

The Pennsylvania State University
The Graduate School
Intercollege Graduate Degree Program in Ecology

**ARE THERE SEX DIFFERENCES IN THE WAY GUPPIES (*POECILIA RETICULATA*)
NAVIGATE THEIR ENVIRONMENT?**

A Thesis in
Ecology
by
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ABSTRACT

Sex differences in spatial cognition have been found in mammalian, avian, and even invertebrate species. Yet, in teleost fish, one of the most diverse vertebrate groups, the consequences of sex-specific life history demands on spatial cognition remain unclear. Many evolutionary hypotheses have been proposed to explain why sex differences in spatial cognition arise, and several of these discuss the natural spatial behavior of the two sexes. Given that remarkably varied life history strategies are present in fish, determining whether sex differences in spatial cognition are present in teleosts may help identify which of the current hypotheses that address sex differences in spatial cognition have the greatest explanatory power. This thesis focuses on understanding sex differences in spatial cognition in a commonly studied teleost fish, the guppy (*Poecilia reticulata*). This species was selected because previous work has described the natural history of the guppy and demonstrated sex differences in mating and dispersal patterns, as well as in regions of the brain responsible for spatial cognition. I first evaluated whether adult male and female guppies show sex-specific patterns in spatial learning or preference for strategy of spatial navigation (the first set of experiments). I found that male and female guppies that were given the opportunity to solve a spatial task using either a landmark or turn-based navigation strategy solved the task in the same number of trials, indicating analogous spatial learning ability. However, when the sexes were tested for their preference of spatial navigation strategy, male guppies, similar to male polygynous mammals, exhibited a preference for a turn-based (egocentric) spatial strategy while female guppies exhibited equal preference for either a turn-based or landmark-based (allocentric) strategy. This suggests that either spatial navigation preference is highly conserved or selective forces have reprised similar sex differences in multiple taxa through convergent evolution. In order to address how these sex differences in

spatial cognition might arise, I ran a further experiment in which male guppies were exposed to a sex steroid hormone that typically produces feminizing effects in male teleosts, 17 β -estradiol. Unexpectedly both the estradiol and vehicle-exposed males exhibited an equal preference for both turn-based and landmark-based navigational strategies. This result is discussed in terms of the possible effects of all male housing (used in experiment 2), compared to mixed sex housing (used in experiment 1), and potential experiments that would address the relative effects of housing and exogenous estradiol exposure are proposed. Although estradiol has been demonstrated to have feminizing effects on somatic traits, it may be that the effects of estradiol on cognition are more complex and cannot be described as feminizing because of sex-specific responses to sex steroids.

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Chapter 1

The use of spatial information is sex-specific in guppies (*Poecilia reticulata*)

1. Introduction

Sex differences in spatial cognition have been among the most widely reported sex differences in cognition for decades and are represented in numerous avian, mammalian, and even invertebrate species (Gaulin & Fitzgerald, 1989; Tommasi & Vallortigara, 2004; Jozet-Alves et al., 2008; Rodríguez et al., 2010; Perdue et al., 2011). Yet, sex differences in spatial cognition are not well understood in the most diverse vertebrate group, teleost fish (Magurran & Garcia, 2000). It seems likely that sex differences in spatial cognition also occur in teleosts because, like mammals and birds, many species of fish exhibit sex-specific life history demands (Holtby & Healey, 1990; Magurran & Garcia, 2000). There are many benefits to exploring sex differences in spatial cognition in teleosts; the remarkably varied life history strategies present both across taxa and in closely related teleost species can enable studies in teleosts to address (i) explanatory power of current evolutionary hypotheses to explain sex differences in spatial cognition – some of which are derived from studies in humans, where measures of fitness are difficult or impossible, and (ii) whether consistent patterns of sex differences in spatial cognition that span multiple taxa are conserved or driven by convergent evolution. Male and female conspecifics may differ in spatial cognition traits because of sex-specific advantages for recalling resource locations, or other sex-specific pressures like mate selection or predation risk (Odling-Smee & Braithwaite, 2003); I will assess whether there are sex differences in spatial navigation in a teleost species, guppies (*Poecilia reticulata*).

There is evidence to suggest that teleosts have sex-specific spatial cognition. Costa et al. (2011) reported that in two species of blenniid fish with exclusively paternal parental care, *Parablennius parvicornis* and *Lipophrys pholis*, males have smaller home ranges and dorsolateral ventral telencephalic nuclei (DLv) compared with females. The DLv is suggested to be homologous to the hippocampus and plays an integral role in cognitive abilities including spatial cognition and spatial/temporal/relational memory (Portavella et al., 2002; Vargas et al., 2009). Similarly, *P. reticulata* have environment-dependent sex differences in another brain region important for spatial cognition, the telencephalon (Burns & Rodd, 2008). It has been suggested that sex differences in neurological traits and behaviors related to spatial cognition (e.g. shoaling in guppies) may be mediated by sex differences in life history strategy that generate sex-specific selective pressures (reviewed in Magurran & Seghers, 1994; Costa et al., 2011; Kotrschal et al., 2012).

Many hypotheses, derived largely from studies in mammals, suggest that sex differences in spatial cognition are mediated by a sex-specific life history demand, such as foraging, dispersal, parental care, mate choice, and male warfare (reviewed in Jones et al., 2003). One of the best supported explanations for sex differences in spatial cognition is the range size hypothesis, which states that a sex with a larger range size (typically males) will have to process more spatial information and so should have greater spatial cognitive abilities (e.g. spatial learning, memory, mental rotation, etc.) compared with a sex with a smaller range size (typically females; Gray & Buffery, 1971; Gaulin & Fitzgerald, 1986; Jones et al., 2003). Another hypothesis that may explain sex differences in spatial cognition is the dispersal hypothesis, which posits that a sex that disperses further should exhibit better spatial abilities (Silverman & Eals, 1992). Data addressing this hypothesis are mixed; male deer mice (*Peromyscus maniculatus*) disperse further and show greater spatial abilities in a Morris-water maze, but only during the

breeding season (Galea et al., 1996). In voles, both *Microtus pennsylvanicus* and *Microtus ochrogaster* males that disperse from their natal sites move about twice as far as female conspecifics that disperse (Boonstra et al., 1987), but only *M. pennsylvanicus* males have a larger home range than female conspecifics while *M. ochrogaster* show no sex difference in home range size (Gaulin & FitzGerald, 1986). Supporting the home range hypothesis, but not the dispersal hypothesis, only *M. pennsylvanicus* show male-biased spatial abilities, while *M. ochrogaster* males and females show similar performance on maze tasks (Gaulin & FitzGerald, 1989). The home range hypothesis has been extended to suggest that range size may also mediate the enhanced object location memory and landmark use (allocentric navigation) documented in female rodents and humans, as compared with male conspecifics who often prefer turn or path integration based navigation strategies (egocentric navigation) which do not require detailed cognitive maps (Kolb & Cioe, 1996; Blokland et al., 2006; Silverman et al., 2007). Given that *P. reticulata* have a polygynous mating system with strong selective pressure on males to maximize their reproductive success by seeking novel females, which drives males to move between sites and shoals more often than females (Croft et al., 2003), the dispersal and home range hypotheses should predict that female guppies will show reduced spatial learning and increased preference for landmark cues. Several of hypotheses addressing sex difference in spatial cognition describe traits that vary across taxa, and differ markedly between humans and guppies. For example, the parental care hypothesis states that females with reduced spatial ability should have enhanced reproductive success because of reduced mobility, lower energy expenditure, and reduced likelihood of accident or attack (Sherry & Hampson, 1997). Given that guppies do not exhibit parental care (Magurran & Garcia, 2000), the parental care hypothesis should predict that guppies will not show sex differences in spatial cognition. Similarly, there are hypotheses that identify division of labor in early humans as a cause of sex differences in spatial ability (i.e. females

primarily gather food while males primarily hunt, Silverman & Eals, 1992); given that male and female guppies do not share labor but rather obtain resources independently, the division of labor hypothesis should also predict that guppies will not show sex differences in spatial cognition. Comparisons of species where sex-differences in these traits vary independently could help further distinguish between these hypotheses.

Understanding whether species exhibit sex differences in spatial learning ability and cue preference is important because differential cue use may affect attention and recall of an environment, and can camouflage differences in spatial learning, particularly in tasks where only one cue type is available (reviewed in Jones et al., 2003). The capacity for both allocentric and egocentric navigation strategies has been demonstrated in goldfish, *Carassius auratus* (Rodriguez et al., 1994), but it is unknown whether fish have sex differences in spatial learning or navigation strategy preference. I will assess spatial learning and navigation strategy preference in male and female *P. reticulata*, a fish species with known neurological sex differences related to spatial navigation (Burns & Rodd, 2008) and male-biased dispersal that both resembles patterns typically found in mammals and is consistent with the dispersal hypothesis (Griffiths & Magurran, 1998; Magurran & Garcia 2000; Piyapong et al., 2009).

The mechanisms underpinning sex differences in spatial cognition remain unclear; one reason is that sex differences in spatial cognition can be present early in life but can also arise later (Choi & Silverman, 2003). For example, sex differences in preferred cue type arise only after rats reach puberty and are strengthened during adolescence in humans - female adult humans and rats prefer landmark cues while male rats and humans, as well as juvenile female rats, prefer egocentric cues (Galea & Kimura, 1993; Rodríguez et al., 2010, 2013). The emergence of sex differences in spatial cognition around puberty suggests that some sex-specific spatial abilities

may be linked to the production of sex steroids. Manipulations of sex steroid levels can affect the use of spatial cues in rats; female rats that have been ovariectomized (terminating sex steroid production) or exposed to hormones that masculinize the fetal brain (estradiol administration during gestation or at 26 days of age) will cause females to adopt male-typical cue use (Williams et al., 1990; McCarthy, 2008; Pleil & Williams, 2010; Rodríguez et al., 2013). In humans, females show a preference for a landmark navigation strategy, compared with males, that arises around puberty (and is present at 12 but not 9 years of age) and persists into adulthood (Choi & Silverman, 1996, 2003). Endogenous fluctuations in sex steroids can also modulate sex differences in spatial cognition; women show enhanced spatial rotation abilities – and perform similar to males – in phases of their menstrual cycle where their hormonal profile most closely resembles males (low estradiol and high androgen levels) compared with performance in other menstrual cycle phases (Silverman & Phillips, 1993; Hausmann et al., 2000). Similarly, female rats in proestrus (when estradiol levels are highest) show a greater preference for landmark cues than during the diestrus and estrus phases of the estrous cycle (Shaikh, 1971; Pleil & Williams, 2010).

The effects of estradiol vary by taxonomic group; estradiol exposure in mammals typically masculinizes the brain, but estradiol has a dramatically different role in teleosts (Nelson, 2005). Male fish exposed to estradiol exhibit changes in gene expression and physical characteristics that are consistent with phenotypic feminization: induction of vitellogenin (an egg yolk precursor protein), reduced gonad growth (including possible oocyte development), and reduced secondary sex characteristics (Brion et al., 2004). Though the reasons for this dichotomy are unclear, it is known that species can differ in the way estradiol is generated or processed; the production of estradiol through the aromatization of androgens is greater (and occurs in more cell types) in teleost fish compared with other vertebrate groups (including mammals), particularly in

the telencephalon/diencephalon (Callard et al., 1990; Garcia-Segura et al., 2003; Forlano et al., 2006). The capacity for estradiol to feminize male fish has been documented in captive and wild settings (Pandian & Sheela, 1995; Black et al., 2005; Blanchfield et al., 2015). In fish hatcheries, estradiol is the most preferred hormone for managing sex ratios because it can convert genetic males into phenotypic females in > 31 fish species including poeciliid, cichlid, salmonid, cyprinid, anabantid, and ictalurid species (Takahashi, 1975; Kavumpurath & Pandian, 1993; reviewed in Pandian & Sheela, 1995). Further, exogenous estrogens can feminize male fish during fertilization, posthatching, and postmaturity (Goetz et al., 1979; Hunter et al., 1986). In wild settings, estradiol in wastewater has contributed to the feminization of free-living male fish of various species across the globe, resulting in widespread decreases in population density (Bjerregaard et al., 2006; Tyler & Jobling, 2008; Liu et al., 2011; Blazer et al., 2012). In some teleost species environmental or social conditions can cause individuals born as males to transition into females (protandrous hermaphroditism) or vice versa (protogynous hermaphroditism) by modulating their conversion of androgens to estrogens and levels of 17 β -estradiol (Cardwell & Liley, 1991a,b; Black et al., 2005; Nelson, 2005). Intriguingly, during a transition in sex, fish can differentially modulate sex steroid concentrations in their body; as female *Lythrypus dalli* become male their levels of aromatase activity decrease in their brain but not in their gonads (Black et al., 2005).

Despite evidence of extensive somatic changes resulting from exposure to estradiol in male fish, the effect of estradiol on male behavior is underexplored (Lavelle & Sorenson, 2011) and effects on cognition are virtually unknown. Exposure to estradiol can affect behavior in *P. reticulata*, exogenous estradiol can restore sexual responsiveness behaviors in ovariectomized female guppies and reduce reproductive behaviors in male guppies (Liley, 1972; Lavelle & Sorenson, 2011). In rodents, exogenous estradiol can enhance consolidation for object

recognition, supporting landmark-based spatial navigation strategies (Luine et al., 2003; reviewed in Andreano & Cahill, 2009). Estrogens unequivocally play a role in modulating spatial cognition and the function of related neural structures in mammals (e.g. the hippocampus, McEwen, 2001; Schöning et al., 2007), and estradiol appears to have feminizing effects on fish, thus, it is possible that an increase in estradiol in fish will have feminizing effects on spatial cognition and increase preference for landmark cues. However, the relationship between sex steroids and spatial cognition is complex; hormone treatments for humans undergoing male-to-female transgender procedures produce substantial somatic changes but do not affect spatial cognition or associated brain activation patterns, suggesting that the influence of feminizing hormones on cognition may be fundamentally different in genetic males and females (Haraldsen et al., 2005; Schöning et al., 2010). The capacity for estradiol to affect cognition is dependent upon sex-specific features of estrogen receptors (ERs) and a type of G protein-coupled receptor (GPR30), including their density and the distribution of receptor subtypes and modulators (Kühnemann et al., 1994, 1995; Prossnitz et al., 2007; Etgen & Pfaff, 2010). Thus, despite the extensive feminizing effects of estradiol on somatic characteristics in numerous fish taxa, it is possible that estradiol may not have feminizing effects on spatial cognition. To address this, I conducted a second experiment in which I exposed male guppies to feminizing sex steroids to determine whether 17 β -estradiol can modulate sex differences in spatial cognition (spatial learning and navigation preference) in fish.

I evaluated sex differences in *P. reticulata* in behavior, spatial learning, and the cues males and females prefer to use to navigate an environment. I hypothesized that female guppies would exhibit a slower spatial learning compared with males and prefer a landmark navigation strategy over a turn-based strategy, while males would prefer a turn-based strategy (similar to prior studies in rodents Ossenkopp et al., 1996; Faraji et al., 2010). I then tested the effects of exposure to exogenous estradiol on somatic traits, behavior, spatial learning, and navigation

strategy preference; I hypothesized that male guppies exposed to 17 β -estradiol would either (a) show somatic and cognitive feminization and exhibit reduced spatial learning and preference for the landmark cues typical of female guppies, or (b) show somatic but not cognitive feminization and exhibit spatial learning and navigation preferences that do not differ from typical males.

2. Methods

2.1. Animals

Domestic guppies, *Poecilia reticulata*, (n=175) were obtained from stock populations of guppies Wild Side Pets in State College, Pennsylvania. The fish were maintained in mixed sex tanks and were allowed to reproduce naturally over at least 12 months prior to the start of the experiments. Adult, sexually mature fish were randomly selected and assigned to a treatment. The fish were kept on a 12:12 light cycle (lights on at 7:30am); lights dimmed for 30 minutes at the start and end of the light phase to simulate dawn and dusk. To control for circadian rhythms, tests were started a minimum of 2 hours after the beginning of the light cycle and completed within 7 hours of the start of the test. Testing order was pseudo-randomized; order was varied but treatment groups were evenly distributed during the first and last hours of the testing session. All experiments were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC), protocol #45265.

In experiment 1, I investigated sex differences in exploratory behavior, motivation to socialize with female conspecifics, and preference for allocentric or egocentric cues while navigating. For the spatial navigation test, guppies were housed in groups of 6 (3 males and 3

females) in a Y-maze. The maze walls were removed to create additional living space between training sessions (Supplementary Fig. 1). The maze was cleaned every week by replacing half the water and suction-cleaning the floor of the tanks. The maze tank contained a cotton, charcoal power filter and a heater to maintain tank temperatures at $22 \pm 1^\circ\text{C}$; both were removed during trials.

In experiment 2, I investigated the effects of estradiol exposure on male somatic characteristics, exploratory behavior, motivation to socialize with female conspecifics, and preference for allocentric or egocentric cues while navigating. For experiment 2, male fish were housed by treatment in groups of 5 in 28cm x 34cm x 53cm glass tanks to prevent exogenous hormones from contaminating the maze and the control, untreated fish. Housing tanks were cleaned every two weeks by replacing half the water and suctioning-cleaning the floor of the tanks. The housing tanks contained a plastic shelter, a cotton charcoal power filter, and a heater to maintain tank temperatures at $22 \pm 1^\circ\text{C}$.

1.2. Estradiol treatment

In *P. reticulata*, as well as at least 30 other fish species, phenotypical feminization can be induced through the consumption of 17 β -estradiol treated food (Goetz et al., 1979; reviewed Yamazaki, 1983; Bjerselius et al., 2001). I treated daily allotments of flake food (0.05g per tank of 5 fish) with 17 β -estradiol (Sigma-Aldrich® Lot# SLBG0383V) at a concentration of 100ug/L. This concentration is congruent with 17 β -estradiol concentrations reported in previous experiments in guppies or similarly sized fish (Zentel et al., 1987; Bjerselius et al., 2001; Toft & Baatrup, 2003), however the current concentration is not as high as those used to induce transitions in sex (Pandian et al., 1994). To calibrate the estradiol concentration and avoid buildup

of uneaten food (possibly leading to toxicity) I monitored food intake volume (while monitoring for illness and mortality) prior to the initiation of the first experiment in a separate cohort of 3 tanks, each containing 5 guppies, over 2 weeks.

Procedures to create the estradiol-treated flake food were modeled on Bjerselius et al., (2001). A stock solution was created by dissolving 10mg of estradiol in 100mL of ethanol (99%, VWR®). A 1g aliquot of flake food (TetraMin®, 47% protein) was placed into a Petri dish (100mm x 15mm) and 10mL of the estradiol stock solution was added. The Petri dishes were dried in a fume hood (approx. 18 hours), then 0.05g daily aliquots were measured for each tank. To control for possible effects from the ethanol, the same procedure, with the estradiol omitted, was used to create ethanol-treated stock solution. Fish were fed treated-flake for a minimum of 2 weeks before training began.

To assess the somatic effects of the estradiol treatment, after completion of the open field, motivation, and spatial tests I measured dry body weight, body condition (weight(g)/length(mm)), and gonopodium length (a modified anal fin that functions in sperm transfer). To measure “dry” body weight, individual fish were briefly placed onto absorbent paper then quickly transferred into a tared Petri dish to be weighed to the thousandth of a gram. The gonopodium was then extended using a probe with the right side of the fish pressed to the dish, and a photo was obtained under standardized lighting conditions. Photos were analyzed for gonopodium length and standard body length (from the tip of the snout to the end of the caudal peduncle on the left side of the fish) using ImageJ®. In *P. reticulata* exposure to estradiol can increase gonopodium size in males (Toft & Baatrup, 2003), but estradiol exposure has also been reported to decrease or have no effect on gonopodium size in *Gambusia holbrooki* (Doyle & Lim, 2002; Doyle & Lim, 2005). In *P. reticulata* and *Gambusia affinis* females prefer longer gonopodia (Langerhans et al.,

2005; Evans et al., 2011), but gonopodium length has an inverse relationship with mobility: long gonopodia reduce swimming burst speed and are less frequent in predator dense environments (Rosen & Tucker, 1961; Langerhans et al., 2005).

2.3. Open field test

I used an open field test based on Archard & Braithwaite (2011) to test sex differences in exploratory behavior (14 untreated male guppies, 14 untreated female guppies) and the effects of estradiol on exploratory behavior (14 estradiol-treated male guppies, 11 control-treated male guppies). These fish were not used for any other behavioral test. Open field tests have been used as a proxy for home range size (Perrot-Sinal et al., 1996; Jozet-Alves et al., 2008); it is suggested that higher levels of activity in the open field suggest greater exploration and space requirements in an ecological context. In the open field test, fish were tested individually in a plastic arena (48cm x 30cm x 28cm) with a water depth of 10 cm (Fig. 1A). The water in the open field arena was replaced between each fish. The open field arena was covered on external sides with black plastic and surrounded by a black curtain. To quantify exploration, the arena was marked with a grid (4cm x 5cm) and a line 4 cm from the sides to delimit edge and center zones (Archard et al., 2012).

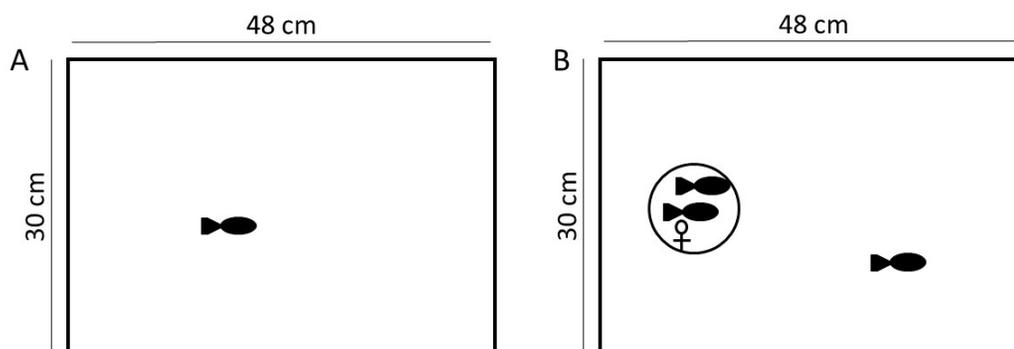


Figure 1-1: Open field (A) and motivation to socialize (B) testing schematics. Testing conditions were the same in the open field test and the motivation test, except in the motivation test fish were given access to a pair of untreated, stimulus females in an enclosed, transparent 6cm diameter plastic cylinder.

To begin the open field test, an individual guppy was placed in a transparent start cylinder (10cm diameter) in the center of the arena. After a 2-minute delay to allow the fish to acclimate to the arena, the start cylinder was removed from behind the curtain using a pulley. The fish moved freely around the open field arena for 5 minutes. Trials were video recorded to allow subsequent analysis by a second experimenter blind to experimental treatment using EthoLog® v. 2.2.5. (Ottoni, 2000). I quantified indicators of boldness (latencies to unfreeze, reach the edge of the arena, return to the center of the arena, and crosses in to the center of the arena) as well as exploration (number of grid squares crossed; Dahlbom et al., 2011; Archard et al., 2012). Indicators of boldness are important for the current study because a bolder sex (or treatment group) may encounter new aspects of the spatial learning task more quickly resulting in an extraneous advantage; sex differences in boldness have previously been documented in zebrafish (Dahlbom et al., 2011).

2.4. Motivation to socialize test

I investigated whether motivation to interact with conspecifics can be affected by sex (using 14 untreated male guppies and 14 untreated female guppies) and the estradiol exposure (using 14 estradiol-treated male guppies and 11 control-treated male guppies). These fish were not used for any other behavioral test. To quantify motivation to interact with conspecifics, individual fish were exposed to 2 adult female stimulus guppies enclosed in a 6cm diameter cylinder in the open field arena (Fig. 1B). Focal fish were released from a start cylinder after a 2-minute delay to facilitate acclimation, and were then allowed to explore the maze and interact with the cylinder containing conspecifics for 5 minutes. Between each trial, the water in the arena was replaced. Females remained in the cylinder for a maximum of 1 hour and were rotated from a group of 12 adult females for successive trials. Trials were video recorded to allow subsequent analysis by a second experimenter blind to treatment conditions using EthoLog® v. 2.2.5. (Ottoni, 2000). I quantified socializing behaviors: latency to socialize (latency to approach within 1 body length of the cylinder containing stimulus females), amount of time spent socializing (total time spent within one body length of the cylinder and oriented towards the females or displaying), and the number of separate socializing bouts (socializing bouts were defined as separate if the fish moved >1 body length away from the cylinder and oriented away from the females >2 seconds before returning again). I also assessed exploratory and boldness behaviors: number of squares crossed, latency to reach the edge of the arena and return to the center.

2.5. Spatial cognition test

For both experiments, to assess spatial cognition and spatial cue preference I tested adult male and female guppies in a glass tank containing a Y-maze framework. To prevent fish from

using cues outside the maze, the exterior of the Y-maze tank was covered in opaque black plastic and the tank was surrounded by opaque black curtains suspended from the ceiling. The Y-maze tank contained light brown gravel and 12cm of water. For experiment 1, a total of 12 untreated males and 12 untreated females were tested in cohorts of 6. Testing order was randomized. Untreated fish in the first experiment were housed in the Y-maze to habituate the fish to the testing arena and to avoid the potential stress of transferring fish between tanks. For experiment 2, I tested 15 estradiol-treated and 15 control-treated males individually in the Y-maze (fish were housed by treatment in groups of 5 in glass tanks). Testing order was randomized within treatment, and treatment testing order was alternated. The Y-maze was cleaned between testing of the estradiol and control-treated males by draining and replacing all water in the maze (and suction-cleaning the gravel) three times and flushing the maze with water for 15 minutes. The maze contained a cotton charcoal power filter and a heater to maintain tank temperatures at $22 \pm 1^{\circ}\text{C}$.

2.5.1. Habituation

In the first experiment, fish were habituated to the testing conditions by allowing fish continuous access to all chambers in the Y-maze for 7 days and by providing food only in the reward cups that would later serve as the reward during testing. Reward cups (28mm diameter; 12mm height) were placed in the center of the runway, equidistant from all chambers, during habituation. Each reward cup contained 3-5 food flakes (TetraMin[®]). Flakes were secured by in the reward cup using a small amount of Vaseline[®] such that the majority of the flake moved freely in the water (Beri et al., 2014).

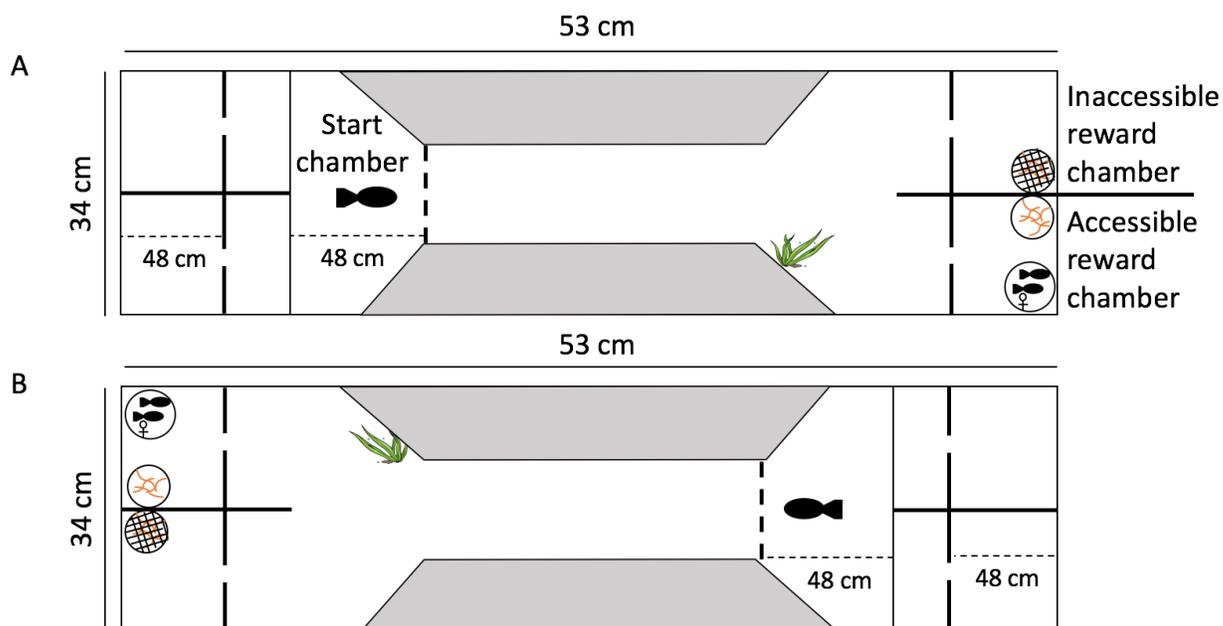


Figure 1-2: Schematic of spatial cognition trial conditions (not to scale). Fish alternated between maze orientations (A) and (B) to so that extraneous cues (other than the plant cue and side) were unreliable indicators of the reward location. During shaping trials, to familiarize fish with the rewarded arm, the accessible food reward cup (shown at the top of the accessible reward chamber in A) was located in the entrance to the accessible reward chamber. This allowed fish to consume the food reward without entering the rewarded chamber, but did not prevent fish from entering the rewarded chamber because of the height of the entrance.

In the second experiment, fish were habituated to the Y-maze individually because exogenous hormone application prevented communal housing. Fish were habituated to the maze by transferring the fish to the start chamber (Fig. 2A). After a 5-minute delay to allow fish to acclimate following transfer, the partitions creating the start chamber were removed giving the fish access to all goal chambers for 20 minutes. During habituation, the Y-maze contained only gravel and was devoid of conspecifics or food rewards. This procedure was repeated, such that fish were habituated to the Y-maze on two occasions separated by a minimum of 24 hours. To habituate fish in the second experiment to consuming food from the reward cups, fish were given

reward cups containing 5 food flakes (TetraMin[®]) in their home tanks once per day for three days, and no other form of food was available.

2.5.2. *Spatial learning: Shaping and training trials*

For both experiments, fish were familiarized with exiting a start chamber to access rewards in the rewarded arm during three sessions of shaping trials (the shaping trials did not require fish to enter the rewarded chamber, only the rewarded arm to obtain a reward, Fig. 2A,B). During the shaping trials, the food reward was placed just in front of the entrance to the rewarded chamber to familiarize fish with consuming a reward in a specific arm of the maze. The rewarded maze arm was marked with an orange plastic plant landmark; an unfamiliar plant shape and color was used to ensure that the landmark cue would be novel. The rewarded arm contained food (flake food cups) and social rewards (a 7.5cm transparent cylinder containing two adult female conspecifics). Social rewards have previously been used for shoaling fish species, including *P. reticulata* (Odling-Smee & Braithwaite, 2003; Burns & Rodd, 2008; Mühlhof et al., 2011). The social reward cylinder did not permit water exchange so that focal fish could not navigate using chemosensory cues. To balance potential chemosensory cues from the food reward, the inaccessible rewarded chamber contained a food cup with the flake food made inaccessible by mesh. To control for potential cues from the mesh, the external sides of the rewarded cups were also covered with mesh. The side of the rewarded arm was constant for each individual fish, but was counterbalanced across sex and treatment to control for side preference. The predictability of the rewarded side and the reliable presence of the landmark allowed fish to learn the spatial task using two navigation strategies, landmark based (recalling the plant cue) and turn based (recalling the side of the arm; Bjerselius et al., 2001).

To begin each session of 3 trials, an individual fish was placed in the start chamber. After a 5-minute delay for acclimation, the fish was released into the maze by removing the start chamber panel (the dashed line in Fig. 2A). All trials lasted 5 minutes, or 1 minute after the fish encountered either the food or social reward. A fish was determined to have found a reward if it contacted or oriented towards the reward from a distance within one body length. After the first trial, the fish was guided into a start chamber constructed on the opposite side of the maze from the prior location of the start chamber (Fig. 2B) for the second trial of the session. The position of the start chamber was alternated to ensure that the only stable landmark cue was the plant, reducing the likelihood that fish would use cues outside of the experimental design, thus minimizing effects from nondemonic intrusion (Hurlbert, 1984). During a 30 second intertrial interval the rewards and landmark were moved into the opposite maze configuration (Fig. 2B). The second trial was initiated by removing the start chamber panel, giving the fish access to the chambers that had previously been blocked by the back of the start chamber (the same arrangement as trial 1, but facing the opposite cardinal direction). After the second trial the fish was again guided into a start chamber constructed on the opposite side of the maze (now in the same configuration as the first trial, Fig. 2A); the fish began the third trial after a 30 second intertrial interval from the same side as the first trial (Fig. 2A). The initial position of the start chamber was alternated across sessions so that fish were trained equally in both starting directions. In experiment 1, fish frequently consumed the flake food reward in the maze and were not given supplementary food. Fish in experiment 2, however, were housed under all-male conditions outside the maze and preferentially interacted with the social reward, very infrequently consuming the food reward in the maze and so were provided supplementary food in their home tank a minimum of 1 hour after testing.

For spatial learning trials in both experiments, fish underwent 3 training trials per session. Training trials were identical to shaping trials except that the rewards were positioned against the inside walls of the rewarded chambers to prevent fish from gaining information about the contents of a chamber until they had entered the chamber. To assess learning rate across training, I measured the number of trials correct in each session. A trial was denoted to be correct if the fish entered the accessible reward chamber first. Fish continued training until they passed a learning criterion of 8 of 9 trials correct in 3 consecutive training sessions.

2.5.4. Spatial navigation strategy preference test

After passing the learning criterion, fish in both experiments were tested to determine whether they navigated using the landmark or turn based navigation strategy. To do this, prior to the beginning of a trial session the plant cue was moved to the previously unrewarded maze arm; the fish could either follow the plant landmark and enter the unrewarded chamber marked by the plant cue (indicating a landmark strategy) or disregard the landmark and enter the chamber that had been rewarded during training (indicating a turn based strategy). To balance chemosensory cues during the navigation strategy preference test, both chambers contained only inaccessible food reward cups (and no social rewards). As with the shaping and training trials, the fish remained in the start chamber for 5 minutes prior to starting the trial and the maze configuration was opposite to the configuration used in the previous trial.

2.6. Data analysis

For the first experiment using untreated males and females, the following data were natural log transformed to meet requirements for parametric analysis: latencies to unfreeze, reach the maze edge, and reach the maze center for both the open field and motivation test. For the second experiment using estradiol and control-treated males, body weight data were natural log transformed to meet the assumptions for parametric analysis. All estradiol-treatment data (body weight, body condition, and gonopodium length) were analyzed with analysis of variance (ANOVA) tests with treatment condition as a fixed effect. For the open field and motivation to socialize test in the second experiment, the following data were natural log transformed to achieve normality: latencies to reach the edge and center for the open field test and latencies to unfreeze, reach the edge and center, and socialize for the motivation test. Following these transformations, all data conformed to the assumptions required for conducting parametric analyses, determined using Levene's Test for Equality of Variances. Open field and motivation test data were analyzed with ANOVAs with a fixed effect of sex (for experiment 1) or treatment condition (for experiment 2). For the spatial learning test in both experiment 1 and experiment 2, the number of training days before passing the learning criterion was also analyzed with ANOVAs using sex or treatment condition as a fixed effect. Spatial navigation strategy preference data were analyzed with a chi-squared test; standard error was calculated for each strategy preference using the formula $SE = \sqrt{(p[1-p]/n)}$, where p = the proportion of fish that chose that strategy, and n = the number of fish represented in the testing group. Analyses were run using IBM® SPSS® Statistics Version 23; values are reported as means \pm 1 standard error.

3. Results

3.1. Estradiol treatment

Exposure to estradiol in male guppies increased body weight by 24% ($F_{1,36} = 7.728$, $P = 0.008$) and body condition (weight/length) by 17% compared with unexposed males ($F_{1,36} = 7.632$, $P = 0.009$; Fig. 3). These findings are consistent with feminization; adult female *P. reticulata* are typically larger than adult male conspecifics (Harris et al., 2010). Estradiol exposure did not affect either gonopodium length ($F_{1,55} = 2.008$, $P = 0.162$; estradiol-treated = 3.6 ± 0.08 g, control-treated = 3.4 ± 0.09 g) or gonopodium length standardized by body length (gonopodium length/body length: $F_{1,55} = 0.375$, $P = 0.543$; estradiol-treated = 0.22 ± 0.01 g, control-treated = 0.21 ± 0.01 g).

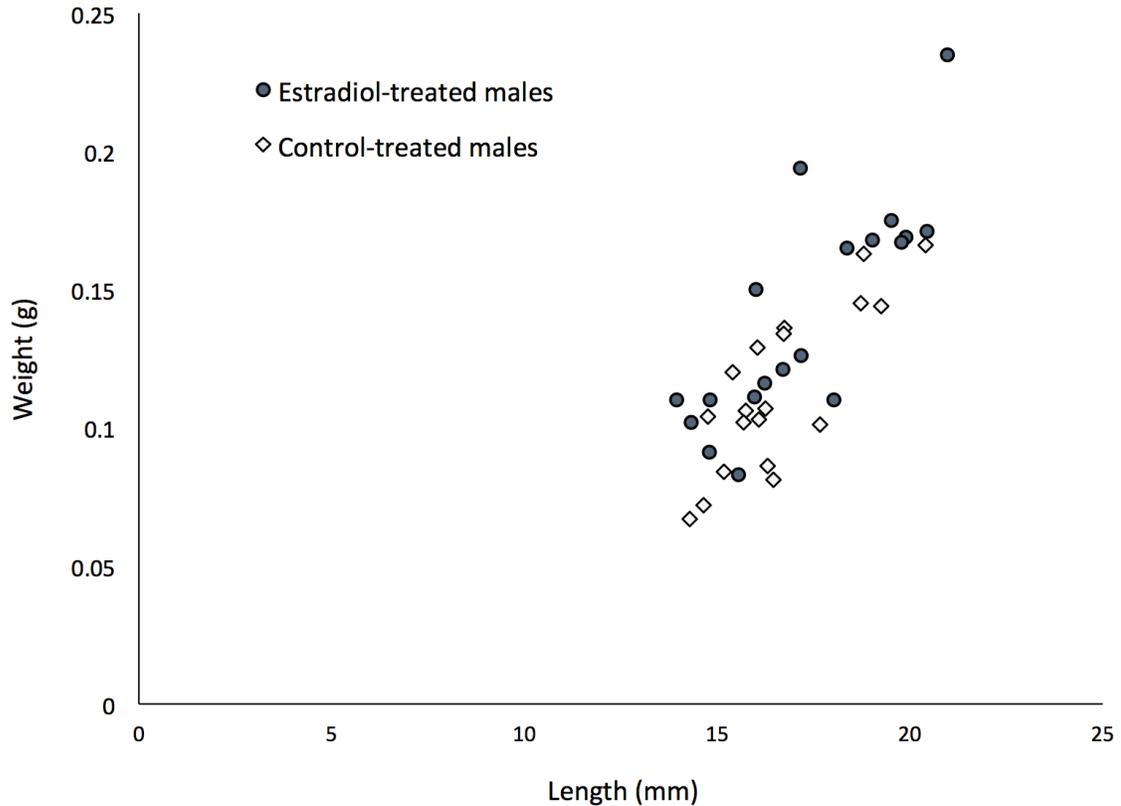


Figure 1-3: Male guppies fed exclusively 17 β -estradiol-treated flake food showed increased body condition (weight(g)/length(mm)) compared with male guppies fed only control (ethanol vehicle)-treated flake food ($F_{1,36} = 7.632$, $P = 0.009$). Each point represents an individual male *P. reticulata*.

3.2. Open field test

In the first experiment, untreated male guppies tended to be more active than female conspecifics ($F_{1,26} = 3.957$, $P = 0.057$; Fig. 4); given that free-living male guppies emigrate more frequently and disperse further than do females, the current findings may support the suggestion that activity in an open field test can reflect territory size in free-living systems (Perrot-Sinal et al., 1996; Croft et al., 2003; Jozet-Alves et al., 2008). No other behaviors in the open field test

were affected by sex (statistics are provided in Table 1). In the second experiment, estradiol-treatment increased a boldness-linked behavior - entries into the center of the arena ($F_{1,26} = 4.826$, $P = 0.038$; Fig. 5). Estradiol-treatment did not significantly affect any other behavior in the open field test (statistics in Table 1).

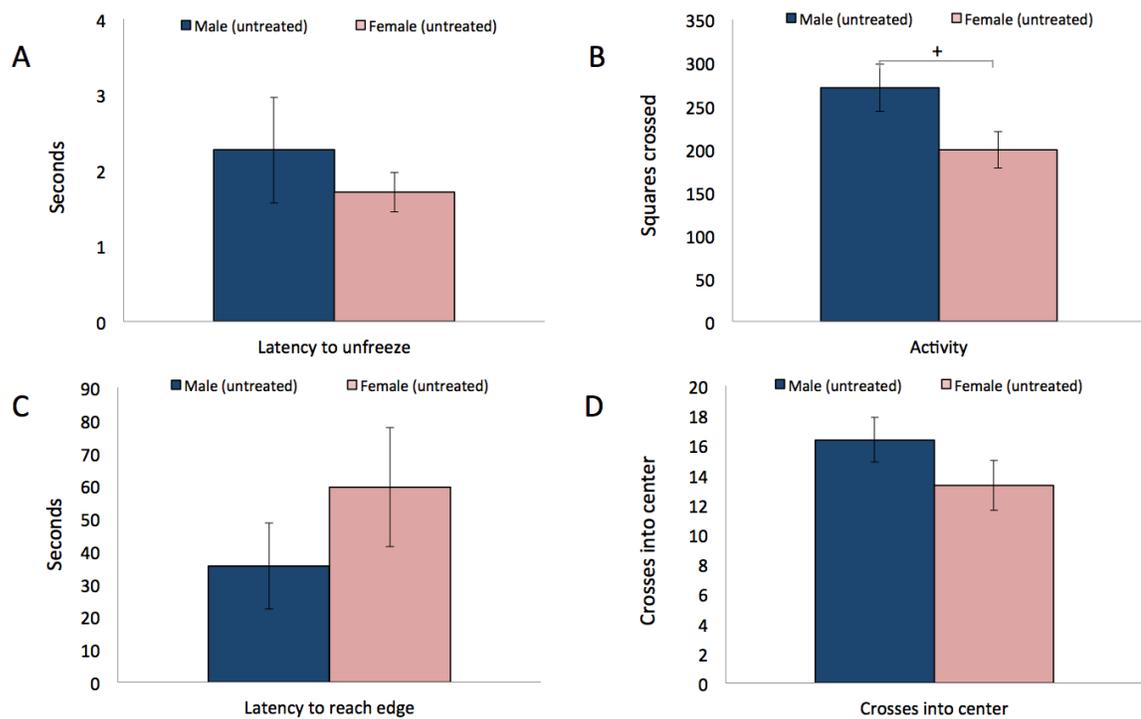


Figure 1-4: Open field behaviors of male and female adult domestic guppies. I measured latency to first movement (A), activity (B), latency reach the edge of the maze (C), and the number of crosses into the center of the maze (D). ⁺Indicates a trend at $P = 0.057$; means ± 1 SE.

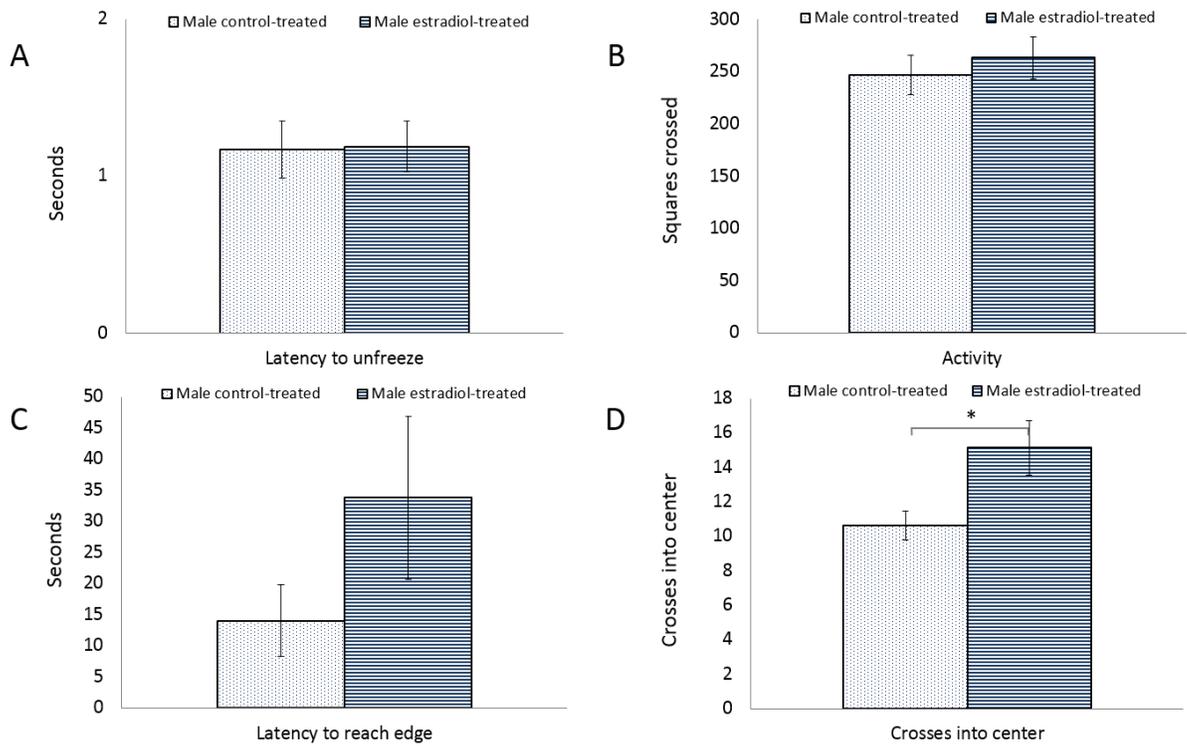


Figure 1-5: Open field behaviors in estradiol-treated and control-treated male adult guppies. I measured latency to first movement (A), activity (B), latency reach the edge of the maze (C), and the number of crosses into the center of the maze (D). *Indicates $P < 0.05$; means ± 1 SE.

3.3. Motivation to socialize

In the motivation to socialize test there were effects of sex and estradiol treatment on behavior. In the first experiment, untreated females spent 76% more time socializing ($F_{1,26} = 5.100$, $P = 0.033$) and moved around the arena 47% less than did untreated males ($F_{1,26} = 9.199$, $P = 0.005$), suggesting that females were more motivated to socialize than untreated males (Fig. 6). No sex differences were detected in latency to socialize, the number of socializing bouts, or the latency to reach the edge or the center of the arena (statistics in Table 2). In the second experiment, compared with control-treated males, estradiol-treated males spent 65% less time

socializing with the stimulus females ($F_{1,23} = 5.053$, $P = 0.035$), had 49% fewer socializing bouts ($F_{1,23} = 4.876$, $P = 0.038$), and moved around the arena 54% more ($F_{1,23} = 7.132$, $P = 0.014$), suggesting that estradiol exposure lowered motivation to interact with females (Fig. 7). No effects of the estradiol treatment were detected on latency to socialize or the latency to reach the edge or the center of the arena (statistics in Table 2).

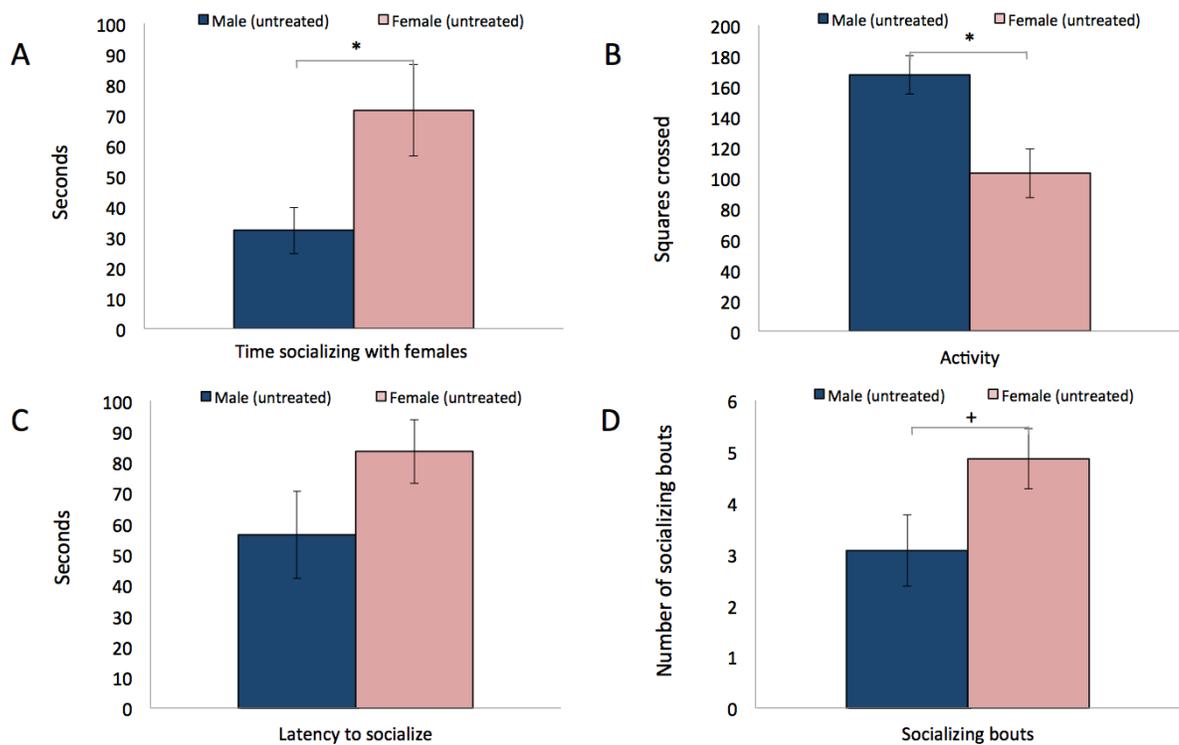


Figure 1-6: Behaviors during a motivation to socialize test in adult male and female domestic guppies. I quantified time spent within one body length of females (A), activity (B), latency to approach females (C), and number of socializing bouts (D). *Indicates significant at $P < 0.05$; +Indicates a trend at $P = 0.07$; bars represent means ± 1 SE.

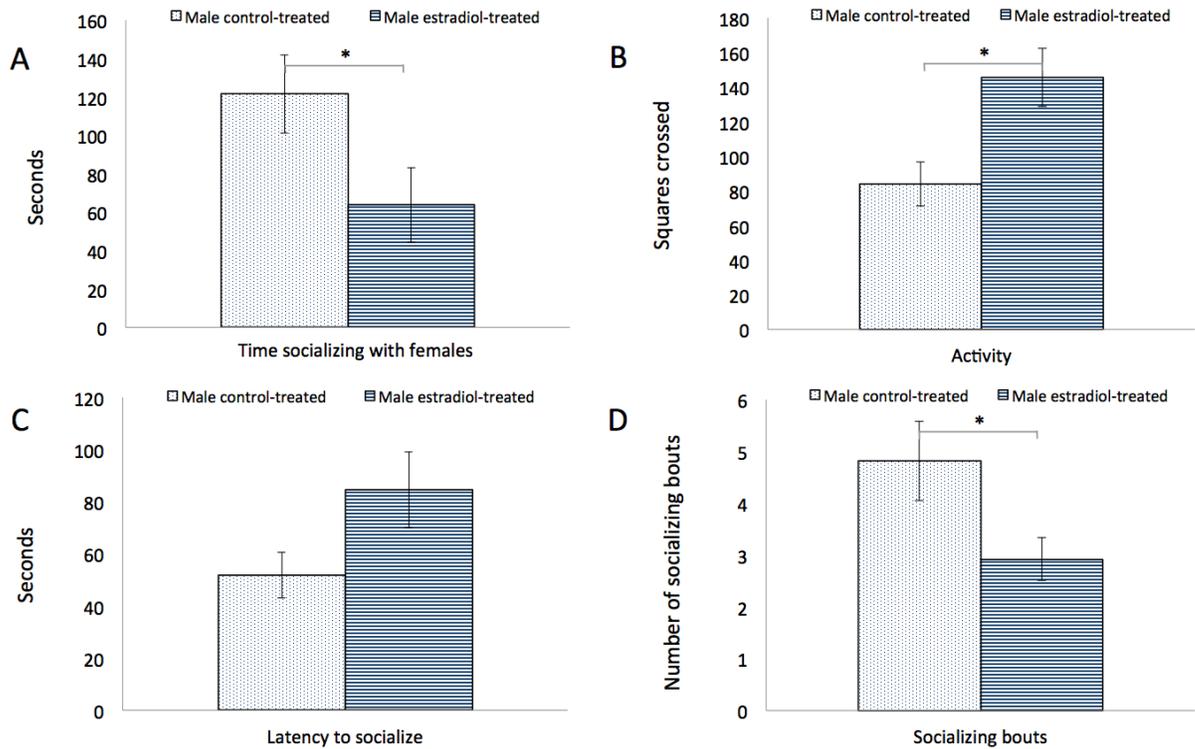


Figure 1-7: Behaviors during a motivation to socialize test in estradiol-treated and vehicle treated adult, domestic guppies. I quantified time spent within one body length of females (A), activity (B), latency to approach females (C), and number of number of socializing bouts (D). *Indicates significant at $P < 0.05$; means ± 1 SE.

3.4.1. Spatial cognition test: Spatial learning

Neither sex nor estradiol-treatment affected spatial learning rate. In the first experiment, 10 untreated males and 10 untreated females passed the learning criterion and so were tested for spatial cognition. Sex did not affect spatial learning rate (number of trial days before passing the learning criterion: males = 9 ± 2 , females = 10 ± 1 ; $F_{1,18} = 0.055$, $P = 0.817$). In the second experiment, 10 estradiol-treated males and 7 control-treated males passed the learning criterion.

Estradiol exposure did not affect learning rate (estradiol-treated males = 14 ± 2 , control-treated males = 14 ± 1 days; $F_{1,15} = 0.002$, $P = 0.964$).

3.4.2. Spatial cognition test: Spatial navigation strategy preference

In the untreated guppies, males showed a strong preference for a turn based navigation strategy while females were equally likely to navigate using the landmark or turn based navigation strategy ($\chi^2_1 = 4.550$, $N = 20$, $P = 0.033$; Fig. 8A). In the second experiment, both estradiol exposed and control-treated male guppies were equally likely to use the landmark or turn based navigation strategy ($\chi^2_1 = 0.486$, $N = 17$, $P = 0.637$; Fig. 8B).

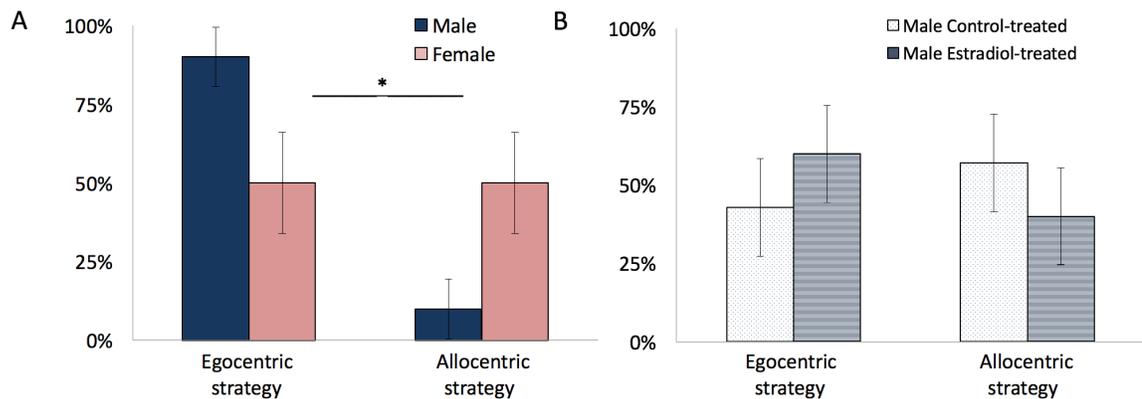


Figure 1-8: Percent of fish that navigate using a plant landmark (allocentric strategy) or a direction-based strategy (egocentric strategy) in (A) untreated male and female guppies and (B) estradiol-treated and vehicle treated male guppies. *Indicates significant at $P < 0.05$; means ± 1 SE. For all treatments $n=10$, except for control-treated males for which $n=7$.

4. Discussion

Overview

Understanding sex differences in teleosts, which have greater variation in life history strategies compared with mammals, could help clarify hypotheses that have been proposed to explain sex differences in spatial cognition in mammalian species (reviewed in Jones et al., 2003). I hypothesized that *Poecilia reticulata* would show sex differences in behavior, spatial learning, and spatial navigation strategy preference, given that similar sex differences have been documented in mammals, birds, and invertebrates (Tommasi & Vallortigara, 2004; Jozet-Alves et al., 2008; Perdue et al., 2011; Rodríguez et al., 2010). I found no difference between male and female guppies in exploratory behavior in an open arena. However, when novel conspecifics are present, I found that female guppies explored less but socialized more compared male guppies. Despite behavioral differences, spatial learning rate was not affected by sex. Spatial navigation strategy preference in untreated male and female guppies was congruent with the sex specific patterns documented in mammals (Rodríguez et al., 2010, 2013); males preferred a turn-based (egocentric) strategy while females were equally likely to use either a turn-based or landmark (allocentric) strategy. I assessed a potential mechanism of this sex difference in spatial navigation strategy preference in a second set of experiments, by manipulating exogenous levels of a potentially feminizing hormone, 17 β -estradiol, in male guppies. I found that estradiol exposure affected the somatic, behavioral, and cognitive traits of male guppies. Estradiol exposure male guppies exhibited an increase in a boldness-linked behavior - entries into the center of the arena. In contrast to expected feminizing effects, estradiol exposure in male guppies decreased motivation to socialize with novel females. Spatial learning rate was not affected estradiol treatment. Unexpectedly, both estradiol exposed and control-treated males showed an equal preference for both spatial navigation strategies; potential reasons are discussed, which include

housing conditions and adverse responses to transfer between tanks prior to experimental procedures.

Sex differences in behavior

I investigated sex differences in exploratory and boldness-linked behaviors in guppies using an open field test. I found no sex differences in behavior that could contribute to sex differences in spatial abilities. Though guppies are sexually dimorphic, male and female guppies show small or no differences in parental care, and fewer differences before sexual maturity – these results emphasize that some sex differences can be minimized by isolating fish in a context devoid of sex-specific cues (Jones et al., 2003). I also assessed motivation to socialize in an open arena in male and female guppies; I found that compared with untreated males, untreated females spent more time socializing and explored the arena less, suggesting that females were more motivated to socialize than untreated males. This inverse relationship between socialization and exploration across the sexes may reflect the increased likelihood of free-living females to shoal with familiar shoals and the increased likelihood of males to emigrate and find new shoals (Magurran & Garcia, 2000). In the context of cognition tests, these findings suggest that sex differences may be masked by differential motivation to access a social reward in guppies. Although the current findings do not support sex differences in spatial learning, females in the current study may have been more motivated to complete the spatial task to gain access to the social reward, which could have obscured a sex difference in learning rate.

Sex differences in spatial learning

Given that we found no difference in spatial associative learning in guppies, a viviparous species that exhibits sexual dimorphism and sex differences in shoaling and emigration patterns (Magurran & Garcia, 2000), it is unlikely that these factors drive sex differences in species where sex difference in spatial associative learning are present. Variance across species in sex difference in spatial cognition is supported by the currently available data; sex differences in spatial associative learning have been reported in horses and meadow voles (male-biased, Kavaliers et al., 1998; Murphy et al., 2004) but equal spatial associative learning performance has been reported for male and female in guinea pigs and zebra finches (Dringenberg et al., 2001; Hodgson et al., 2007). It should be noted that although the results show that guppies do not exhibit sex difference in spatial learning, sex differences in spatial learning may be present in other teleost species, given that teleost species show diversity in mating system, degree of sexual dimorphism, territoriality, habitat, and range size. For example, *Salaria fluviatilis* are a species of blenny in which females have a larger home range compared with males during the breeding season, as males care for their nesting sites independently (Costa et al., 2011; Fabre et al., 2014). When given a spatial learning task requiring the use of landmarks, 80% of males *S. fluviatilis* learn the task compared with only 30% of females (Fabre et al., 2014). This study also emphasizes that tests of sex-differences in performance, including spatial abilities, may be complicated by the cues available in the testing condition.

Sex differences in spatial navigation strategy preference

The results that female guppies show a greater preference for landmark cues while navigating compared with male guppies, which parallels sex-specific preferences seen in

mammals (Rodríguez et al., 2010) and other taxa (Tommasi & Vallortigara, 2004). Many hypotheses, derived largely from studies in mammals, suggest that sex differences in spatial cognition are mediated by a sex-specific life history demand, such as dispersal, mate choice, gender roles in foraging (male hunting and female gathering), parental care, and male warfare (reviewed in Jones et al., 2003). The current results suggest that sex differences in parental care, warfare, and gender roles in foraging are not primary drivers of sex differences in spatial cognition because guppies exhibit sex-specific spatial navigation strategies but do not form pair bonds facilitating division of labor or exhibit either parental care or male warfare (Magurran & Garcia, 2000). The current results support the dispersal hypothesis; male guppies have been shown on multiple occasions to be more likely to disperse and move between schools (largely in search of novel mates), while female guppies prefer to school with familiar females (Magurran et al., 1992; Magurran, 1998; Griffiths & Magurran, 1998). Female guppies may prefer landmark cues in order to navigate back to a familiar school – landmark use may enable recognition of prior locations she visited with a school, whereas dispersing males may favor directional cues to navigate to novel schools in novel environments (where landmark cues will be unfamiliar). Sex differences in guppies in home range is less well understood, but it is thought that males may have larger home ranges, and so the current results also support the home range hypothesis (although this link is tentative, given that there are not conclusive data showing sex differences home range size, Magurran & Garcia, 2000). Additional information about guppy home range size, or investigation of a species in which the sexes differ in home range size but not in dispersal patterns, could help distinguish between the dispersal and home range hypotheses. Further, studies comparing closely related fish species that vary in degree of sexual dimorphism, or have female-dominant social systems, variable home ranges, or seasonal variation in home range size

could help shed light on the ultimate and proximate causes for sex differences in spatial cognition.

Estradiol treatment on somatic traits

The emergence of sex differences in spatial cognition around puberty in humans and rodents suggests that some sex-specific spatial abilities may be linked to the production of sex steroids (Galea & Kimura, 1993; Rodríguez et al., 2010, 2013). I found that exposure to estradiol, which can have feminizing effects on guppies, increased body weight and body condition (weight/length) in male guppies. These findings are consistent with feminization, given that mature female guppies are typically larger than mature males (Harris et al., 2010). Although 17 β -estradiol exposure can cause a transition in sex from male to female in many teleost species (with higher concentrations than those used here; Pandian et al., 1994), in the current experiment estradiol exposure did not affect gonopodium length (either raw length or length standardized by total body length). Changes in gonopodium length caused by exposure to exogenous estradiol below the levels required to cause a transition in sex are variable across teleosts, and include increases (*P. reticulata*, Toft & Baatrup, 2003), decreases (juvenile *Gambusia holbrooki*, Doyle & Lim, 2002), and no change (adult *Gambusia holbrooki*, Doyle & Lim, 2005). I found no evidence of toxicity from estradiol exposure – fish in the estradiol treatment exhibited no differences in learning or mortality rates. Further investigation is needed to understand the dose-dependent somatic effects of 17 β -estradiol on male teleosts.

Estradiol treatment and open field trials

In the second experiment, estradiol-exposure affected boldness-linked behavior but not exploratory behavior in the open field test. Estradiol-treatment increased entries into the center of the arena, which is suggested to correlate positively with boldness, but the increase in boldness behavior was not caused by a general increase in activity, given that estradiol-treatment did not affect overall activity (Maximino et al., 2010). In other contexts, exogenous estradiol can increase activity rate (e.g. running wheels available to rats, Gentry & Wade, 1976). Understanding how estradiol affects activity and boldness behaviors is important because these traits can affect more complex behaviors. In male *P. reticulata*, bolder males (measured with a predator inspection task) exhibited greater associative learning (Dugatkin & Alfieri, 2003). Similarly, boldness is associated with faster associative learning rates in rainbow trout (*Oncorhynchus mykiss*), suggesting that the effects of estradiol on boldness could account for variation in spatial learning, and could potentially have masked learning deficits caused by estradiol exposure.

Estradiol treatment and motivation to socialize

Estradiol-exposure also affected behavior in the motivation test; however, as with the open field test, estradiol-exposure did not induce female-like behaviors; untreated female guppies spent more time socializing with female conspecifics than untreated male guppies, but the estradiol-treatment decreased motivation to socialize. This suggests that estradiol may affect male differently than females, possibly because males have not evolved to process estradiol in the same manner as females (reviewed in Gillies & McArthur, 2010). The relationship between sex steroids and cognition is complex; humans undergoing hormone treatments for male-to-female gender reassignment (with 228% higher 17 β -estradiol and 70% less testosterone than untreated males) undergo substantial somatic changes but no changes in mental rotation ability (Schöning

et al., 2007). Spatial rotation performance is typically higher in males than in females, and rotation ability in females has a positive relationship with testosterone and a negative relationship with estradiol, suggesting that the influence of feminizing hormones on cognition may be fundamentally different in genetic males and females (Hausmann et al., 2000). Exposure to estradiol in the current experiment (below the levels used to induce a transition in sex in fish) affected somatic and secondary sexual characteristics, yet it is possible that changes in these traits may be poor indicators of changes in cognitive or behavioral states.

Effects of estradiol treatment on spatial learning

We found no effect of sex or estradiol exposure on associative spatial learning, suggesting that sex differences in associative spatial learning detected in other species, including horses and meadow voles (male-biased, Kavaliers et al., 1998; Murphy et al., 2004) may not be mediated by estradiol levels. Further studies examining how the sexes differ in how estradiol is processed are necessary. Such studies might shed light on why increases in sex steroids such as estradiol can have sex-specific effects (Hausmann et al., 2000; Lund et al., 2001; Schöning et al., 2009).

Estradiol treatment and navigation preference

In the current study, both estradiol and control-treated males, unexpectedly showed a spatial navigation strategy preference that differed from untreated males from the first experiment (but resembled those exhibited by the untreated females). I suggest three possible reasons for this, each of which will be discussed in more detail: (i) artificial same-sex housing conditions in the second experiment, (ii) effects from the ethanol vehicle used to create both the estradiol and

control-treated flake food, and (iii) stress caused by transfer between the housing and testing tanks (fish in the first experiment were housed in the testing tank).

Male *P. reticulata* aggressively court and display to female conspecifics almost continuously in laboratory conditions (Farr, 1989; Griffiths, 1996); in the absence of females, both the control and estradiol-treatment males were frequently observed courting and displaying to other males. Further, exogenous estrogens can influence dominance hierarchies (Filby et al., 2012). In *P. reticulata*, plasticity in the brain allows adult males housed with a female conspecific to increase brain size in approximately 5 weeks (with no change in body size) compared with males housed with only a male social partner (possibly for courtship, as female brain size is not affected by mixed vs same sex housing, but female guppies prefer faster learning males and fitness outcomes are far more variable for male guppies than for females, Magurran & Garcia, 2000; Shoet & Watt, 2009; Kotrschal et al., 2012). In the current study, untreated males in the first experiment were continuously exposed to females – in rodents the presence of female conspecifics can increase male testosterone and LH levels (Purvis & Haynes, 1974; Batty, 1978; Bronson & Desjardins, 1982). Given that same vs. mixed-sex housing can differentially modulate behavior (Piyamong et al., 2010) and sex steroid levels (Bronson & Desjardins, 1982), housing conditions could both affect the control-treated males and mask effects of the estradiol-treatment. Thus, it seems that the unexpected change in spatial navigation preference in both treatment conditions in experiment 2 is most likely the result of extended all-male social conditions (> 30 days). It is also possible that exposure to ethanol-treated food contributed to these findings, though the use of ethanol solutions is the most common method for creating steroid-treated food for fish, I am not aware of any previous report or investigation of side effects (Yamazaki, 1983; Goetz et al., 1979; Hunter et al., 1986; Bjerselius et al., 2001). Although transferring fish between housing and testing tanks is also common, it has previously been noted to cause stress and

changes in behavior (Brydges et al., 2009). I therefore cannot eliminate the possibility that either ethanol exposure or transfer between tanks contributed to the contrasting spatial navigation strategy preferences detected in untreated male guppies compared with both control and estradiol-treated male guppies. However, the results from the open field and motivation tests indicate that estradiol exposure in male guppies may have effects that are more complex than feminization, possibly mediated by sex differences in the capacity to sequester or process estradiol in the brain (e.g. a-fetoprotein concentrations; Bakker et al., 2006). Further studies are needed to address the interaction between behavioral, cognitive, and somatic effects of changes in estradiol in teleost fish where sex can be plastic even in adulthood (Warner, 1984).

Overall, the current results show that male and female guppies differ in spatial navigation strategy but not spatial associative learning, which demonstrates that sex differences in spatial cognition in teleosts are context specific. The results also inform an interdisciplinary effort to identify the ultimate and proximate causes of sex differences in spatial cognition; our findings support the dispersal hypothesis and demonstrate that sex differences in spatial cognition are not isolated to mammals, but are also present in teleosts. I found no effect of estradiol exposure on spatial associative learning or navigation strategy preference. This suggests that, for species that do show sex-specific patterns in these traits, sex differences in these aspects of spatial cognition are likely not mediated by estradiol levels. Given that teleosts have remarkable variation in degrees of sexual dimorphism as well as life history and mating strategies, future studies in fish species have great potential to expand our understanding of which factors drive sex differences in spatial cognition.

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Appendices

Appendix A

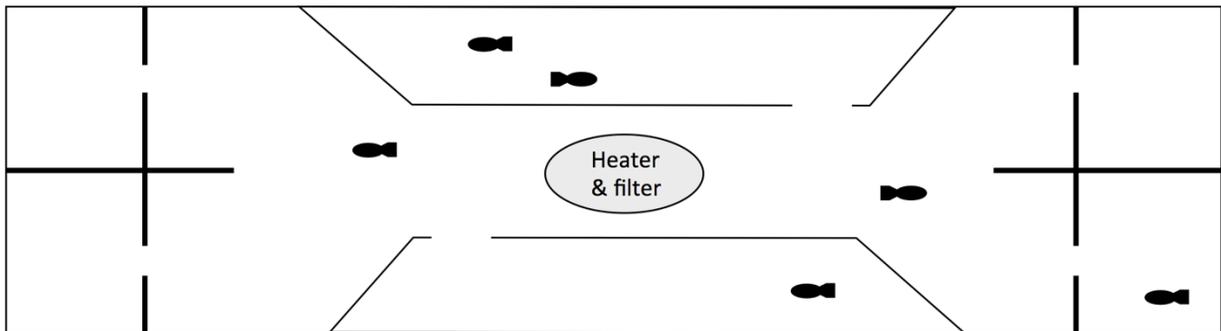
Tables

Table 1: Open field behavior in male, female, estradiol-treated male, and control-treated male guppies.

<i>Measure</i>	<i>Effect of sex in untreated fish</i>	<i>Effect of estradiol-treatment</i>
Latency to unfreeze	$F_{1,26} < 0.001$, $P = 0.991$	$F_{1,23} = 0.015$, $P = 0.903$
Activity	$F_{1,26} = 3.957$, $P = 0.057^+$	$F_{1,23} = 0.289$, $P = 0.596$
Latency to reach edge	$F_{1,26} = 0.589$, $P = 0.450$	$F_{1,23} = 0.950$, $P = 0.340$
Latency to reach center	$F_{1,26} = 2.146$, $P = 0.155$	$F_{1,23} = 1.279$, $P = 0.270$
Crosses into center	$F_{1,26} = 1.745$, $P = 0.198$	$F_{1,23} = 4.826$, $P = 0.038^*$

Table 2: Motivation test behavior in male, female, estradiol-treated male, and control-treated male guppies.

<i>Measure</i>	<i>Effect of sex in untreated fish</i>	<i>Effect of estradiol-treatment</i>
Latency to socialize	$F_{1,26} = 1.331$, $P = 0.261$	$F_{1,23} = 2.859$, $P = 0.105$
Time spent socializing	$F_{1,26} = 5.100$, $P = 0.033^*$	$F_{1,23} = 5.053$, $P = 0.035^*$
Socializing bouts	$F_{1,26} = 3.568$, $P = 0.070^+$	$F_{1,23} = 4.876$, $P = 0.038^*$
Activity	$F_{1,26} = 9.199$, $P = 0.005^*$	$F_{1,23} = 7.132$, $P = 0.014^*$
Latency to reach edge	$F_{1,26} = 0.322$, $P = 0.575$	$F_{1,23} = 0.217$, $P = 0.646$
Latency to reach center	$F_{1,26} = 3.843$, $P = 0.061^+$	$F_{1,23} = 0.225$, $P = 0.640$

Appendix B**Supplementary materials**

Supplementary figure 1: In experiment 1, untreated male and female guppies were housed in the Y-maze and moved freely between all chambers before and after spatial testing