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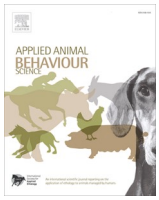

# Is social buffering consistent across the time of the day? A study in a diurnal (Nile tilapia *Oreochromis niloticus*) and a nocturnal fish species (tench *Tinca tinca)*

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ABSTRACT

In social animals, interactions with conspecifics provide several benefits that ultimately enhance welfare and fitness, including the attenuation of individuals' responses to environmental challenges often referred to as social buffering. This effect has been reported across several fish taxa. However, evidence suggests that social buffering is remarkably variable in fish, depending on intrinsic factors of species and environmental conditions. In this research, we explored whether social buffering effects vary throughout the time of the day in two fish species with opposed activity patterns: the diurnal Nile tilapia (*Oreochromis niloticus)* and the nocturnal tench (*Tinca tinca)*. To this goal, we collected behavioural indicators of welfare in fish subjected to two different social conditions (i.e., isolation or groups of five fish) for 24 h every 4 h. These indicators were based on well-reported behaviours associated with stress in fish, such as bottom-dwelling, freezing, activity and erratic movements. Our results revealed social buffering effects in both species in almost all analysed indicators, showing increased welfare in the presence of conspecifics. Critically, while the social buffering effect was constant throughout the day in tench, it varied between day and night in tilapia. Furthermore, the isolation condition disrupted daily rhythmicity only in tilapia, but not in tench, highlighting interspecific variation in the influence of conspecifics on behavioural rhythms. Overall, our findings supported the presence of social companions to improve fish welfare in captivity and underscored the importance of considering species-specific behavioural rhythms for tailoring management practices that minimise stress.

## 1. Introduction

In social animals, the opportunity to interact with conspecifics plays a key role in the well-being of the individuals. For example, the presence of social companions can increase foraging efficiency (Costa et al., 2015; Velázquez-Martínez et al., 2010), reduce the risk of predation (Crane et al., 2013; Lung et al., 2007), and attenuate stress responses to environmental challenges (Eisenberger et al., 2007; Hennessy et al., 2009). These benefits enhance the overall welfare of the individuals and ultimately improve their fitness (Snyder-Mackler et al., 2020). In mammals, several studies have documented that individuals housed in isolation displayed greater physiological responses to stress than those housed in pairs or in groups. For instance, squirrel monkeys *Saimiri sciureus* separated from conspecifics exhibited abnormal, prolonged peaks of circulating cortisol (Mendoza et al., 1992; Lyons et al., 1994) and stronger physiological responses to threats (Stanton et al., 1985). Guinea pigs *Cavia porcellus* showed increased cortisol responses to novelty in isolation than in presence of conspecifics (Hennessy et al., 2008). This phenomenon, often referred to as social buffering, has recently been described in a wide range of taxa, including insects (Oliveira and Faustino, 2017; Tian et al., 2017), reptiles (Martin et al., 2023) and birds (Edgar et al., 2015).

In fish, the existence of social buffering has been documented across various species, particularly in those commonly reared in captivity (Culbert et al., 2019; Gilmour and Bard, 2022; Pavlidis et al., 2013). Consequently, many authors have proposed social buffering as a strategy

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to refine the impact of husbandry procedures and improve the welfare conditions of fish held captive (Arechavala-Lopez et al., 2022; Jones et al., 2023; Yusishen et al., 2020). Nevertheless, the effect of conspecifics on individuals' welfare showed considerable inter- (Pintos et al., 2024b) and intra-specific variation in relation to sex (Akinrinade et al., 2023), developmental stage (Hesse et al., 2015), and stocking density (Abdel Fattah et al., 2020; Turnbull et al., 2005). This suggests that social interactions and their benefit are variable, and as such, they should be carefully evaluated in each species (Barreto et al., 2011; Brown et al., 1992).

One factor known to significantly affect fish welfare is the time of day. Its effect is mainly due to the daily modulation of the stress response, which is well-reported in various species. Fish responses are frequently stronger when the stressor occurs during the resting period of a given species (Figueiredo et al., 2020; López-Olmeda et al., 2013; Vera et al., 2014). Reports on circadian regulation of stress responses in fish are mostly based on physiological traits (Sánchez-Vázquez et al., 2019; Hernández-Pérez et al., 2019) and are generally conducted in isolated fish (Cousineau et al., 2014; Vera et al., 2014). Although this daily variation has recently been documented in both model and farmed fish species through behavioural stress indicators (Pintos et al., 2023; Pintos et al., 2024a; Pintos et al., 2025), it remains unclear how social buffering might influence welfare throughout the day. This possibility has been suggested in other taxa, including primates (Mendoza et al., 1991; Lyons et al., 1995) and birds (Chaturvedi et al., 2024).

In our experiment, we analysed the effect of social companions on behavioural welfare indicators across the day in two fish species with opposed activity patterns. Our study species were the Nile tilapia (*Oreochromis niloticus*) and the tench (*Tinca tinca*). Nile tilapia is a diurnal, highly social cichlid that forms dominance hierarchies within groups (Fortes-Silva et al., 2010; Gonçalves-de-Freitas et al., 2019), whereas tench is a nocturnal species described as shy and less gregarious in the wild (Herrero et al., 2003; Kennedy and Fitzmaurice, 1970; Pintos et al., 2024a; Vanovac et al., 2021). These species are commonly farmed for food production purposes and face welfare challenges when reared in captivity (El-Sayed and Fitzsimmons, 2023; Flajšhans et al., 2010; Gonçalves-de-Freitas et al., 2019; Owen et al., 2010). We collected different behavioural indicators in fish subjected to two social conditions (i.e., isolation or groups of five fish) for 24 h every 4 h. These behaviours included activity, mostly considered positive welfare indicator, and freezing, erratic movements, and bottom-dwelling, considered negative welfare indicators (Martins et al., 2012; White et al., 2017). All these behaviours are characterised by daily variations throughout the 24 h cycle in both studied species (Pintos et al., 2024a). We hypothesised that 1) the presence of social companions increases behavioural indicators of welfare in both species, 2) the social buffering effect exhibits daily variations and 3) the effect of social companions on daily behaviour is different (i.e., opposed) across the day between diurnal and nocturnal species.

## 2. Materials and methods

Fish were reared following Spanish legislation on Animal Welfare and Laboratory Practices. Experimental protocols were performed following the Guidelines of the European Union (2010/63/UE) and Spanish legislation (RD 53/2013 and Law 32/2007) for the use of laboratory animals. They were also approved by the Committee of the University of Murcia on Ethics and Animal Welfare (A13230303).

### 2.1. Experimental subjects

Juvenile tilapia (length: 4.67 ± 1.39 cm; n = 52) and tench (length: 5.48 ± 1.04 cm; n = 52) were reared separately in the fish facility of the University of Murcia. At these developmental stages, individuals of both species are considered sexually immature (de Alba et al., 2021; Pompei et al., 2012), hence potential confounding effects associated with sex were expected to be negligible. Tilapia subjects were obtained through the spawning of adult individuals obtained from an aquaculture company (Fishgen, Swansea, Wales, UK). Tench were purchased at the fry stage from a commercial supplier (Tencas-Atanasio S. L., Badajoz, Spain). Each housing tank (28 × 25 × 15 cm; 10.5 L) contained 10–11 individuals. The water in the housing tanks was kept at a constant temperature: 26 ± 1 °C for tilapia and 21 ± 1 °C for tench. Housing tanks were exposed to a 12:12 h light-dark (LD) artificial photoperiod with lights on at 09.00 am ("Zeitgeber" time 0; ZT0). All tanks were provided with constant aeration and mechanical and biological filters and were kept barren to simulate the typical conditions of commercial facilities.

All fish were fed to satiety once per day for 20 days prior to behavioural testing. The feed was randomly administered during the active phase of species to avoid influencing fish behaviour by determining feeding rhythms (López-Olmeda and Sánchez-Vázquez, 2010). Whereas tilapia has been classified as a diurnal species (Fortes-Silva et al., 2010; Pintos et al., 2024a), showing feeding activity from dawn until dusk (Piet and Guruge, 1997), tench has been described as nocturnal with foraging behaviour peaking at dawn and dusk (Herrero et al., 2003; Pintos et al., 2024a; Vanovac et al., 2021). Therefore, the feed was administered during the light phase for tilapia (ZT0 to ZT12) and during the dark phase for tench (ZT12 to ZT0).

To preliminarily confirm the diurnal (i.e., tilapia) and nocturnal (i.e., tench) activity pattern of species (Pintos et al., 2024a), four housing tanks (n = 2/species) were equipped with one infrared photocell (Omron, mod E3S-AD62, Kyoto, Japan) to record daily locomotor activity of experimental subjects before conducting the behavioural experiments. Photocells were placed in the centre of the front wall of housing tanks and were connected to a computer. Every time a fish interrupted the infrared light beam, they produced an output signal that was recorded and stored in 10-minute intervals using specialised software (DIO98USB; University of Murcia, Spain).

### 2.2. Testing conditions

#### 2.2.1. Experimental settings

The behavioural test was performed following a well-established paradigm (Blaser and Rosemberg, 2012; Cachat et al., 2010; Tran and Gerlai, 2016). This paradigm exploited the innate aversion of fish to explore unfamiliar and potentially threatening arenas. When small fish are exposed to novel environments, they often reduce their activity and descend close to the bottom of their habitat, where they are likely less visible to eventual predators (Brown et al., 2004; Templeton and Shriner, 2004; Wisenden and Sargent, 1997). These responses have been observed across several teleost species (Chin et al., 2018; Levin et al., 2007; Maximino et al., 2010; Qiu et al., 2017; Thompson et al., 2016), including tench and tilapia (Pintos et al., 2024a).

For the behavioural testing, we used a plastic arena filled with dechlorinated tap water aged 24 h. This arena was equipped with constant aeration and mechanical and biological filtration to ensure constant water conditions. The water was changed between each trial to prevent exposure to the chemical cues from the previous experimental subject. The size of the arena in the isolation condition was as follows: 25 × 15 × 15 cm with 13 cm of water (4.88 L). In the group condition, the size of the arena was increased as much as possible within logistical constraints: 30 × 20 × 18 cm filled with 16 cm of water (9.6 L). The arena used for the isolation condition has been shown to elicit anxiety-like behaviours in isolated individuals of both species (Pintos et al., 2024a), while the group condition arena was designed to approximate the dimensions used for the housing tanks. The walls of the arena were opaque from behind and transparent from the front and sides. A digital full-HD camera (Visiotech WebcamIP-W002WA) was located 40 cm from the front of the experimental tank. This camera was sensitive to infrared light. An infrared backlight panel irradiated the arena from behind, providing the necessary light for the camera to work during the dark phase. A white LED strip (AquaRay, natural daylight, 6500 K)

illuminated the arena from above during light-hour trials (ZT0–12).

### 2.2.2. Isolation condition

Twelve fish from each species (n = 12/species) were randomly selected from the housing tank and individually tested in the arena. These subjects were gently transported by means of an opaque jar. The fish were acclimated to the arena for 24 h. During this period, food was randomly administered in one meal during the light phase for tilapia and during the dark phase for tench. While acclimatisation likely reduced the intensity of the stress response to the novel environment during the behavioural measurements, it allowed us to study indicators with greater applied value, as they may better resemble those routinely measured in captivity conditions. After the acclimatisation, the behaviour of the subjects was recorded for 1 h at different times of the day, every 4 h: at ZT 2, 6, 10, 14, 18 and 22. The video recordings were then analysed offline using the software Ethovision XT®. This software allowed us to track the position of the subject in the arena during each minute of the recording. Using the tracking data, the software provided us with the following variables: 1) the time spent in the lower half of the arena (bottom-dwelling; negative indicator), which usually increases when fish' welfare is reduced for example due to stress (Blaser and Rosemberg, 2012; Cachat et al., 2010); 2) the distance moved (activity; mostly considered positive indicator, see Discussion), a common proxy of stress in fish (Levin et al., 2007); 3) the time spent not moving (freezing; negative indicator) with a speed of 0.7 cm/s as threshold for tench and 0.5 cm/s for tilapia (Pintos et al., 2024a), as immobility is a typical antipredator behaviour displayed by several teleost species (Barreto et al., 2013; Egan et al., 2009); and 4) the angular velocity of the path (erratic movement; negative indicator), which is often assumed to increase in response to stressors (Blaser et al., 2010).

### 2.2.3. Group condition

Eight groups of 5 fish from each species (n = 8 groups/species) were used in the experiment. This group size was chosen based on previous evidence suggesting that this number effectively increased welfare in fish while minimising the likelihood of aggressive interactions (Pintos et al., 2024b). These fish were randomly selected from different housing tanks. After being moved into the arena, fish were randomly fed once during their active phase and left undisturbed for 24 h. Then, the recording began with the same conditions described in the previous section (i.e., 6 recordings, 1 each 4 h at ZT2, 6, 10, 14, 18 and 22). Group condition recordings were then analysed offline in two sequential steps. As a first step, an experimenter reviewed all the recordings played back on a computer at increased speed to check for aggressive behaviours. After confirming the absence of aggressive interactions (e.g., bites), the same variables analysed in the isolation condition were obtained from

**Table 1**
Cosinor parameters of behavioural indicators collected from tench and tilapia individuals over the 24 h cycle and according to social condition. Data are presented as mean ± C.I. 95 % and acrophases in time values (ZT).

| SPECIES | BEHAVIOUR | SOCIAL CONDITION | ACROPHASE | AMPLITUDE | MESOR |
|---|---|---|---|---|---|
| Tench | Bottom dwelling | ISOLATION | 5.43 ± 0.74 | 11.21 ± 4.27 | 89.10 ± 2.41 |
| Tench | Bottom dwelling | GROUP | 5.08 ± 2.45 | 17.26 ± 10.41 | 67.81 ± 5.86 |
| Tench | Activity | ISOLATION | 17.77 ± 1.18 | 4761.77 ± 1461.89 | 5659.48 ± 824.28 |
| Tench | Activity | GROUP | 18.30 ± 1.67 | 4392.82 ± 1856.19 | 8871.90 ± 1044.29 |
| Tench | Freezing | ISOLATION | 5.73 ± 1.07 | 38.70 ± 10.75 | 52.50 ± 6.06 |
| Tench | Freezing | GROUP | 6.42 ± 1.22 | 22.77 ± 7.20 | 30.96 ± 4.04 |
| Tench | Erratic movement | ISOLATION | 6.15 ± 2.52 | 48.92 ± 29.97 | 251.38 ± 16.90 |
| Tench | Erratic movement | GROUP | 5.43 ± 2.78 | 78.50 ± 52.40 | 306.59 ± 29.48 |
| Tilapia | Bottom dwelling | ISOLATION | 4.97 ± 3.50 | 10.81 ± 8.58 | 54.22 ± 4.83 |
| Tilapia | Bottom dwelling | GROUP | 6.22 ± 2.22 | 10.86 ± 5.97 | 50.01 ± 3.36 |
| Tilapia | Activity | ISOLATION | N/A | N/A | N/A |
| Tilapia | Activity | GROUP | 3.97 ± 3.28 | 1515.03 ± 1150.16 | 6730.36 ± 647.07 |
| Tilapia | Freezing | ISOLATION | N/A | N/A | N/A |
| Tilapia | Freezing | GROUP | 17.35 ± 3.55 | 8.82 ± 7.04 | 30.66 ± 3.96 |
| Tilapia | Erratic movement | ISOLATION | 18.60 ± 1.58 | 43.56 ± 17.43 | 203.86 ± 9.83 |
| Tilapia | Erratic movement | GROUP | 18.10 ± 1.32 | 97.29 ± 32.67 | 315.46 ± 18.38 |

the tracking analyses. The Ethovision XT® provided as the output the average values of all the fish in the group for each analysed behaviour.

### 2.3. Statistical analysis

Statistical analysis was performed in R Statistical software version 4.0.1 (The R foundation for Statistical Computing Vienna Austria http://www.r-project.org). We conducted a two-step analysis using complementary strategies with distinct goals. First, all behaviours were subjected to Cosinor analysis, performed separately for each combination of species (i.e., tilapia and tench) and social condition (i.e., isolation and group). This allowed us to assess the presence of daily rhythmicity in fish behaviour and was implemented using the *cosinor2* R package (Mutak, 2018). Cosinor analysis employs least-squares regressions to model cosine curves, which are useful to describe circadian variations (Nelson, 1979) and estimates a circadian rhythm through a zero-amplitude test, in which $p < 0.05$ constitutes evidence for a statistically significant rhythm of the given period under consideration (i.e., 24 h). Rhythm parameters such as mesor and acrophase were calculated for each behavioural rhythm (Table 1) and compared between social conditions by the *circacompare* R package (Parsons et al., 2020). All acrophases were corrected to locate them in the correct quadrant (Cornélissen and Halberg, 2014) and subsequently transformed from radians to time values (i.e., ZT).

As a second step, we conducted an analysis using linear mixed-effects models (LMMs; Pinheiro et al., 2017), with ZT and social condition as fixed effects (*nlme* R package). While this approach does not estimate rhythmic parameters like the Cosinor analysis, it allows us to directly compare the behaviour of subjects across all experimental conditions, specifically across the six ZT levels (2, 6, 10, 14, 18, and 22) and two social conditions (isolation and group). The LMM comparison identifies differences in mean behaviours rather than in rhythmic parameters. Therefore, this LMM analysis provided complementary insights into the effects of social condition and time of day on fish behaviours, supplementing the rhythmic information obtained from the Cosinor analysis. The models also included the experimental subjects' or shoals' IDs as a random effect to account for repeated measurements over time. The interaction between the two fixed effects was evaluated as it would indicate social buffering effects that vary through the day. If necessary, relevant pairwise comparisons (e.g., between ZT levels or the combinations of both factors) were performed with the Tukey HSD test. Model assumptions were verified by the Shapiro-Wilk test and Q-Q plot (normality) and by Levene's test (homoscedasticity). Behavioural data that did not meet the assumptions for parametric analysis were transformed through square (bottom-dwelling in tench), square root (angular velocity in tench) and logarithmic (freezing in tench and activity in tilapia) functions.

## 3. Results

### Tench

### 3.1. Daily locomotor activity

In housing tanks, tench exhibited a nocturnal activity pattern, displaying most of their locomotor activity during the dark phase (91.98 ± 0.55 %, Table S1).

### 3.2. Bottom-dwelling

On average, tench from the isolation condition spent 89.10 ± 1.51 % of the testing time in the lower half of the arena, whereas tench from the group condition showed this behaviour in 67.81 ± 3.35 % of the testing time. Both experimental groups denoted more time spent in the lower half of the arena than expected by random movements (one sample *t*-test: isolation condition: $t_{71} = 25.89$, $p < 0.01$; group: $t_{48} = 5.31$, $p < 0.01$). Cosinor analysis showed that bottom-dwelling behaviour exhibited significant daily variations for both social conditions (isolation condition: $p < 0.01$; group condition: $p < 0.01$), indicating similar diurnal acrophases (isolation condition = 5.43 ZT; group condition= 5.08 ZT; $p_{\text{acrophase}} = 0.74$; Fig. 1A), but lower mesor in the group condition as compared to the isolation condition ($p_{\text{mesor}} < 0.01$).

In line with the Cosinor analyses, the LMM on bottom-dwelling behaviour showed significant main effects of ZT ($F_{5,97} = 26.23$, $p < 0.01$). Bottom-dwelling was higher during the light phase when compared to the dark phase (Tukey post-hoc test: Fig. 2A). Moreover, the LMM found a significant effect of social condition ($F_{1,97} = 87.48$, $p < 0.01$), indicating a social buffering effect: tench of the group condition spent less time in the lower half of the arena when compared to tench of the isolation condition (Fig. 2A). The interaction between the two fixed factors was not significant ($F_{5,97} = 1.53$, $p = 0.18$), suggesting no daily variation in the social buffering effect.

### 3.3. Activity

On average, tench moved 5659.48 ± 570.60 cm across the experimental arena in the isolation condition and 8871.90 ± 680.20 cm in the group condition. Cosinor analysis showed significant daily variations in activity for both social conditions (isolation condition: $p < 0.01$; group condition: $p < 0.01$), with similar acrophases close to the middle of the dark phase (isolation condition = 17.76 ZT; group condition = 18.30 ZT; $p_{\text{acrophase}} = 0.49$; Fig. 1B), but increased mesor in tench of the group condition compared to tench of the isolation condition ($p_{\text{mesor}} < 0.01$).

The LMM on activity registered a significant main effect of ZT ($F_{5,97} = 37.49$, $p < 0.01$), due to higher activity during the dark phase as compared to the light phase (Tukey post-hoc test: Fig. 2B). Moreover, the effect of social condition was also significant ($F_{1,97} = 43.10$, $p < 0.01$), because of higher activity in the group condition as compared to the isolation condition (Fig. 2B). The interaction between the two factors was not significant ($F_{5,97} = 0.45$, $p = 0.81$), suggesting no daily variation in the buffering effect.

### 3.4. Freezing

On average, tench from the isolation condition spent 52.50 ± 4.41 % of testing time displaying freezing behaviour, whereas tench from the group condition displayed freezing for 30.95 ± 3.06 % of testing time. Cosinor analysis showed that freezing behaviour exhibited significant daily variations for both social conditions (isolation condition: $p < 0.01$; group condition: $p < 0.01$), indicating similar acrophases close to the middle of the light phase (isolation condition = 5.73 ZT; group condition = 6.42 ZT; $p_{\text{acrophase}} = 0.41$; Fig. 1C) but increased mesor in the isolation condition as compared to the group condition ($p_{\text{mesor}} < 0.01$).

The LMM test on freezing revealed a significant main effect of both ZT ($F_{5,97} = 46.11$, $p < 0.01$) and social condition ($F_{1,97} = 13.98$, $p < 0.01$), and no significant interaction between fixed factors ($F_{5,97} = 1.83$, $p = 0.11$). Post-hoc analyses revealed increased freezing in the light phase compared to the dark phase (Tukey post-hoc test: Fig. 2C). Moreover, consistently with the presence of social buffering, we found reduced freezing in the group condition as compared to the isolation condition (Fig. 2C).

### 3.5. Erratic movement

Tench from the isolation condition showed an average angular velocity of 251.38 ± 9.30 deg/s, while tench in the group condition displayed an average angular velocity of 306.6 ± 16.45 deg/s. Cosinor analysis showed that this behaviour exhibited significant daily variations for both social conditions (isolation condition: $p < 0.01$; group condition: $p < 0.01$), indicating similar acrophases close to the middle of the light phase (isolation condition = 6.15 ZT; group condition= 5.44 ZT; $p_{\text{acrophase}} = 0.60$;; Fig. 1D) but increased mesor in the group

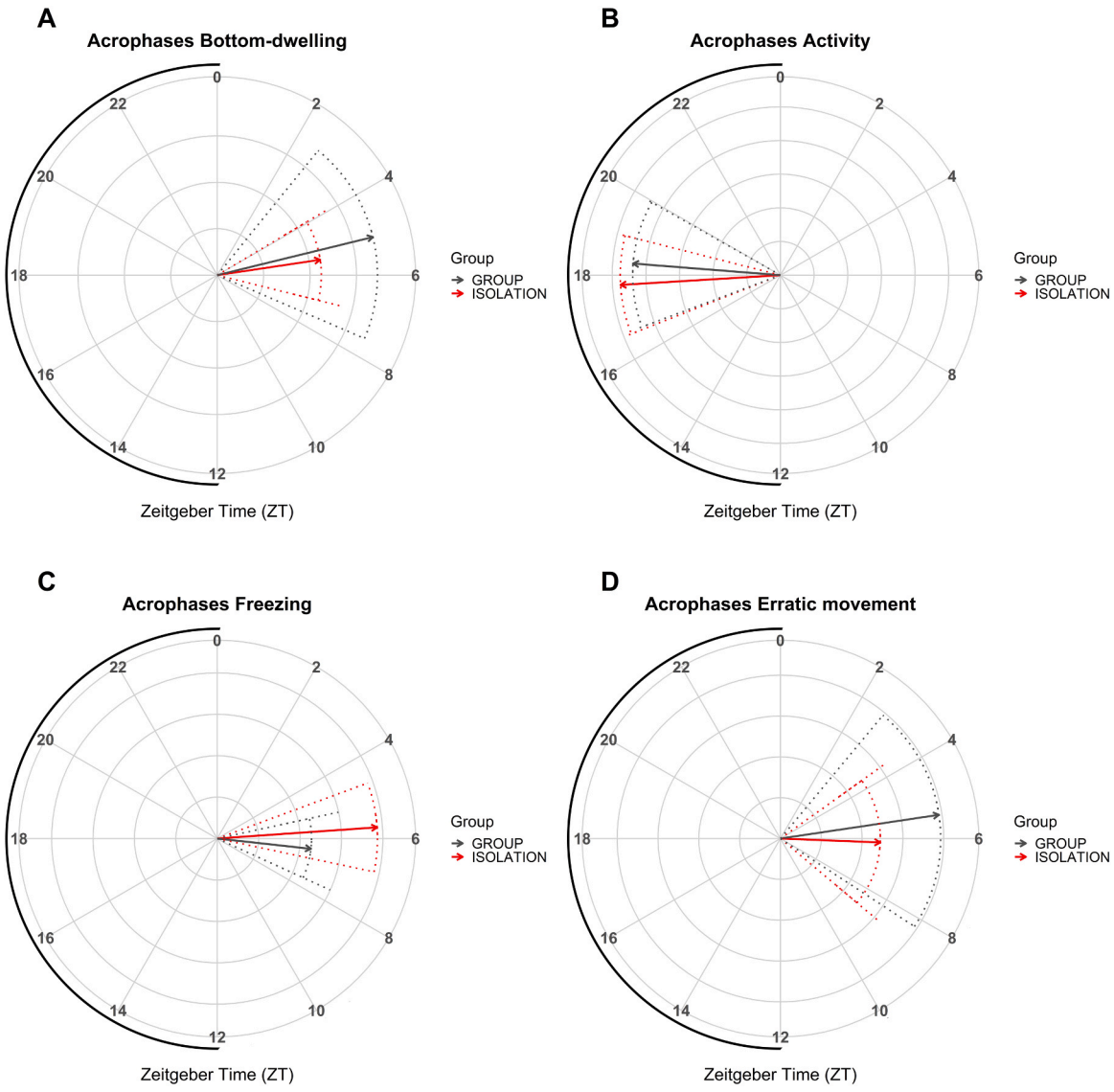


**Fig. 1.** Polarograms of estimated acrophases for significant daily behaviours in tench (Cosinor, $p < 0.05$). **A.** Bottom-dwelling. **B.** Activity. **C.** Freezing and **D.** Erratic movement. Solid arrows indicate the mean acrophase and dotted lines indicate the confidence interval (set at 95 %). The radial axis represents the time of the day (ZT) and the vector length represents the amplitude of the rhythm. The black line above radial axis represents the dark phase.

condition as compared to the isolation condition ($p_{mesor} < 0.01$).

The LMM on angular velocity revealed a main effect of both ZT ($F_{5,97} = 10.98$, $p < 0.01$) and social condition ($F_{1,97} = 8.86$, $p < 0.01$). Post-hoc analyses revealed increased erratic movement during the light phase compared to the dark phase (Tukey post-hoc test: Fig. 2D). Moreover, higher erratic movement was found in the group condition when compared to the isolation condition (Fig. 2D). The interaction between these factors was not significant ($F_{5,97} = 0.51$, $p = 0.76$), indicating no circadian variation of the social buffering effect.

**Tilapia**

### 3.6. Daily locomotor activity

In housing tanks, tilapia exhibited a diurnal activity pattern, displaying most of their locomotor activity during the light phase (71.5 ± 1.39 %, Table S1).

### 3.7. Bottom dwelling

On average, tilapia from the isolation condition spent 54.22 ± 2.55 % of the testing time in the lower half of the arena, whereas tilapia from the group condition spent 50.00 ± 1.97 % of the testing time. Therefore, the fish from the two experimental social conditions did not display a significant preference for the lower part of the arena (one sample *t*-test: isolation condition: $t_{71} = 1.65$, $p = 0.10$; group condition: $t_{48} < 0.01$, $p = 0.99$). Cosinor analysis showed bottom-dwelling behaviour exhibited significant daily variations for both social conditions (isolation condition: $p < 0.01$; group: $p < 0.01$), with similar acrophases close to the middle of the light phase (isolation condition = 4.96 ZT; group condition= 6.23 ZT; $p_{acrophase} = 0.44$; Fig. 3A) and no significant differences in the mesor ($p_{mesor} = 0.20$).

The LMM on bottom-dwelling behaviour showed a significant main effect of ZT ($F_{5,97} = 6.18$, $p < 0.01$). This indicated that bottom-dwelling varied across the time of the day. In particular, tilapia spent more time in the lower half of the arena at the end of the light phase as compared to the beginning of the dark phase (Tukey post-hoc test: Fig. 4A). There was no significant effect of social condition ($F_{1,97} = 1.55$, $p = 0.21$) and no significant interaction between social condition and time of the day ($F_{5,97} = 0.65$, $p = 0.65$).

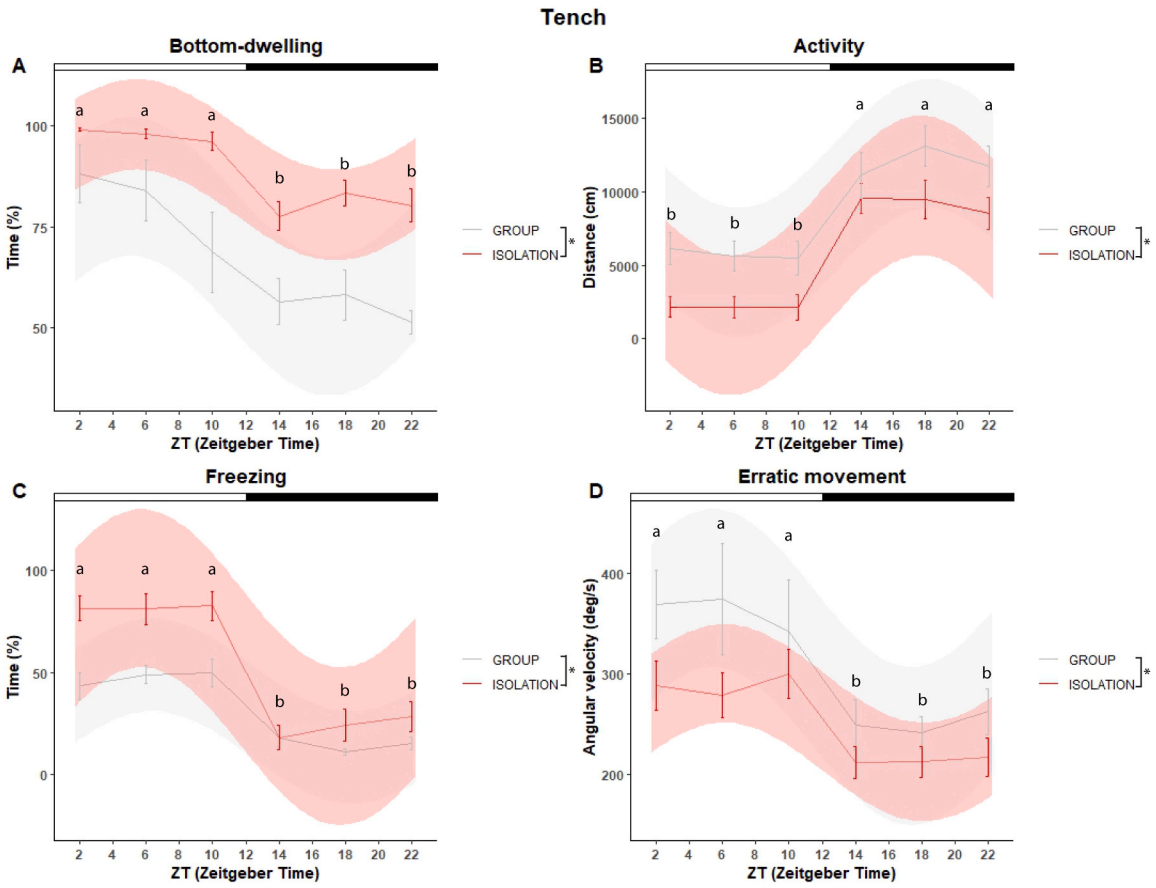


**Fig. 2.** Daily variation of tench behaviour according to social condition (i.e., isolation or group). **A.** Bottom-dwelling. **B.** Activity. **C.** Freezing. **D.** Erratic movement. Data points represent mean ± standard error. Different letters (a,b) indicate statistical differences between ZTs by Tukey HSD test performed on linear mixed models. Asterisks (*) indicate a significant main effect of social condition. Wave area plots represented predicted data based on significant Cosinor models. White and black bars above each graph represent light and dark phases, respectively.

### 3.8. Activity

Tilapia moved an average of 4792.90 ± 223.63 cm across the diving test arena in the isolation condition and 6730.36 ± 351.05 cm in the group condition. Cosinor analysis revealed daily rhythms in activity only for tilapia of the group condition (isolation condition: $p = 0.20$; group condition: $p < 0.01$). The acrophase of activity behaviour for tilapia in the group condition was located close to the middle of the light phase (3.96 ZT; Fig. 3B).

The LMM on activity registered significant main effects of ZT ($F_{5,97} = 3.20$, $p = 0.01$) and social condition ($F_{1,97} = 51.43$, $p < 0.01$). This indicated two independent effects of ZT time and social condition on activity behaviour. Post hoc analysis showed that tilapia exhibited higher activity during the beginning of the light phase as compared to the middle and the end of the dark phase (Tukey post-hoc test: Fig. 4B). Moreover, higher activity was found in the group condition when compared to the isolation condition (Fig. 4B). The interaction between the two factors was not significant ($F_{5,97} = 1.85$, $p = 0.10$).

### 3.9. Freezing

On average, tilapia from the isolation condition displayed freezing behaviour at 39.09 ± 1.87 % of the testing time. Similarly, tilapia from the group condition spent 30.66 ± 2.12 % of testing time displaying freezing behaviour. Cosinor analysis showed significant daily rhythms in freezing only for tilapia of the group condition (isolation condition: $p = 0.61$; group condition: $p = 0.01$;). In the group condition, freezing acrophase was located close to the middle of the dark phase (17.35 ZT; Fig. 3C).

The LMM test on freezing behaviour found a significant main effect of ZT ($F_{5,97} = 2.41$, $p = 0.04$) and social condition ($F_{1,97} = 36.06$, $p < 0.01$). Furthermore, the interaction between these factors was significant ($F_{1,97} = 3.97$, $p < 0.01$). This indicated diverging effects of ZT on the social buffering of freezing. Post-hoc analysis showed that, during daylight trials, tilapia of the group condition exhibited lower freezing than tilapia of the isolation condition (Tukey post-hoc test: Fig. 4C).

### 3.10. Erratic movement

On average, tilapia from the isolation condition showed an angular velocity of 203.85 ± 6.07 deg/s, whereas tilapia from the group condition exhibited an angular velocity of 315.45 ± 13.43 deg/s. Cosinor analysis found significant daily rhythms in erratic movement behaviour for both social conditions (isolation condition: $p < 0.01$; group condition: $p = 0.03$). The acrophases were similarly located between the two social conditions, with maximum values close to the middle of the dark phase (isolation condition = 18.61 ZT; group condition = 18.10 ZT; $p_{acrophase} = 0.54$; Fig. 3D). Furthermore, increased mesor was observed in the group condition compared to the isolation condition ($p_{mesor} < 0.01$).

The LMM on angular velocity revealed a main effect of ZT ($F_{5,97} = 26.65$, $p < 0.01$) and social condition ($F_{1,97} = 143.21$, $p < 0.01$). Furthermore, the interaction between these factors was significant ($F_{1,97} = 4.54$, $p < 0.01$), suggesting daily variation in the social buffering effect. However, post-hoc analysis showed higher angular velocity values in tilapia of the group condition compared to tilapia of the isolation

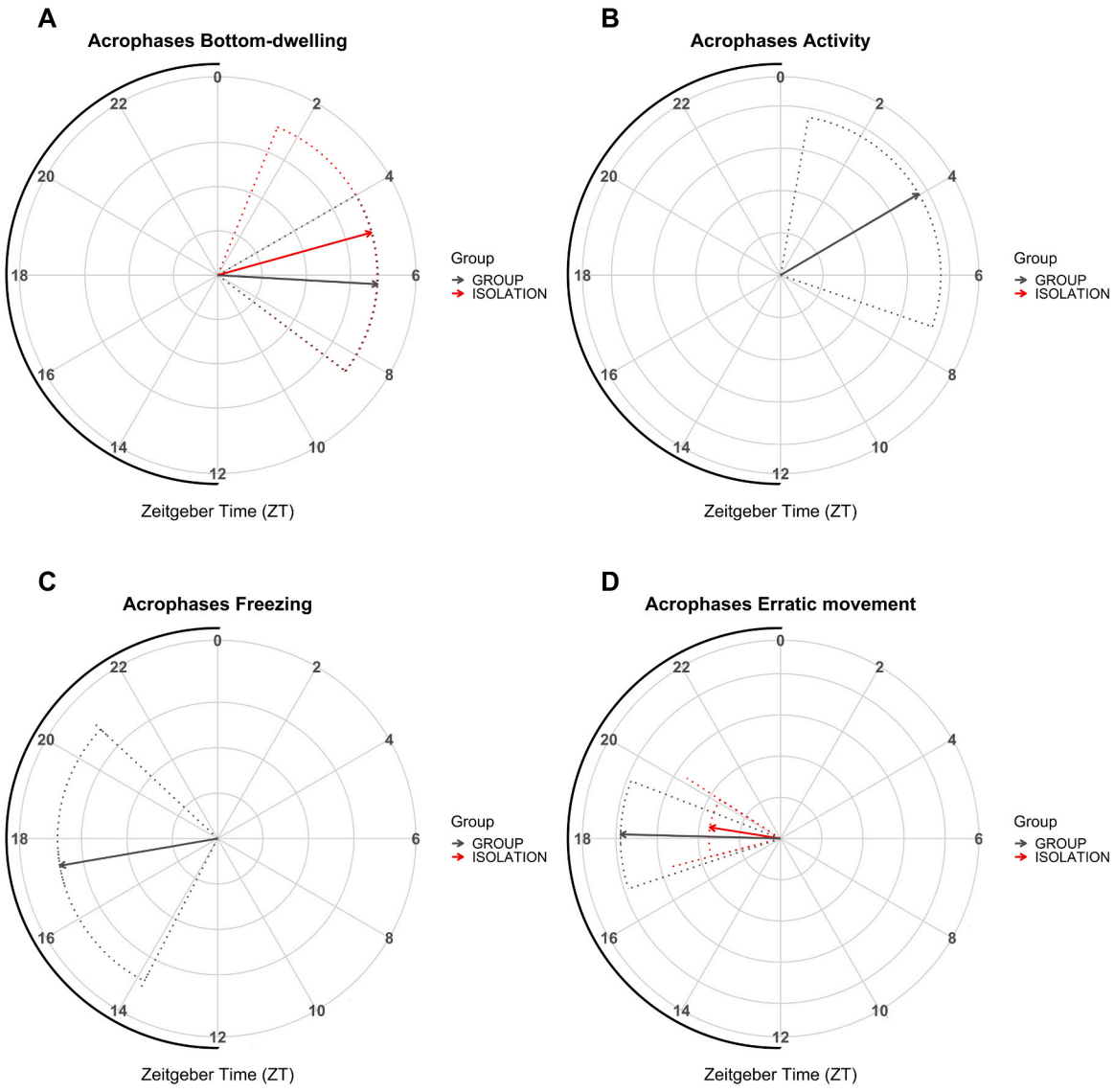


**Fig. 3.** Polarograms of estimated acrophases for significant daily behaviours in tilapia (Cosinor, $p < 0.05$). **A.** Bottom-dwelling. **B.** Activity. **C.** Freezing and **D.** Erratic movement. Solid arrows indicate the mean acrophase and dotted lines indicate the confidence interval (set at 95 %). The radial axis represents the time of the day (ZT) and the vector length represents the amplitude of the rhythm. The black line above radial axis represents the dark phase.

condition for all sampled time points (Tukey post-hoc test: Fig. 4D).

## 4. Discussion

This study investigated the buffering effect provided by social companions on the behaviour of two farmed species with opposed activity patterns (Nile tilapia and tench) throughout the daily cycle. We found general support for the presence of social buffering: group testing affected welfare indicators commonly used in fish for both tench and tilapia in 7 out of 8 cases. Moreover, isolation conditions disrupted daily rhythmicity in two behavioural indicators in tilapia. Critically, while in tench the social buffering was constant throughout the day, in tilapia social buffering varied between day and night in two indicators. We conclude that social buffering might vary throughout the 24 h cycle, but only in some species and considering certain indicators. These findings offer valuable insights into the welfare requirements of farmed species and highlight the importance of adapting management strategies to species-specific behavioural rhythms.

In tench, cosinor analyses found daily rhythms for all analysed behavioural indicators, both in group and isolation conditions. In line with previous literature, including observations in nature (Perrow et al., 1996), and with our preliminary recordings (Table S1), we conclude that the tench were mostly nocturnal in their activity. Note that some activities, such as feeding, have been reported to occur during the light phase, at least in the early morning hours (e.g., Bezmaternykh and Shcherbina, 2018; Herrero et al., 2005). However, due to our experimental approach based on specific ZTs, we could not detect this subtle behavioural variation. The acrophases were similar between the two social conditions, suggesting that the presence of social companions did not influence the rhythmic pattern of the behavioural indicators analysed.

In line with the presence of social buffering effects, the social condition affected the overall levels of the behavioural indicators over the 24 h cycle in tench, as evidenced by mesor comparisons and LMMs. In general, the effect indicated that social companions enhanced welfare by decreasing negative and increasing positive welfare indicators. Bottom-dwelling and freezing, two behaviours indicative of stress and negative welfare in teleost species (Chin et al., 2018; Levin et al., 2007; Maximino et al., 2010; Qiu et al., 2017; Thompson et al., 2016) and proposed as stress indicators in tench (Pintos et al., 2024a), decreased in the group

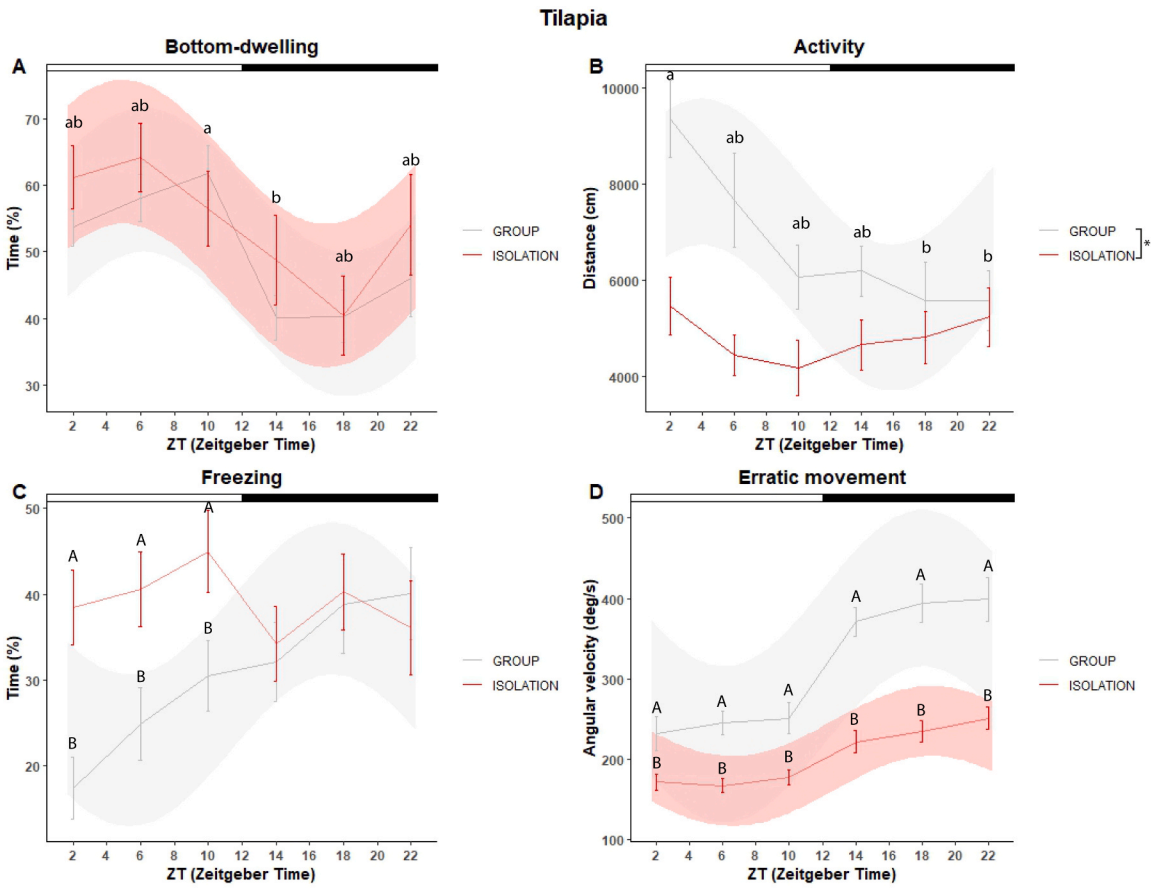


**Fig. 4.** Daily variation of tilapia behaviour according to social condition (i.e., isolation or group). A. Bottom-dwelling. B. Activity. C. Freezing. D. Erratic movement. Data points represent mean ± standard error. Asterisks (*) indicate significant main effects of social condition. Lowercase letters (a, b) indicate significant differences between ZTs. Uppercase letters (A, B) indicate significant differences between social conditions at the same ZT. Post-hoc differences were obtained by the Tukey HSD test performed on linear mixed models. Wave area plots represented predicted data based on significant Cosinor models. White and black bars above each graph represent light and dark phases, respectively.

condition. Moreover, the activity, which under some circumstances has been considered evidence of positive welfare in teleost species such as zebrafish *Danio rerio*, the mackerel *Scomber japonicus*, the bloodfin tetra *Aphyocharax anisitsi*, and the Nile tilapia (Golla et al., 2020; Nakamura et al., 2022; Pintos et al., 2021; Pintos et al., 2024b), was increased in the social condition. Therefore, these three indicators suggested that the group condition reduced stress behaviours and increased welfare in tench. Interestingly, erratic movement apparently deviated from the aforementioned pattern: whereas some studies supported it as an anxiety indicator in fish (Sireeni et al., 2020; Speedie and Gerlai, 2008), our findings detected increased values in grouped fish. However, this variable has evidenced controversial results and a weaker relation to anxiety (Blaser et al., 2010; Blaser and Gerlai, 2006; Gerlai et al., 2006). Therefore, it is possible this proxy was mainly influenced by other factors such as activity levels (Tran and Gerlai, 2016) or even the shoaling behaviour of the group rather than their stress level. Critically, all the effects of social condition observed in the tench were visible across the entire day (i.e., we did not detect significant interactions between social condition and time of the day). The general conclusion from the tench data is the presence of a social buffering effect that is consistent across the entire day, including the resting phase of the species.

Note that our findings on social buffering may appear to contrast with studies describing tench as not particularly social, showing little intra- or interspecific clustering (Vanovac et al., 2021). We interpret this divergence as reflecting context-specific variation in social behaviour. For example, Hoare et al. (2004) experimentally demonstrated that banded killifish (*Fundulus diaphanus*) tend to avoid social groups during foraging to reduce competition, but seek the safety of large groups in the presence of predator cues. Applied to our system, it is possible that tench are relatively unsocial under normal, safe conditions, but under potentially stressful circumstances, such as the experimental arena in our study, or captive environments more generally (Owen et al., 2010), they increase their reliance on social groups. Thus, the social buffering effects observed in this study may be partly explained by a greater reliance on conspecifics to cope with captivity-related stressors (Culbert et al., 2019). Future studies should explore long-term behaviour in tench exposed to different social conditions and further elucidate the role of conspecifics in improving welfare for this farmed species.

In the case of tilapia, behavioural indicators exhibited circadian rhythmicity in the group condition, indicating a diurnal pattern in line with natural observations (Piet and Guruge, 1997), previous laboratory studies (Fortes-Silva et al., 2010; Pintos et al., 2024a), and our preliminary recordings (Table S1). However, two indicators did not show rhythmicity in the isolation condition (i.e., activity and freezing). For instance, in the presence of social companions, tilapia followed the circadian activity pattern expected for a diurnal species (Iigo and Tabata, 1996; del Pozo et al., 2011; Ueda and Oishi, 1982). This rhythmicity was, however, absent in the isolation condition. In agreement, several studies showed that stressors (e.g., social isolation) can disrupt circadian processes across taxa, including locomotor activity patterns (mammals: Albrecht, 2010; birds: Chaturvedi et al., 2024; fish: Leliavski et al., 2014; López-Patiño et al., 2014; Ota et al., 2018; Pintos et al., 2024a). This is consistent with a group-living species such as Nile tilapia, which establishes complex social hierarchies and in which the

absence of this social structure has been linked to increased stress and poor welfare (Barreto et al., 2015; Gonçalves-de-Freitas et al., 2019). Together, these findings suggest that stable social conditions and social buffering effects might be important for developing natural behavioural rhythmicity and improving welfare in tilapia.

Furthermore, social buffering was evident in three behavioural indicators previously identified as sensitive to social companions in tilapia and as potential welfare measures: activity, presumed to reflect positive welfare, and freezing and erratic movements, which are usually associated with negative welfare (Pintos et al., 2024b). However, the effect was consistent throughout the day only in one indicator. In particular, activity was increased by group testing across the 24-hour cycle. Conversely, freezing was reduced by the social condition only during the light phase and erratic movement was increased by the group condition mostly at night. Whereas we believe that the effect of the erratic movement is difficult to interpret because of the arguments reported for the results on tench, the effect of freezing clearly demonstrates that the social buffering effect might vary across the time of the day. Certainly, our work suggests that this daily modulation of social buffering occurs only in limited circumstances.

Ecological and evolutionary factors may underpin the interspecific differences observed in the circadian modulation of social buffering. Some fish studies revealed that nocturnal benthic species often exhibit strict daily behavioural patterns, with no plasticity in response to different environmental conditions. For instance, two bottom dwellers and nocturnal teleost species, the burbot (*Lota lota*) and the stone loach (*Barbatula barbatula*), did not display changes in their nocturnal behaviour (i.e., foraging and activity) either in the presence/absence of predation risk and under starvation (Fischer, 2004). Similarly, our results showed that tench did not alter its behavioural rhythmicity regardless of social environment. Furthermore, the observed rhythmicity was consistent with that reported in tench under acute stress throughout the 24-h cycle (Pintos et al., 2024a). Therefore, this evidence would collectively suggest strict behavioural patterns and limited plasticity in nocturnal and bottom-dwellers fish species (Alaş et al., 2010; Erguden et al., 2010). Such low behavioural plasticity may help explain the inherently high stress responsiveness of tench in captivity and the difficulties in rearing this species under intensive farming conditions (e.g., overcrowding, barren environments; Owen et al., 2010).

In contrast, diurnal species often show a strong plasticity in their behavioural rhythms (Reebs, 2002). For instance, both seabass *Dicentrarchus labrax* and seabream *Sparus aurata* individuals that showed diurnal feeding could be turned into nocturnal by restricting food availability to the night-time (Sánchez-Vázquez et al., 1995; Vera et al., 2013). Similarly, the Atlantic salmon *Salmo salar* switch between diurnal and nocturnal foraging solely in response to environmental temperature (Fraser et al., 1993). Indeed, previous studies in Nile tilapia reported intraspecific variation and even nocturnal activity rhythms (Vera et al., 2009). Accordingly, our results also documented behavioural plasticity over the day in a diurnal species, underscoring for the first time its dependence on social conditions. Future work on tilapia behaviour should explicitly consider such time-of-day effects, particularly when assessing long-term social isolation (e.g., Waheed et al., 2023). Similarly, further studies should gather data on this species-specific behavioural plasticity in other fish species with different temporal habits (i.e., crepuscular) and social structures, including solitary species.

Besides these potential ecological explanations for the interspecific differences we observed, it is important to note that, although group size (i.e., the number of fish) was controlled in this study, stocking density (i.e., biomass per unit area) across conditions was not. Different species may respond differently not only to the number of conspecifics but also to the spatial constraints and perceived crowding associated with density. Therefore, we cannot rule out the possibility that some of the observed behavioural differences were influenced, at least in part, by species-specific responses to changes in density rather than purely by social interactions. This potential confound should be carefully considered in future research aiming to disentangle the effects of social buffering from density-related influences. Designing experiments that independently manipulate group size and density could provide more precise insight into how social and environmental factors interact to shape behaviour.

Our study bears three implications relevant to the welfare of captive fish. First, both species evidenced a social buffering effect by reducing behavioural indicators of welfare in the presence of conspecifics. As supported by previous studies, social rearing is an important enrichment strategy that can increase welfare in captive conditions and decrease the negative effects of manipulation and other farm stressful procedures in fish (Arechavala-Lopez et al., 2022; Cavallino et al., 2023; Saraiva et al., 2016; da Silva et al., 2020). Second, our work supports the hypothesis that the social buffering effect might vary across the day, although not in all species and not for all indicators. This effect requires specific investigations for each species of interest. Third, the behavioural indicators typically adopted to assess welfare in fish vary through the time of the day, and this variation depends on the diurnal versus nocturnal habit of the species. Therefore, caution should be adopted when using behavioural indicators of welfare in applied conditions.

## Author contributions

*SP*, *GDA* and *LMV* conceived the ideas and designed the methodology. *SP*, *GDA* and *FGC* collected the data. *SP* and *TLX* analysed the data and led the writing of the manuscript with contributions from all other authors. *FJSV*, *CB* and *LMV* supervised all stages of this work. All authors contributed critically to the drafts and gave final approval for publication.

## CRediT authorship contribution statement

**Cristiano Bertolucci:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Luisa María Vera:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Francisco Javier Sánchez-Vázquez:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Gonzalo De Alba:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Francelly Geralda Campos:** Writing – review & editing, Methodology. **Santiago Pintos:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tyrone Lucon-Xiccato:** Writing – original draft, Formal analysis, Data curation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2025.106895.

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