected to the monitors allowed for the size of the iDVD projection to be adjusted properly. The display size of each female was measured to ensure it fell within the range of female standard lengths from this population (Female 1 = 40.5 mm, Female 2 = 44 mm, Female 3 = 42.5 mm; Population $X \pm \text{SE} = 41.11 \pm 0.73$). The front of the tank was covered with black perforated viewing film.

Each trial consisted of a five minute acclimation period during which the male was placed in a clear square 5" plastic holding tube in the center of the tank followed by a simultaneous presentation of the two videos. The male was shown a two minute lead-in sequence followed by a three minute assessment period. The footage during the assessment period was similar to that of the recorded trial period. Data collection was continued only if the test male swam to both sides of the holding tube during the 3-minute assessment period. After the assessment period, the holding tube was removed from the tank and the five minute trial period began. We recorded the time spent associating with each video in the 'choice' zones, number of courtship displays performed, number of head down postures, and the percent time gaze at the videos. Proportion of time a male gazed at the videos was defined as the time a male spent within 10 cm of the video display in the choice zone and within 45° of perpendicularly facing the monitor (90° total, 45° to either side). After the first 5 minute trial, the male was returned to the center holding tube, the videos were switched to the opposite sides, and the male was acclimated for 5 minutes. After the 5 minute acclimation period, the videos were started and the same

methodology used in the first trial was repeated. We averaged the number of courtship displays, headstands/pecks performed by the test male, and the amount of time each male associated with the female stimuli across these two trials.

Results

Two-tailed paired t-tests were used to compare the differences in association time, percent time spent gazing at females, courtship displays and headstands performed by males when associating with either a video of a freely swimming female or a video of the same female performing intermittent headstands/pecks. The results were considered statistically significant at $p < 0.05$. There was no significant difference in association time between the freely swimming female and the pecking female ($t_{21} = 0.52$, $N = 22$, $P = 0.48$). There was also no significant difference in the percentage of time that males spent gazing toward the freely swimming or pecking female ($t_{21} = 0.33$, $P = 0.57$). Finally, males did not direct more courtship behaviours or perform more headstand displays when associating with freely swimming or pecking females (courtship displays, $t_{21} = 0.50$, $p = 0.49$; headstands $t_{21} = 1.688$, $P = 0.21$).

During behavioural observations we noted that the behaviour of the larger males was different from that of the smaller males. Unlike *X. nigrensis* and *X. multilineatus* in which there are distinct, genetically determined size classes of males (Kallman 1989), *X. cortezi* males range in size across a normal distribution. Therefore, to test whether larger males and smaller males responded differently when exposed to identical experimental conditions we measured the average standard length of each male and divided the subject pool into those that were above the mean ("larger males" ≥ 40 mm, $N = 14$, $X \pm SE = 43.17 \pm 0.49$) and those that were below the mean ("smaller males" < 40 mm, $N = 8$, $X \pm SE = 37.58 \pm 0.59$). The data were analyzed for each of the four dependent variables (association time, percent time gaze, courtship displays and headstands) using two-tailed two-way mixed factorial ANOVAs. Our results show that the larger and smaller males were indeed behaving differently. Though there was no main effect of treatment (freely swimming or pecking female) on association time, there was a significant interaction effect indicating that the larger males associated more with intermittently pecking females whereas the smaller males associated more with the freely swimming females ($F_{1,20} = 4.57$, $P = 0.045$; Fig. 3a). There was also no main effect of treatment on the mean percentage of time males spent gazing toward each female ($F_{1,20} = 1.49$, $P = 0.24$). There was a significant interaction effect for the mean percentage of time males spent gazing toward each female such that larger males spent more time gazing toward the pecking females and smaller males spent more time gazing toward the freely swimming females ($F_{1,20} = 5.15$, $P = 0.04$). Finally, there was no main effect of treatment (freely swimming vs. pecking female) on the performance of courtship displays ($F_{1,20} = 0.07$, $P = 0.93$). However, there was an interaction effect such that larger males performed more courtship displays in front of pecking females than in front of freely swimming females, whereas smaller males performed courtship displays in front of freely swimming females and no courtship displays in front of pecking females ($F_{1,20} = 6.07$, $P = 0.02$; Fig. 3b). There was no significant main effect ($F_{1,20} = 0.84$, $P = 0.37$) or interaction effect ($F_{1,20} = 1.70$, $P = 0.21$) of treatment on the total number of headstands performed by males in either choice zone.

DISCUSSION

The purpose of this study was to systematically examine a previously undescribed behaviour performed by female *Xiphophorus cortezi*. Three lines of evidence from this study suggest that headstands and pecks toward the substrate performed by female *X. cortezi* function as mating signals. First, females only performed headstands/pecks when associating with males. Second, as reported in several other species of female poeciliids (*X. nigrensis*, Ryan & Wagner 1987, Ryan et al. 1990; *Poecilia reticulata*, Endler & Houde 1995; *X. multilineatus*, Morris et al.

1995; *Poecilia latipinna*, Gabor 1999; *X. pygmaeus*, Hankison & Morris 2002), *X. cortezi* females preferred larger males and in our study performed more headstands/pecks in the presence of these males than smaller males. Finally, males responded to females performing this signal differently depending on their size. Specifically, larger males courted and associated significantly more with pecking females, whereas, smaller males spent more time with and exclusively courted the freely swimming females.

One possible explanation for why these behaviours have not been previously described in the literature as signals of a female's willingness to mate is that, to an observer not specifically interested in mating signals, the female appears to be foraging. The results from our first experiment indicate that headstands/pecks can better be explained from a social perspective. First, to control for hunger, females were tested after they had been fed to satiation so that they would not be motivated to feed during trials. Second, if headstands/pecks were simply attempts at foraging it would be expected that females would indiscriminately perform the behaviour under a variety of social conditions. In addition, headstands/pecks were rarely performed in the neutral zone outside of the choice zones. These results suggest that headstands/pecks are not foraging behaviours but rather social displays. In addition, we anecdotally observed that headstands/pecks were most likely to follow male courtship displays. Future studies should be conducted to examine the temporal relationship between male courtship displays and this female behaviour.

Interestingly, the size of the males influenced their response to the headstands/pecks. Smaller males discriminated against females who pecked the substrate and only performed courtship displays in the presence of freely swimming females. Similarly, Sumner et al. (1994) found that male *P. latipinna* can recognize receptive females using olfactory signals and that males varied in their response to receptive females depending on their size such that small males spent more time with unreceptive females. Not only did the smaller males in our study spend more time with non-signaling *X. cortezi* females, these males also courted the freely swimming females at the same rate that the larger males courted signaling females, indicating that they do not use alternative mating strategies as has been described in *X. nigrensis* and *X. multilineatus* (Ryan & Causey 1989; Morris & Ryan 1992). It has been hypothesized that female fertility advertisement would favor larger males because, being the most dominant and aggressive, they would be able to fend off smaller males and prevent them from courting receptive females (Farr & Travis 1986). In a closely related species, *X. nigrensis*, larger males interact more with females, chase away smaller males, and dominate access to females in both natural and laboratory conditions (Morris et al. 1992). This is important because, although females are able to store sperm, the most recently deposited sperm fertilize the most offspring (Constantz 1989), particularly if insemination occurs when the females are most fertile (Farr & Travis 1986).

There are several reasons why it would benefit *X. cortezi* females to engage in a head-down posture when signaling their willingness to mate. For example, it is possible that the head-down posture facilitates successful insemination by allowing males to more clearly view the darkly pigmented gonopore of females (Constantz 1989). This gonoporal pigmentation could guide the orientation of the gonopodium toward the genital area of the female (Peden 1973), signal receptivity (Peden 1973; Farr & Travis 1986; Rowland et al. 1991; McLennan 1993; McLennan 1994; Rowland et al. 2002), provide a signal of the female's fecundity and overall physiological condition (Peden 1973) or facilitate gonoporal nibbling, a courtship behaviour performed by males, which may allow a male to determine female receptivity via olfactory cues (Farr & Travis 1986). Researchers in our laboratory (Robinson, personal communication) have observed that females turn and face away from the males when they are performing this behavior, presenting males with their ventral surface. Moreover, Benson (2007) has shown that *Xiphophorus helleri* males prefer females with larger brood patches, indicating that they use

this pigmentation to focus their behaviour on select females and that this pigmentation is important in male/female interactions.

Performing headstands/pecks could also benefit females by attracting larger males. Larger male poeciliids (*P. latipinna*) have more sperm available and produce more sperm in the presence of females, particularly large females, than smaller males (Aspbury & Gabor 2004). Moreover, Schlupp and Ryan (1996) have shown that sailfin molly males (*P. latipinna*) spend more time with a female that is being courted by other males. If the same were true within *Xiphophorus*, it would allow females the opportunity to compare and choose among several larger *X. cortezi* males simultaneously on either their physical characteristics or ability to win contests. It would also indirectly result in the exclusion of smaller, less preferred males. Future studies should be conducted to examine whether these headstand/peck behaviours in female *X. cortezi* do in fact cause larger males to approach and engage in competitive interactions.

Despite the statistical significance of the results, the rate of headstand/pecking behaviours performed by females was low in each of our experiments. One limitation of our study is that, because we did not use postpartum females (i.e. known reproductive state) in our first and second experiment, we cannot definitively conclude that the headstand/pecking behaviour is a signal of receptivity. Because female poeciliids are able to store sperm, most studies of mating preferences test females within 48 hours of giving birth to ensure that they are receptive (Sumner et al. 1994; Ptacek & Travis 1997; Gabor & Page 2003). This was not possible in our study because we have experienced significant problems with *X. cortezi* females cannibalizing their fry within a short time after giving birth. Without constant surveillance, it is difficult to know with certainty if females had dropped fry. We have also had minimal success with gravid females dropping fry while in brood traps. Therefore, if headstands/pecks are a signal of receptivity, then variation in the ovulatory cycles of the test females may explain the low frequency of female courtship behavior. However, female poeciliids may still benefit by signaling their willingness to mate with large males regardless of their reproductive state, particularly if larger males deliver more sperm that could be stored and used in the future (Aspbury & Gabor 2004). Future studies should be conducted in which the reproductive state of the females is known. If headstands/pecks are a signal of receptivity, we would hypothesize that the frequency of these behaviours would increase sharply when females are ovulating.

Due in part to their unique life history, live-bearing fishes have drawn considerable interest in studies of sexual selection. Despite the theoretical importance of female advertisement of receptivity given their unique reproductive biology (Farr 1989), reports of potential female mate solicitation behaviours have been relatively rare (Peden 1973; Farr & Travis 1986; Cummings & Mollaghan 2006). In *Xiphophorus*, Cummings and Mollaghan (2006) were the first to experimentally identify a female behaviour, the glide response, performed more often by females in the presence of preferred, large males. Our study is unique in that we not only experimentally identified a behaviour that is more likely to be performed in the presence of preferred males than in other social contexts, but we also experimentally showed that males respond to the signal. Historically, the detection and repeatability of male mate preference in barrier trials that prevent olfactory communication has been difficult (Herdman et al. 2004, Hoysak & Godin 2007). The detection of male mate choice in *X. cortezi*, without the aid of olfactory cues, underscores the importance of headstands/pecks as a visual signal in mate selection. Our results also provide further evidence that *Xiphophorus* males are choosy, which has been reported in only one other study (*X. helleri*, Benson 2007). This contributes to the growing evidence of mutual mate choice in livebearers. Further studies of female receptivity behaviour and male choosiness are vital to continue our understanding sexual selection and evolution of poeciliids. Because both males and females perform headstand behaviors in mating contexts, investigation into the evolution of this signal and its design would be of considerable importance.

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Figure 1.

Mean (+ SE) association time (Primary Y axis, solid bars) and frequency of headstands/pecks (Secondary Y axis, striped bar) performed in association with no stimulus fish, a female stimulus fish or a male stimulus fish. Focal females did not perform any headstands/pecks in the no stimulus fish and female stimulus fish trials.

Figure 2.

Mean (+ SE) (a) association time and (b) frequency of headstands/pecks in association with either a large male (> 40 mm SL) or a small male (< 40 mm SL).

Figure 3.

Mean (+ SE) (a) association time and (b) frequency of courtship displays performed by large males and small males in association with either video playback of a freely swimming female or the same freely swimming female with bouts of pecking at the substrate inserted intermittently into the video.