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Sex differences in landmark-based spatial learning in the sailfin tetra *Crenuchus spilurus* (Characiformes: Crenuchidae)

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Spatial navigation is a central component of fish ecology, influencing foraging, mate choice, and territorial defense, and may differ between sexes under distinct ecological pressures. We investigated whether the Amazonian sailfin tetra *Crenuchus spilurus*, a sexually dimorphic and dichromatic species, uses visual landmarks for orientation and whether sex differences occur in spatial learning. In its natural habitat, solitary males are territorial and relatively sedentary, defending nests where they provide exclusive paternal care, while females move in shoals in search of food and mates. We tested individuals in a maze in which they had to locate the correct exit using a visual cue, allowing us to assess learning and cognitive flexibility. Both sexes showed a progressive reduction in resolution time; however, only females exhibited clear evidence of learning based on accuracy measures, while males maintained low accuracy even after repeated trials. Additionally, females completed the task more quickly and showed higher accuracy in route correction, indicating greater cognitive flexibility and behavioral updating capacity, possibly associated with the need to explore new reproductive sites. Males, in contrast, displayed a more rigid and less efficient navigational style, consistent with territoriality and spatial fidelity. These results indicate that female *C. spilurus* use visual landmarks to solve spatial tasks and reveal sex-specific cognitive strategies.

Keywords: Cognition, Navigation, Sex-based differences, Spatial abilities, Visual cues.

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A navegação espacial é um componente central da ecologia de peixes, influenciando forrageamento, escolha de parceiros e defesa territorial, e pode diferir entre os sexos sob pressões ecológicas distintas. Investigamos se o tetra amazônico *Crenuchus spilurus*, uma espécie sexualmente dimórfica e dicromática, utiliza marcos visuais para orientação e se há diferenças sexuais na aprendizagem espacial. Em seu habitat natural, machos solitários são territorialistas e relativamente sedentários, defendendo ninhos onde realizam cuidado paternal exclusivo, enquanto as fêmeas deslocam-se em cardumes em busca de alimento e parceiros. Testamos indivíduos em um labirinto no qual deveriam localizar a saída correta orientando-se por um estímulo visual, permitindo avaliar aprendizagem e flexibilidade. Ambos os sexos apresentaram redução progressiva no tempo de resolução; contudo, somente as fêmeas mostraram evidências claras de aprendizagem baseada em acurácia, enquanto os machos mantiveram baixa precisão mesmo após repetições. Além disso, fêmeas completaram a tarefa mais rapidamente e com maior acurácia na correção de rota, indicando maior flexibilidade cognitiva e capacidade de atualização comportamental, possivelmente associadas à necessidade de explorar novos locais de reprodução. Machos, em contraste, exibiram um estilo de navegação mais rígido e menos eficiente, consistente com territorialidade e fidelidade espacial. Esses resultados indicam que fêmeas de *C. spilurus* utilizam marcos visuais para resolver tarefas espaciais e revelam estratégias cognitivas sexualmente diferenciadas.

Palavras-chave: Cognição, Diferenças baseadas no sexo, Habilidades espaciais, Navegação, Pistas visuais.

INTRODUCTION

Efficient and safe locomotion is crucial for vital activities such as foraging (Warburton, 2003; Rosati, 2017), searching for reproductive partners (Kotrschal *et al*., 2015a; Fuss, 2021), and escaping predators (Brown, Braithwaite, 2005; Kelley, Brown, 2011). Animals use multiple sensory cues as sources of directional information to orient themselves and navigate (Akre, Johnsen, 2014; Green *et al*., 2020), while also integrating past experiences into their spatial behavior, which leads to adaptation and learning processes (Kotrschal, Taborsky, 2010). Spatial orientation in environments with constantly changing sensory cues presents a challenge that drives the evolution of navigational skills. These skills require attention to signal variation, risk assessment, memory of stimuli, and making inferences from incomplete information, thereby supporting adaptive behavior across different contexts (Brydges *et al*., 2008; Mettke-Hofmann, 2014; White, Brown, 2015; Reichert, Quinn, 2017; Cauchoix *et al*., 2020).

Animals employ diverse navigation strategies, ranging from simple movement rules to complex spatial mapping, which can be analyzed both in terms of behavior and the underlying neural mechanisms (Broglio, Salas, 2003; Rodríguez *et al*., 2021; Vinepinsky, Segev, 2023). Among these strategies, egocentric navigation stands out, in which individuals follow directional instructions (such as turning right or left) based on previous experiences, and allocentric navigation, in which they rely on global landmarks such as visual cues in the environment (Tolman, 1948; Rodriguez *et al*., 1994; Salas *et al*., 2017). Allocentric navigation allows the use of visual landmarks as direct, indirect, or sequential references, integrating information from different directions and establishing complex geometric relationships for spatial localization (Perera, Garcia, 2003; Perera, 2004; Braithwaite, Perera, 2006).

Sexual differences in spatial abilities have been documented in several animal groups (Jones *et al*., 2003; Healy, Hurly, 2004; Jozet-Alves *et al*., 2008; Perdue *et al*., 2011; Guigueno *et al*., 2014; Lucon-Xiccato, Bisazza, 2017a; Wallace *et al*., 2020). Males and females often make different decisions, even within the same social context (Van der Bijl *et al*., 2015). These differences frequently reflect distinct ecological pressures and sex-specific fitness demands (Watson, Platt, 2008; Kotrschal *et al*., 2015b; Keagy *et al*., 2019; Corral-López *et al*., 2020). For example, in some bird and fish species, females exhibit greater mobility than males due to the need to locate spawning sites (Costa *et al*., 2011; Guigueno *et al*., 2014). This behavioral requirement in females is correlated with increased volume in brain regions linked to spatial cognition, whereas males of the same species, which do not need to engage in extensive searching for spawning sites, do not show the same enlargement in these brain areas (Guigueno *et al*., 2014). Conversely, in territorial species, across several vertebrate groups, including fishes, birds, and mammals, competition for mates has been shown to enhance spatial learning and cognitive performance, with the more territorial sex (generally males) displaying superior spatial performance (Jones *et al*., 2003; Saucier *et al*., 2008; Carazo *et al*., 2014). These findings highlight the significant influence of ecological and social pressures on sex differences in cognition.

Crenuchus spilurus Günther, 1863 is a sexually dimorphic and dichromatic species that inhabits clear-water Amazonian streams with structurally complex habitats (Pires *et al*., 2016). During reproduction, males display hypertrophied fins and bold courtship behaviors, whereas females indicate receptivity through abdominal darkening. Mate choice is mutual, and visual cues play a key role in this process (Pinto *et al*., 2021; Borghezan *et al*., 2023). Males are highly territorial and show strong site fidelity, defending fixed shelters that function both as refuges and nesting sites, where they provide exclusive paternal care (Pires *et al*., 2016, 2021). Males also exhibit fine visual discrimination when assessing rivals, avoiding escalated aggression with larger and/or more ornamented individuals (Pires *et al*., 2021). Previous work suggests that *C. spilurus* tends to rely on egocentric navigation when solving maze tasks (Pinto *et al*., 2021), but the species inhabits clear waters with low turbidity and abundant structurally complex (Pires *et al*., 2016) conditions in which visual landmarks may be ecologically relevant for orientation (Braithwaite, Perera, 2006; Sovrano *et al*., 2020). Given this ecological and behavioral context, we aimed to investigate whether *C. spilurus* can use visual landmarks for spatial navigation and whether males and females differ in this ability. Based on their territorial behavior and site fidelity, we hypothesized that males would exhibit greater spatial navigational ability than females. To test this, we employed a spatial task in which individuals had to follow visual cues to locate the exit of a maze.

MATERIAL AND METHODS

Subjects. Adult *Crenuchus spilurus* of both sexes (Figs. 1A–B) were collected with small fine-mesh hand nets in a stream located in an urban forest area within the city of Manaus, Amazonas, Brazil (03°06'22.7"S 59°58'40.5"W) in December 2021. Our laboratory is located approximately 3 km from the sampling site, which allowed the transport of specimens from the field to the laboratory aquaria in less than 1 h, minimizing stress and potential injuries. To ensure welfare during transport, the fish were placed in plastic bags with water inside buckets equipped with portable aeration devices. Voucher specimens were deposited in the Fish Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), municipality of Manaus, Amazonas State, Brazil, as *Crenuchus spilurus* from the Negro River (INPA-ICT 61815). Fish were housed in the laboratory until the beginning of the experiments, which were conducted between April and September 2022.

FIGURE 1 | Representative images of the sexually dimorphic Amazonian fish *Crenuchus spilurus*. **A.** Male; **B.** Female. Images reproduced from Borghezan *et al.* (2019).

Housing. Following housing procedures previously adopted in experimental studies with this species (Pinto *et al*., 2021; Pires *et al*., 2021), males and females were housed separately in aquaria (60 Length × 30 Width × 30 Depth cm), with a maximum density of 40 individuals per tank. This approach was used to reduce overt male-male aggressive interactions associated with territorial defense during the acclimation period. To simulate the natural environment, natural and artificial plants were added to each tank, along with approximately 10 sections of PVC tubes (10 cm length and 25 mm diameter) to provide shelter. Individuals of this species do not move extensively within the aquaria, and males defend territories only around the PVC tubes; therefore, aggressive interactions were rarely observed in the holding tanks. Each tank was equipped with a mechanical filter and an air pump, and weekly water changes were carried out to maintain water quality. Laboratory water supply originated from groundwater, and the only difference compared to stream water quality was the lack of dissolved organic carbon. To compensate for this, we regularly collected dead leaves from the forest stream and added them to a 100-liter reservoir, simulating the natural incorporation of dissolved organic carbon into the water, following procedures previously adopted in studies from our group (Pinto *et al*., 2021; Pires *et al*., 2021). Water was kept in this reservoir for at least three days before being used in the tanks. The laboratory received indirect natural light through large glass windows, and since it is located in an equatorial region (Manaus, Amazonas State, Brazil; 03°05’45”S 59°59’21”W), it followed a natural 12:12 h light-dark cycle. On experimental days, artificial lights were turned on from 08:00 to 18:00 to ensure consistent illumination during setup and testing. On non-experimental days, tanks were exposed primarily to the natural daylight entering the room. The room was maintained at a constant temperature of 24 °C with air conditioning, simulating the prevailing water temperature of Central Amazonian forest streams. Fish were fed twice daily with high-quality commercial ornamental fish food (Sera GVG Mix, granules), which was readily consumed by the individuals. Only adult individuals were included in the study.

Apparatus. The experiments were conducted in a glass tank (70 L × 30 W × 20 D cm) filled with a 15 cm water column (Fig. 2). To ensure that no visual cues were available beyond those provided in the experimental setup, the tank walls were externally covered with opaque cloth. The experimental apparatus (50 L × 25 W × 3 D cm) was built using green plastic bricks (Lego Bricks, Billund, Denmark), positioned at the center of the tank. The apparatus consisted of an initial corridor leading to a first sector (9 L × 22 W × 3 D cm) with two exits: one to the right and one to the left. One exit led to a dead end, while the other led to a second sector, with the same dimensions, which also had two exits, one leading to a dead end and the other to the goal zone. The correct paths connecting sector 1 to sector 2 and sector 2 to the goal zone were marked with a red visual cue, which was always placed in opposite positions in each sector. A glass plate was placed on top of the apparatus, maintaining a 3 cm water column to restrict the vertical movement of the fish. Restricting water column height functions as a stimulus for fish to swim forward and solve the spatial task (Kellogg, Gavin, 1960; Lucon-Xiccato, Bisazza, 2017b). The remaining spaces of approximately 10 × 30 cm at each end of the tank were used as the initial sector, where the fish was placed before the trial, and as the goal sector, reached upon solving the task. These two sectors were

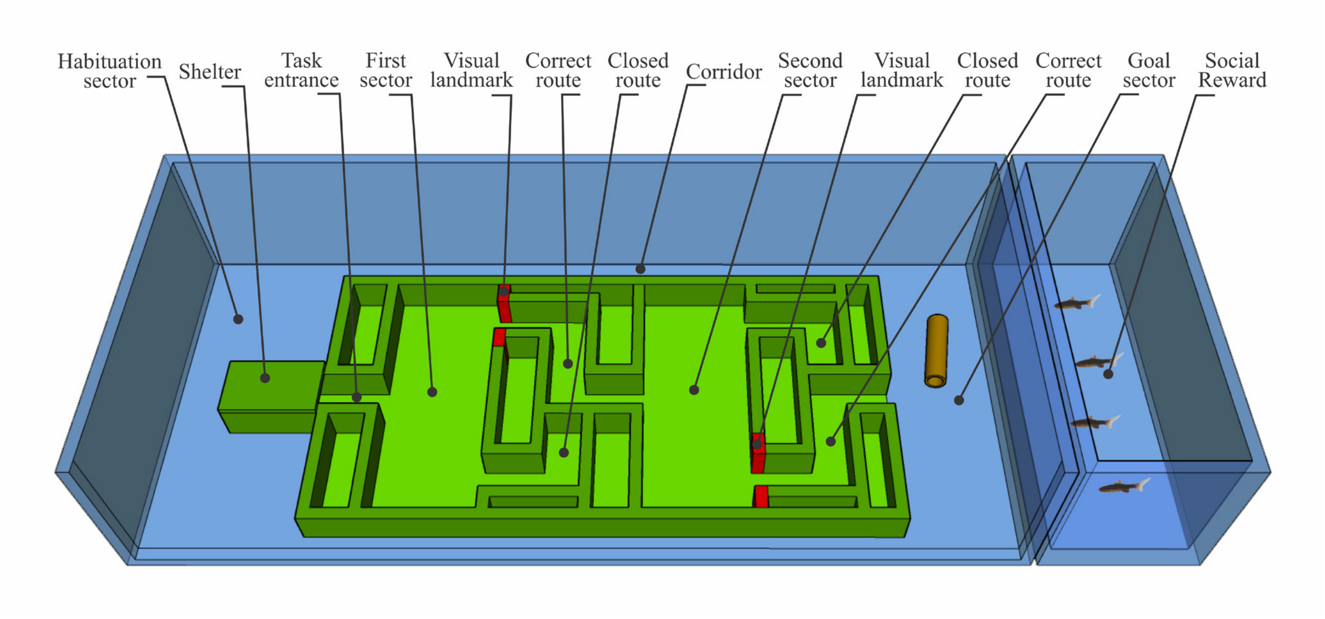

FIGURE 2 | 3D schematic of the task used in the spatial learning experiment.

connected by a lateral corridor approximately 5 cm wide, allowing the fish to be guided back from the goal sector to the initial sector without being removed from the water. In the initial sector, we built a shelter with two open ends made of green plastic bricks (3 L × 6 W × 3 D cm), directly attached to the experimental apparatus, to facilitate guiding the individuals into the initial corridor. All individuals used this structure from the start of habituation. In the goal sector, located at the opposite side of the tank, we placed as a reward a PVC tube identical to those in the housing aquaria. Additionally, the goal sector was adjacent to a smaller aquarium (30 L × 20 W × 25 D cm, not connected to the apparatus) containing social stimuli (four females of *C. spilurus*), which served as an additional reward. We used four females to provide a stronger and more stable social stimulus, as males of this species are highly motivated to approach and court potential reproductive partners, and females are naturally gregarious and tend to remain in shoals with other females (Pires *et al*., 2016). The laboratory space used to house the apparatus was surrounded by opaque cloth to prevent visual contact with the experimenter and was indirectly illuminated by room lights. A video camera (Logitech C920) mounted above the tank recorded the trials.

Procedure. Initially, an individual was transferred from the holding aquarium to the initial sector of the apparatus 24 h before testing for habituation. Access to the test area was blocked by an opaque sliding panel, which also prevented visual access to stimuli. After the habituation period, with the individual inside the shelter leading to the test area, the opaque panel was removed, and a plastic brick was gently inserted at the opposite end of the shelter, encouraging the fish to slowly swim into the maze. At this point, with the fish free to decide when to advance to the next sectors, recording began. The experimenter observed the test remotely via a monitor connected to the camera. After the individual reached the goal sector, it remained there for 5 min close to the social reward in the adjacent aquarium. Individuals who took longer than 20 min to complete the test (two males and three females) were removed from the experiment and replaced by naïve individuals from the holding aquarium (see Video **S1** for a representative example of the task). The sample size was 25 males (Standard length, SL 3.92 ± 0.18 cm) and 25 females (SL 3.67 ± 0.14 cm). At the end of each trial, the tested individual was gently guided back to the initial sector through the lateral corridor while remaining underwater. Then, the glass plate covering the top of the apparatus was removed, and with the aid of a brush, the water inside the apparatus was gently mixed to homogenize potential chemical cues. The spatial task was then reconfigured. First, the corridors were switched in both sectors, but the correct paths were always maintained on opposite sides of the two sectors. The visual cue was also repositioned, always indicating the correct corridor. When correct choices have remained fixed in the environment, it becomes difficult to determine whether animals are using egocentric learning (repeating a directional turn) or allocentric learning (using environmental landmarks) (Tolman, 1948). Therefore, by alternating the correct side in each training session, learning could only occur if individuals relied on the relative position of the visual landmark rather than on previously correct directional patterns. In this framework, route correction refers to the fish's ability to avoid repeating a direction that had been correct in previous trials and instead adjust its trajectory based on the visual cue presented in the current session. All experimental trials were conducted between 10:00 and 18:00. After these procedures, the experimental session was repeated again after 1 h, and all five trials for each individual were conducted on the same day. Thus, each fish completed five consecutive trials within a single day, after which individuals were photographed and returned to their respective housing aquaria (males or females).

Statistical analyses. From the video recordings, we verified whether the first route chosen by each individual was correct or incorrect to calculate choice accuracy. We also recorded the time to solve the task, defined as the time in seconds from the moment the fish entered the first sector of the test until it reached the goal sector. To analyze task resolution time, we used a linear mixed-effects model (LMM) with log-transformed time as the dependent variable. In this model, trial number (1 to 5) and sex (male or female) were considered fixed effects, and individual identity was included as a random effect. To assess choice accuracy, we fitted a generalized linear mixed-effects model (GLMM) using the R package lme4 (Bates *et al*., 2015). The dependent variable was the binary choice of each individual in each trial (correct or incorrect). Trial (1 to 5), sex (male or female), and sector (first or second) were included as fixed effects. Individual identity was included as a random effect to control for the repeated-measures structure. We did not include fish standard length in either model because males are generally larger than females in this species, resulting in collinearity between sex and standard length (Pires *et al*., 2016). Additionally, in a previous study, we found an effect of male ornamentation in spatial learning (Pinto *et al*., 2021). Since the ornamentation degree is only available for males, we tested its effect on time and accuracy beforehand. As it showed no significant effect (time: $F_{1,23} = 0.32$, $p = 0.577$; accuracy: $\chi^2(1) = 0.156$, $p = 0.692$), ornamentation was excluded from the final models, allowing symmetric comparisons between sexes with the same fixed predictors. Finally, to evaluate route correction, *i.e.*, accuracy specifically in the second sector, we assessed the influence of sex using a generalized linear mixed-effects model (GLMM) with a binomial distribution and logit link function. The dependent variable was the binary response (correct or incorrect) in the second sector, which directly reflects route-correction performance, and sex was included as the explanatory factor. Individual identity was again included as a random effect to control for repeated measures on the same animal. Predicted accuracy was obtained from model coefficients using a logistic transformation. All statistical analyses and graphical representations were conducted in R software v. 4.2.0 (R Development Core Team, 2022). Statistical tests were two-tailed, with significance set at $p = 0.05$.

RESULTS

Time to solve the task. The interaction between test sequence (1 to 5) and sex was not significant ($F_{4,442} = 0.793$, $p = 0.529$), indicating that males and females showed a similar pattern of improvement across trials. Considering the main effects, resolution time progressively decreased across tests ($F_{4,442} = 5.618$, $p < 0.001$), reflecting improved performance with repetition. Additionally, males took significantly longer to complete the task than females ($F_{1,48} = 9.299$, $p = 0.003$). Together, these results show that although both sexes improved with experience, females consistently solved the task more quickly (Fig. 3A; see also Fig. **S2** for individual-level distributions and Tab. 1 for a summary of statistical effects).

Accuracy. The GLMM conducted to evaluate route choice accuracy revealed no significant effect of test sequence ($\chi^2(4) = 7.725$, $p = 0.102$), sex ($\chi^2(1) = 0.655$, $p = 0.418$), or sector ($\chi^2(1) = 0.460$, $p = 0.497$). However, there was a significant interaction between test and sex ($\chi^2(4) = 10.417$, $p = 0.033$), suggesting that the pattern of accuracy

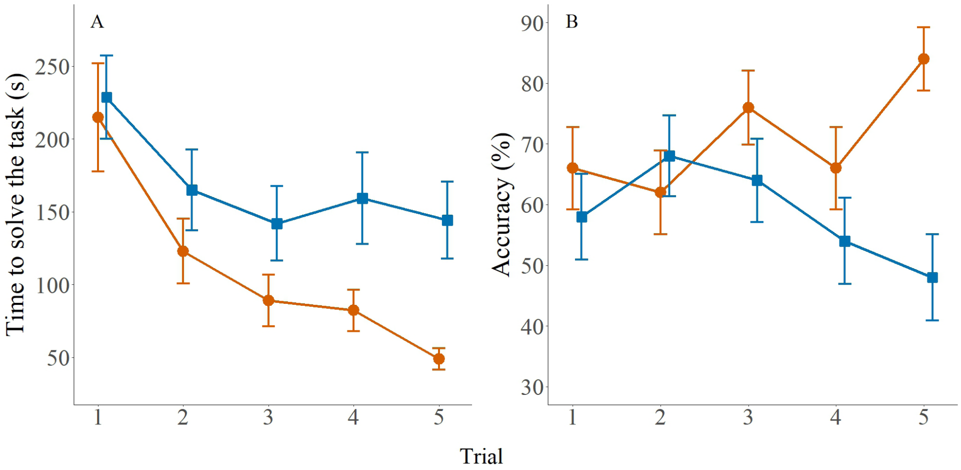

FIGURE 3 | Spatial performance of male and female *Crenuchus spilurus* across five trials. **A.** Time to solve the task. **B.** Accuracy (%) of choices across trials. Individual data points are shown for each trial, with horizontal lines representing mean values and vertical bars indicating SEM. Males are shown in blue and females in red. Sample size: N = 25 males and 25 females.

across trials differed between males and females (Fig. 3B). Therefore, we performed two separate GLMMs for males and females. In both models, the effect of test was not significant (males: $\chi^2(4) = 7.148$, $p = 0.128$; females: $\chi^2(4) = 6.374$, $p = 0.173$), nor was the interaction between test and sector (males: $\chi^2(4) = 3.714$, $p = 0.446$; females: $\chi^2(4) = 9.241$, $p = 0.055$). For females, the test-by-sector interaction was close to, but did not reach, the significance threshold defined in our statistical procedures. Although this pattern hints at a possible tendency, it does not provide sufficiently clear evidence to support conclusions about learning across trials. The effect of sector was not significant for males ($\chi^2(1) = 0.088$, $p = 0.766$), but was significant for females ($\chi^2(1) = 4.159$, $p = 0.041$), which showed increased accuracy in the second sector (see Tab. 1 for a summary of statistical effects).

Route correction. The GLMM analyzing the influence of sex on accuracy in the second sector revealed a significant effect on the probability of a correct choice ($\chi^2(1) = 7.86$, $p = 0.005$, Fig. 4), indicating that males exhibited lower accuracy compared to females. Females showed an estimated probability of success of approximately 72%, while for males this probability was about 54%. The variance of the random effect by individual was low ($\sigma^2 = 0.014$), suggesting that most of the variation in performance was explained by sex (see Fig. **S3** for individual accuracy distributions and Tab. 1 for a summary of statistical effects).

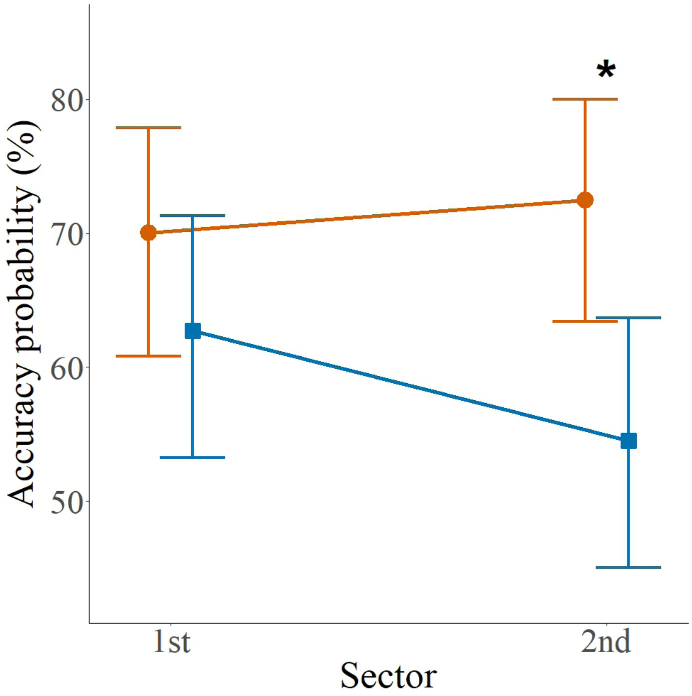

FIGURE 4 | Interaction between sex and sector in the accuracy of choices by *Crenuchus spilurus* individuals. Points and vertical bars represent the mean probability of correct responses (with 95% confidence intervals) for males (blue) and females (red) in the two experimental sectors. Lines connect sectors for each sex, and the asterisk indicates a significant effect.

TABLE 1 | Summary of the statistical effects of sex, test sequence, sector, and their interactions on performance measures (resolution time, accuracy, and route correction) during the spatial navigation task. Significant effects are indicated with an asterisk. df = degrees of freedom.

Factors	Statistic	df	*p*-value
Resolution time			
Test sequence	F = 5.618	4.442	< 0.001 *
Sex	F = 9.299	1.48	0.003 *
Test x Sex	F = 0.793	4.442	0.529
Accuracy (overall)			
Test sequence	$\chi^2 = 7.725$	4	0.102
Sex	$\chi^2 = 0.655$	1	0.418
Sector	$\chi^2 = 0.460$	1	0.497
Test x Sex	$\chi^2 = 10.417$	4	0.033 *
Accuracy (males)			
Test sequence	$\chi^2 = 7.148$	4	0.128
Sector	$\chi^2 = 0.088$	1	0.766
Test x Sector	$\chi^2 = 3.714$	4	0.446
Accuracy (females)			
Test sequence	$\chi^2 = 6.374$	4	0.173
Sector	$\chi^2 = 4.159$	1	0.041 *
Test x Sector	$\chi^2 = 9.241$	4	0.055
Route correction			
Sex	$\chi^2 = 7.86$	1	0.005 *

DISCUSSION

Our results indicate that female *Crenuchus spilurus* are capable of using visual landmarks to solve spatial navigation tasks, and that males and females differ in how they process and integrate spatial information. While both sexes showed a progressive decrease in resolution time across trials, males consistently took longer to complete the task and did not improve in accuracy across sessions. Accuracy, a most robust measure of learning, as it is less influenced by habituation or motivational changes, revealed clear evidence of landmark-based learning only in females. Males, in contrast, maintained low accuracy even after repeated exposure, indicating that they did not learn the visual cue. This pattern aligns with the species' ecology, as *C. spilurus* inhabits clear-water streams rich in vegetation (Pires *et al*., 2016), environments that favor the use of stable visual references (Odling-Smee, Braithwaite, 2003; Akre, Johnsen, 2014). Our findings reinforce the broader evidence that spatial cognition is well developed in teleost fishes, especially in ecologically relevant contexts (Brown, Laland, 2003; Salas *et al*., 2006; Sovrano *et al*., 2020).

Although both sexes reduced their resolution time across trials, only females showed accuracy patterns consistent with learning the landmark-based task. Contrary to our initial assumption, females completed the task faster and with higher accuracy when correcting their route in the second sector, a pattern suggesting greater cognitive flexibility, potentially linked to broader ecological demands for spatial exploration. In many species, including fishes and birds, females are responsible for locating suitable spawning or foraging sites and are therefore under selective pressure to develop adaptable and efficient navigation strategies (Healy, Hurly, 2004; Guigueno *et al*., 2014). In this study, our interpretation of "mobility" refers specifically to ecological mobility, broader space uses, and exploratory range in the natural habitat, rather than increased locomotor activity inside the experimental apparatus. Field observations support this distinction: females of *C. spilurus* exhibit greater ecological mobility in the wild, whereas males show territorial fidelity and remain within small structured areas (Pires *et al*., 2016). Thus, we do not suggest that females performed better because they physically moved more within the apparatus, but rather that long-term ecological differences in space use may shape sex-specific spatial strategies. Consistent with this interpretation, the test-by-sector interaction in females approached, but did not reach, the predefined statistical threshold; although not conclusive, this trend aligns with the broader pattern observed and may indicate a subtle tendency toward more efficient integration of visual information across task phases, warranting further investigation in future studies with larger samples or refined designs.

Interestingly, males showed lower accuracy, particularly in the phase requiring integration of new visual information after an initial choice (*i.e*., the second sector of the maze). This limitation may reflect a cognitive bias toward more rigid, possibly egocentric, strategies in which trajectories are based on prior movements rather than on environmental landmarks (Braithwaite, Perera, 2006; Salas *et al*., 2006). In territorial species such as male *C. spilurus*, this style may suffice for solving spatial problems in familiar areas but is less effective when the task requires flexibility and constant information updating. This distinction between sexual cognitive styles, with females favoring allocentric navigation and males relying more on fixed-route (egocentric) navigation, is consistent with widely documented patterns in vertebrates, including rodents, birds, and humans (Saucier *et al*., 2002; Jonasson, 2005; Lucon-Xiccato, Bisazza, 2017c).

On the other hand, previous studies with *C. spilurus* have shown that more ornamented males, though more cautious, learn quickly in maze contexts (without visual cues), making fewer errors over time (Pinto *et al*., 2021). This combination of hesitation and efficient learning may reflect the "asset protection" principle (Clark, 1994), according to which individuals that have substantial ongoing reproductive investment, such as male *C. spilurus*, who defend territories and provide paternal care, tend to adopt more conservative, though not necessarily less effective, strategies in challenging contexts. Thus, the longer resolution times observed in males in this study do not necessarily indicate lower cognitive ability but may reflect a deliberative style that enhances safety. This interpretation reinforces the importance of considering personality traits and individual reproductive value as intra-sexual modulators of cognition, as has been evidenced in several animal groups (Gosling, 2008; Guillette *et al*., 2017).

An additional consideration concerns the potential difference in the salience of the social reward used in the task. The reward consisted of access to a sheltered PVC tube in an adjacent aquarium containing four conspecific females, a stimulus that likely carries distinct ecological meanings for each sex. For males, the presence of multiple females may represent a direct reproductive opportunity, whereas for females, it more likely provides social companionship and safety, consistent with the shoaling tendencies known for this species (Pires *et al*., 2016). Although the motivational salience of this reward may therefore differ between sexes, it represents a positive stimulus for both males and females. Importantly, there is no evidence of female-female competition for access to males in *C. spilurus*, nor indications that females are less motivated by social stimuli. Thus, differences in reward salience alone are unlikely to account for the observed sex differences in accuracy, learning patterns, and route correction. Instead, the results are more parsimoniously explained by sex-specific spatial strategies and differences in cognitive flexibility shaped by ecological and reproductive roles.

Furthermore, our results suggest that males and females adopted distinct problem-solving strategies across trials. Females were more efficient in adapting to spatial changes in the task, particularly when it was necessary to correct choices based on new visual information. This improved performance is consistent with greater behavioral flexibility and more effective inhibition of previously learned but incorrect responses, patterns that have been repeatedly reported in other teleosts (Lucon-Xiccato, Bisazza, 2014; Miletto Petrazzini *et al*., 2017; Lucon-Xiccato *et al*., 2020). Such inhibitory control is closely linked to executive functions, including cognitive flexibility and behavioral updating, as emphasized in recent reviews on sex differences in animal cognition (Lucon-Xiccato, 2022). The involvement of working memory and contextual integration mechanisms, cognitive processes essential for navigation in complex or unpredictable environments, may also contribute to female performance (Cauchoix *et al*., 2017; Vinepinsky, Segev, 2023). Males, in contrast, appear to rely on more fixed and less adaptive (egocentric) spatial rules, which may be advantageous in stable environments but hinder performance in uncertainty. In this context, it is noteworthy that *C. spilurus* shows higher abundance in partially dammed streams (Pires *et al*., 2016), where reduced water flow and lower structural rearrangement may create a more stable scenario. Under such conditions, the advantages associated with more flexible navigation may be attenuated, reducing the functional consequences of sexual differences in navigation and potentially favoring population-level reproductive success. Although speculative, this hypothesis underscores the importance of investigating how environmental stability modulates the expression and adaptive relevance of sex-specific cognitive strategies.

From an ecological and evolutionary perspective, the sex differences observed in this study may result from distinct pressures related to space use and social organization. In *C. spilurus*, male courtship and nest defense behavior depend strongly on maintaining fixed territories, often associated with specific structures such as submerged palm leaves or tubular shelters (Pires *et al*., 2021). Females, in contrast, need to locate and evaluate multiple territories and potential mates, which may favor greater versatility in navigation skills. This pattern of sexual specialization aligns with theoretical frameworks proposing that cognitive dimorphism can emerge when males and females consistently occupy different spatial or ecological niches (Gaulin, 1992; Watson, Platt, 2008).

This aligns with the "sexually dimorphic adaptive cognition hypothesis", according to which distinct selective pressures shape cognitive specializations between sexes when their ecological demands consistently diverge. Geary (1996) argued that sex differences in cognitive skills arise from sexual selection and contrasting reproductive strategies between males and females, favoring, for example, greater efficiency in navigation/space use in the more mobile or competitive sex. Complementarily, Jacobs (1996) proposed the "sex-based-dispersal hypothesis", suggesting that differences in spatial mobility between sexes, such as greater male dispersal or the need for females to return to the nest, may impose distinct pressures on navigation and spatial memory. In the case of *C. spilurus*, while males are territorial, site-faithful, and perform exclusive paternal care, females must explore the environment in search of feeding grounds and mates, fitting the model predicted by these theories. Accordingly, the differences observed here may reflect the action of natural selection shaping sex-specific cognitive profiles that are potentially adapted to the ecological challenges experienced by each sex in its respective niche.

The results presented here contribute to a broader understanding of how cognition, behavior, and ecology interact in the evolution of sex differences. They reinforce the role of fishes as valuable models for investigating adaptive cognition, highlighting that even small-bodied and cryptic species such as *C. spilurus* display sophisticated cognitive repertoires, sensitive to ecological context and differential selective pressures between sexes. Moreover, our data suggest that spatial cognition should not be assessed in a generalized manner but should take into account not only sex and environment but also the functional context of the task, individual cognitive style, and the species' ecological-evolutionary history.

In conclusion, this study reveals that female *C. spilurus* exhibits navigation skills based on visual cues, while males did not show accuracy improvement and therefore did not demonstrate learning of the landmark-based task. The results also reveal modulation by individual experience and sex differences in spatial learning. Females demonstrate greater flexibility and accuracy in spatial tasks, a pattern that suggests a predominantly allocentric navigation strategy, whereas the combination of lower accuracy and longer resolution times in males may suggest that they rely more heavily on egocentric navigation and potentially engage in more cautious or conservative exploratory strategies. Such conclusions underscore the value of ecological and comparative approaches in understanding the evolution of animal cognition and position *C. spilurus* as a promising model for further studies on sexually differentiated cognition. Future studies exposing *C. spilurus* to reversal learning tasks may reveal with greater detail the sex differences in cognitive flexibility and behavioral updating capacity under changing contingencies, as suggested by the present results.

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AUTHORS' CONTRIBUTION

Kalebe S. Pinto: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing-original draft, Writing-review and editing.

Jansen Zuanon: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

Tiago H. S. Pires: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing-original draft, Writing-review and editing.

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ETHICAL STATEMENT

Animal collection was conducted under SISBIO permit 10199–1, issued on October 16, 2007. This study was approved by the Ethics Committee for Animal Use (CEUA-INPA, protocol nº 046/2016), in accordance with the guidelines of the Brazilian National Council for Animal Experimentation Control (CONCEA). After the experiments, most fish were released back at their collection site. Fish that were not returned to the habitat were kept in the laboratory for other experiments. No procedure was considered invasive or harmful to the fish, and no individuals died or were injured as a result of the experimental procedures.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available in the GitHub repository (https://github.com/kalebepinto/NI-2025-0160).

AI STATEMENT

Artificial intelligence (AI) tools (ChatGPT, OpenAI) were used to translate the manuscript from Portuguese to English and to assist with grammar correction.

COMPETING INTERESTS

The authors declare no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary Material File

PEER REVIEW

Peer Review File

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