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Social buffering of behavioural stress response in two fish species, Nile tilapia (*Oreochromis niloticus*) and koi carp (*Cyprinus carpio*)

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Abstract

The presence of conspecifics plays a crucial role in mitigating stress responses in social teleost species and holds potential for enhancing welfare in captive fish. While studies on social buffering effects have typically focussed on single species, marked interspecific differences can exist. Here, we conducted an analysis of social buffering of stress response in two of the most extensively farmed fish, the Nile tilapia and the koi carp. Subjects were exposed to a behavioural stress response assay (open-field test) in three conditions simulating increasing levels of social enrichment: isolation, pairs, or shoals of five fish. We obtained five stress indicators from the assay: thigmotaxis, freezing, activity, erratic movements and interindividual distance in conditions with more than one fish. In both species, erratic movements significantly decreased with increasing levels of social enrichment, suggesting a similar social buffering effect. However, other indicators revealed species differences. Koi carp, but not Nile tilapia, showed a socially-mediated reduction in thigmotaxis, whereas Nile tilapia, but not Koi carp, showed a socially-mediated reduction in freezing behaviour. Furthermore, social enrichment determined opposite effects on the activity of the two species: Nile tilapia were more active as group size increased, whereas the opposite trend was found in koi carp. Finally, Nile tilapia showed increased interindividual distance with increasing social group size, whereas no changes were observed for koi carp. Our study indicates that the buffering effects of social enrichment on the behavioural stress response do not completely overlap between different fish species, highlighting the importance of developing finely-tuned species-specific enrichments and welfare indicators.

KEYWORDS
anxiety-like behaviour, *Cyprinus carpio* koi, fish behaviour, open field test, *Oreochromis niloticus*, social buffering

1 | INTRODUCTION

Social interactions have long been recognised as a fundamental aspect of animal behaviour, mainly due to their key role in individual fitness and processes such as reproduction, foraging and predator avoidance (Armitage, 2010; Cacioppo & Decety, 2011; Cote et al., 2008). An interesting example of this relevance is the reduction of stress responses typically observed in the presence of conspecifics, known as social buffering (Edgar et al., 2015; Hennessy et al., 2009; Kikusui et al., 2006). Social buffering is evident at multiple levels, including physiological (e.g., reduction of circulating stress hormones and neural activation of the brain stress axis; Hennessy et al., 2015; Hodges et al., 2014) and behavioural changes (reduction of anxiety-like behaviour; Bowen et al., 2013; Donovan et al., 2018). Several studies have described social buffering effects in teleost fish (reviewed in Faustino et al., 2017; Gilmour & Bard, 2022). For instance, cichlid fish exposed to handling stressor recover better in the presence of conspecifics than in isolation, as displayed by reduced cortisol levels in the former condition (Culbert et al., 2019). A similar effect was reported in a species of sturgeon exposed to thermal stress (Yusishen et al., 2020). Evidence of social buffering effects is also visible in baseline conditions, without administering acute stressors (Nadler et al., 2016).

It has been proposed that the opportunity to interact with conspecifics and the resulting social buffering may be used as a strategy to improve welfare for animals kept under captive conditions (Arechavala-Lopez et al., 2022; Bolt & George, 2019; Cavallino et al., 2023; Lucon-Xiccato, Cattelan, et al., 2022; Näslund & Johnsson, 2016; Orihuela et al., 2019; Pintos et al., 2021; Zhang, Gao, et al., 2022). This gains particular relevance in ornamental fish species, which are often kept in isolation, pairs or small groups. Social companion effects in fish and their application to farming conditions are however rather complex. Part of the problem is related to the fact that excessively high stock density may determine negative effects (Turnbull et al., 2008), which can worsen other known welfare issues such as the lack of physical (Näslund & Johnsson, 2016) and cognitive enrichments (Varracchio et al., 2024). Moreover, reports have suggested that the effects of social enrichment may differ across fish species (Jones, Alexander, et al., 2023; Jones, Cortese, et al., 2023; Zhang, Fu, et al., 2022), which, if confirmed by direct testing, would require careful species-specific evaluation for welfare applications.

This study aims to investigate whether social buffering of behavioural stress response varies across fish species. We focussed on two social species, the Nile tilapia *Oreochromis niloticus* and the koi carp *Cyprinus carpio*, which are among the most extensively farmed fish globally for food production and ornamental purposes, respectively (Chan et al., 2019; Evers et al., 2019; FAO, 2022). Despite this importance, there is a knowledge gap regarding the behavioural indicators of welfare in these species. We analysed these two teleosts under the same conditions and with the same paradigm, in order to detect eventual interspecific differences in social buffering. The fact that tilapia and koi carp differ significantly in their ecology and life histories suggests that these two species might show at least some differences in social buffering, thereby facilitating the scope of this study, i.e., providing the first behavioural evidence of this effect across species. However, the choice of the study species based on their common occurrence in captivity and the lack of literature prevents us from formally testing hypotheses on the factors determining the interspecific differences.

In our experiment, isolated individuals, pairs and 5-individual groups of fish from both species were observed in the open-field test (hereafter, OFt). This is a well-established test for assessing behavioural stress response in fish that exploits exposure to a novel environment as the stressor. In the last few years, novel environment paradigms have been extensively used in welfare research to describe the effect of enrichment strategies (Brunet et al., 2022; Dias et al., 2023; Forsatkar et al., 2017; Mezzomo et al., 2016; Valcarce et al., 2020; Xu et al., 2022). Moreover, behavioural indicators of stress are growing in importance in assessing animal welfare because they can be easily implemented, also in commercial facilities, without the need of collecting samples from the subjects or to conduct individual analyses (Martins et al., 2012). We expected to observe a decreasing stress response in the presence of conspecifics and with increasing number of conspecifics in both social species. As for our main goal, we also expected to observe differences between the species in the social buffering of behavioural responses.

2 | MATERIALS AND METHODS

2.1 | Experimental subjects

Juvenile Nile tilapia (weight: 72.39 ± 13.61 g; length: 14.48 ± 0.97 cm; $n = 76$) and koi carp (weight: 25.61 ± 6.21 g; length: 10.01 ± 1.16 cm; $n = 76$) were reared in the facility of the University of Trás-os-Montes and Alto Douro (UTAD) separated by species. Tilapia specimens used in this study were obtained from Til-aqua (Someren, the Netherlands) at the fry stage (~3 g) and then reared in UTAD facilities. Koi carp individuals were obtained through spawning induction of reproducers previously reared at UTAD facilities and originally acquired from local suppliers. Therefore, our experimental subjects belonged to a population that underwent a long history of domestication and resembled individuals currently bred in captivity for commercial activities. Fish were approximately 4–5 months old at the time of the experiment. Housing tanks ($70 \times 60 \times 60$ cm; 250 L) contained 20–25 individuals and were kept at constant temperature of $25 \pm 0.5°$C and exposed to a 12:12 h light–dark (LD) artificial photoperiod with lights on at 09.00 am. All the tanks were equipped with constant aeration and supplied with filtered freshwater water from a mechanical and a biological filter and were kept barren to simulate the typical conditions of commercial facilities. Fish were fed twice per day, at 10:00 a.m. and 2:00 p.m., with a commercial diet at 2% of body weight (3.5 mm; Crude Protein: 25% for tilapia and 23% for carp). The subjects used in the experiments were randomly collected from the housing tanks.

2.2 | STRANGE framework

Related to the potential experimental biases outlined by Rutz and Webster (2021) in the STRANGE framework, all the relevant information concerning the experimental subjects is disclosed in the methods section. Since all behavioural trials were conducted with fish of the same age, sampling bias can be excluded for the age factor. We could not control the sex factor due to the fact that fish were juveniles. However, having randomly selected the experimental subjects from the housing tanks, the sex ratio was expected to be consistent across experimental conditions. Therefore, sampling bias could also be excluded for the sex factor. The sample might not be representative of wild Nile tilapia and carp populations since experimental subjects were obtained from farms and these two species are among the oldest farmed fish, thereby subjected to a long domestication process (Fabrice, 2018). This may explain some of the observed results in this study and has been commented in the discussion. However, our results will likely hold for similarly farmed populations.

2.3 | Open-field arena

The OFt was conducted in an experimental apparatus consisting of a white plastic arena (120×100×40 cm) filled with 15 cm of water (Burns, 2008; Lucon-Xiccato et al., 2022b; Pintos et al., 2023). The arena was empty, with no physical enrichments. A white LED tube (6500 K; 1600 lumens) illuminated the arena from above and all experiments were performed between 10:00 a.m. and 1:00 p.m. A full-HD camera (ELP USBFHD08S-MFV, Shenzhen Ailipu Technology Co. Ltd, Shenzhen, China) was placed 2 m above the arena to record the experiments in 1920×1080 pixels of resolution and 30 frames per second.

2.4 | Testing procedure

Fish were tested in the open field (OF) arena in three experimental social conditions (individual, in pairs, in groups of 5 individuals) to assess the effects of social buffering. The general testing procedure of the OFt was the same for all the experimental conditions, with the exception of the number of fish simultaneously assayed. In the individual experimental condition, 12 fish of each species ($n = 12$ fish per species) were individually and independently tested in the OFt. In the second condition (pairs), 24 fish of each species were exposed in pairs to the OFt, resulting in 12 trials ($n = 12$ pairs per species). In the third condition (groups), the behavioural stress response in the OFt was assessed in groups of 5 fish ($n = 8$ groups per species; $N = 40$ fish per species). The experimental design is summarised in Figure 1.

All fish were fed 30 min before the experiment, to avoid conditioning their behaviour with hunger states. At the beginning of each OF trial, the experimenter collected the subject/s from the maintenance tanks and immediately released it/them into the middle of the

OF arena using a net. This was possible because the housing tanks were situated next to the experimental arena. Once in the OF arena, the behaviour of the subjects was recorded for 10 min using a camera. The water was changed between each trial to prevent exposure to the chemical cues from the previous experimental subject.

2.5 | Behavioural parameters

The recordings were analysed in two sequential steps. First, an experimenter scored all the recordings played back on a computer at increased speed to check for aggressive behaviours among individuals tested in pairs and in groups. After ensuring the absence of aggressive interactions such as chases and bites, the recordings were analysed with computer software for automatic tracking (Ethovision XT, Noldus, Wageningen, The Netherlands). The software was used to extract behavioural parameters typically used to study fish stress response (Maximino et al., 2010; Pintos et al., 2023; Sireeni et al., 2020). These parameters included: thigmotaxis as the time spent in the outer part of the arena (centre: 60×50 cm), which usually increases when the fish are stressed (Champagne et al., 2010); activity measured as the distance travelled and as a proxy for the arena exploration (Levin et al., 2007); the freezing behaviour measured as time spent not moving with a speed lower than 1 cm/s for Nile tilapia and 0.7 cm/s for koi carp, which is often linked to anxiety states in model fish species (Egan et al., 2009); and the erratic movements as the angular velocity of the paths, which generally represents how erratic fish trajectory is and an anxiety indicator in fish (Blaser et al., 2010). For the pair and group conditions, we also obtained distance between subjects as indicative of shoal cohesion. This parameter is often used as a proxy of anxiety in fish because small social species tend to increase cohesion and therefore reduce inter-individual distance, when exposed to threats (Alfonso et al., 2020; Morgan, 1988; Speedie & Gerlai, 2008). For the second and third condition, the EthoVision XT produced as the output the average values of individual fish for each behavioural variable. We chose the aforementioned behavioural parameters because they have been often showed to covary with physiological indicators of stress (Archard et al., 2012; Egan et al., 2009; Lara & Vasconcelos, 2021), although the limited literature on the study species suggests caution when interpreting the results.

2.6 | Statistical analyses

Statistical analyses were performed using R Statistical software version 4.0.1 (The R foundation for Statistical Computing Vienna Austria http://www.r-project.org) and all analyses were conducted by functions from the R base package. To analyse the effect of the social condition (three levels: isolated, in pair and in group) and the species (two levels: tilapia and carp) on fish anxiety-like and exploratory behaviours, a two-way analysis of variance (ANOVA) was performed considering both as fixed factors and the interaction
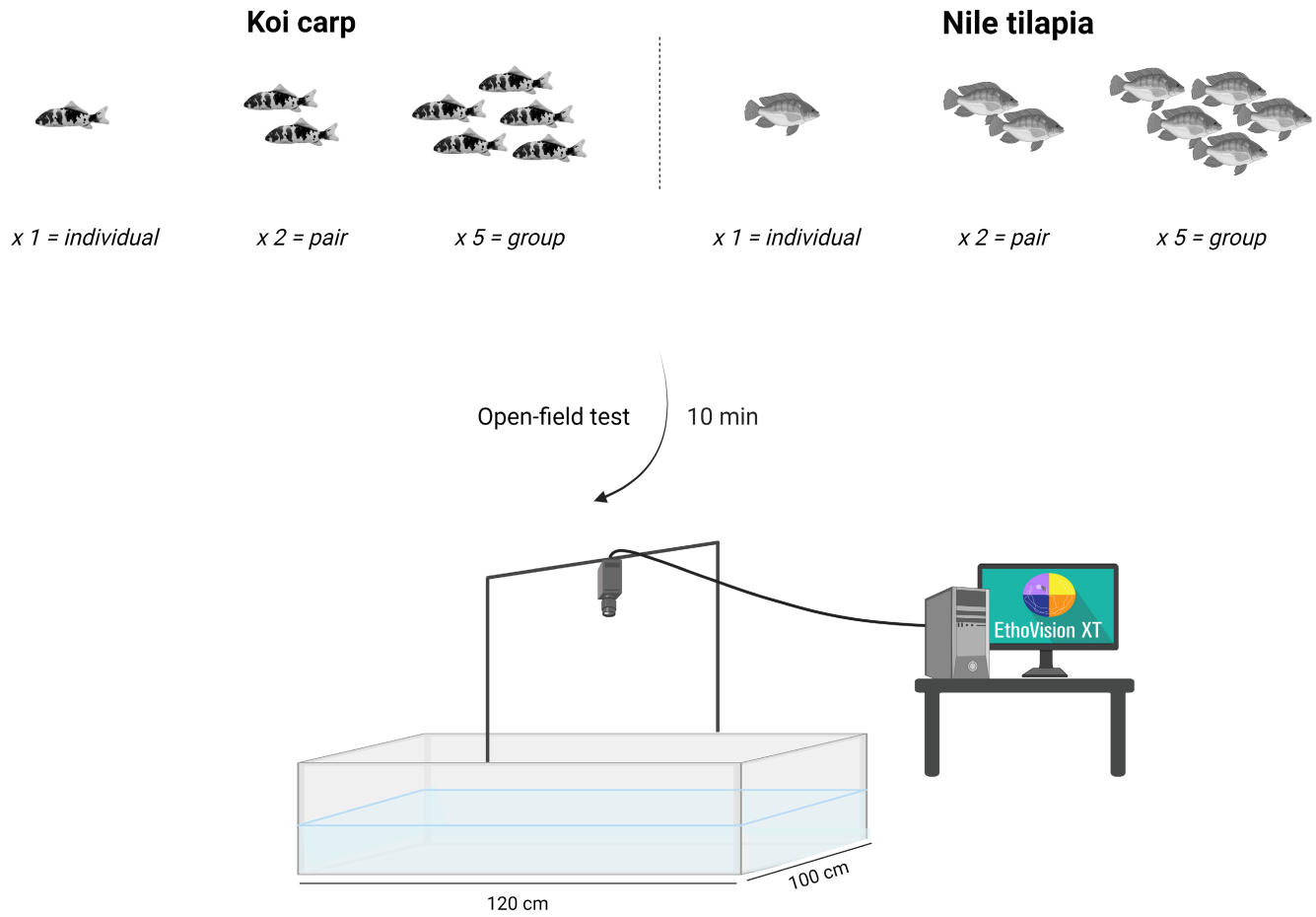
FIGURE 1 Illustrative scheme of the experimental apparatus and design. Juveniles from both species (Nile tilapia and koi carp) were subjected to the open-field test according to the following group sizes: (1) isolated individuals, (2) pairs and (3) groups of five individuals. Their behaviour was recorded for 10 min and fish were tracked by the Ethovision XT software. The experimental arena consisted of a white plastic rectangular tank (120×100×40 cm).

between them. When a significant interaction was found, the dataset was split by species to assess the effect of group size separately by one-way ANOVA or *t*-test (i.e., social behaviour). Furthermore, Tukey's HSD tests were performed as post-hoc statistical analyses to conduct pairwise comparisons between the levels of the social condition factor. Orthogonal polynomial contrasts were performed to test whether significant effects of group size followed a linear trend. Moreover, one sample *t*-tests were performed to study if time in edge values of both species are driven by thigmotaxis responses or associated with random movements across the experimental arena (time in edge expected by chance considering the experimental arena size; 75%). Normality and suitability for the tests were verified by the D'Agostino and Pearson test and QQ plot. Behavioural data that did not meet normality were transformed through logarithmic (i.e., freezing, distance between subjects and erratic movements) or square (i.e., thigmotaxis) transformations. Outliers were identified by the interquartile range (IQR) technique (sample size; Freezing: $n=64$; Activity: $n=63$; Erratic movements: $n=64$; Thigmotaxis: $n=61$; Distance between subjects: $n=39$). Descriptive statistics is represented in the text as mean±SD and the significance level was set at $p=0.05$.

3 | RESULTS

3.1 | Thigmotaxis

Figure 2 shows representative traces and heat maps of fish behaviour according to species and social condition. On average, both Nile tilapia and koi carp showed the expected thigmotaxis in the OFt, evidenced by a high percentage of time spent in the edge of the arena (mean±standard error: 94.76±5.51% for tilapia and 88.58±12.62% for carp). For both species, the time spent in the edge was significantly greater than that expected in the case of random movements in the arena (*t*-test: tilapia, $t_{29}=19.61$, $p<.01$; carp, $t_{30}=5.99$, $p<.01$).

The ANOVA test comparing thigmotaxis between the two species revealed a significant interaction between social condition and species ($F_{2,55}=6.24$, $p<.01$). The main effects of both fixed factors were also significant (species: $F_{2,55}=6.75$, $p<.01$; social condition: $F_{1,55}=10.24$, $p<.01$, respectively). This pattern of results (i.e., significant interaction) indicated that the social condition had different effects on thigmotaxis between the two species. The analysis separated by species denoted that Nile tilapia did not vary their thigmotaxis response with social condition (ANOVA: $F_{2,27}=0.06$, $p=.93$).

Koi carp
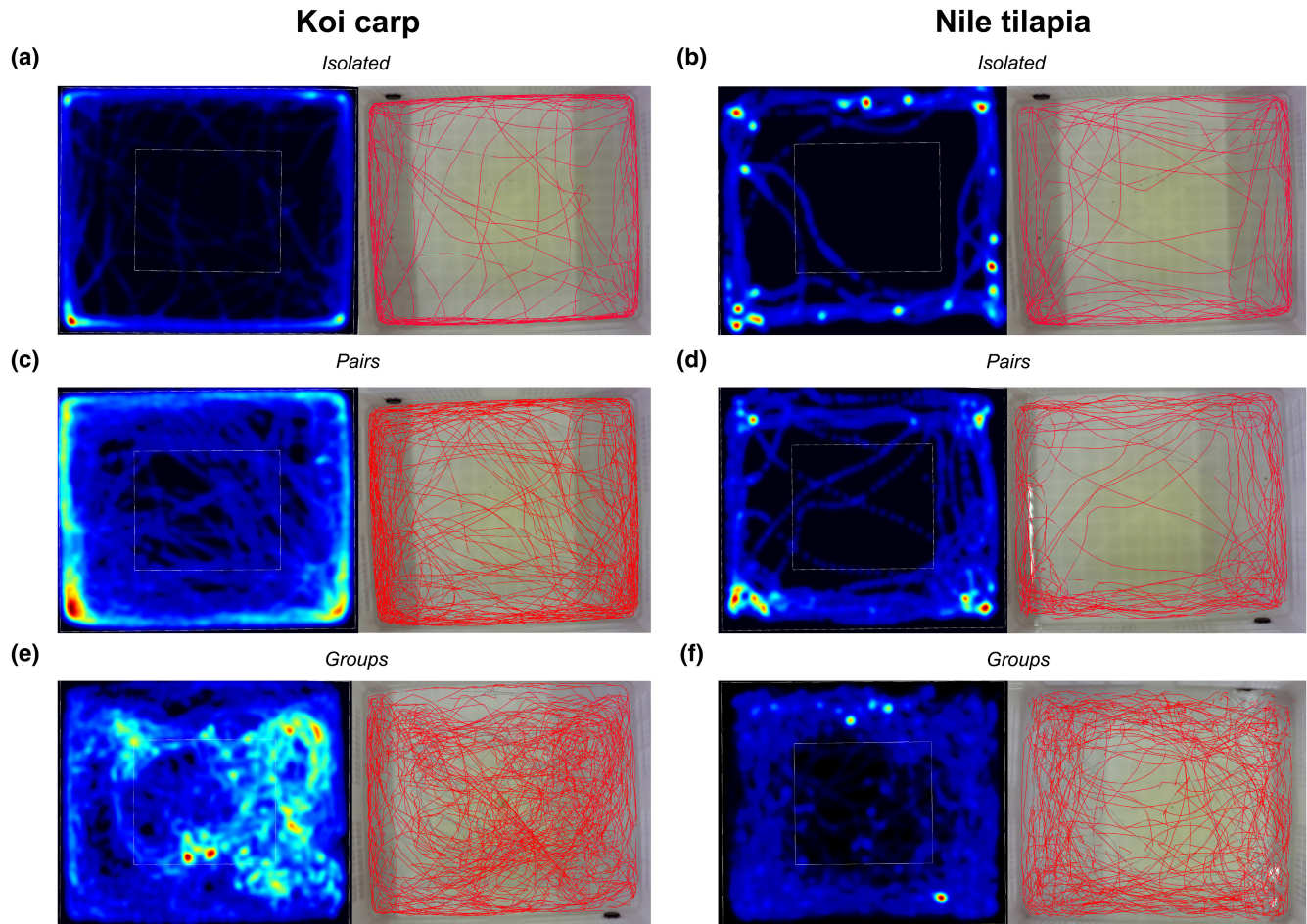
Nile tilapia

FIGURE 2 Representative heatmaps and traces of the open-field arena exploration by isolated (a, b), pairs (c, d) and groups of five individuals (e,f) of both Nile tilapia and koi carp. Heatmaps show the minimum (dark blue) to maximum (dark red) amount of time that fish spent in each pixel, while trace (red) plots denote fish trajectory across the experimental arena.

Conversely, thigmotaxis was significantly affected by group size in koi carp (ANOVA: $F_{2,28} = 9.06$, $p < .01$). Post-hoc testing in koi carp indicated that thigmotaxis was lower in 5-individual shoals and in pairs as compared to isolated individuals (Tukey HSD: isolated vs. pairs; $p = .04$; isolated vs. groups; $p < .01$; pairs vs. groups; $p = .15$; Figure 3a). Orthogonal polynomial contrasts analysis in koi carp's data indicated that thigmotaxis decreased linearly as group size increased ($p < .01$).
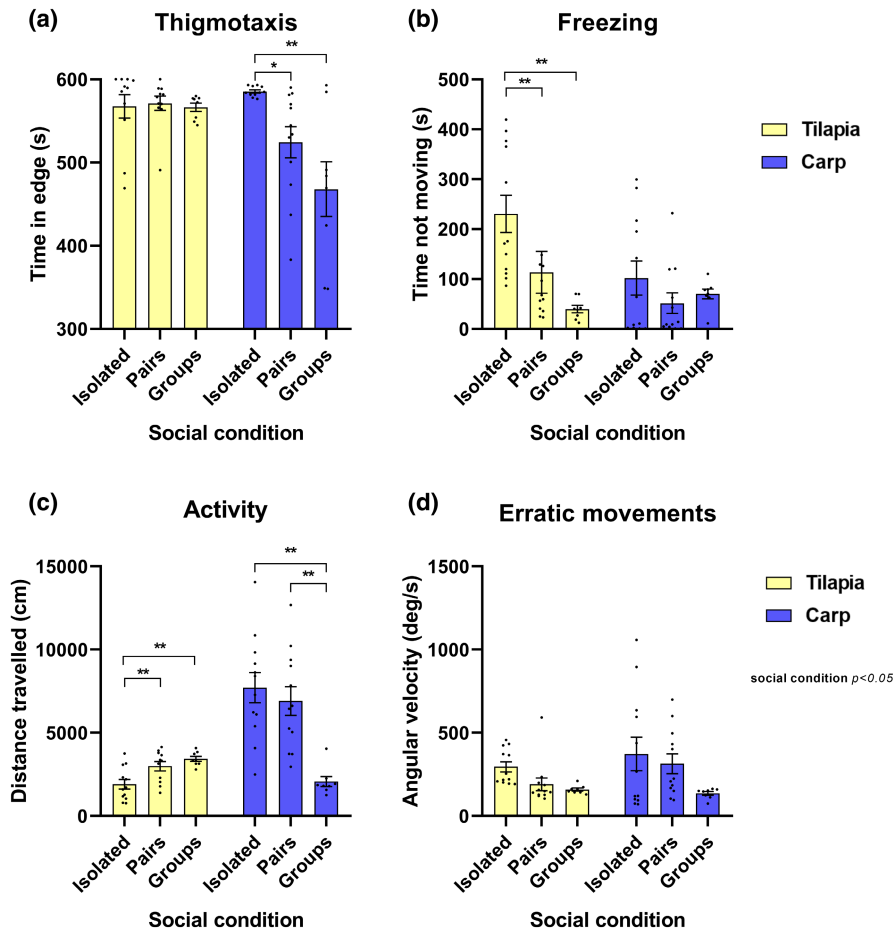
3.2 | Freezing

On average, Nile tilapia fish spent $21.03 \pm 16.03\%$ of the testing time motionless, whereas koi carp did so for $12.43 \pm 4.23\%$ of the testing time. The ANOVA on the freezing behaviour revealed a statistically significant interaction between species and social condition ($F_{2,58} = 4.62$, $p = .01$). The main effect of species was also significant ($F_{1,58} = 8.67$, $p < .01$) but the main effect of social condition was not ($F_{2,58} = 1.18$, $p = .31$). This pattern of result also implied that social enrichment elicited different effects depending on the species. A separate analysis on Nile tilapia evidenced that social condition

had a significant effect on freezing (ANOVA: $F_{2,29} = 14.64$, $p < .01$). However, the presence of social companions did not affect freezing in koi carp (ANOVA: $F_{2,29} = 1.03$, $p = .36$). The post-hoc analysis on Nile tilapia showed that the presence of at least one social companion significantly decreased freezing behaviour compared to the individual testing condition (Tukey HSD: isolated vs. pairs; $p < .01$; isolated vs. groups; $p < .01$; pairs vs. groups; $p = .07$; Figure 3b). Additionally, the orthogonal polynomial contrasts analysis in Nile tilapia's data indicated that freezing decreased linearly as group size increased ($p < .01$).

3.3 | Activity

Nile tilapia moved an average of 2776.18 ± 784.53 cm during the OFt, whereas koi carp moved 5562.25 ± 3055.44 cm. The ANOVA on activity revealed a statistically significant interaction between species and social condition ($F_{2,57} = 15.53$, $p < .01$) along with significant main effects of both social condition ($F_{2,57} = 6.64$, $p < .01$) and species ($F_{1,57} = 28.91$, $p < .01$). The significant interaction denoted a diverse impact of social condition in the activity of the two

FIGURE 3 Anxiety-like behaviours of Nile tilapia and koi carp in response to the open field test and according to group size (i.e., in isolation [$n=12$ per species], pairs [$n=12$ per species] and groups [$n=8$ per species]). (a) Thigmotaxis; (b) Freezing; (c) Activity; (d) erratic movement. Data points are presented as mean $\pm$ standard error. Asterisks indicate statistical differences among the experimental groups (*$p<.05$; **$p<.01$).

species. In particular, analysis on Nile tilapia' data indicated a significant effect of social condition on activity (ANOVA: $F_{2,28}=8.41$, $p<.01$). This was due to an increase in distance moved in pairs and 5-individual shoals compared to isolated fish (Tukey HSD: isolated vs. pairs; $p=.01$; isolated vs. groups; $p<.01$; pairs vs. groups; $p=.3$; Figure 3c). The orthogonal contrast analysis on Nile tilapia' data confirmed this trend, suggesting that activity increased linearly with increasing group size ($p<.01$). Koi carp also showed an effect of social condition (ANOVA: $F_{2,29}=11.57$, $p<.01$), which was however due to lower distance travelled in 5-individual groups compared to both pairs and isolated fish (Tukey HSD: isolated vs. pairs; $p=.75$; isolated vs. groups; $p<.01$; pairs vs. groups; $p<.01$; Figure 3c). According to the orthogonal contrasts analysis on koi carp, the activity of this species decreased linearly with increasing group size ($p<.01$).

3.4 | Erratic movements

The average angular velocity registered was 214.6 ± 71.43 °/s and 274 ± 123.4°/s for Nile tilapia and koi carp, respectively. The ANOVA on erratic movements data indicated a significant effect of social condition ($F_{2,58}=4.47$, $p=.01$), which was irrespective of the species (interaction: $F_{2,58}=1.82$, $p=.17$; species: $F_{1,58}=0.03$, $p=.85$). The post-hoc analysis showed significantly lower angular velocity in

5-individual groups compared to isolated fish (Tukey HSD: isolated vs. pairs; $p=.43$; isolated vs. groups; $p=.01$; pairs vs. groups; $p=.15$; Figure 3d). The orthogonal contrasts analysis indicated that angular velocity decreased linearly as group size increased (linear: $p<.01$).

3.5 | Distance between subjects

In Nile tilapia, the average distance between subjects in the pair and 5-individual conditions was 24.70 ± 9.21 cm. In koi carp, it was 16.22 ± 0.01 cm. The ANOVA on this variable showed a significant interaction between group size and species ($F_{1,35}=7.90$, $p<.01$), while the main effect of group size ($F_{1,35}=10.41$, $p<.01$) and the main effect species were also significant ($F_{1,35}=16.61$, $p<.01$). The analysis separated by species showed that individual proximity did not vary within group size in koi carp (t-test: $t_{17}=0.27$, $p=.78$; Figure 4). In Nile tilapia, the analysis evidenced an increase in the distance between fish when they were in the 5-individual condition compared to the pair condition ($t_{18}=4.49$, $p<.01$).

4 | DISCUSSION

This study showed how two freshwater fish species evidenced social buffering effects on the behavioural stress response to a novel

Distance between subjects
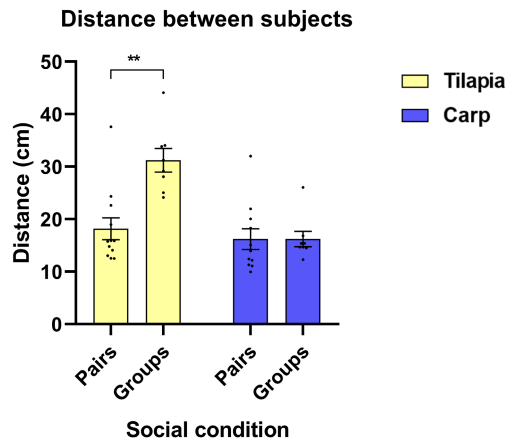
FIGURE 4 Distance between subjects in pairs ($n = 12$ per species) and groups ($n = 8$ per species) of both Nile tilapia (yellow) and koi carp (blue) in response to the open field test. Data points are presented as mean ± standard error. Asterisks indicate statistical differences among the experimental groups (*$p < .05$; **$p < .01$).

environment. Critically, the buffering effects were similar for one of the behavioural indicators of stress considered (i.e., erratic movements), but remarkably different for the others (i.e., thigmotaxis, activity and freezing).

The presence of social companions generally decreased erratic movements in both Nile tilapia and koi carp. Highly erratic paths, identified by high angular velocity values, have been described as indicative of anxiety and stress responses in one extensively studied fish species, the zebrafish (Sireeni et al., 2020; Tran & Gerlai, 2016) and some evidence support the same interpretation in other species (Brunet et al., 2022). Accordingly, the observed reduction of angular velocity in the study species might indicate lower stress responses in the presence of conspecifics. This interpretation based on erratic movement as behavioural indicator of stress is in line with the presence of a social buffering effect consistent across the study in both species, although a validation of this indicator outside the zebrafish is required.

Considering the other four behavioural variables, we found evidence of social buffering that was not consistent in the two species. Both species exhibited a pronounced thigmotaxis response, preferring the outer part of the OF arena, close to the edges, for over 85% of the testing time. This tendency is a typical and well-described anxiety-like response in various animals, including several species of fish (Godwin et al., 2012; Maximino et al., 2010; Schnörr et al., 2012; Watanabe et al., 2021) such as Nile tilapia (Benhaïm et al., 2017; Cerqueira et al., 2016). Moreover, thigmotaxis has been validated as a behavioural indicator of stress (Schnörr et al., 2012) that also covaries with physiological parameters (van den Bos et al., 2019; Wilson et al., 2013, 2016). In our experiment, the presence of conspecifics determined a general reduction of thigmotaxis in koi carp. Considering the robust data on this indicator in the literature, this reduced thigmotaxis in koi carp likely implies lower stress response in individuals exposed to the OFt in pairs and in 5-individual groups compared to those tested in isolation (Pintos et al., 2023; Sharma

et al., 2009). In Nile tilapia, thigmotaxis response did not vary depending on the social condition and remains similar regardless of companions. We therefore conclude that thigmotaxis revealed social buffering effects only for the koi carp.

An analogous conclusion can be drawn based on freezing behaviour, which was reduced in the presence of companions for Nile tilapia, but did not show any difference related to the social context in koi carp. Freezing behaviour has been considered as a stereotyped anti-predator and stress-related behaviour in *O. niloticus* (Barreto et al., 2010; Barreto et al., 2013; de Oliveira Mesquita & Young, 2007; Saraiva et al., 2021) and other teleost fish (Godwin et al., 2012; Hallgren et al., 2011; Qiu et al., 2017), but data on koi carps are not available in the literature. Therefore, the observed behavioural shift from a freeze-hide to a more active reaction in the presence of conspecifics likely indicated reduced stress in the tilapia fish (Jesuthasan, 2012; Speedie & Gerlai, 2008). Our analyses of freezing results are compatible with evidence of a social buffering effect only in Nile tilapia although, due to the limited literature on this indicator in koi carp, it is difficult to draw conclusions on both study species. Similarly, the social variable collected in the pair and 5-individual condition was in line with this trend: Nile tilapia increased interindividual distance with increasing group size, but koi carp did not show such effect. This suggested that larger group sizes decreased stress only in Nile tilapia, since this behaviour has been previously used in other fish species as an anxiety-like behaviour (Alfonso et al., 2020; Rosa et al., 2020). Overall, results of thigmotaxis, freezing and interindividual distance indicate that social buffering does not affect all the behavioural indicators of stress in the various species.

The sharpest difference between species in the social buffering regards the activity parameter. Being in presence of conspecifics increased activity in Nile tilapia, but decreased it in koi carp. The contrasting effects on activity in the two species may be interpreted in several ways. Considering that in most species low levels of activity are generally associated with an antipredator response and are therefore considered evidence of stress (Barreto et al., 2010; Blaser et al., 2010; Johnson et al., 2023; Yoshida, 2021), one may speculate that social buffering occurred only for Nile tilapia. This interpretation is however inconsistent with the results of the other variables, which suggest social buffering also for koi carp. According to another interpretation, hyperactivity responses may be linked to elevated anxiety and stress (Audira et al., 2018; López-Patiño et al., 2008; Qiu et al., 2017), evincing a proactive and energetically expensive strategy such as flight response (Koolhaas et al., 1999; Korte et al., 2005). One should also consider that the activity of the two species could be differently affected by various methodological parameters. For instance, we assayed the subjects of the two species in the same arena and the koi carps were on average larger than the Nile tilapia. It has been shown that the relative size of the arena might affect behavioural responses (e.g., Jones, Alexander, et al., 2023; Jones, Cortese, et al., 2023; Lovin et al., 2023; Näslund et al., 2015). An additional methodological factor potentially involved is water temperature (Forsatkar et al., 2016), which we also kept constant for both species in spite of their different

optimum (El-Sayed & Kawanna, 2008; Watson et al., 2004). However, while it is conceivable that parameters such as temperature and arena size determined differences in the average behaviour between the two species (e.g., Forsatkar et al., 2016; Näslund et al., 2015), the fact that these parameters were kept constant within species makes it more difficult to hypothesise their effects on within-species behavioural variation due to the social context. Therefore, the more likely interpretation for our data on activity is that both species showed social buffering, but that this indicator has a different meaning due to different behavioural strategies in the two species. It is worth considering this interpretation also in light of the findings on the freezing behaviour: activity measures in the OFt appear not completely dependent on the amount of freezing (i.e., koi carp), but probably also strongly affected by swimming velocity.

It would be interesting to understand whether the observed interspecific differences in social buffering might be explained by distinct ecological and evolutionary backgrounds. In their natural environments, tilapia and carp are likely exposed, for instance, to different predation rates (e.g., Crivelli, 1981; Kolding, 1993; Mauck & Coble, 1971; Weber et al., 2012) and different prey types (García-Berthou, 2001; Temesgen et al., 2022). However, it is not possible to draw conclusions on the role of these factors, given for our study we selected species based on their common use in aquaculture and not for a controlled comparative analysis. Moreover, the fact that subjects of both species derived from populations raised in captivity for many generations further complicates the interpretation of ecological and evolutionary causes of behaviour (Saraiva et al., 2018).

From a practical point of view, this research highlights the potential importance of conspecifics in mitigating stress responses of Nile tilapia and koi carp. Fish rearing practices that require isolation should be avoided in these and similar species (e.g., other cyprinids and cichlids) and conversely, social enrichments can be provided to increase welfare and recovery from stressful procedures, such as fish transportation and other handling operations. It is worth noting this study assessed social buffering effects using short paradigms following the standard method to obtain behavioural indicators of stress, which is based on the response to novel environments (Colson et al., 2019; Egan et al., 2009; Rosemberg et al., 2011). When considering social buffering for enrichment purposes, it is worth considering that the situation might change on the long term. For instance, several fish species, such as Nile tilapia, exhibit aggressive behaviours that are expected to emerge after habituation to the novel environment and can negatively impact welfare and health (Giaquinto & Volpato, 1997; Gonçalves-de-Freitas et al., 2019). Moreover, our study suggests that the welfare solution based on social enrichment should be analysed carefully in each fish species due to interspecific differences in behavioural responses to stress and the significance of the behavioural indicators. This requires more comprehensive investigations exploiting multiple behavioural indicators based on the response to other stimuli such as foraging opportunities and predation threats. The association of behavioural indicators with other indicators, such as physiological, molecular and immunological ones, may further help to confirm the reliability of

the behavioural proxies. Indeed, while some of the indicators used in this study have been extensively applied and validated, for other indicators the literature, especially in non-model species, is quite limited. In conclusion, our study supports the current concerns about the necessity to develop species-specific indicators and enrichments for improving the welfare assessment of fish held captive (Browning, 2023; Saraiva et al., 2018; Toni et al., 2019).

CONFLICT OF INTEREST STATEMENT
The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in the Supplementary Material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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