

Number of individuals, but not habitat complexity, influences the antipredator behavior of an Amazonian floodplain fish

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Trophic relationships shape ecosystem structure by regulating energy flow and nutrient cycling, impacting prey consumption and prompting behavioral adaptations in prey species to mitigate risks. While much is known about fish antipredator behavior in marine environments, less is understood about these dynamics in freshwater environments. We investigated the antipredator behavior of an Amazonian floodplain fish, *Crenuchus spilurus*, across different habitat structural complexities. Through experiments, we examined how the number of individuals (1 and 4 individuals) and habitat structural complexity (low, medium, high, and very high) influence the fish response to predator presence. Small groups showed longer flight initiation distance (FID) than solitary individuals, suggesting a possible collective defense strategy against approaching predators. The structural complexity of the habitat did not have a direct effect on FID. Additionally, we noted variations in refuge usage by the fish across different habitat structural complexities. These findings underscore the interplay between behavioral and environmental factors in prey adaptation to predation risk in aquatic ecosystems.

Keywords: *Crenuchus spilurus*, Ecological interactions, Ethology, Flight initiation distance, Predation.

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Relações tróficas moldam a estrutura dos ecossistemas ao regular o fluxo de energia e o ciclo de nutrientes, impactando o consumo de presas e promovendo adaptações comportamentais nas espécies de presas para mitigar riscos. Embora muito seja conhecido sobre o comportamento antipredador de peixes em ambientes marinhos, pouco se sabe sobre essas dinâmicas em ambientes de água doce. Investigamos o comportamento antipredador de um peixe da planície de inundação amazônica, *Crenuchus spilurus*, em diferentes complexidades estruturais de habitat. Através de experimentos, examinamos como o número de indivíduos (1 e 4 indivíduos) e a complexidade estrutural do habitat (baixa, média, alta e muito alta) influenciam a resposta do peixe à presença de predadores. Grupos pequenos apresentaram maior distância de iniciação de fuga (DIF) do que indivíduos solitários, sugerindo uma estratégia de defesa coletiva contra predadores em aproximação. A complexidade estrutural do habitat não teve um efeito direto sobre a DIF. Além disso, observamos variações no uso de refúgios pelos peixes em diferentes complexidades estruturais de habitat. Esses achados ressaltam a interação entre fatores comportamentais e ambientais na adaptação das presas ao risco de predação em ecossistemas aquáticos.

Palavras-chave: *Crenuchus spilurus*, Distância de iniciação de fuga, Etologia, Interações ecológicas, Predação.

INTRODUCTION

Trophic relationships shape ecosystem structure by regulating energy flow and nutrient cycling. Predation refers to the consumption of an organism (the prey) by another (the predator), resulting in the transfer of energy and matter between trophic levels. Such interaction impacts prey consumption and prompts behavioral adaptations in prey species to mitigate risks (Preston *et al.*, 1998). While reducing predation risks, prey defensive behaviors can affect prey demography, fitness, and morphology (Parry *et al.*, 2022). For instance, vigilance and escape mechanisms can increase survival rates, affecting population density and age structure (Sansom *et al.*, 2009). However, these behaviors demand energy and time investments, potentially detracting from foraging and reproductive efforts, thereby impacting individual fitness (Lind, 2004; Sansom *et al.*, 2009). Morphologically, prey species may evolve traits like cryptic coloration or physical defenses that complement behavioral strategies, illustrating an adaptive response to predation pressure (Sherratt, Franks, 2005). Antipredator behavior is quantified by metrics like flight initiation distance (FID), used to study the escape behavior of the prey when approached by potential predators or perceived threats (Cooper, 2008). The FID refers to the distance at which an animal starts to flee from a perceived approaching threat (Blumstein, 2003). FID can provide information about the trade-offs prey make between the costs of fleeing (energy expenditure and lost foraging and other beneficial opportunities) and the perceived risk of predation (Benevides *et al.*, 2016; Giglio *et al.*, 2020). It can also address the influence of ecological attributes like habitat structure, predator density, and prior experience with predators influence escape behavior. Overall,

FID may be used to understand prey's adaptive strategies to minimize predation risk in their natural environments.

In addition to trophic interactions, changes in environmental conditions such as water temperature, pH and light penetration play a pivotal role in shaping the physiology and behavior of organisms, influencing community structuration (Dunison, Travis, 1991; Ward, Morton-Starner, 2015; Gragnolati *et al.*, 2024). Temperature is linked to key developmental processes in ectothermic organisms. This includes impacts on growth, swimming abilities, and vulnerability to predation, underscoring the intricate relationship between environmental conditions and biological responses (Anderson *et al.*, 2001). Furthermore, the structural complexity of aquatic habitats may influence the behavior of fishes. Habitats with greater complexity offer more hiding places, leading to shorter FID, as fish in these environments may feel more secure (Nunes *et al.*, 2015). Social attributes such as group size may also be crucial (Benevides *et al.*, 2016). The relationship between group size and flight distance, often studied among birds (Ydenberg, Dill, 1986), has also been extended to aquatic organisms, utilizing similar metrics. Shoaling or schooling fish, which often engage in collective defense strategies, demonstrate that larger groups can exhibit shorter FIDs due to the confusion effect, complicating predators' attempts to single out individual fish (Ioannou *et al.*, 2012). This web of environmental, physiological, and social factors highlights the complex dynamics at play in aquatic ecosystems.

The body of knowledge about fish antipredator behavior is mostly from marine reef systems (*e.g.*, Nunes *et al.*, 2015; Benevides *et al.*, 2016; Meekan *et al.*, 2018; Bond *et al.*, 2019; Samia *et al.*, 2019). Given the clear water and diversity of reefs, researchers can dive into the fish habitat and collect data *in situ*. However, such an approach is impracticable in most freshwater ecosystems mainly due to low visibility conditions, especially when studying small and cryptic fish in small water bodies where a diver would not go unnoticed by the fishes. In such a context, the use of experimental frameworks is an alternative approach to studying the behavior of freshwater fishes.

The sailfin tetra, *Crenuchus spilurus* Günther, 1863, is a small floodplain Amazonian fish distributed in the Orinoco and Amazon basins, and coastal rivers of the Guianas (Géry, 1963). The species reaches a maximum body size of 5.7 cm, typically display solitary and territorial behavior, and live in temperatures ranging from 23 to 29 °C. Populations are generally small, with only a few exceptions where they are relatively large, and have low population densities. Its preferential habitats are characterized by partially dammed regions that form pools. These specific habitat conditions, less common in untouched Amazonian forest streams, favor the establishment of large local populations due to a combination of slow water flow and mild temperatures (Pires *et al.*, 2016).

The sailfin tetra is a micropredator that primarily consume invertebrates and diverse forms of zooplankton. Its diet plasticity allow it to utilize locally abundant food sources. Notably, the species stands out among Amazonian fish for its strong dichromatism, where males and females engage in mutual signaling during courtship. Moreover, males exhibit exclusive parental care of eggs and early larvae, underscoring unique reproductive behaviors (Pires *et al.*, 2016).

We investigate the influence of structural habitat complexity and number of individuals in the antipredator behavior in a small floodplain Amazonian fish, *C. spilurus*. We hypothesize that higher structural habitat complexity delays antipredator behavior,

providing more refuge for prey against predators. Furthermore, we anticipate that individual fish exhibit fewer cautionary behaviors than fish in small schools, potentially due to the protective strategy of schooling against predators.

MATERIAL AND METHODS

Experimental design. Forty individuals of sailfin tetra, with an average size of 29 mm, were collected exclusively for this study from their natural habitat in a floodplain within the Trombetas basin (01°45'07.0"S 55°50'25.7"W). Individuals were acclimated over 15 days in the laboratory to adapt to experimental aquariums. The experiment was conducted using eight glass aquariums with 60 x 30 cm and 50 L. Each aquarium featured a closed recirculation environment through hang-on filters with independent biological, chemical and mechanical filtering.

A procedural control was integrated into our study, where an additional aquarium was maintained under stable conditions of temperature, pH, nitrite, ammonia, etc., throughout the experiment. By ensuring uniform environmental conditions in the control aquarium, we aimed to isolate and attribute any observed changes in fish behavior, particularly flight initiation distance (FID), specifically to the manipulated structural variables.

In the experimental aquariums, the FID of fishes was evaluated under four different structural complexities. The levels of complexity were manipulated by altering the number of structures within the aquariums: only the substrate (low complexity); substrate, one small rock and one artificial plant (medium complexity); substrate, one small rock and two artificial plants (high complexity); substrate, two small rocks and two artificial plants (very high complexity) (Fig. 1). A plastic replica of a 13 cm piranha (*Serrasalmus* sp.) was used to simulate a natural predator, closely mimicking its colors and size to enhance the realism of the experimental setup. The fish predator replica was introduced into the water at a depth of 4 cm along the aquarium's side (Fig. 1), approaching the fish individuals to simulate constant swimming. To determine the constant speed of the predator during the experiments, we timed the replica's traversal across the aquarium, which took approximately 10 sec. The fish predator replica was moved manually during the experiment using a monofilament fishing line attached to a lightweight aluminum bar, which provided better control over its movements. This manipulation ensured that there were no influences such as shadows from the manipulator, as all handling occurred above the aquarium lights. The manipulators positioned themselves at the sides of the aquariums, which were covered with black cardboard to prevent the fish from noticing any additional stimuli. The FID of sailfin tetras was sampled in the four different habitat structural complexities in experimental frames with a solitary individual and a group with four individuals (Fig. 1).

The FID sampling was recorded through a video recorder to eliminate observer bias. Cameras were placed in front of the aquarium and supported on a tripod placed at a distance of 80 cm, ensuring optimal framing of the aquariums in the footage. In the laboratory, we analyzed the recordings frame by frame using the software ImageJ (Schneider *et al.*, 2012) to quantify the FID. We investigated three behavioral responses to predation: i) FID against the fish predator, ii) shelter use after swimming away from

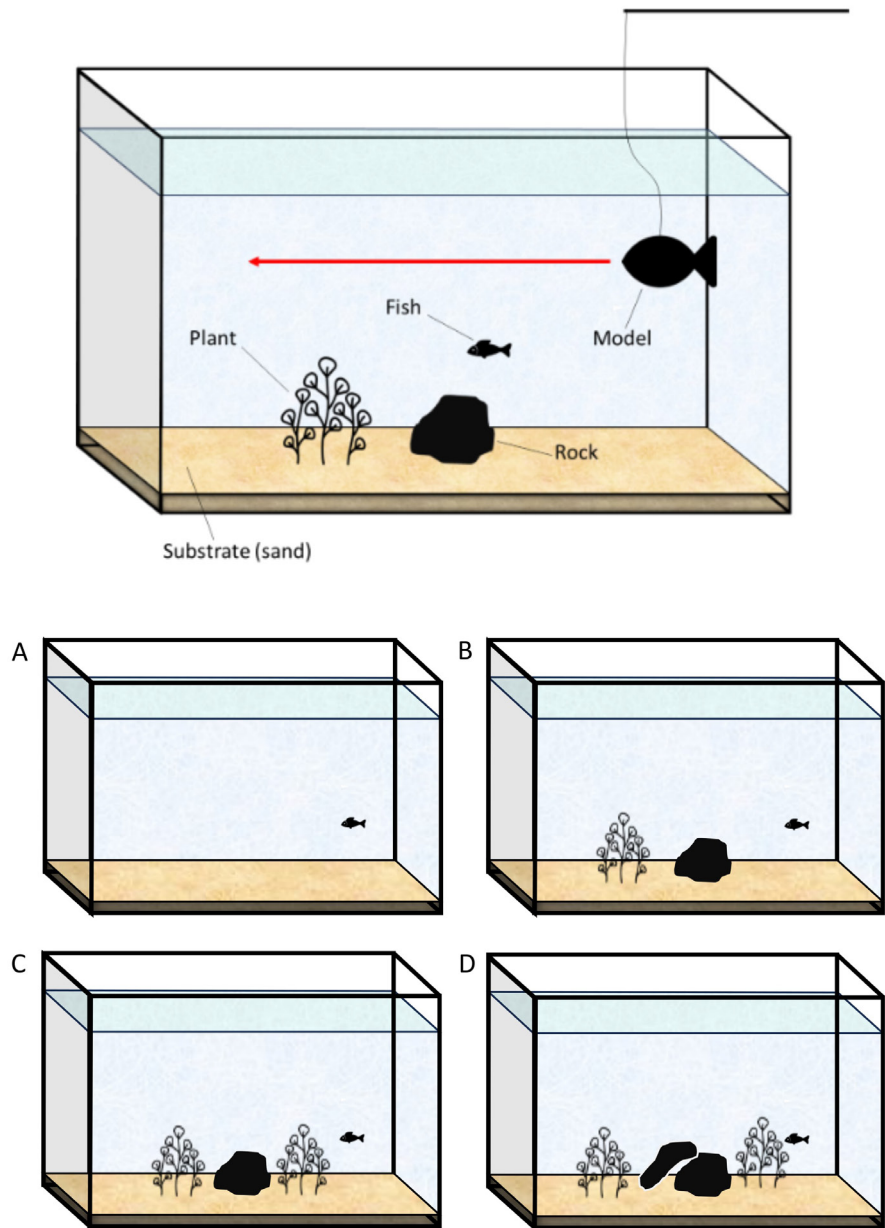


FIGURE 1 | Experimental design showing the four displays of habitat structural complexity used in the experiment. **A.** Low complexity, **B.** Medium complexity, **C.** High complexity, and **D.** Very high complexity.

the predator, and iii) vertical positioning relative to the substrate at the FID moment. The behaviors observed in the experiment included swimming away, seeking refuge in rocks, algae and holes. The FID and the vertical positioning were quantified in centimeters. A tape measure (cm level) was added to the external bottom of each aquarium to serve as a scale for the measurements in the videos. The experiment was conducted between August and September 2023, took place from 3 pm to 4 pm, with each recording lasting approximately 4 min.

Data analysis. Non-parametric ANOVA (Kruskal-Wallis test) was fitted to verify differences in antipredator behavior, measured as the FID among structural complexities. To investigate the effects of both structural complexity and the number of individuals in FID, we conducted a two-way ANOVA addressing the interaction of complexity and the number of individuals as predictors and FID as response variables. Since the data did not meet homoscedasticity and normality assumptions, we applied a natural logarithm transformation to the FID values, which has been shown to stabilize variance and improve normality in skewed distributions. A Spearman Rank correlation was fitted to verify if FID was related to the height of the observed fish when it swam away. Analyses were conducted using R software v. 3.5.1 (R Development Core Team, 2018).

RESULTS

A total of 388 fish predator-approaching simulations were sampled. The overall FID of the sailfin tetra varied from 2 to 35 cm and the average FID was 6.8 ± 0.4 cm (\pm S.E.). The FID differed significantly among the number of individuals (Tab. 1; Fig. 2A). The treatment with four individuals had a greater FID than those with a solitary individual (average FID = 9.0 ± 0.84 and 5.8 ± 0.43 cm, respectively). The overall FID did not differ among the four habitat structural complexities (Tab. 1; Fig. 2B). The comparison of the FID for number of individuals among the four different habitat structural complexities revealed significant differences for medium ($p = H = 12.61$, 0.0004) and high complexities ($H = 9.79$, $p = 0.002$; Fig. 3). A significant effect was found for the interaction between the structural complexity and the number of individuals in the FID of sailfin tetra (Tab. 1).

Swimming away from the predator model was the most observed behavior response against fish predator, representing 100% of the events for low ($n = 66$) and medium ($n = 42$) habitat complexities and 57% and 84% in high and very high complexities (Fig. 4). Individuals in high complexity seek refuge in algae with higher frequency (37%, $n = 18$), followed by very high complexity (13%, $n = 8$). Refuge in rocky was used a few instances in high and very high complexities. The detailed description of each behavior is described in Tab. 2. The height of individuals in the experimental aquarium when the FID was recorded varied from 1 to 7.3 cm and the average was 1.68 ± 0.05 cm. A positive significant correlation between FID and height was found for samples with 1 individual (Spearman Rank correlation $S = 2677610$, $\rho = 0.2358$, $p < 0.0001$; Fig. 5).

TABLE 1 | Summary output of two-way ANOVA fitting the flight initiation distance of sailfin tetra as response variable. Df = Degrees of freedom; Sum Sq = Sum of Squares; and Mean Sq = Mean Squares. Asterisks indicate significance level. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Variable	Df	Sum Sq	Mean Sq	F-value	p-value
Habitat complexity	3	9.47	3.15	2.26	0.08
Number of individuals	1	22.34	22.34	16.05	<0.0001***
Complexity x number of individuals	3	11.63	3.87	2.78	0.04*
Residuals	380	528.79	1.39		

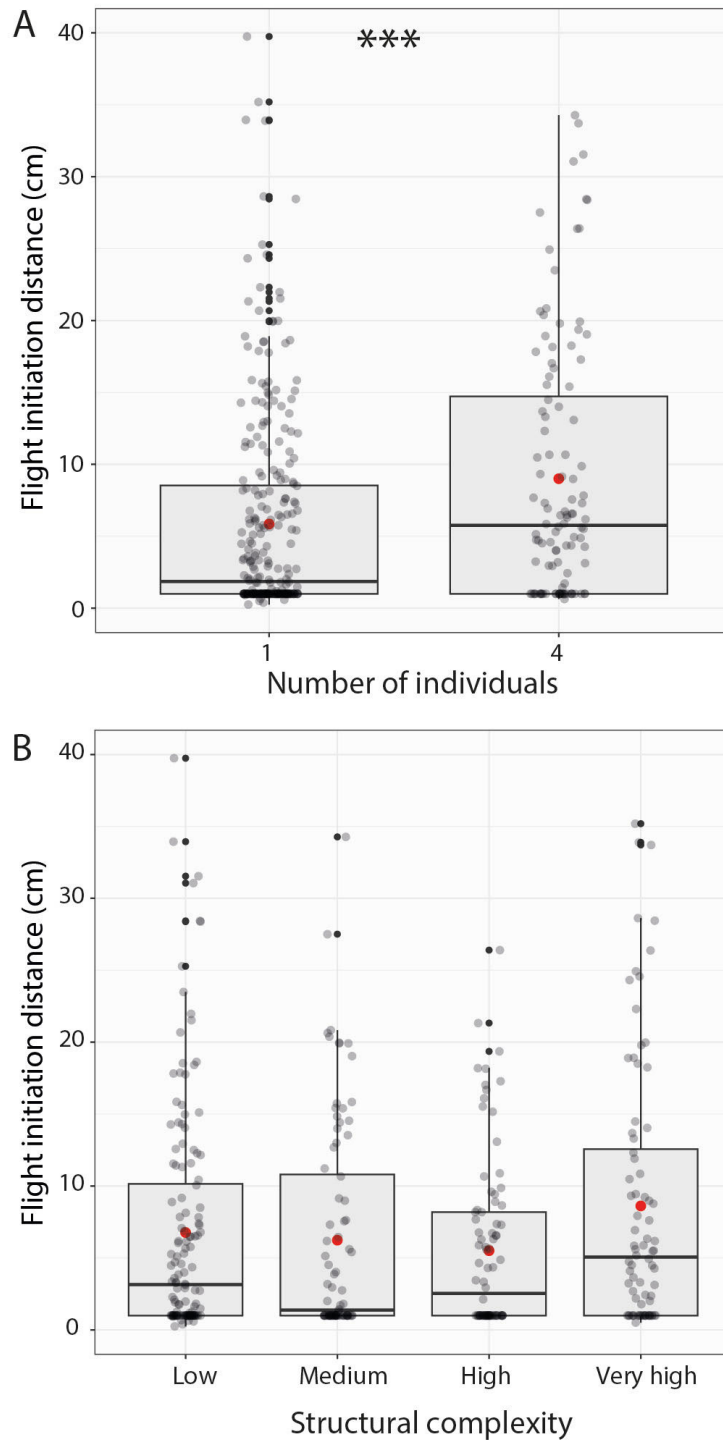


FIGURE 2 | Flight initiation distance measures of sailfin tetra, *Crenuchus spilurus*, among habitat structural complexities (A) and number of individuals (B). The black line represents the median, the gray box is the range between the 1st and 3rd quartiles. The whiskers end at the minimum and maximum values of the data, excluding outliers. Gray dots are the raw data, the red dot is the mean, and black dots are outliers. Outliers were identified as data points falling outside 1.5 times the interquartile range from the first and third quartiles. Asterisks above boxplots indicate significance level. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

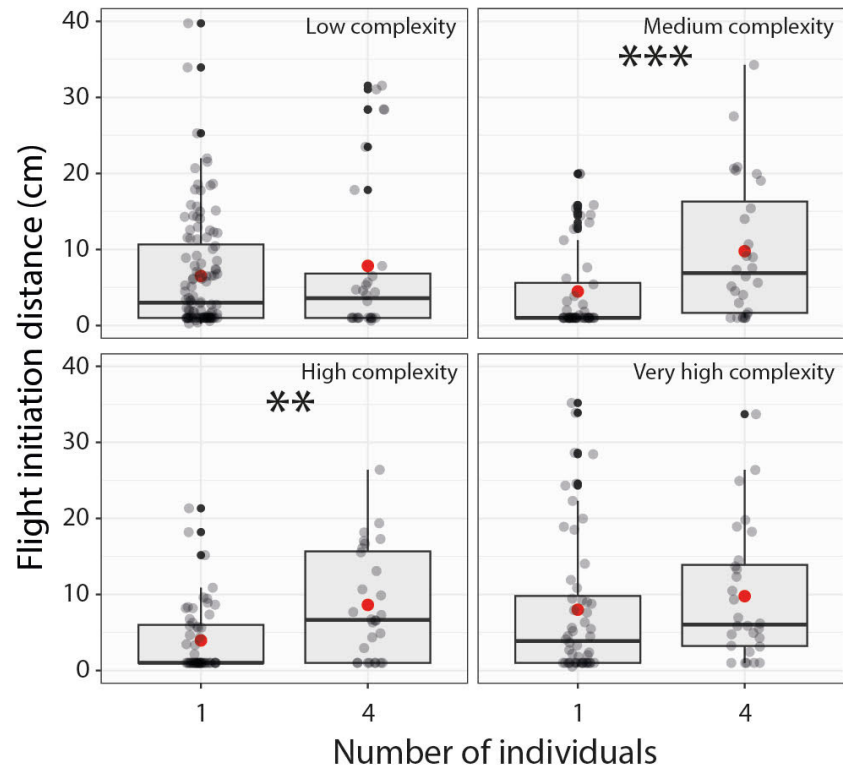


FIGURE 3 | Flight initiation distance comparison of sailfin tetra, *Crenuchus spilurus*, according to habitat structural complexities and number of individuals. The black line represents the median, the gray box is the range between the 1st and 3rd quartiles. The whiskers end at the minimum and maximum values of the data, excluding outliers. Gray dots are the raw data, the red dot is the mean, and black dots are outliers. Outliers were identified as data points falling outside 1.5 times the interquartile range from the first and third quartiles. Asterisks above boxplots indicate significance level. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

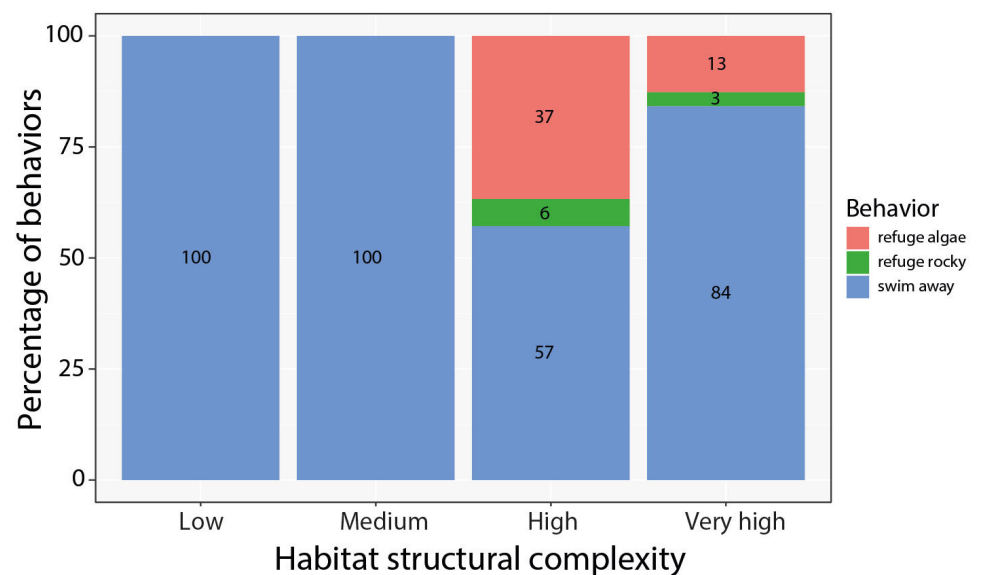


FIGURE 4 | Behavioral display of sailfin tetra, *Crenuchus spilurus* against fish predator among the habitat structural complexities.

TABLE 2 | Descriptions of observed behaviors in fish when exposed to a predator stimulus.

Behavior	Description
Refuge algae	Upon detecting the presence of the predator, the individual sought refuge among artificial plants.
Refuge rocky	Upon detecting the presence of the predator, the individual sought refuge among small rocks.
Swim away	Upon detecting the presence of the predator, the individual swam away without seeking shelter among small rocks or artificial plants.

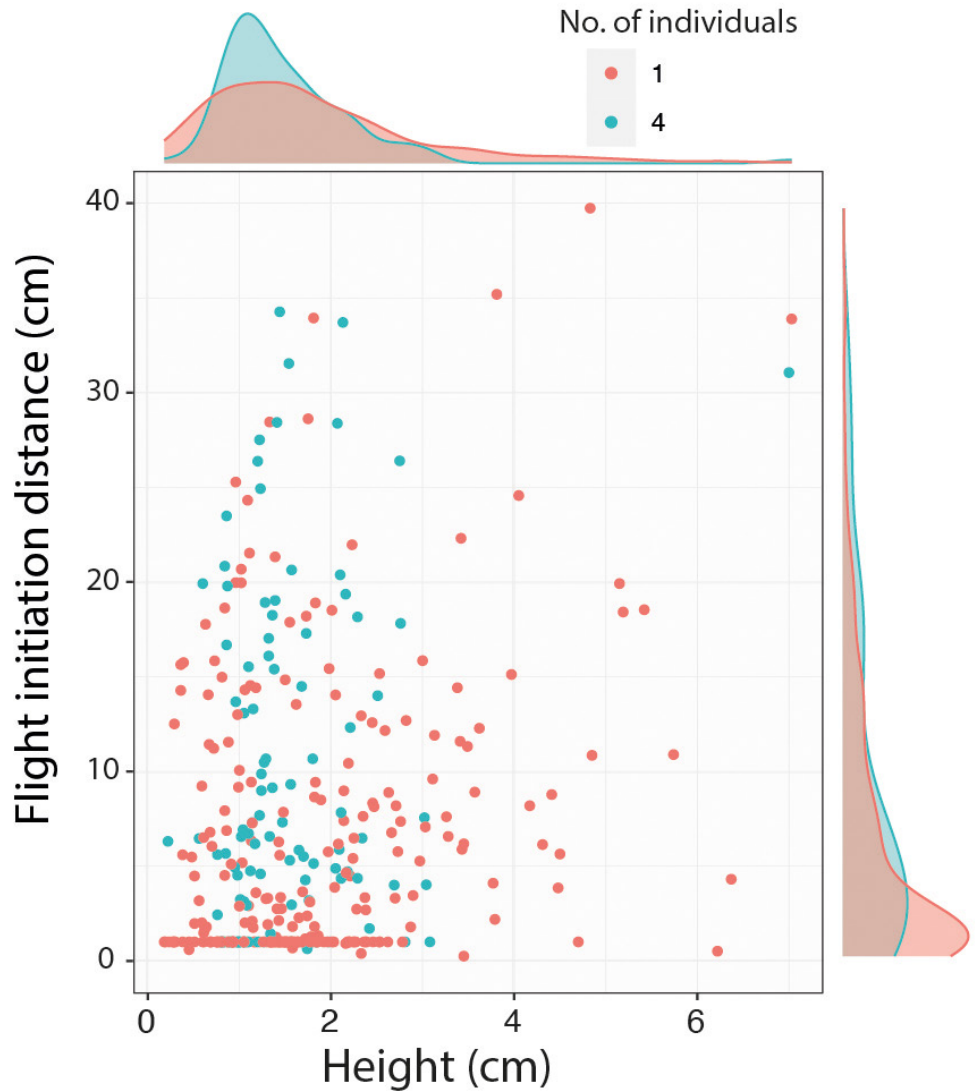


FIGURE 5 | Relationship among the flight initiation distance of sailfin tetra, *Crenuchus spilurus*, and the height of individuals in the aquarium regarding substrate at the flight initiation distance moment. Spearman Rank correlation for FID samples with 1 sailfin tetra individual (S = 2677610, rho = 0.2358, p < 0.0001).

DISCUSSION

We investigated the effects of structural habitat complexity and the number of individuals in the antipredator behavior of sailfin tetra, *Crenuchus spilurus*, through flight initiation distance (FID) metric. Contrary to our initial hypothesis, our findings indicate that habitat structural complexity does not influence the antipredator behavior. This result contrasts with previous findings that described more structurally complex environments providing more effective refuges against predators, potentially reducing the need for antipredator responses (Lima, Dill, 1990; Turner, Montgomery, 2003). It is often observed that fish in habitats with greater structural complexity show an increased tolerance to the proximity of predators (e.g., Quadros *et al.*, 2019). An explanation for such discrepancy may lie in the study's habitat and species specificity (Eklöv, Hamrin, 1989), demonstrating that the impact of habitat structural complexity on ecological dynamics is not uniform but varies depending on the species involved (e.g., Nunes *et al.*, 2015). Eklöv, Hamrin (1989) observed a decline in the foraging success of pike (*Esox lucius* Linnaeus, 1758) preying on Eurasian perch (*Perca fluviatilis* Linnaeus, 1758) and rudd (*Scardinius erythrophthalmus* (Linnaeus, 1758)) as habitat complexity increased. Furthermore, Amazonian floodplain fishes live within a continuum of habitat structural complexities and may have evolved adaptive strategies that do not correlate directly with the structural complexity of their environment.

Moreover, the ability of predators to penetrate refuges in complex habitats may not decrease as linearly with increased complexity as previously assumed (Sih, Wooster, 1994). This pattern might reflect the interplay between the increased costs of vigilance in densely structured environments and the enhanced availability of refuges, indicating that habitat complexity's role as a defense mechanism is modulated by other factors like refuge quality and predator navigability (Heithaus *et al.*, 2009). This interaction underscores the need to consider species characteristics and broader ecological context in understanding antipredator strategies. Simultaneously, our results support the hypothesis that group living augments escape behaviors, as seen in the significant increase in FID for group *vs.* solitary individuals, supporting the risk dilution and “many eyes” theories (Hamilton, 1971; Pulliam, 1973; Lima, Dill, 1990; Samia *et al.*, 2019). This collective efficiency in predator detection underscores the complex interplay of social and environmental elements in shaping antipredator responses (Krause, Ruxton, 2002; Hammer *et al.*, 2023). Being in bigger groups may enhance the ability to remain alert, thereby boosting the capability to notice predators (Elgar, 1989; Lima, 1995). The concept of collective detection suggests that the entire group becomes aware of a predator as soon as one member spots it (Lima, 1995).

We show the influence of the number of individuals on FID, supporting the role of social dynamics in predation risk modulation, consistent with foundational theories (Hamilton, 1971; Krause, Ruxton, 2002). This evidence supports the concept that collective behavior augments individual predator detection efficiency and risk distribution. Although our experimental study revealed that habitat structural complexity did not influence FID, suggesting a nuanced interaction possibly unique to this species, the interaction between habitat complexity and the number of individuals was significant. Such findings suggest that habitat structural complexity influence on defensive behavior is modulated by social context, illustrating an adaptive strategy that integrates environmental and social cues to optimize antipredator responses.

Our study revealed links between antipredator behavior, habitat complexity, and the height of fish from the substrate. With higher availability of structural complexity, fish use algae and rocks as refuges and resort to evasion in simpler ones due the lack of availability, aligning with theories on habitat structure's role in predation risk reduction (Lima, Dill, 1990; Turner, Montgomery, 2003). A positive correlation between FID and fish height from the substrate indicates that fish positioned higher are more likely to initiate escape earlier, highlighting vertical positioning as a key component of their antipredator strategy (Krause, Ruxton, 2002; Scheuerell, Schindler, 2003). This behavior underscores the adaptive use of environmental features and vertical space to mitigate predation risks, showcasing the species' behavioral flexibility in response to environmental variability (Thiriet *et al.*, 2022). The term "height from the substrate" captures this aspect of their spatial behavior, emphasizing its importance in understanding antipredator mechanisms in aquatic environments.

In conclusion, we investigated the dynamics of antipredator behavior in sailfin tetra, showing that habitat complexity alone does not influence such behavioral response. Instead, the attribute enhancing survival strategies is the social structure, notably through group living, which supports risk dilution and increased vigilance theories. The interplay between habitat complexity and the number of individuals, alongside the strategic use of vertical space, highlights the importance of considering both environmental and social factors in behavioral ecology. This study contributes to understanding prey-predator relationships in floodplains, demonstrating the adaptive mechanisms employed against predation within their complex floodplain ecosystem.

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AUTHORS' CONTRIBUTION

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ETHICAL STATEMENT

The experimental procedures received approval from the Animal Ethics Committee of the Universidade Federal do Oeste do Pará (approval number 0220230241). The specimen collection was authorized by Sistema de Autorização e Informação em Biodiversidade (SISBIO permit number 88729–1). After the experiment, all individuals were euthanized using benzocaine and deposited in the zoological collection of the Universidade Federal do Oeste do Pará - CORI under voucher number 0002.

COMPETING INTERESTS

The author declares no competing interests.

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