



Sex differences in cognitive performance and style across domains in mosquitofish (*Gambusia affinis*)

Kelly J. Wallace¹ · Richie T. Rausch¹ · Mary E. Ramsey¹ · Molly E. Cummings¹

Received: 3 October 2019 / Revised: 13 February 2020 / Accepted: 26 February 2020 / Published online: 12 March 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Given that the sexes often differ in their ecological and sexual selection pressures, sex differences in cognitive properties are likely. While research on sexually dimorphic cognition often focuses on performance, it commonly overlooks how sexes diverge across cognitive domains and in behaviors exhibited during a cognitive task (cognitive style). We tested male and female western mosquitofish (*Gambusia affinis*) in three cognitive tasks: associative learning (numerical discrimination), cognitive flexibility (detour task), and spatio-temporal learning (shuttlebox). We characterized statistical relationships between cognitive performances and cognitive style during the associative learning task with measures of anxiety, boldness, exploration, reaction time, and activity. We found sex differences in performance, cognitive style, and the relationships between cognitive domains. Females outperformed males in the spatio-temporal learning task, while the sexes performed equally in associative learning and cognitive flexibility assays. Females (but not males) exhibited a ‘fast-exploratory’ cognitive style during associative learning trials. Meanwhile, only males showed a significant positive relationship between domains (associative learning and cognitive flexibility). We propose that these sexually dimorphic cognitive traits result from strong sexual conflict in this taxon; and emphasize the need to explore suites of sex-specific cognitive traits and broader comparative work examining sexual selection and cognition.

Keywords Sex differences · Cognitive style · Numerical discrimination · Cognitive flexibility · *Poeciliidae* · *Gambusia affinis*

Introduction

Individuals vary not only in their level of performance on a cognitive task (accuracy of behavioral output, Shettleworth 2010), but also in how they approach the cognitive task itself [e.g., cognitive style (Sih and Del Giudice 2012; Thornton and Lukas 2012)]. For example, individuals may differ in how they sample an environment or their hastiness to make a decision, and these cognitive styles may or may not be independent of their resulting performance. Additionally, this relationship between style and performance may vary across

cognitive domains [different cognitive domains are those that require different types of cognitive performance (Deary et al. 2010)]. These attributes—performance, cognitive style, and cognitive domain—are often examined individually, but recent efforts have begun to identify how they may be inter-related (Carere and Locurto 2011; Sih and Del Giudice 2012; Guillelte et al. 2017). For instance, some of the early work in this arena has suggested that how quickly an animal makes a decision and how much it explores its environment (e.g., a fast-exploring cognitive style) should predict an individual’s performance (accuracy) in a learning task (Sih and Del Giudice 2012). Empirical studies have shown variable support for this hypothesis with some taxa revealing a positive relationship (Burns and Rodd 2008) between fast-exploratory styles and learning performance, others revealing a negative relationship (Madden et al. 2018; Guillelte et al. 2011), and yet other studies find that a fast-exploratory cognitive style is unrelated to performance (Lucon-Xiccato et al. 2016). Furthermore, relationships between cognitive style and performance are often domain-specific (Dougherty

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10071-020-01367-2>) contains supplementary material, which is available to authorized users.

✉ Kelly J. Wallace
kwallace@utexas.edu

¹ Department of Integrative Biology, University of Texas, 1 University Station C0990, Austin, TX 78712, USA

and Guillette 2018). Understanding how and why these relationships vary across taxa and between domains is a current challenge in cognitive studies. However, a factor that is emerging as one of the predominant predictors of variation across cognition is sex (Dougherty and Guillette 2018).

A recent meta-analysis by Dougherty and Guillette (2018) identified sex as the only categorical variable that improved the fit of a Bayesian model explaining variation across animal studies in cognition–personality relationships (Dougherty and Guillette 2018). The meta-analysis empirically evaluated the relationship between measures of personality (such as boldness, aggression, sociability, and exploration) with measures of performance (such as errors and reversal speed) in a phylogenetically controlled manner across 19 species spanning mammals, birds, reptiles, fish, and insects. Their result is unsurprising given fitness benefits for particular cognitive traits are often sex-dependent (Maille and Schradin 2016; Shohet and Watt 2009; Smith et al. 2015; Boogert et al. 2011; Farrell et al. 2012), leading to differential selective pressures favoring certain cognitive traits in one sex and potentiating sexual dimorphism. While cognitive sex differences are accumulating across the literature (but see Lucon-Xiccato and Bisazza 2014; Healy et al. 1999), there exists a great deal of variation between and within taxa (Shaw and Schmelz 2017) and very little work examining how these sex differences bear out across cognitive domains. Sex differences in cognitive performance are frequently domain-specific (Petrazzini et al. 2017; Lucon-Xiccato and Bisazza 2014; 2017a, b; Brust et al. 2013; Carazo et al. 2014; Laland and Reader 1999; Kolata et al. 2008), and even when learning performance is equivalent between the sexes, the behavioral predictors that underlie individual variation can differ (Mamuneas et al. 2015; Lucon-Xiccato and Bisazza 2016; Etheredge et al. 2018; Titulaer et al. 2012). The present study is designed to specifically examine sex differences in cognitive performance, cognitive style, and relationships between cognitive domains in a taxon with well-defined sexually dimorphic behaviors (Magurran and Macías 2000; Heinen-Kay et al. 2016; Lucon-Xiccato and Bisazza 2017a, b; Cummings 2018).

The *Poeciliidae* family of live-bearing freshwater fish—which includes the guppies, mollies, mosquitofish, and swordtails—exhibits a wealth of natural variation in sexual selection pressures (Magurran and Macías 2000; Dadda 2015) and cognition (Lucon-Xiccato and Bisazza 2017a, b), making this family a uniquely suitable system to address sex differences in cognition (Cummings 2018). Poeciliids are famous for sexually divergent reproductive roles as males provide only sperm via an intromittent organ (gonopodium) and females undergo a monthlong internal gestation period. Mosquitofish (*Gambusia* sp.) are characterized by a high degree of sexual conflict, with males exhibiting some of the highest rates of sexual harassment across the Poeciliidae

family and females adopting strong avoidance responses including shoaling to reduce male harassment (Dadda 2015; Pilastro et al. 2003). Artificial selection experiments on male gonopodial length in *Gambusia holbrooki* resulted in larger female (but not male) brain size (Buechel et al. 2016), suggesting that larger brains might be an evolutionary counter-adaptation for female defense against male harassment. More recent experiments could find no advantage for a larger brain in *G. holbrooki* females from natural populations in defending themselves against male harassment (Culumber et al. 2020). Other experiments have shown that larger-brained female guppies (*Poecilia reticulata*) displayed greater cognitive flexibility (assayed by reversal learning) than smaller brained conspecific females (Buechel et al. 2018). Taken together, these studies suggest that sexual conflict may impact cognition. However, the specific relationship between the two remains unclear.

In our study, we investigated sexual differences in cognitive performance, cognitive style, and domain relationships in the western mosquitofish (*Gambusia affinis*). We assessed performance in three cognitive domains—associative learning (numerical discrimination assay), cognitive flexibility (detour task), and spatio-temporal learning (shuttlebox assay)—as these domains have been shown to utilize different underlying neural mechanisms (López et al. 2000; Wallis et al. 2001; Dalley et al. 2004; Eisenhardt and Menzel 2007). We evaluated cognitive style by measuring the relationship between associative learning performance and a variety of behaviors displayed during the numerosity discrimination test trials. We then examined whether performance and behavior in one cognitive domain predicted performance and behavior in another, and whether these relationships varied by sex.

Associative learning—learning that results from experiencing predictive relationships between stimuli and outcome (Shettleworth 2010), has been extensively tested across many taxa through many unique paradigms. Here, we use a numerical discrimination assay to test a fish's ability to associate a specific numerical contrast (higher or lower number of shapes) with a food reward. Cognitive flexibility is the ability to modify behavior in response to variations in consequences or context of the environment (Bond et al. 2007; Easton 2004). Cognitive flexibility has commonly been assessed either via reversal learning paradigms or through a detour task, and here, we utilize a single-trial detour task paradigm (Thompson et al. 1984). Studies in primates have shown that single-trial detour tasks invoke distinct neural mechanisms from those involved in multiple training trials (Wallis et al. 2001). This single-trial design distinguishes itself in our set of tasks as a unique “problem-solving” complement to the associative learning paradigm of numerical discrimination that involves multi-trial conditioning. Spatio-temporal learning (the learning of a time-place

pattern, Schatz et al. 1999) has frequently been tested using shuttlebox assays with rodents (Bovet et al. 1968), but only once in fish (Pather and Gerlai 2009). While numerical discrimination and detour reaching tasks have both been conducted on numerous poeciliid species (Agrillo et al. 2012b, 2017; Etheredge et al. 2018), performance on the shuttlebox task has yet to be tested in this system.

By examining sex differences in cognitive performance across associative learning, cognitive flexibility, and spatio-temporal learning, we can begin to determine how divergent sexual selection pressures influence these cognitive domains. Based on previous studies with this species, we expect similar numerical discrimination performances between the sexes (Etheredge et al. 2018). Based on work with guppies, we predicted that females will outperform males in a detour task (Petrizzini et al. 2017; Lucon-Xiccato and Bisazza 2017a, b). Our spatio-temporal assay (shuttlebox) has only previously been performed on one other fish taxon (zebrafish, see Pather and Gerlai 2009) with no sex-dependent effect. However, given the strong selection pressures on females to find refuge from male harassment, we predicted that female *G. affinis* are likely to outperform male *G. affinis* in a spatio-temporal learning assay that varies time and place of a shoal group. Moreover, we predicted that the sexes would diverge in cognitive style, where a ‘fast-exploratory’ learning type (e.g., faster decision-making and shorter latencies to sample) would be more associated with males, as has been found in other poeciliids (Lucon-Xiccato and Dadda 2016). Finally, we predicted that the sexes would diverge in their relationship between cognitive domains. While some studies have found negative relationships between associative learning and cognitive flexibility (Bebus et al. 2016), studies in poeciliids thus far have not (Petrizzini et al. 2017; Buechel et al. 2018). Domain relationships between spatio-temporal learning and associative learning have not yet been explored in poeciliids. However, we hypothesized that *Gambusia affinis* females will exhibit a positive relationship between these two domains as both are critical to shoaling decisions.

Materials and methods

Housing

Wild-caught western mosquitofish *Gambusia affinis* (27 female, 27 male) from outdoor ponds at Brackenridge Field Laboratories in Austin, TX were group housed in 132 L aquaria at 24.4–26.6 °C on a 13–11 light cycle. Prior to testing, individuals were socially isolated for 2 days in 9 L aquaria. Individuals participated in 13 days of cognitive assay testing with assay order balanced across individuals and sexes, and 24 h intervals between assays.

Numerical discrimination experimental design

We modeled our numerical discrimination experimental design after Agrillo et al. (2012a). Agrillo et al. (2012a) introduced an innovative associative learning paradigm that differed from the traditional operant conditioning experiments in that it trained and tested small fish (*Gambusia holbrooki*) in experimental housing aquaria to eliminate moving subjects between trials, employed a relatively short training period (5 days), and evaluated performance using time spent with reinforced stimuli in only a few probe trials. They validated that results collected with their learning criterion (60% time spent with reinforced stimulus in no reward trials) with this shorter paradigm were consistent with results for *Gambusia holbrooki* following 10 days of training and up to 60 training trials with classic operant conditioning (Agrillo 2010; Agrillo et al. 2009). Furthermore, they used this paradigm to identify variation in cognitive performance across five small fish species (Agrillo et al. 2012b). With our numerical discrimination experiment, we adopted the same visual stimuli (assortment of black geometric shapes on white background), ratios during training (5 vs 10 and 6 vs 12) and testing (7 vs 14, 8 vs 12, and 9 vs 12), and learning criterion (60% association time with reinforced stimuli) as Agrillo et al. (2012a, b), but added automation of stimuli presentation and video recording (Fig. 1). By adopting this validated approach, we aimed to identify possible individual and/or sex variation in cognitive performance with *G. affinis*. In addition, the association time metric provides a continuous measure to compare with behavioral measures for our cognitive style evaluation.

To test associative learning, individuals were placed in a modified 38-L automated numerical discrimination experimental tank for an 11 day assay including 2 days of habituation (with 5 trials per day for a total of 10 habituation trials), 6 days of training (with 5 trials/day for a total of 30 training trials), and 3 days of testing (with 3 test trials per day interspersed with 3 reinforced trials each day to prevent extinction for a total of 9 test trials) (Fig. 1a, b). Stimuli were geometric shapes (adapted from Etheredge et al. 2018) presented on LCD screens attached to the end sides of each tank and controlled with raspberry pi boards (see Supplementary Methods for complete description on our automated numerical discrimination operation). Version control of the automation scripts is available at <https://github.com/jenkins-cummingslab/ethoStim>. These visual stimuli were controlled for non-numerical cues including cumulative surface area and the overall space that the objects occupy (Stevens et al. 2007; Agrillo et al. 2017) by varying the position and sizes of shapes in the stimulus set. Using the visual stimuli from (Etheredge et al. 2018), we were able to minimize the difference in cumulative surface area between numerical stimuli to less than 10%.

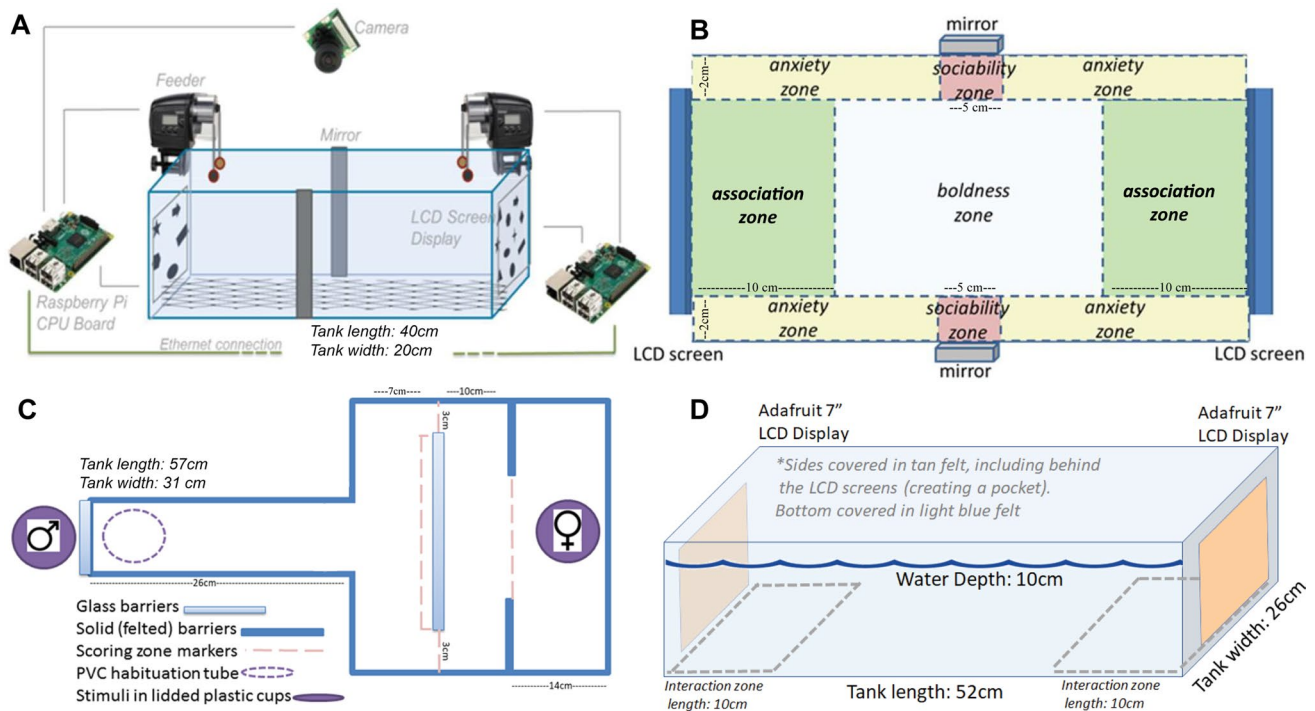


Fig. 1 Experimental designs of the numerical discrimination, detour task, and shuttlebox task. **a** Twelve automated tanks were designed for the numerical discrimination task. The electronic equipment per tank consisted of two LCD displays, a camera, and two modified fish feeders that were controlled using two Raspberry Pi Computer Boards. The computer boards were connected via ethernet to separate desktops to perform trials without human interference. **b** In addition to performance, behavior in the numerical discrimination assay

was assessed via zone designations in the tank. **c** In the detour task, a male was placed at the near end of the starting alley in a clear lidded plastic cup behind a pane of UV-pass glass, and a female reward was placed behind the glass barrier in a clear lidded plastic cup. **d** The shuttlebox apparatus consisted of a tank with two LCD screens at either end. These screens alternated between showing a video of a five-female conspecific shoal and an empty tank

During habituation, a food reward (a netted weight containing Cargill Aquaxcel Starter Food[®]) was submerged simultaneously in front of both screens projecting blank (white) backgrounds and remained in the tank continuously for 4 min. Following this 4 min, the weight was pulled up out of the tank, making the food unavailable. Meshing placed approximately 1 cm above the tank floor allowed any submerged food that escaped the netted weight to fall through to the bottom of the tank and become unreachable. During training, individuals were presented two 1:2 ratios (5 versus 10 shapes or 6 versus 12 shapes) haphazardly alternating between left and right reward sides across training trials. During a training trial, the numeric stimuli first appeared on screens for 10 s. Following this 10 s (and with the numeric stimuli still present), the food reward was submerged into the tank for 10 s in front of the rewarded screen, with a control weight simultaneously submerged in front of the unrewarded screen. Half of the subject males and females received food reward on the side with the greater quantity on the screen, and half of the subject males and females received food reward on the lesser quantity side. Following 30 total training trials, individuals were then administered a total of nine

testing trials (no food reward) over the course of 3 days. On each testing day, subjects were tested three times with each test representing a different ratio of varying difficulties and with novel numerical contrasts that they had not experienced during training: 1:2 (7 vs 14 shapes), 2:3 (8 vs 12 shapes), and 3:4 (9 vs 12 shapes). A rewarded reinforcement trial with one of the original training stimuli (6 vs 12 or 5 vs 10 shapes) was administered following each test trial to minimize extinction effects. At the end of testing, individuals had undergone each test ratio three times with the order of test ratio presentation counterbalanced across testing days.

All test trials were recorded with an overhead video camera (Fig. 1a). We assessed learning performance during the initial 20 s of each test trial in which stimuli were presented on the screens, which corresponded to the time before and during reward administration during training (though no food was given during testing). Performance was measured as the proportion of time spent in the correct screen area (representing ~20% of the tank, Fig. 1b) of the total time fish spent in both screen areas. We calculated the median proportion of time spent with the correct stimulus across the three replicate test trials for each test ratio, and employed the *G*.

holbrooki validated learning criterion of 60% of time associating with the reinforced stimulus (Agrillo et al. 2012a, b). Specifically, our learning criterion required individuals to achieve a median association time $> 60\%$ in at least one of the three test ratios. Because our study aimed to capture individual variation in learning, we additionally employed a stricter ‘high performance’ learning criterion to identify individuals who successfully generalized the learning rule of attending to higher or lower numerical contrasts for a food reward across multiple novel ratios. For this ‘high performance’ learning criterion, we require individuals to achieve a $> 82\%$ median score of association time with the reinforced stimulus in at least two of the three novel ratios. This high-performance criterion required a fish to exhibit highly selective behavior in 2 out of 3 replicate ratio test trials across two distinct test ratios, and is statistically robust to those who show high performance in two ratios and lower performance in a third ratio ($> 82\%$ in 4 out of 9 trials, with a binomial probability of $p=0.049$). This more difficult level of evaluation allows us to identify individuals who can generalize learned associations across novel ratios and meet the high levels of performance over a much shorter timeframe than what is typically achieved after extensive training trials common in operant conditioning paradigms. We used the median performance within test ratios to evaluate whether individuals were classified as either non-learners ($< 60\%$ median in each test ratio), low-performing learners ($> 60\%$ median in at least 1 test ratio), or high-performing learners ($> 82\%$ median in at least 2 test ratios). To compare a continuous metric of learning performance with behavioral measures, we evaluated each individual’s average numerical performance (average proportion of time spent near the correct screen out of total screen time within the first 20 s of all 9 test trials).

Cognitive flexibility (detour task) experimental design

An experimental detour task tank (filled to 13 cm) was subdivided into a starting alley (26×14 cm), center section (14×31 cm), and reward section (14×31 cm), see Fig. 1c. Because male *Gambusia* exhibit high rates of copulation attempts with females (Dadda 2015) and because female *Gambusia affinis* shoal with other females to reduce male harassment (Pilastro et al. 2003; Dadda 2015; Dadda et al. 2005), we used a male social activator and a female social reward for all focal individuals. The male was placed behind the starting alley in a visible container and remained there for the length of the trial. The male activator is expected to motivate both males and female subjects to move away from the social activator male and towards the female reward. Female subjects are likely to move away to minimize harassment, and male subjects are likely to move away to minimize

competition. The center section contained a 25 cm-wide glass barrier which prevented individuals traveling in a direct line from reaching the social reward. The goal of the task was for individuals to change their behavior upon encountering the consequence (unsuccessful passage) of approaching the reward head-on. The social reward was a female in a clear container during the entirety of the trial. Individuals could solve the detour task by turning away from the direct line and traveling through the unobstructed zones (3 cm) at each side of the glass barrier to reach the social reward (Fig. 1c). The focal individual habituated for 5 min in an opaque tube and then swam freely in the apparatus for the 10 min trial with social activator male and female reward stimuli visible. Motivation was recorded as the latency to reach the barrier, and solution speed was recorded as the time difference between arrival at the barrier and arrival at the social reward.

Temporal learning (shuttlebox) experimental design

Our experimental shuttlebox tank was 52×26 cm filled to a depth of 10 cm, with an Adafruit 7" LCD Display at either end (Fig. 1d). The focal individual swam freely during a 5-min habituation in which both screens displayed a video of an empty tank. After habituation, the fish was allowed to continue swimming in the apparatus as one screen presented a 20 s video of five conspecific females, followed by a 90 s inter-stimulus interval (ISI) of the empty tank on both screens and then by the conspecific video on the opposite side (Supplementary Table 1). The video of five conspecific females was a recording of live individuals placed in a container the approximate size window of the screen to ensure realistic swimming behavior. This alternating stimuli-ISI pattern lasted 1 h as in Pather and Gerlai (2009).

An individual must have transited between the two interaction zones (10 cm in front of each screen, Fig. 1d) at least once during ISI’s 4–9 to be considered a participant. Learners in the shuttlebox were evaluated as participants who spent $> 50\%$ of interaction time in the correct region (within 10 cm of the screen with an imminent shoal group appearing) during the final 30 s of the ISI for three consecutive trials following the 4th ISI. This criterion required individuals to traverse the middle 62% of the tank in two consecutive ISIs to reach these interaction areas at the far end of the tank, each of which only account for 19% of the tank area.

Video scoring

Human observers scored time spent in regions of the numerical discrimination tank (Fig. 1b) (using CowLog 3.0.2 and a python-generated grid overlay see “make_a_grid_script.py” in <https://github.com/kellyjwallace/Comparative-Cognition-R-scripts>). Additionally, we recorded latency to change

regions following the image presentation (reaction time), number of unique zones visited (exploration), and total transits between zones (activity). One author (KW) and twelve undergraduate students independently scored 315 numerosity discrimination videos (Single Score Intraclass Correlation between eleven scorers $p = 5.41 \times 10^{-90}$). Recordings for the detour task and shuttlebox assays were taken using Debut Video Capture Software and LifeCam cameras. Detour videos were independently scored by hand by two undergraduate student scorers and compared to the co-author (KW) scorer ($p = 4.83 \times 10^{-45}$ and $p = 2.03 \times 10^{-35}$). Shuttlebox videos were hand scored by three student scorers using a python program developed by Luke Reding (see https://github.com/lukereding/shuttlebox/blob/master/track/shuttlebox_hand_track.py) that evaluated the position of the fish at 5 s intervals.

Statistics

Data analyses and visualizations were conducted in R version 3.5.0 (2018) and evaluated at $\alpha = 0.05$. We conducted standard multiple linear regressions using the R package “lm()” to determine correlations and effect sizes of relationships between continuous independent variables using the formula $\text{lm}(\times 1 \sim \times 2)$. To identify the significance of sex and learning performance category in our models, we additionally ran linear models that included interaction terms using the formula $\text{lm}(\times 1 \sim \times 2 * \times 3)$. We conducted an unpaired t test or unpaired Wilcoxon signed-rank test (determined by a Shapiro–Wilk normality test) for continuous data split into two categories, or a one-way ANOVA or Kruskal–Wallis test depending on normality for continuous data split into > 2 categories. For categorical data, we conducted Chi-squared tests on data with > 5 observations per category and Fisher’s exact test for data which had < 5 observations in a category. Multivariate analyses were conducted using principal component analysis via the R package “prcomp”. Data analysis coding scripts, original data, and protocols can be found at <https://github.com/kellyjwallace/Comparative-Cognition-R-scripts>.

Results

Fifty-four individual *G. affinis* were run through a series of three cognitive assays (numerosity discrimination, detour task, and shuttlebox). However, due to technical errors, not all individuals completed all assays. Completion rates across assays varied with 36 individuals completing numerosity discrimination (18 males, 18 females), 52 individuals completing detour task (25 males, 27 females), and 26 individuals completing shuttlebox (9 males, 17 females).

Associative learning performance (numerical discrimination)

In the numerical discrimination task, 18 individuals were removed from analysis due to component malfunctions; thus, 36 individuals were used for analysis. 23 individuals successfully met the learning criterion (12F/11M), and 13 individuals did not (6F/7M) (Fig. 2a). This proportion of learners is similar to the proportion found in the previous studies employing this experimental design and with different poeciliid species (Agrillo et al. 2012a, b; Etheredge et al. 2018). Six of the twenty-three learners (3F/3M) additionally met the high-performance learning criterion. Sex did not influence the distribution of learners to non-learners ($\chi^2 = 0$; $p = 1.00$, Fig. 2a). Our metric of performance correlated significantly with several others measured [Supplementary Fig. 1; e.g., first side chosen ($r^2 = 0.175$, $p = 0.013$), and latency to enter the correct side ($r^2 = 0.349$, $p = 0.0004$)]. There was no difference in performance between individuals trained to the higher quantity or lower quantity ($p = 0.465$, $t = -0.738$) or across ratios ($F = 0.302$, $p = 0.7399$). The sexes did not differ significantly in their learning performance ($t = 1.483$, $p = 0.148$, Supplementary Fig. 3). Body size (standard length, a proxy for age in female *G. affinis* (Hughes 1986)) did not predict learning performance (females: $p = 0.719$, Pearson’s $r = -0.094$, males: $p = 0.770$, Pearson’s $r = -0.074$, combined: $p = 0.589$, Pearson’s $r = -0.095$, see Table 1).

Cognitive style in associative learning (numerical discrimination)

Given the differences in scoring methods across the three assays, the most extensive data on cognitive style activity were in the numerical discrimination. In the numerical discrimination assay, males and females exhibited the same levels of exploration ($W = 192$, $p = 0.350$), reaction time ($W = 121$, $p = 0.203$), sociability ($W = 202$, $p = 0.214$), boldness ($t = 1.5621$, $p = 0.128$), and activity ($W = 217.5$, $p = 0.082$). Males exhibited a higher proportion of time in regions associated with anxiety (thigmotaxis) than females ($W = 97$, $p = 0.040$, Supplementary Fig. 2). Female standard length was significantly positively correlated with exploration ($r = 0.539$, $p = 0.021$) and higher activity ($r = 0.464$, $p = 0.052$), but negatively correlated with boldness ($r = -0.471$, $p = 0.048$, see Table 1). Male standard length showed no significant relationship to any of these behaviors (Table 1). Female learning performance was significantly correlated with reaction time ($r = -0.541$, $p = 0.025$, Fig. 3a) and exploration ($r = 0.510$, $p = 0.036$, Fig. 3b), and marginally significant with activity $r = 0.477$, ($p = 0.053$, Fig. 3c). Male behaviors did not relate to performance (reaction time:

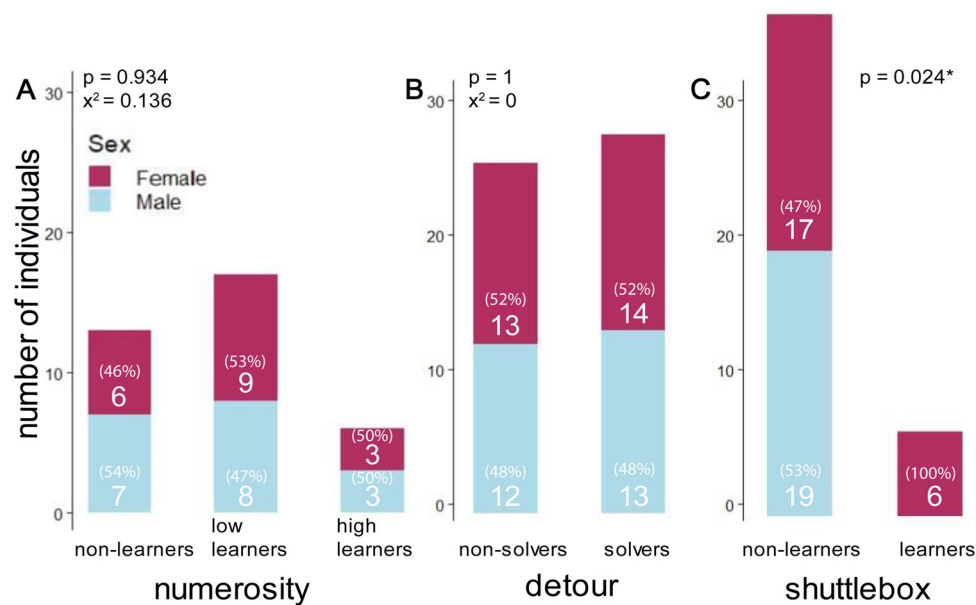


Fig. 2 Learning performance differs between the sexes in the shuttlebox assay, but not the numerical discrimination or detour tasks. **a** In the numerical discrimination assay, the sexes show roughly a 3:2 distribution of learners to non-learners (learners reaching a minimum median performance of 60% for one of the three test ratios (7:14, 8 vs 12, 9 vs 12)). Equal numbers of male and females (3M/3F) reached the high-performance criterion (82% minimum median performance

across 2 of the 3 test ratios). **b** Detour task solvers versus non-solvers also are distributed equally across the sexes. **c** Females reached the learning criterion in the shuttlebox assay (learners exhibiting three consecutive trials in which a majority of interaction time during the last 30 s of the ISI was spent within 10 cm of the correct screen) more than males (Fisher's exact test, $p = 0.024$)

Table 1 Female size predicts cognitive style behaviors in the numerical discrimination task

Behavior	Females p	Females effect size	Males p	Males effect size	Both sexes p	Both sexes effect size
Numerical discrimination performance	0.719	−0.094	0.770	−0.074	0.589	−0.095
Numerical discrimination learning category*	0.687	−0.204	0.595	0.267	0.839	−0.068
Detour solve category*	0.641	0.225	0.139	0.770	0.284	0.364
Detour latency to solve	0.344	−0.358	0.219	−0.454	0.537	−0.156
Detour motivation	0.438	−0.217	0.455	0.218	0.795	−0.050
Shuttlebox learning category*	0.434	6.676	na	na	0.034	5.504
Numerical discrimination exploration	0.021	0.539	0.182	0.329	0.009	0.430
Numerical discrimination boldness	0.048	−0.471	0.706	−0.096	0.423	−0.138
Numerical discrimination anxiety	0.234	0.295	0.733	−0.086	0.434	−0.135
Numerical discrimination activity	0.052	0.464	0.122	0.378	0.0008	0.535
Numerical discrimination reaction time	0.144	−0.358	0.148	−0.355	0.024	−0.376

The relationship between standard length (a proxy for age) and the behavior listed is shown for three data sets: only females, only males, and all individuals combined. Continuous behavioral data results were determined using a multiple linear regression, and the reported effect size is Pearson's correlation coefficient. Categorical behavioral data results (denoted with *) were determined using a t test, and the reported effect size is Cohen's D . Significant p values are bolded.

$p = 0.689$, exploration: $p = 0.332$, activity: $p = 0.489$). Interaction effects of sex were marginally significant for reactivity ($p = 0.099$) and exploration ($p = 0.077$). A principal component analysis of behavior (reaction time, exploration, and activity) significantly predicts female

learning ($p = 0.007$, $r^2 = 0.399$) but not male learning ($p = 0.439$, $r^2 = 0.038$) with a significant interaction effect of sex ($p = 0.037$) (Fig. 3d, e).

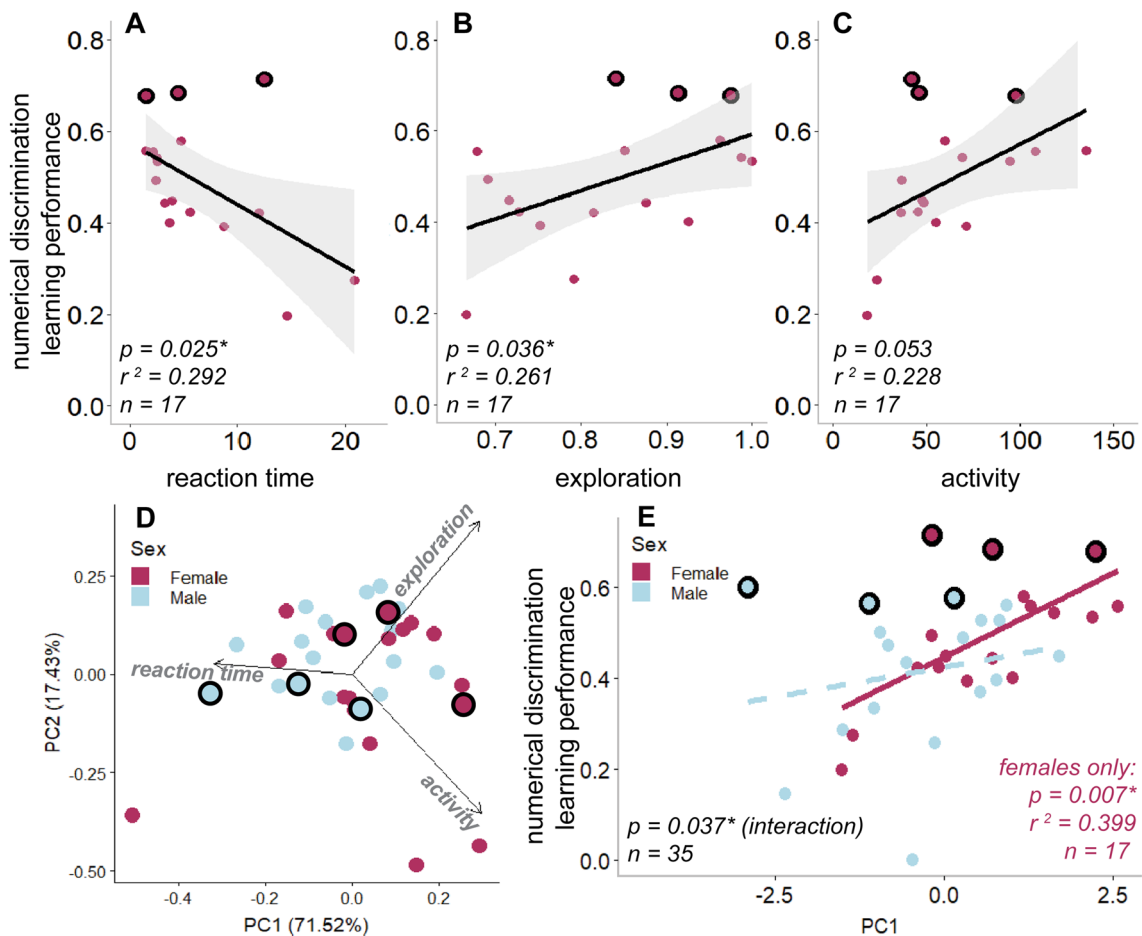


Fig. 3 Cognitive style in the numerical discrimination task predicts performance. Female learning performance (average proportion of screen time spent in front of the correct screen during initial 20 s across 9 test trials) is predicted by **a** female reaction time ($p=0.025$), **b** exploration ($p=0.036$), and **c** activity ($p=0.053$) displayed during test trials; whereas no significant correlations were found between these measures in males. **d** A principal component analysis of these three behaviors (reaction time, exploration, and activity) resulted

in two major axes that collectively describe 88.95% of variation in behavior. The first principal component axis was primarily described by reaction time, and the second axis was primarily described by exploration and activity. **e** Associative learning performance in the numerical discrimination task is significantly predicted by PC1 in females ($r=0.631$; $p=0.007$) but not males ($r=0.194$; $p=0.439$) with a significant interaction effect ($p=0.037$). High-performance learners are denoted by black outlining

Cognitive flexibility performance (detour task)

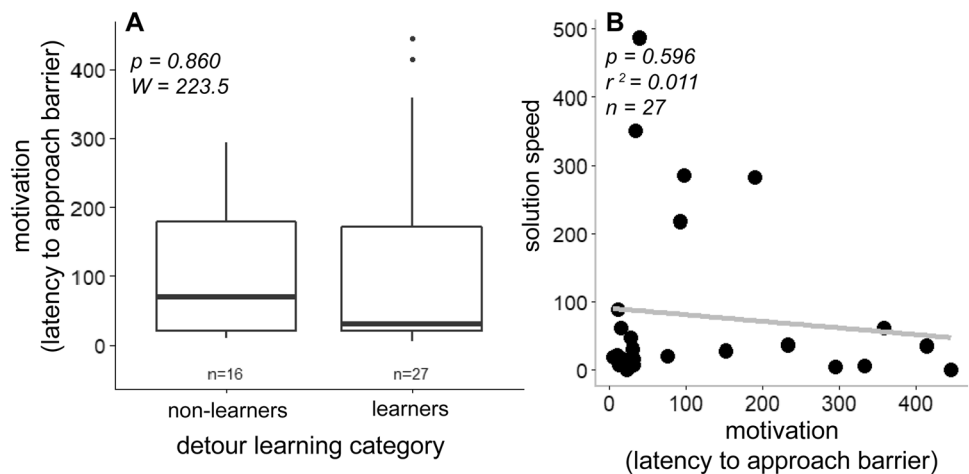
In the detour task, 27 individuals (14F/13M) successfully navigated around the transparent barrier, 25 did not (13F/12M) (Fig. 2b). Of the 25 individuals who did not navigate around the barrier, 9 (4F/5M) did not approach the barrier and were thus removed from further analysis. Size did not differ between solvers and non-solvers (Table 1). On average, it took solvers 80 s (SE=24 s) to solve the task upon reaching the barrier. Latency to solve the task was not predicted by sex ($W=101$, $p=0.645$, Supplementary Fig. 4), size (females: $r=-0.358$, $p=0.344$, males: $r=-0.454$, $p=0.219$, combined: $r=-0.156$, $p=0.537$), or motivation (latency to reach the barrier, $r=-0.107$, $p=0.596$, Fig. 4). Motivation did not differ by sex ($W=221.5$, $p=0.846$) or size (females: $r=-0.217$, $p=0.438$, males: $r=0.218$,

$p=0.455$, combined: $r=-0.050$, $p=0.795$) and did not influence whether an individual successfully navigated around the barrier ($W=223.5$, $p=0.860$, Fig. 4a) or solution speed (linear model $p=0.596$, $r^2=0.011$, quadratic model $p=0.279$, Fig. 4b).

Spatio-temporal learning performance (shuttlebox)

Of the 54 individuals tested in the shuttlebox assay, three were removed due to technical malfunctions, and nine were removed from analysis for nonparticipation. We identified six learners (all female) and 36 non-learners (19M/17F) (Fig. 2c). A significant sex difference in performance was found, where females reached the learning criterion more often than males (Fisher's exact test, $p=0.024$, Fig. 2c).

Fig. 4 Motivation in the detour task does not predict performance. **a** Individuals who solve the detour task do not exhibit higher motivation to reach the barrier than non-solving individuals. **b** Solution speed in the detour task is not predicted by motivation (latency to reach the barrier)



Cognitive style across tasks

In addition to cognitive style measures collected in the numerical discrimination task, we collected measures of cognitive style in the detour task and shuttlebox assay. We assessed exploration in the shuttlebox as the number of inter-stimulus-intervals—of 30 possible ISIs—that an individual transited across the length of the tank from one interaction area to the other. Reaction time in the numerical discrimination task correlates with motivation in the detour task (latency to approach the transparent barrier) in a sex-general manner (combined sexes: $r = 0.391$; $p = 0.036$; males only: $r = 0.181$; $p = 0.536$; females only: $r = 0.596$, $p = 0.019$, Fig. 5a). Exploration in the numerical discrimination correlated positively with motivation in the detour task in a sex-general manner (combined sexes: $r = -0.453$; $p = 0.014$; males only: $r = -0.45$; $p = 0.105$; females only: $r = -0.471$, $p = 0.077$, Fig. 5b). Activity in the numerical discrimination (number of entries into all zones) correlated positively with exploration in the shuttlebox task in males ($r = 0.519$, $p = 0.039$, Fig. 5c) but not females ($r = -0.311$, $p = 0.209$), with a marginally significant interaction effect of sex ($p = 0.050$) (Fig. 5d).

Relationships in performance across domains

Individuals who solve the detour task exhibit significantly higher numerical discrimination performance (sexes combined: $p = 0.046$, $t = -2.080$, Fig. 6). While there is no significant interaction effect of sex and detour solve category ($p = 0.248$), males who solved the detour task exhibited significantly higher numerical discrimination performance than non-solvers ($t = -2.361$, $p = 0.035$; Fig. 6).

Discussion

We identified sex differences emerging across multiple attributes of cognition: in performance within a given task, in cognitive style across domains, and in how cognitive styles predict performance in the numerical task.

Sex differences in cognitive performance

Similar to findings in previous poeciliid studies utilizing numerical, color, and shape discrimination, we observed no sex differences in associative learning in our numerical discrimination assay (Etheredge et al. 2018; Lucon-Xiccato and Bisazza 2016). We found similar ratios of learners to non-learners as other numerical discrimination experiments with *G. affinis* (two-thirds learners, Etheredge et al. 2018). We found no sex difference in cognitive flexibility as measured via our detour task, which is contrary to findings in a related poeciliid (*Poecilia reticulata*) where females outperform males in this task (Petrizzini et al. 2017). Confirming our prediction, we found a significant sex difference in performance in the spatio-temporal learning assay, with females being more likely to reach the learning criterion than males. The only previous examination of this cognitive task in teleosts showed no sex differences in zebrafish (Pather and Gerlai 2009).

The greater spatio-temporal learning performance in female *G. affinis* may be driven by sexually dimorphic motivations to shoal. Females from poeciliid species with high levels of sexual coercion like *Gambusia* employ shoaling as a strategy to reduce male harassment (Dadda 2015), and *Gambusia* females shoal more than males (Etheredge et al. 2018; Heinen-Kay et al. 2016). Female *G. affinis* have been shown to choose shoal group size in a rational manner (Reding and Cummings 2019); and in *Gambusia*, the benefit for

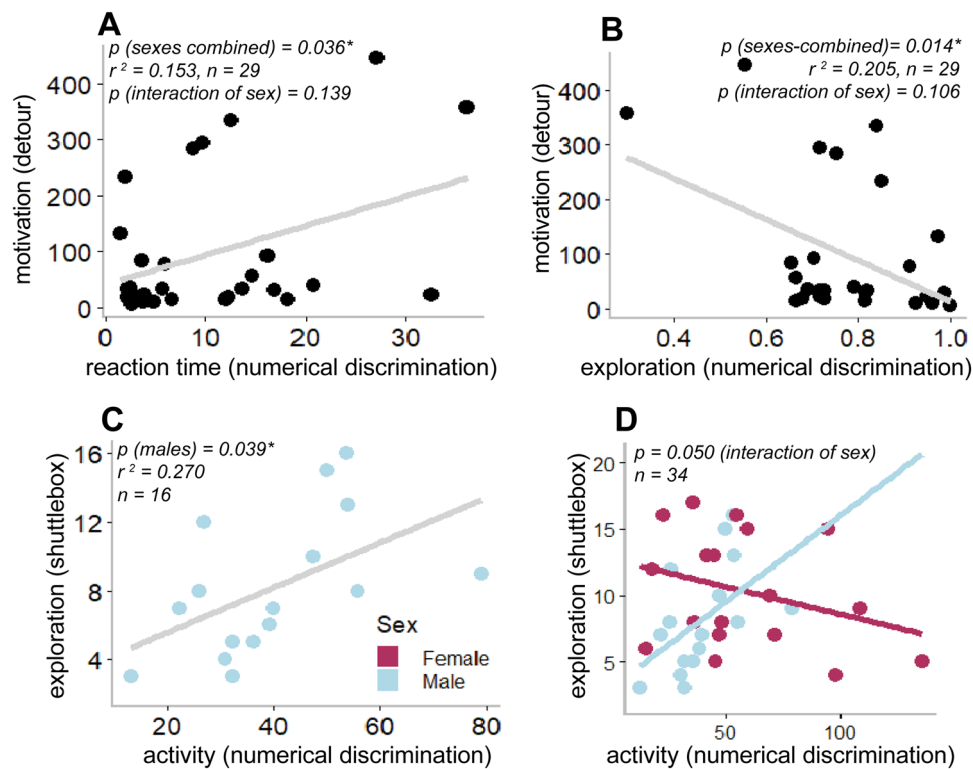
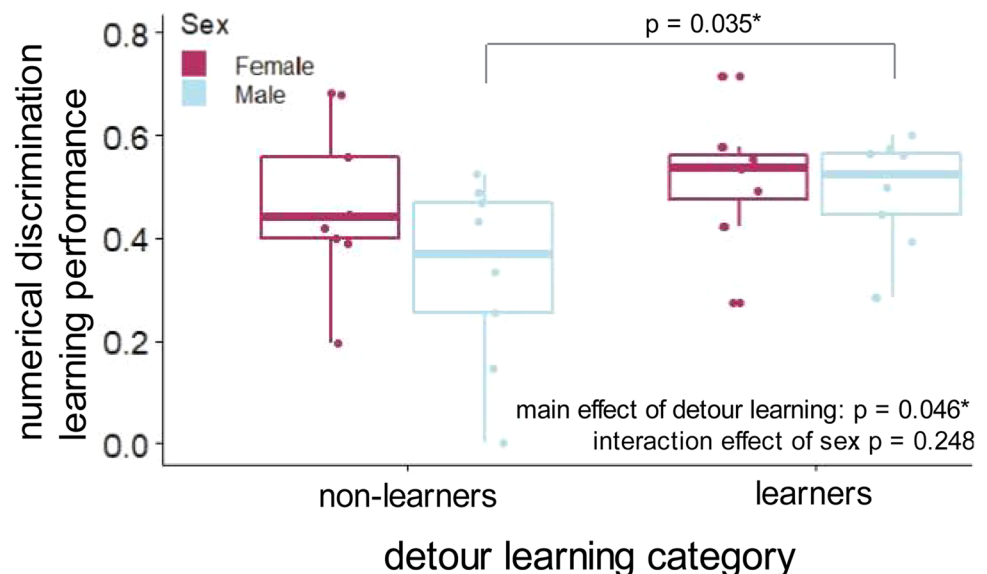


Fig. 5 Cognitive style is consistent across tasks. **a** Reaction time in the numerical discrimination task correlates with exploration in the detour task in a sex-general manner ($p=0.036$), suggesting that individuals who were quick to move towards conspecifics in the detour task were also quick to make decisions in the numerical discrimination task. **b** Exploration in the numerical discrimination task correlates with exploration in the detour task in a sex-general manner ($p=0.014$), though this relationship is non-significant when we

remove the single individual with exploration values less than 0.4 ($p=0.139$). **c** Male activity in the numerical discrimination task correlates with exploration in the shuttlebox task ($p=0.039$). **d** While female activity in numerical discrimination task did not significantly correlate with exploration in the shuttlebox task ($r=-0.311$, $p=0.209$), we find a near-significant interaction effect of sex ($p=0.050$)

Fig. 6 Male performance is predicted across cognitive domains. Individuals who solve the detour task exhibit higher performance on the numerical discrimination task ($p=0.046$). While there is no significant interaction effect of sex on this relationship ($p=0.24$), males who solved the cognitive flexibility task (detour task) exhibited significantly higher associative learning (numerical discrimination) performance than males who did not solve the cognitive flexibility task ($p=0.035$)



females to select larger shoals includes reduced male harassment, increased male–male agonistic interactions, and increased female foraging efficiency, (Pilastro et al. 2003; Smith 2005). Increased shoaling tendencies in females may drive the shoaling-related cognitive advantage observed in *G. affinis* females as seen in our spatio-temporal learning task in which females were more successful than males in predicting the time and place of the shoal. This sexual dimorphism in shoaling—and the sexual conflict that drives it—may manifest in sex differences in spatio-temporal cognitive tasks that emulate shoaling decisions.

Relationships between cognitive style and performance in the numerical discrimination task

While performance did not differ between the sexes in the numerical discrimination task, the relationship between cognitive style and performance differed between the sexes. This suggests that males and females exhibit different approaches (likely driven by different sex-specific selection pressures) by which they reach equal levels of ability. Performance in numerosity discrimination tasks was predicted by a suite of related behaviors exhibited during the test trials by females but not males. Female mosquitofish that exhibited a ‘fast-exploratory’ cognitive style in the numerical discrimination assay exhibited higher associative learning performance. Specifically, females that explored a greater area of the tank, reacted faster, and moved more demonstrated higher numerical discrimination performances. A positive association between ‘fast behavioral’ type and associative learning is documented across multiple taxa particularly in response to predation (Dougherty and Guillette 2018), including black-capped chickadees (*Poecile atricapillus*) (Guillette et al. 2009), sticklebacks (*Gasterosteus aculeatus*) (Bensky et al. 2017), and Panamanian bishop fish (*Brachyrhaphis episcopi*) (DePasquale et al. 2014). The ecological pressures that might lead to individuals adopting fast behavioral types may stem from being exposed to threatening environments (high predation), which places a selective pressure on the speed at which they sample their environment.

In some populations of guppies, intense male harassment has driven females into different habitats with greater predation levels than males (Croft et al. 2006). Have the intense social pressures found in *Gambusia* driven sex-specific habitat differentiation and thus shaped female fast-exploratory cognitive styles? In previous fish studies examining sex differences in cognitive styles during associative learning assays with food rewards (Mamuneas et al. 2015), and during visual discrimination of shapes and colors (Lucon-Xiccato and Dadda 2016), researchers have found a fast-exploratory cognitive style associated with males, not females. Only during shoal discrimination, tasks in poeciliids (guppies)

have females been shown to make faster decisions than males (Lucon-Xiccato et al. 2016). If strong sexual selection pressures have shaped female *Gambusia* shoaling decision-making processes, perhaps, this has influenced the cognitive style in numerical discrimination tasks in general.

The cognitive style that is identified in females during the numerosity discrimination task in this experiment is comprised of behaviors that tend to covary with female size (Table 1). Poeciliid females experience indeterminant growth, and therefore, standard length is often a proxy for female age. While larger/older females were no better at numerosity discrimination than smaller females, they did trend towards being more exploratory, reacting faster and moving more in the test trials (Table 1). This reflects findings showing that larger females tend to disperse farther (Brown 1985). Are female *G. affinis* developing a cognitive style as they age? Age-dependent decision-making processes have been documented among female poeciliids. For instance, relative to smaller females, larger and presumably older female El Abra swordtails show stronger preference for courting phenotypes over coercive phenotypes (Wong et al. 2011) and exhibit less transitivity in mate choice decisions involving male size (Reding and Cummings 2018). Whether the increased exposure to male harassment over a female’s lifetime contributes to the development of fast-exploratory female cognitive style can only be determined with manipulative social experiments.

It is imperative to caution that the causal relationship between cognitive style and cognitive performance is unknown (Sih and Del Giudice 2012; Dougherty and Guillette 2018). In addition to implying that our ‘high learning individuals’ could be the result of individual differences in activity and exploratory tendencies, we must acknowledge that the behavior observed in the numerical discrimination testing trials could be a result of cognitive performance—i.e., those individuals who quickly learn the task may more quickly habituate and thus become more active and exploratory. Further studies assessing the developmental sources of variation in cognitive style and cognitive performance would help us to understand the causal relationship between behavior and cognitive ability.

Consistent cognitive styles across tasks

Expanding beyond the behaviors displayed in the associative learning task, we find consistent behavioral relationships across tasks. When we compared cognitive style behaviors across the assays, we found consistent variation across individuals. Reaction time to stimulus presentation in the numerical discrimination task correlated with motivation behavior in the detour task across sexes (Fig. 5a). Males and females that were the fastest to approach conspecifics in the detour task made faster decisions in the associative learning trials.

These results suggest consistent individual variation along a “fast-slow” axis of behavior not only within the associative learning task, but across contexts. This ‘fast-exploratory’ cognitive style observed in these mosquitofish appears to share attributes of previously described ‘fast behavioral’ type individuals (more exploratory, active, bold, and aggressive, see Sih and Del Giudice 2012) in other taxa. Even within this consistent behavioral variation across contexts, we find emerging sex differences: activity in the numerical discrimination task correlates positively with exploration in the shuttlebox task in males but not females (Fig. 5c, d). This suggests that males are displaying consistent movement-based behaviors (exploration and activity levels) across different contexts. These consistent cross-context individual differences in male movement behaviors possibly reflect underlying genetic variation in male exploratory strategies. While many poeciliid species have a diverse array of genetically determined alternative male reproductive strategies, *Gambusia affinis* males exhibit a single strategy (coercion). Yet, the individual variation in male exploratory behavior observed here may suggest that these coercive may represent a different array of exploratory strategies.

Performance across cognitive domains

In addition to performance and cognitive style, we found consistent performance across domains: individuals who successfully solved the cognitive flexibility task (detour task) showed significantly higher associative learning performance. This relationship has not previously been described in poeciliid fish. Given that acquisition and other measures of cognitive flexibility (e.g., reversal learning) utilize different neural mechanisms (López et al. 2000; Dalley et al. 2004; Eisenhardt and Menzel 2007), our finding of a positive relationship in performance across these domains is an interesting result. *Gambusia* are extremely invasive (found in over 40 countries, Welcomme 1992) and, thus, frequently experience highly variable environments. Invasive species may utilize both associative learning and cognitive flexibility as a strategy to succeed in these dynamic environments. This concept, known as the “adaptive flexibility hypothesis”, emphasizes that cognitive flexibility is an adaptive response to a changing physical environment (Wright et al. 2010) or social (Bond et al. 2007) environment. In *Gambusia affinis*, an investment in a positive domain relationship between associative learning and cognitive flexibility may predominantly benefit males given that they are more likely to disperse than females (Brown 1985) and disperse farther (Wright et al. 2010; Cote et al. 2010). The greater dispersal tendency in male *Gambusia affinis* may be contributing to the putatively stronger relationship between associative learning performance and cognitive flexibility in males

relative to females (Fig. 6). However, further studies with larger sampling sizes are necessary to resolve this intriguing possibility.

Conclusion

Our study identified new sex differences in spatio-temporal learning, sex-specific cognitive styles within associative learning, consistent individual variation in behavior across tasks, and a positive relationship between performances across cognitive domains. A wealth of literature has identified sex differences in cognitive performance in mammals (Rice et al. 2017), birds (Brust et al. 2013), reptiles (Carazo et al. 2014), and fish (Lucon-Xiccato and Bisazza 2017a, b; Cummings 2018). But here, we find that sex differences extend beyond performance into other cognitive attributes such as cognitive style and cross-domain relationships, suggesting that more comprehensive characterization of cognition is important. Our experimental design in which the same individuals were assessed for cognitive performance and style across domains allowed us to detect previously undescribed sex differences in cognition in *Gambusia affinis*. Fish exhibit a wealth of sex-specific ecological (Laland and Reader 1999; Lucon-Xiccato and Dadda 2017) and sexual selection pressures (Magurran and Maciás 2000; Cummings 2018; Dadda 2015); therefore, we can expect fish to continue to be an insightful taxonomic group in uncovering predictive patterns of sex differences in cognition (Cummings 2018). Further studies, particularly those utilizing more extensive suites of cognitive testing, investigating neural mechanisms, and identifying developmental bases of these relationships, will be critical to elucidate mechanisms governing the patterns observed in this study. In addition, comparisons of related species that differ in degree of sexual conflict and ecological pressures will be an important next step in distinguishing the factors that drive individual variation in cognitive performance.

Acknowledgements The authors would like to thank Luke Reding for contributions to the shuttlebox data analysis, the many undergraduates who assisted in data collection on this project including Matt Armstrong, Lauren Borland, Rahi Dakwala, Caleb Fleischer, Daniel Hauser, Amogh Kashyap, Presley Mackey, Claire Mayorga, Jessika McFarland, Lily Parsi, Huynh Pham, Sylvestre Pineau, Adam Redmer, Vishaal Sakthivelnathan, Eduardo Saucedo, Madison Schumm, Ben Whelan, and Melody Ziari, and the very helpful comments and assistance from all Cummings’ laboratory members. We thank the University of Texas’ Brackenridge Field Laboratory for animal care facilities.

Author contributions MC and KW conceived of the study. KW, MR, and RR designed and constructed the experimental setup and data collection procedures. KW collected the data and performed statistical analyses. KW and MC interpreted the results and wrote the manuscript. All authors gave final approval or publication.

Funding This work was funded by an NSF BEACON Award (26-3509-2650) to MC.

Data accessibility Raw data (Microsoft Excel) as well as analysis documentation are available at the following archived Github repository: <https://github.com/kellyjwallace/Comparative-Cognition-R-scripts>.

Compliance with ethical standards

Conflict of interest The authors have no competing interests.

Ethical approval The authors certify that this work followed ethical treatment of animals outlined in their IACUC protocol (AUP-2016-00246).

References

- Agrillo C, Dadda M, Serena G, Bisazza A (2009) Use of number by fish. *PLoS ONE* 4(3):e4786
- Agrillo C (2010) Large number discrimination by mosquitofish. *PLoS ONE* 5(12):e15232
- Agrillo C, Miletto Petrazzini ME, Piffer L, Dadda M, Bisazza A (2012a) A new training procedure for studying discrimination learning in fish. *Behav Brain Res* 230:343–348. <https://doi.org/10.1016/j.bbr.2012.02.025>
- Agrillo C, Miletto Petrazzini ME, Tagliapietra C, Bisazza A (2012b) Inter-specific differences in numerical abilities among teleost fish. *Front Psychol* 3:1–9. <https://doi.org/10.3389/fpsyg.2012.00483>
- Agrillo C, Miletto Petrazzini ME, Bisazza A (2017) Numerical abilities in fish: a methodological review. *Behav Process* 141(2):161–171. <https://doi.org/10.1016/j.beproc.2017.02.001>
- Bebus SE, Small TW, Jones BC, Elderbrock EK, Schoech SJ (2016) Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. *Anim Behav* 111:251–260. <https://doi.org/10.1016/j.anbehav.2015.10.027>
- Bensky MK, Paitz R, Pereira L, Bell AM (2017) Testing the predictions of coping styles theory in threespined sticklebacks. *Behav Process* 136:1–10. <https://doi.org/10.1016/j.beproc.2016.12.011>
- Bond AB, Kamil AC, Balda RP (2007) Serial reversal learning and the evolution of behavioral flexibility in three species of North American corvids (*Gymnorhinus cyanocephalus*, *Nucifraga columbiana*, *Aphelocoma californica*). *J Comp Psychol* 121:372–379. <https://doi.org/10.1037/0735-7036.121.4.372>
- Boogert NJ, Anderson RC, Peters S, Searcy WA, Nowicki S (2011) Song repertoire size in male song sparrows correlates with detour reaching, but not with other cognitive measures. *Anim Behav* 81:1209–1216. <https://doi.org/10.1016/j.anbehav.2011.03.004>
- Bovet D, Bovet-Nitti F, Oliverio A (1968) Memory and consolidation mechanisms in avoidance learning of inbred mice. *Brain Res* 10:168–182
- Brown KL (1985) Demographic and genetic characteristics of dispersal in the mosquitofish, *Gambusia affinis* (Pisces: Poeciliidae). *Copeia* 1985(3):597–612
- Brust V, Wuerz Y, Krüger O (2013) Behavioural flexibility and personality in zebra finches. *Ethology* 119:559–569. <https://doi.org/10.1111/eth.12095>
- Buechel SD, Booksmythe I, Kotrschal A, Jennions MD, Kolm N (2016) Artificial selection on male genitalia length alters female brain size. *Proc R Soc B Biol Sci* 283:20161796. <https://doi.org/10.1098/rspb.2016.1796>
- Buechel SD, Boussard A, Kotrschal A, van Der Bijl W, Kolm N (2018) Brain size affects performance in a reversal-learning test. *Proc R Soc B Biol Sci* 285:20172031. <https://doi.org/10.1098/rspb.2017.2031>
- Burns JG, Rodd FH (2008) Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Anim Behav* 76:911–922. <https://doi.org/10.1016/j.anbehav.2008.02.017>
- Carazo P, Noble DWA, Chandrasoma D, Whiting MJ (2014) Sex and boldness explain individual differences in spatial learning in a lizard. *Proc R Soc B Biol Sci* 281:20133275. <https://doi.org/10.1098/rspb.2013.3275>
- Carere C, Locurto C (2011) Interaction between animal personality and animal cognition. *Curr Zool* 57:491–498. <https://doi.org/10.1093/czoolo/57.4.491>
- Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proc Biol Sci* 277:1571–1579. <https://doi.org/10.1098/rspb.2009.2128>
- Croft DP, Morrell LJ, Wade AS, Piyapong C, Ioannou CC, Dyer JRG, Chapman BB, Wong Y, Krause J (2006) Predation risk as a driving force for sexual segregation: a cross-population comparison. *Am Nat* 167(6):867–878. <https://doi.org/10.2307/3844743>
- Culumber ZW, Engel N, Travis J, Hughes KA (2020) Larger female brains do not reduce male sexual coercion. *Anim Behav* 160:15–24
- Cummings ME (2018) Sexual conflict and sexually dimorphic cognition—reviewing their relationship in poeciliid fishes. *Behav Ecol Sociobiol* 72:73. <https://doi.org/10.1007/s00265-018-2483-9>
- Dadda M, Pilastro A, Bisazza A (2005) Male sexual harassment and female schooling behaviour in the eastern mosquitofish. *Anim Behav* 70:463–471. <https://doi.org/10.1016/j.anbehav.2004.12.010>
- Dadda M (2015) Female social response to male sexual harassment in poeciliid fish: a comparison of six species. *Front Psychol* 6:1–9. <https://doi.org/10.3389/fpsyg.2015.01453>
- Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev* 28:771–784. <https://doi.org/10.1016/j.neubiorev.2004.09.006>
- Deary IJ, Penke L, Johnson W (2010) The neuroscience of human intelligence differences. *Nat Rev Neurosci* 11:201–211. <https://doi.org/10.1038/nrn2793>
- DePasquale C, Wagner T, Archard GA, Ferguson B, Braithwaite VA (2014) Learning rate and temperament in a high predation risk environment. *Oecologia* 176:661–667. <https://doi.org/10.1007/s00442-014-3099-z>
- Dougherty LR, Guillette LM (2018) Linking personality and cognition: a meta-analysis. *Philos Trans R Soc B Biol Sci*. <https://doi.org/10.1098/rstb.2017.0282>
- Easton A (2004) Behavioural flexibility, social learning, and the frontal cortex. The cognitive neuroscience of social behaviour. Psychology Press, Routledge, pp 71–92
- Eisenhardt D, Menzel R (2007) Extinction learning, reconsolidation and the internal reinforcement hypothesis. *Neurobiol Learn Mem* 87:167–173. <https://doi.org/10.1016/j.nlm.2006.09.005>
- Etheredge RI, Avenas C, Armstrong MJ, Cummings ME (2018) Sex-specific cognitive—behavioural profiles emerging from individual variation in numerosity discrimination in *Gambusia affinis*. *Anim Cogn* 21:37–53. <https://doi.org/10.1007/s10071-017-1134-2>
- Farrell TM, Weaver K, An YS, MacDougall-Shackleton SA (2012) Song bout length is indicative of spatial learning in European starlings. *Behav Ecol* 23:101–111. <https://doi.org/10.1093/behec o/arr162>
- Guillette LM, Reddon AR, Hurd PL, Sturdy CB (2009) Exploration of a novel space is associated with individual differences in learning speed in black-capped chickadees *Poecile atricapillus*. *Behav Process* 82:265–270. <https://doi.org/10.1016/j.beproc.2009.07.005>

- Guillette LM, Reddon AR, Hoeschele M, Sturdy CB (2011) Sometimes slower is better: slow-exploring birds are more sensitive to changes in a vocal discrimination task. *Proc Biol Sci* 278:767–773. <https://doi.org/10.1098/rspb.2010.1669>
- Guillette LM, Naguib M, Griffin AS (2017) Individual differences in cognition and personality. *Behav Process* 134:1–3. <https://doi.org/10.1016/j.beproc.2016.12.001>
- Healy SD, Braham SR, Braithwaite VA (1999) Spatial working memory in rats: no differences between the sexes. *Proc R Soc B* 266:2303–2308
- Heinen-Kay JL, Schmidt DA, Stafford AT, Costa MT, Pererson MN, Kern EMA, Langerhans B (2016) Predicting multifarious behavioural divergence in the wild. *Anim Behav* 121:3–10
- Hughes AL (1986) Growth of adult mosquitofish *Gambusia affinis* in the laboratory. *Copeia* 1986(2):534–536
- Kolata S, Light K, Matzel LD (2008) Domain-specific and domain-general learning factors are expressed in genetically heterogeneous CD-1 mice. *Intelligence* 36:619–629. <https://doi.org/10.1016/j.intell.2007.12.001>
- Laland KN, Reader SM (1999) Foraging innovation in the guppy. *Anim Behav* 57:331–340. <https://doi.org/10.1006/anbe.1998.0967>
- López JC, Broglio C, Rodríguez F, Thinus-Blanc C, Salas C (2000) Reversal learning deficit in a spatial task but not in a cued one after telencephalic ablation in goldfish. *Behav Brain Res* 109:91–98. [https://doi.org/10.1016/S0166-4328\(99\)00167-9](https://doi.org/10.1016/S0166-4328(99)00167-9)
- Lucon-Xiccato T, Bisazza A (2014) Discrimination reversal learning reveals greater female behavioural flexibility in guppies. *Biol Lett* 10:20140206. <https://doi.org/10.1098/rsbl.2014.0206>
- Lucon-Xiccato T, Dadda M, Bisazza A (2016) Sex differences in discrimination of shoal size in the Guppy (*Poecilia reticulata*). *Ethology* 122:481–491. <https://doi.org/10.1111/eth.12498>
- Lucon-Xiccato T, Bisazza A (2016) Male and female guppies differ in speed but not in accuracy in visual discrimination learning. *Anim Cogn* 19(4):733–744. <https://doi.org/10.1007/s10071-016-0969-2>
- Lucon-Xiccato T, Dadda M (2016) Guppies show behavioural but not cognitive sex differences in a novel object recognition test. *PLoS ONE* 11(6):e0156589. <https://doi.org/10.1371/journal.pone.0156589>
- Lucon-Xiccato T, Bisazza A (2017a) Sex differences in spatial abilities and cognitive flexibility in the guppy. *Anim Behav* 123:53–60. <https://doi.org/10.1016/j.anbehav.2016.10.026>
- Lucon-Xiccato T, Bisazza A (2017b) Individual differences in cognition among teleost fishes. *Behav Process* 141:184–195. <https://doi.org/10.1016/j.beproc.2017.01.015>
- Lucon-Xiccato T, Dadda M (2017) Individual guppies differ in quantity discrimination performance across antipredator and foraging contexts. *Behav Ecol Sociobiol* 71:1–9. <https://doi.org/10.1007/s00265-016-2231-y>
- Madden JR, Langley EJG, Whiteside MA, Beardsworth CE, Van Horik JO (2018) The quick are the dead: Pheasants that are slow to reverse a learned association survive for longer in the wild. *Philos Trans R Soc B Biol Sci*. <https://doi.org/10.1098/rstb.2017.0297>
- Magurran AE, Maciás GC (2000) Sex differences in behaviour as an indirect consequence of mating system. *J Fish Biol* 57:839–857. <https://doi.org/10.1006/jfbi.2000.1391>
- Maille A, Schradin C (2016) Survival is linked with reaction time and spatial memory in African striped mice. *Biol Lett* 12:20160346. <https://doi.org/10.1098/rsbl.2016.0346>
- Mamuneas D, Spence AJ, Manica A, King AJ (2015) Bolder stick-leback fish make faster decisions, but they are not less accurate. *Behav Ecol* 26:91–96. <https://doi.org/10.1093/beheco/aru160>
- Petrazzini ME, Bisazza A, Agrillo C, Lucon-Xiccato T (2017) Sex differences in discrimination reversal learning in the guppy. *Anim Cogn* 20:1081–1091. <https://doi.org/10.1007/s10071-017-1124-4>
- Pather S, Gerlai R (2009) Shuttle box learning in zebrafish (*Danio rerio*). *Behav Brain Res* 196:323–327. <https://doi.org/10.1016/j.bbr.2008.09.013>
- Pilastro A, Benetton S, Bisazza A (2003) Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. *Anim Behav* 65:1161–1167. <https://doi.org/10.1006/anbe.2003.2118>
- Reding L, Cummings ME (2018) Rational mate choice decisions vary with female age and multidimensional male signals in swordtails. *Ethology* 124:641–649. <https://doi.org/10.1111/eth.12769>
- Reding L, Cummings ME (2019) Rational choice of social group size in mosquitofish. *Biol Lett* 15:20180693. <https://doi.org/10.1098/rsbl.2018.0693>
- Rice MA, Hobbs LE, Wallace KJ, Ophir AG (2017) Cryptic sexual dimorphism in spatial memory and hippocampal oxytocin receptors in prairie voles (*Microtus ochrogaster*). *Horm Behav* 95:94–102. <https://doi.org/10.1016/j.yhbeh.2017.08.003>
- Schatz B, Lachaud JP, Beugnon G (1999) Spatio-temporal learning by the ant *Ectatomma ruidum*. *J Exp Biol* 202:1897–1907
- Shaw RC, Schmelz M (2017) Cognitive test batteries in animal cognition research: evaluating the past, present and future of comparative psychometrics. *Anim Cogn* 20:1003–1018. <https://doi.org/10.1007/s10071-017-1135-1>
- Shohet AJ, Watt PJ (2009) Female guppies *Poecilia reticulata* prefer males that can learn fast. *J Fish Biol* 75:1323–1330. <https://doi.org/10.1111/j.1095-8649.2009.02366.x>
- Shettleworth SJ (2010) Cognition, evolution, and behaviour, 2nd edn. Oxford University Press, Oxford
- Sih A, Del Giudice M (2012) Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Philos Trans R Soc Lond B Biol Sci* 367:2762–2772. <https://doi.org/10.1098/rstb.2012.0216>
- Smith CC (2005) Sexual conflict and density dependence in the western mosquitofish, *Gambusia affinis* (poeciliidae). University of Kentucky Master's Theses. 207. https://uknowledge.uky.edu/gradschool_theses/207
- Smith C, Phillips A, Reichard M (2015) Cognitive ability is heritable and predicts the success of an alternative mating tactic. *Proc R Soc B* 282:20151046. <https://doi.org/10.1098/rspb.2015.1046>
- Stevens JR, Wood JN, Hauser MD (2007) When quantity trumps number: discrimination experiments in cotton-top tamarins (*Saguinus oedipus*) and common marmosets (*Callithrix jacchus*). *Anim Cogn* 10:429–437. <https://doi.org/10.1007/s10071-007-0081-8>
- Thompson R, Harmon D, Yu J (1984) Detour problem-solving behavior in rats with neocortical and hippocampal lesions: a study of response flexibility. *Phys Psych* 12(2):116–124
- Thornton A, Lukas D (2012) Individual variation in cognitive performance: developmental and evolutionary perspectives. *Philos Trans R Soc B Biol Sci* 367:2773–2783. <https://doi.org/10.1098/rstb.2012.0214>
- Titulaer M, van Oers K, Naguib M (2012) Personality affects learning performance in difficult tasks in a sex-dependent way. *Anim Behav* 83:723–730. <https://doi.org/10.1016/j.anbehav.2011.12.020>
- Wallis JD, Dias R, Robbins TW, Roberts AC (2001) Dissociable contributions of the orbitofrontal and lateral prefrontal cortex of the marmoset to performance on a detour reaching task. *Eur J Neurosci* 13:1797–1808
- Welcomme RL (1992) A history of international introductions of inland aquatic species. *ICES Mar Sci Symp* 194:3–14. <https://doi.org/10.2307/1445460>

- Wong RY, So P, Cummings ME (2011) How female size and male displays influence mate preference in a swordtail. *Anim Behav* 82:691–697. <https://doi.org/10.1016/j.anbehav.2011.06.024>
- Wright TF, Eberhard JR, Hobson EA, Avery ML, Russello MA (2010) Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethol Ecol Evol* 22:393–404. <https://doi.org/10.1080/03949370.2010.505580>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.