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Daily rhythms in the behavioural stress response of the zebrafish *Danio rerio*

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ABSTRACT

In nature, animals are exposed to stressors that occur with different likelihood throughout the day, such as risk of predation and human disturbance. Hence, the stress response is expected to vary plastically to adaptively match these challenges. Several studies have supported this hypothesis in a wide range of vertebrate species, including some teleost fish, mostly through evidence of circadian variation in physiology. However, in teleost fish, circadian variation in behavioural stress responses is less understood. Here, we investigated the daily rhythm of stress response at the behavioural level in the zebrafish *Danio rerio*. We exposed individuals and shoals to an open field test every 4 h over a 24 h cycle, recording three behavioural indicators of stress and anxiety levels in novel environments (thigmotaxis, activity and freezing). Thigmotaxis and activity significantly varied throughout the day with a similar pattern, in line with a stronger stress response in the night phase. The same was suggested by analysis of freezing in shoals, but not in individual fish, in which variation appeared mostly driven by a single peak in the light phase. In a control experiment, we observed a set of subjects after familiarisation with the open-field apparatus. This experiment indicated that activity and freezing might present a daily rhythmicity that is unrelated to environmental novelty, and thus to stress responses. However, the thigmotaxis was constant through the day in the control condition, suggesting that the daily variation of this indicator is mostly attributable to the stress response. Overall, this research indicates that behavioural stress response of zebrafish does follow a daily rhythm, although this may be masked using behavioural indicators other than thigmotaxis. This rhythmicity can be relevant to improve welfare in aquaculture and reliability of behavioural research in fish models.

1. Introduction

For many of the stressors that animals have to cope with, such as predation risk, intraspecific competition, and even human disturbance, marked variations throughout the day in occurrence and intensity have been reported [1–4]. To adaptively cope with these challenges, stress response might plastically vary according to a circadian rhythm. Several studies have provided support for this hypothesis in a range of taxa [5–7]. Most of this evidence consists of alterations in physiological traits [8–11], but circadian variation has also been reported in behavioural stress response [12–14]. For instance, Bilu and Kronfeld-Schor [15] have found that three nocturnal rodents display lower behavioural stress response during the night, whereas a diurnal species showed an inverse pattern of rhythmicity.

In fish, data on the influence of time-of-day on the behavioural response to stress is still scarce [16–18]. This is surprising considering that fish species such as the zebrafish *Danio rerio* have been gaining popularity as study models in neuroscience, genetics, biomedicine and toxicology, partly due to a highly conserved neural circuitry related to anxiety phenotypes and underlying mechanisms [19,20]. In these studies, behavioural paradigms measuring stress and anxiety-like states [21–23] are routinely used, but the influence of biological rhythms has been generally overlooked [16], thereby determining a potential confounding effect for research. Moreover, teleost fish are the vertebrates with more individuals held in captivity for alimentary purposes [24], and pisciculture is projected to expand substantially in the near future [25]. This determines the need to investigate the presence of daily variations in fish stress response to develop aquaculture protocols with reduced impact on fish welfare. For instance, if the stress response is lower at a certain time of the day, manipulation protocols might be performed in that period with reduced stress for the fish.

One of the most widely adopted behavioural paradigm to measure stress response in fish is the Open Field test (hereafter, OFt) [26–30]. In the OFt, the behavioural response evoked by a novel environment (i.e., an empty, unfamiliar arena) is assessed through different behaviours such as freezing, swimming activity, and the preference for the outer

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perimeter of the arena (i.e., thigmotaxis) [21,23,31,32]. The present study exploited the OFt to investigate the presence of daily rhythms in the behavioural stress response of zebrafish. With this aim, we observed individual zebrafish (experiment 1) and shoals formed by 5 individuals (experiment 2) in the OFt at different times of the day, every 4 h over a 24 h cycle. Thereafter, we performed a control experiment (experiment 3) in groups of zebrafish after they familiarised with the arena. This was done to determine whether the effects observed in the previous assays were due to the stress response elicited by the novel environment or to basal behavioural rhythms. We hypothesized that if zebrafish behavioural stress response to a novel environment varies depending on the time of the day, we should observe a different daily pattern between the first two experiments and the latter, control experiment.

2. Material and methods

2.1. Experimental subjects

Adult zebrafish (6–7 months old; $N = 341$) obtained from the fish facility of the University of Ferrara were exposed to a 12:12 h light-dark (LD) cycle (light on at 08:00 ["zeitgeber" time 0; ZT0] and light off at 20:00 [ZT12]) in community tanks (200 L, $N = 70$–80 fish per tank) maintained at constant temperature of 27 ± 1 °C. This exposure was performed in the Chronolab of the University of Ferrara, located in a room isolated from the facility and potential external synchronisers. In addition, the walls and the lid of each community tank were completely covered with black panels to avoid exposure of the zebrafish to external visual stimuli. All tanks were equipped with constant aeration and supplied with mechanical and biological filters. Fish were fed twice daily with *Artemia salina* nauplii in the morning (at ZT1; one hour after lights on), and commercial fish pellets (Vipan Nature, Sera GmbH, Heinsberg, Germany) in the afternoon (at ZT8; four hours after light off). Subjects used in the following experiments were randomly collected from the aforementioned fish.

2.2. Experiment 1 – individual zebrafish

Ninety-six zebrafish were tested in this experiment. Each subject was randomly assigned to one of the 6 testing time points: ZT2, 6, 10, 14, 18, 22. This resulted in 6 groups of 16 zebrafish individually tested every 4 h. ZT2 corresponded to 2 h after light on and 1 h after the first food administration of the day. Each fish was tested only once.

The experimental apparatus for experiment 1 consisted of a white plastic square arena (Open Field arena, OF; 40 × 40 × 8 cm) filled with 6 cm of dechlorinated tap water. This arena was placed on a backlight table illuminated with infrared LEDs (λ > 980 nm; Noldus Information Technology, Wageningen, The Netherlands). The experimental room was kept in darkness during the experiments. However, a white LED strip (Superlight Technology Co. Ltd., Shenzhen, China) was placed 1 m above the table and was switched on only during the daytime (ZT0–12). An infrared camera (Monochrome GigE camera, Basler, Germany; resolution: 1280 × 1024) was placed 1 m above the arena to record the experiments at 5 frames per second. A computer running the EthoVision XT software (Noldus Information Technology, Wageningen, The

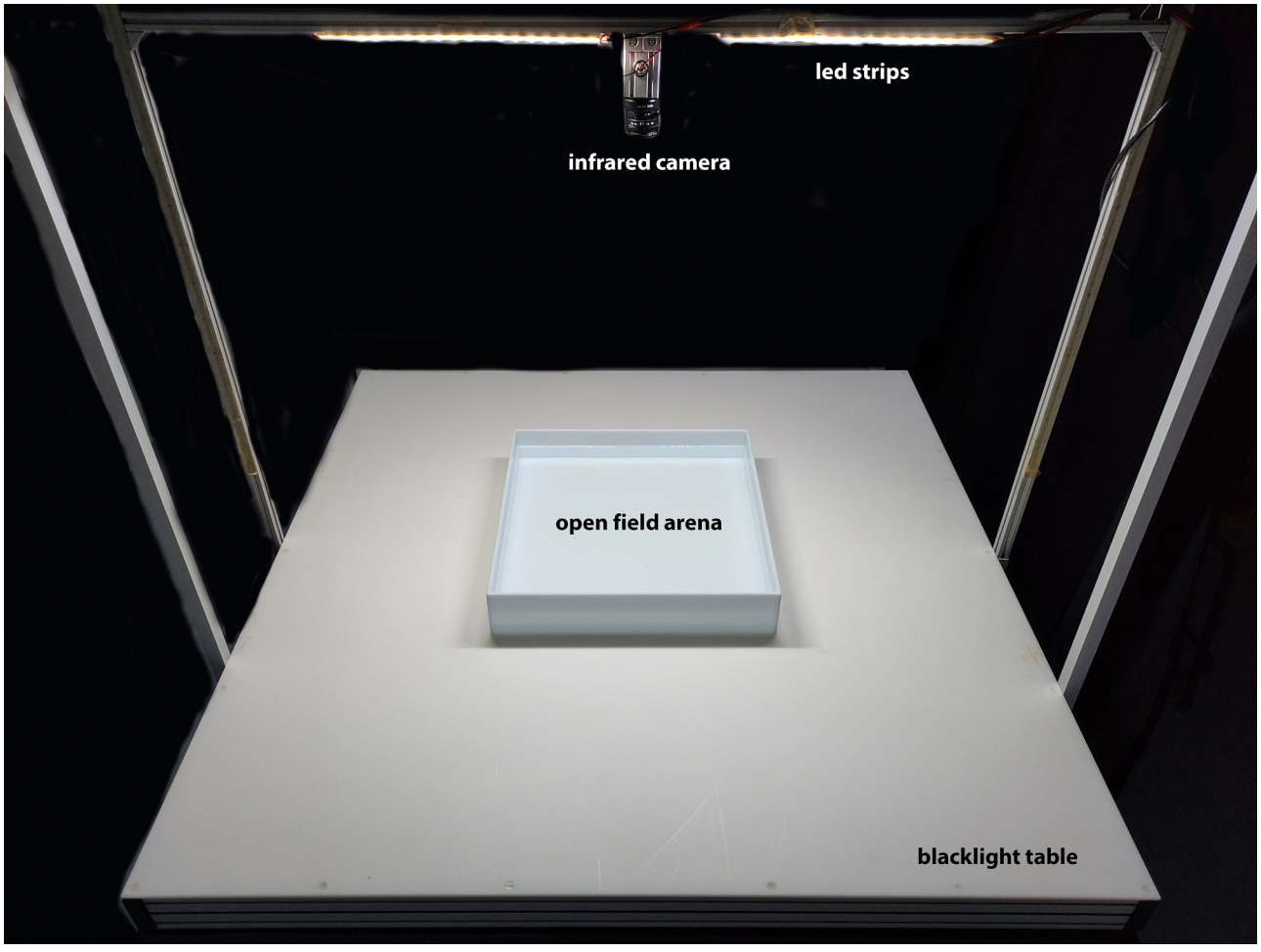

Fig. 1. Diagram of the experimental setup for the open field paradigm. A white plastic 40 × 40 × 8 cm square arena was placed on a blacklight table illuminated with infrared LEDs. An infrared camera was placed above the arena to record fish behaviour during the trials.

Netherlands) performed live tracking of subjects' behaviour (Fig. 1).

At the beginning of each trial, the subject was transported to the arena using an opaque jar, trying to minimise stress. The behaviour of each fish was collected for 10 min after the release into the arena and different dependant variables typically used to study fish anxiety-like behaviours under the OFt were measured by the EthoVision XT software [23,33]. These variables included the time spent in the outer part of the experimental arena considering the centre as a 20 × 20 cm square (thigmotaxis), the distance moved (activity) and the time spent not moving with a speed of 0.40 cm/s as threshold (freezing). The water was changed between trials to prevent exposure of a certain subject to the chemical cues of the previous subjects.

2.3. Experiment 2 – shoals

Forty-two shoals of 5 zebrafish were divided into 6 groups and tested according to each ZT point previously used in experiment 1 (ZT2, 6, 10, 14, 18, 22). Each subject was tested only once. The final sample size of the experiment consisted of 7 shoals per each ZT tested. The water was changed between trials. All the details of the testing conditions were kept as described in the previous experiment. The behaviour of each shoal was collected for 10 min after the release into the arena and the same variables used to describe anxiety-like behaviours in experiment 1 were measured by the EthoVision XT software. The software produced as an output the average of individual fish values for each behavioural variable.

2.4. Experiment 3 – control test in familiar arena

The control experiment was performed to evaluate shoal behaviour in the OF arena using fish previously acclimatised and in groups, which was expected to be the less stressing condition for a social species such as the zebrafish [34]. The comparison with experiments 1 and 2 was expected to allow interpreting the stress response due to exposure to the novel environment. Seven shoals of 5 zebrafish were acclimatised for 24 h in the experimental arena (40 × 40 × 8 cm; white plastic walls) and then, the behaviour of each shoal was independently recorded from ZT2 to ZT22. The same variables analysed in experiment 1 and 2 were measured for each ZT point (2, 6, 10, 14, 18 and 22) to evaluate control OFt performance throughout the time ($N = 7$ shoals/ZT). All behaviours were relativised to 10 min values and represented as an average of individual fish values.

2.5. 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). First, all behaviours analysed were subjected to the Cosinor analysis to evaluate daily activity rhythm using *cosinor* [35] and *cosinor2* [36] R packages. Cosinor analysis employs least-squares regressions to model cosine curves, which are useful to describe circadian variations [37]. Cosinor analysis 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). Rhythms parameters such as the mesor, amplitude and acrophase were calculated for each behavioural rhythm (Table 1). All acrophases were corrected to locate them in the correct quadrant [38] and subsequently transformed from radians to time values (i.e., ZT).

In addition, differences between time points (ZT) were studied by means of ANOVAs. One-way ANOVAs were conducted with the ZT as a fixed effect to analyse data of experiment 1 and 2. For experiment 3, a repeated measures ANOVA was performed to deal with the fact that each shoal was observed at different ZTs [nlme R package; [39]]. Model assumptions were verified by 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 rank-based (experiment 1; time in edge and freezing), logarithmic (experiment 2; distance travelled and freezing) or square root (experiment 3; freezing) transformation. When significant effects of ZT were found with the ANOVAs, Tukey *HSD* post-hoc tests were performed to identify the presence of marked differences between individual ZTs. Results of the post-hoc tests were reported in the relevant figures. Eta-squared (η^2) effect sizes were calculated to determine the magnitude of variation across ZTs. All data were represented as mean ± SEM and the significance level was set at $p = 0.05$. To explore differences in behaviour between experiments 1, 2 and 3, *t*-tests were performed comparing average values during the light and dark phases.

2.6. Ethical note

Experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the law of the country in which they were performed (Italy, D.L. 4 Marzo 2014, n. 26). The Ethical Committee of University of Ferrara reviewed and approved all the experimental procedures (protocol n. TLX-2019–1).

3. Results

3.1. Experiment 1 – individual zebrafish

On average, individual zebrafish spent 89.67 ± 1.38% (mean ± standard error) of the time close to the edges of the apparatus during the light phase and 94.06 ± 0.58% during the dark phase, denoting the expected thigmotaxis behaviour in both phases. Moreover, the zebrafish spent 5.16 ± 1.27% time not moving (i.e., freezing behaviour) during the light phase and 1.39 ± 0.60% during the dark phase. When moving, the overall distance (activity) per subject was 3345.18 ± 170.50 cm during the light phase and 2606.20 ± 119.35 cm during the dark phase.

The three behaviours analysed presented significant daily rhythms (all Cosinor $p < 0.01$; Table 1). Time in edge showed the acrophase close to the middle of the dark phase (ZT = 17.96). The ANOVA generally suggested less time spent at the edge of the arena during the light phase compared to the dark phase ($F_{5,90} = 2.36$, $p = 0.04$, Effect size $\eta^2 = 0.11$; Fig. 2A), although post-hoc tests did not highlight differences between pairs of ZTs (Fig. 2A).

Zebrafish showed a significant diurnal activity pattern under OFt in

Table 1
Cosinor parameters of behavioural indicators collected in experiments 1, 2 and 3 and obtained by Cosinor analysis. Data are presented as mean ± C.I. 95% and acrophases in time values (ZT). NS: non-significant.

	Time in edge (s)			Distance travelled (cm)			Freezing (s)		
Experiment	1	2	3	1	2	3	1	2	3
Rhythmicity	$p < 0.01$	$p < 0.01$	NS	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$
MESOR	551.19 ± 8.86	545.56 ± 7.67	–	2975.69 ± 193.85	2239.38 ± 169.46	1947.56 ± 141.76	19.93 ± 8.2	11.76 ± 5.59	55.65 ± 13.58
Amplitude	20.41 ± 12.49	20.01 ± 10.85	–	704.44 ± 274.15	753.09 ± 239.66	1256.08 ± 200.48	20.83 ± 11.57	10.71 ± 5.41	47.79 ± 19.21
Acrophase (ZT)	17.96 ± 3.47	17.23 ± 2.49	–	2.25 ± 1.5	6.08 ± 1	4.07 ± 0.91	9.78 ± 1.64	18.67 ± 2.34	16.57 ± 1.60

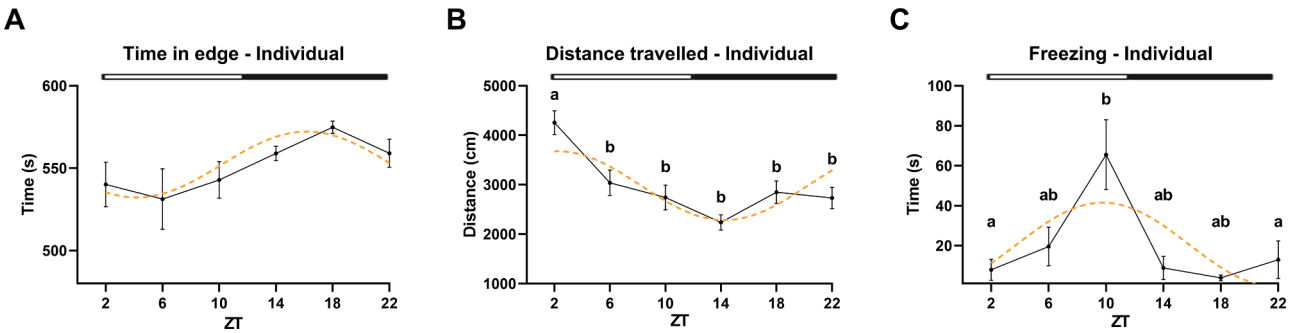

Fig. 2. Daily variations of individuals' behaviour in the open field test (experiment 1). **A.** Time in edge of the arena; **B.** Distance travelled; **C.** Freezing behaviour (time spent motionless). Dotted orange lines indicate significant daily rhythms ($p < 0.05$) based on best-fitting models calculated by Cosinor analysis. Data points are mean ± SEM. Different letters indicate statistical differences by means of Tukey *HSD* test. White and black bars above each graph represent light and dark phases, respectively.

terms of more distance travelled during the light phase than during the dark phase. The acrophase of activity was approximately at the beginning of the light phase (ZT = 2.25). The ANOVA on activity revealed significant differences between ZTs ($F_{5,90} = 3.75$, $p < 0.01$, Effect size $\eta^2 = 0.33$), which were mostly due to higher levels of activity at ZT2 (post-hoc tests: Fig. 2B).

The acrophase of freezing behaviour was approximately at the end of the light phase (ZT = 9.78). The ANOVA on freezing behaviour revealed significant differences between ZTs ($F_{5,90} = 8.96$, $p < 0.01$, Effect size $\eta^2 = 0.17$). This effect was due to higher levels of freezing at the end of the light phase, in correspondence of ZT10 (post-hoc tests: Fig. 2C).

3.2. Experiment 2 – shoals

On average, zebrafish shoals spent 88.10 ± 1.09% of time in the edges of the apparatus during the light phase and 93.74 ± 0.47% during the dark phase. In addition, shoals spent 0.70 ± 0.12% time not moving during the light phase (i.e., freezing behaviour) and 3.22 ± 0.61% during the dark phase. Last, shoal travelled 2804.62 ± 145.16 cm during the light phase and 1674.13 ± 67.01 cm during the dark phase. Freezing time during the light phase was lower in experiment 2 compared to experiment 1, and activity during the dark phase was lower in experiment 2 compared to experiment 1 (t-test; $p < 0.05$). No differences between the two experiments were observed for the thigmotaxis behaviour.

Significant daily rhythms were found for all the behaviours analysed (all Cosinor $p < 0.01$; Table 1). For the time spent in the edge, the acrophase was observed in correspondence of the middle of the dark phase (ZT = 17.23). In general, the rhythmicity was characterised by significantly higher values of time spent close to the edge during the dark phase compared to the light phase (ANOVA: $F_{5,36} = 5.31$, $p < 0.01$, Effect size $\eta^2 = 0.42$; post-hoc tests: Fig. 3A).

The shoals of zebrafish showed a diurnal activity pattern with an acrophase close to the middle of the light phase (ZT = 6.08). The ANOVA found significantly more distance travelled during the light phase than during the dark phase ($F_{5,36} = 10.75$, $p < 0.01$, Effect size $\eta^2 = 0.59$; Fig. 3B).

For the freezing behaviour, the acrophase was approximately in the middle of the dark phase (ZT = 18.67). The ANOVA further indicated significant rhythmicity in freezing behaviour in terms of differences between ZTs ($F_{5,36} = 7.06$, $p < 0.01$, Effect size $\eta^2 = 0.49$). The post-hoc tests (Fig. 3C) suggested that the largest differences were between ZTs of the light and those of the dark phase.

3.3. Experiment 3 – control test in familiar arena

On average, the shoals of the control experiment spent 84.46 ± 1.61% of time in the edges of the apparatus during the light phase and 85.33 ± 0.99% during the dark phase. Moreover, shoals spent 5.21 ± 0.90% time not moving (i.e., freezing behaviour) during the light phase and 13.34 ± 2.42% during the dark phase. When moving, the zebrafish swam 2728.86 ± 151.68 cm during the light phase and 1166.26 ± 123.44 cm during the dark phase. Thigmotaxis and activity values were lower compared to those observed in experiments 1 (in both light and dark phase) and 2 (in the dark phase), whereas freezing values were higher than in experiments 1 (in the dark phase) and 2 (in both phases) (t-test; $p < 0.05$).

The time spent in the edge did not exhibit daily rhythms (Table 1). Similarly, the ANOVA on this variable did not detect significant variation across the ZTs (ANOVA: $F_{5,36} = 0.02$, $p = 0.88$; Fig. 4A).

Significant daily rhythms were found for distance travelled and freezing behaviour (Cosinor $p < 0.01$; Table 1). The distance travelled

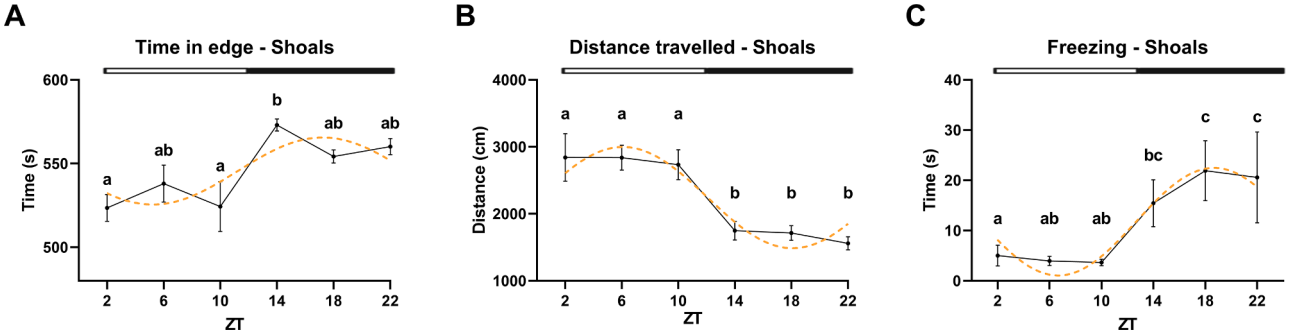

Fig. 3. Daily variations of zebrafish shoals' behaviour in the open field test (experiment 2). **A.** Time in edge of the arena; **B.** Distance travelled; **C.** Freezing behaviour (time spent motionless). Dotted orange lines indicate significant daily rhythms ($p < 0.05$) based on best-fitting models calculated by Cosinor analysis. Data points are mean ± SEM. Different letters indicate statistical differences by means of Tukey *HSD* tests. White and black bars above each graph represent light and dark phases, respectively.

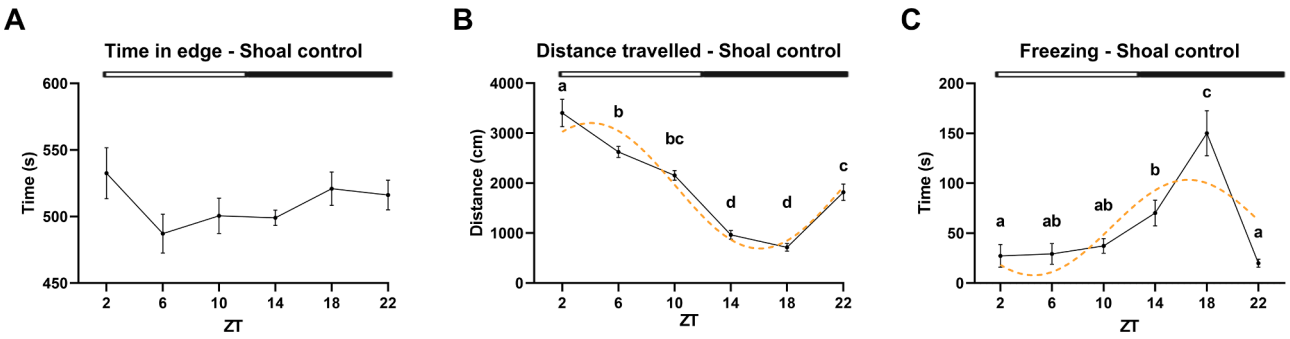

Fig. 4. Daily variations of shoals' behaviour after familiarisation with the open field arena (experiment 3). **A.** Time in edge of the arena; **B.** Distance travelled; **C.** Freezing behaviour (time spent motionless). Dotted orange lines indicate significant daily rhythms ($p < 0.05$) based on best-fitting models calculated by Cosinor analysis. Data points are presented as mean ± SEM. Different letters indicate statistical differences by means of Tukey *HSD* tests. White and black bars above each graph represent light and dark phases, respectively.

revealed a diurnal activity pattern with an acrophase at the beginning of the light phase (ZT = 4.07). The analysis also found significantly lower distance travelled in the dark phase (ANOVA: $F_{5,36} = 44.87$, $p < 0.01$, Effect size $\eta^2 = 0.86$; post-hoc tests: Fig. 4B).

Last, the rhythmicity of the freezing behaviour presented its acrophase close to the middle of the dark phase (ZT = 16.57), reaching approximately 25% of time (150 ± 59.72 s) at ZT18. The rhythmicity was also detected by the ANOVA ($F_{5,36} = 4.86$, $p = 0.03$, Effect size $\eta^2 = 0.10$) and the post-hoc tests (Fig. 4C) suggested marked differences in freezing between ZT18 and the other ZT points.

4. Discussion

This study investigated how well-established behavioural indicators of stress and anxiety measured in the OFt vary according to the time of the day in a teleost fish, the zebrafish *D. rerio*. Results support the presence of circadian rhythmicity in the behavioural stress response in zebrafish, although they also point out that some behavioural indicators may be unreliable for this type of analysis.

Our first two experiments showed that the three behavioural indicators measured following exposure to a novel environment exhibited daily variations. This was true for zebrafish tested both individually and in shoal. For thigmotaxis behaviour, probably the most used anxiety-like behaviour in open field paradigms [23,33], the responsiveness appeared stronger during the night compared to the day; i.e., the maximum values of thigmotaxis were found during the dark phase. Thigmotaxis is operatively measured as time spent in the edge of the apparatus. Therefore, at night, the zebrafish were less likely to leave the edges of the apparatus, which they probably perceived as safer [31,40-42]. This apparently suggests increased stress and/or anxiety during the dark phase. Although overall significant, the behavioural rhythmicity was possibly smoother for the individual subjects compared to the shoals, as evinced from the lack of significant differences between individual ZTs in experiment 2. Regarding activity, in experiments 1 and 2 zebrafish showed lowest values during the dark phase with significantly more distance travelled during the day. These differences may be interpreted as evidence of greater responsiveness to the OFt at night [43-45; see also 46-47]. The last behavioural indicator measured, freezing as time spent motionless, showed results aligned with thigmotaxis and activity data for experiment 2, but a less clear pattern for experiment 1. While freezing was higher at night for the shoals of experiment 2, it was higher at the evening for individual zebrafish of experiment 1. As the former two parameters, freezing is also considered an anxiety and stress response induced by the OFt performed during daytime [23,33,48]. Therefore, these results would suggest a greater stress response in individual fish during the evening which was not observed in grouped zebrafish across daytime. Although we cannot be certain of the cause of this discrepancy, we speculate that it could be driven by the calming effect of conspecifics in social fish species: the zebrafish tested individually in experiment 1 were probably exposed not only to the stress derived from the novel environment but also from that due to social isolation [34,49-51]. However, considering that the circadian rhythmicity in freezing of experiment 1 was possibly due to a peak on a single ZT, experimental confounds cannot be ruled out.

Before interpreting the results of experiments 1 and 2, it is worth considering that some behavioural measures collected in the OFt may vary during the day also because of biological factors not related to stress. For instance, activity of diurnal species such as the zebrafish is generally higher during the day as compared to the night [52–57]. Activity and freezing might also be significantly affected and biased by sleeping patterns during the resting phase of the species [58,59]. We therefore performed experiment 3 with zebrafish familiarised in the OF arena before the behavioural recording. Hence, this experiment allowed us to record eventual rhythmicity that is not due to stress and compare it with that observed in the previous experiments. When comparing the results of experiment 3 with those of experiments 1 and 2, it emerged that activity followed roughly the same daily pattern despite average values in both phases appear to reflect the effect of stress between familiar and unfamiliar arena conditions. The same could be concluded considering freezing behaviour in experiments 2 and 3. Critically, in experiment 3, we found no significant daily rhythmicity for the thigmotaxis behaviour along with lower average values of this variable. Taken together, results of these comparisons suggested us to refrain from interpreting the daily pattern of activity and freezing in the light of stress response, because they are likely affected by other rhythmicity that occurs in non-stressing situations (i.e., testing in a familiar environment). However, the thigmotaxis daily pattern of experiments 1 and 2 can be reasonably attributed to stress response because it was absent in a less stressing condition of testing as the familiar environment (experiment 3). This suggests that there is no differential preference for the edges throughout the day, but daily variations in thigmotaxis are triggered in response to stressful conditions.

Based on the thigmotaxis measures, as discussed above, the main conclusion of our study is that zebrafish might be more behaviourally responsive to stressors presented in the dark phase, which represents the resting phase for this diurnal species. This conclusion relies on the interpretation that thigmotaxis is a reliable anxiety-like behaviour from a chronobiological approach, which derives from experiment 3′s results. In support to our interpretation, a recent study has revealed that activity and immobility variables were not consistent across different behaviour tests in the zebrafish, cautioning against their use as proxies for anxiety in fish [60]. However, other studies have highlighted that thigmotaxis might not always accurately reflect anxiety states [e.g., 21,61]. The uncertainty on the significance of behavioural indicators of stress can be at least partially overcome with a research approach that includes also other types of indicators. While we did not collect alternative stress

indicators, an earlier study on zebrafish did so. Manuel et al. [62] found increased whole-body cortisol and expression levels of corticoid receptor genes in zebrafish in response to stress experienced during the resting phase compared to the diurnal phase. Therefore, behavioural and physiological indicators converge in supporting greater sensitivity to stress during the night in zebrafish. Interestingly, in the sole *Solea senegalensis* and the sea bream *Sparus aurata*, the physiological response to an acute stress event was also higher during the resting phase of the species and presented daily variations [63,64]. However, this pattern differed in other fish species. Some studies in fish have indicated that the daily cycle has little influence on the acute stress response [Chinook salmon, *Oncorhynchus tshawytscha*; 65] or more impact during the active phase of the species [Green sturgeon, *Acipenser medirostris*; 66]. Therefore, daily rhythms of the stress response are likely species-specific, calling for further research on its evolutionary and ecological causes.

The implications for the daily variation in stress responsiveness in teleosts are mostly two. First, given the large use of species such as the zebrafish in applied research with behavioural tests measuring stress-related responses such as the open field [67–70], it became critical to control for the time of the day at which experiments are performed to avoid confounding effects. Second, considering the stronger impact of stress during specific phases of the day, it is possible to hypothesise that welfare of individuals held captive in aquaculture conditions would be improved if manipulations were performed in moments of less responsiveness. Future studies on fish welfare should attempt to verify this possibility. Moreover, our study described the rhythmicity in stress response but did not analyse the synchronising mechanism, while one may be tempted to suggest that the light/dark alternation triggered the synchronisation of the behavioural stress response. However, evidence suggests that this parameter might be also sensitive to feeding [71,72]. It is therefore possible that the time of the day at which the food was delivered determined the observed daily variation in zebrafish stress response, a hypothesis that can be investigated with mismatch experiments.

Declaration of Competing Interest

The authors declare no competing or financial interests.

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