Differential impact of artificial light at night on cognitive flexibility in visual and spatial reversal learning tasks

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Artificial light at night (ALAN) is a pervasive environmental pollutant with multiple adverse effects on animal biology. As the vertebrate nervous system is particularly sensitive to light effects, this study explores the potential negative impact of ALAN on cognition in adult zebrafish, *Danio rerio*. Fish from mesocosm populations exposed to either ALAN or control conditions underwent visual and spatial learning tasks, along with an evaluation of cognitive flexibility with visual and spatial reversal learning tasks. Contrary to our initial prediction of a general negative impact, learning performance was not affected by the ALAN treatment. The analysis of reversal learning revealed task-dependent effects on cognitive flexibility: fish exposed to ALAN performed worse than control fish in the visual reversal learning task, but displayed better performance in the spatial reversal learning task. We propose that the influence of ALAN on cognition might differ between different cognitive functions or involve noncognitive factors that play different roles in the testing paradigms. The study underscores the importance of task specificity in nonadaptive cognitive plasticity and calls for ecological assessments to quantify fitness consequences of ALAN in natural settings.

Artificial light at night (ALAN) is recognized as a pervasive source of pollution across terrestrial, freshwater and marine ecosystems (Cox & Gaston, 2023; Marangoni et al., 2022; Moore et al., 2006). The list of negative impacts on animals' biology due to exposure to ALAN is growing rapidly (Gaston et al., 2015, 2021; Maggi et al., 2020; Sanders et al., 2021). For instance, impacts on reproduction (Fobert et al., 2019), migration (Vowles & Kemp, 2021), circadian activity (Duarte et al., 2019; Pulgar et al., 2019; Wolkoff et al., 2023), behaviour (Czarnecka et al., 2022; Kurvers et al., 2018; Pulgar et al., 2023) and metabolism (Velasque et al., 2023) are known. Considering the sensitivity of the vertebrate nervous system to environmental light signals (Lee et al., 2020; Vandewalle et al., 2009), ALAN's potential impact on cognition is also concerning (Kumar et al., 2023; Liu et al., 2022; Sangma & Trivedi, 2023). Cognition plays a crucial role in animals' interactions with the environment and is often linked to fitness (Cole et al., 2012; Huebner et al., 2018; Rochais et al., 2023; Smith et al., 2015), which makes such concerns significant.

A recent study found that Indian house crows, *Corvus splendens*, exposed to ALAN required more training sessions compared to subjects exposed to standard conditions (i.e. no light at night) to learn the position of a food reward on an array of six locations, both in the presence and in the absence of visual cues (Buniyaadi et al., 2022). These results suggest that spatial learning was impacted by ALAN. The same study found that ALAN increased the latency of crows to remove a dish hiding a food reward, indicating a further potential impairment in problem solving (Buniyaadi et al., 2022). Conversely, there was no impact of ALAN on problem solving in other avian species, such as the peafowl, *Pavo cristatus* (Yorzinski et al., 2017). In great tits, *Parus major*, ALAN exposure led to reduced performance in a task measuring inhibitory control, which is the ability to withhold a behaviour not adapted to the situation (Meijdam et al., 2023). In another vertebrate taxon, a recent study demonstrated that ALAN impacted a simple learning function,

habituation learning, in larvae of a teleost fish, the zebrafish, *Danio rerio* (Lucon-Xiccato, De Russi, et al., 2023).

The aforementioned results collectively suggest that the impact of ALAN on cognition may vary depending on the type of task and/or cognitive function involved. They additionally indicate that ALAN affects not only the learning functions but also a family of cognitive functions referred to as executive functions, which include inhibitory control and problem solving (Diamond, 2013). In humans, executive functions are mostly considered nonspecific processes. In other words, a certain executive function can be activated in a range of different tasks (Diamond, 2013; Packwood et al., 2011). However, no consensus has been reached on this point in the literature (Bobrowicz & Greiff, 2022). In the present study, we aimed to elucidate the function-specific impact of ALAN on animal cognition by simultaneously analysing multiple traits in zebrafish. In contrast to the earlier study in this species (Lucon-Xiccato, De Russi, et al., 2023), we assayed adult individuals instead of larvae. This was done because larval zebrafish display a limited behavioural repertoire that prevents assaying several cognitive traits. Teleost fish exhibit remarkable cognitive plasticity across various functions (Brandão et al., 2019; Fontana et al., 2021; Kotrschal & Taborsky, 2010; Lucon-Xiccato, Montalbano, & Bertolucci, 2023; Montalbano et al., 2022; Salvanes et al., 2013; Spence et al., 2011; Toni et al., 2019; Triki et al., 2024), including responses to environmental stressors (Domenici et al., 2012; Knecht et al., 2017; Lucon-Xiccato et al., 2014; Lucon-Xiccato, Savaşçı, et al., 2023; Moreira et al., 2023; Naderi et al., 2018; Pilehvar et al., 2020; Silveira et al., 2023; Sørensen et al., 2013). Moreover, fish retain abundant neurogenesis and neural plasticity as adults (Pushchina et al., 2024). Therefore, we anticipated that our study species would respond to the ALAN treatment with alterations in cognitive performance even at the adult stage.

In this study, we exposed mesocosm populations of zebrafish to either ALAN or control conditions and subsequently characterized the cognitive phenotype of the subjects. Characterization involved assessment of learning in the context of visual and spatial discrimination along with an executive function, cognitive flexibility, for visual and spatial tasks (Arthur & Levin, 2001; Culbert et al., 2021; Fuss & Witte, 2019; Lucon-Xiccato & Bisazza, 2014; Miletto Petrazzini et al., 2017; Montalbano et al., 2022; Vila Pouca et al., 2021). We expected that, because of the stress of living in an environment with an altered light cycle, ALAN-exposed fish would perform worse than control fish in the cognitive assays (Maille & Schradin, 2017). Our approach allowed us to explore the effects of ALAN on individual cognitive differences as well, by analysing covariation between performance in different tasks (Lucon-Xiccato & Dadda, 2017; Montalbano et al., 2020; Prentice et al., 2022; Vila Pouca et al., 2022; Wallace & Hofmann, 2021). Individual differences are indeed predicted to be affected by environmental stressors (Killen et al., 2013).

METHODS

Ethical Note

This study was approved by the Institutional Animal Care and Use Committee of the University of Ferrara (auth. n. TLX/2022-1). The behavioural observations were designed to avoid stress and discomfort to the subjects and followed the ASAB/ABS Guidelines for the Use of Animals in Research (https://doi.org/10.1016/j.anbehav.2019.11.002).

The subjects were monitored throughout the experiment to ensure that they did not show signs of distress. Environmental enrichments, such as natural gravel and continuous oxygenation, were provided to improve fish welfare. The setting ensured minimal contact between the subjects and experimenter to reduce stress. All fish used in the experiment were naïve to the experimental protocol. After completion of the experiment, all fish were released in maintenance tanks.

Subjects

We examined 24 adult zebrafish (8–10 months old) from a wild-type population ('Ariosto' strain) bred in the laboratories of the University of Ferrara. This population was established in 2011, starting with 100 individuals purchased from a local shop and currently comprises around 500 individuals housed in 200-litre aquaria equipped with mechanical, biological and chemical filters. The water temperature was maintained at 27 ± 1 °C, and the fish were fed twice a day with commercial flakes (Vipan Nature, Sera, Germany) and live brine shrimp nauplii, *Artemia salina*.

Experimental Treatments

Before conducting the cognitive testing, we exposed the zebrafish to the treatment conditions for 2 months. Previous studies showed that this period is long enough to observe the photic entrainment of this species (Idda et al., 2012; Morbiato et al., 2019). The fish were randomly collected from the maintenance tanks and moved into four treatment aquaria (55 × 33 cm and 33 cm high; water level: 28 cm; N = 20 subjects per aquarium). The aquaria walls were covered in black plastic and a black plastic lid was used to prevent external disturbances; the aquaria were kept in an isolated, dark room at 27 ± 1 °C. We exposed two treatment aquaria to control conditions (12:12 h light:dark [LD] cycle) and two treatment aquaria to ALAN conditions (12:12 h light:ALAN cycle). The illumination was provided by white light-emitting diode (LED) strips (TMR, ELCART, Italy) glued to the lid of the aquaria. During the light phase (i.e. day), in both control and ALAN aquaria, one large LED strip was turned on, providing 45.3 lx (0.358 W/m²). In the control aquaria, the LEDs were turned off during the dark phase (i.e. night). In the ALAN aquaria, a small LED strip was turned on during the night phase, providing 3.3 lx (0.026 W/m²) in line with field measures reported for urban freshwater habitats impacted by ALAN (Brüning et al., 2015; Perkin et al., 2014). All treatment aquaria were equipped with water filters and aeration, and food was provided as described for the maintenance conditions.

Apparatus for the Cognitive Testing

To conduct the cognitive assays, we individually transferred six zebrafish per treatment aquarium (total N = 24 zebrafish) into apparatuses made of dark grey plastic (30 × 40 cm and 22 cm high; water level: 18 cm). Each apparatus housed a single subject to allow individual testing. The apparatus was provided with an aerator and had a gravel bottom. The subjects from the two treatments (control and ALAN) were housed in these apparatuses under the respective treatment condition (control treatment: 12:12 h LD; ALAN treatment: light:ALAN 12:12 h) for the entire experimental period. We maintained the apparatuses with the fish from the two treatments in two separate rooms to allow for the different illumination. The temperature in the experimental rooms was set at 27 ± 1 °C. During the intervals between experimental sessions, we covered the apparatuses with a net to prevent the fish from jumping out. The net and the aerator were removed from each apparatus 1 h before the start of the testing sessions. Water was changed every 7 days and when required due to evaporation.

Procedure of the Visual Discrimination Learning Task

The ability to discriminate colours has been previously reported in zebrafish (Colwill et al., 2005; Gatto et al., 2020; Lucon-Xiccato et al., 2022; Parker et al., 2012). In our experiment, each subject underwent a series of trials in which we presented two colour stimuli. The subject had to choose the predetermined correct colour to obtain a food reward. The duration of the task was not fixed but varied according to the performance of the subject (i.e. it lasted until the subject reached a learning criterion, described below in this section).

The task started 6 days after the subject was introduced into the apparatus for habituation. On the sixth day, we placed two transparent plastic sectors with a trapezoidal shape inside each apparatus (Fig. 1a). These sectors divided the apparatus into two large main sectors connected by a narrow central corridor. The central corridor served to force the subject to swim through the centre of the apparatus before choosing between the two stimuli. After the insertion of the sectors, the subject was fed and left undisturbed until the following day. We then performed 2 days of habituation to the procedure. In the first day of habituation, we presented the rewarded stimulus (i.e. only a single colour) to each subject for 12 trials. The 12 trials were divided into two sessions separated by a 3 h interval, with six trials in the morning session (0900—1100 hours) and six trials in the afternoon session (1400—1600 hours). Within a session, consecutive trials took place after a 10 min interval from the previous trial. The stimulus consisted of a yellow or blue plastic disc (diameter = 1.0 cm) glued at the end of a transparent plastic support (26 × 2 cm). The colour of the rewarded stimulus was randomly assigned and counterbalanced across subjects and treatments. In a control analysis, we found that the rewarded colour assigned did not significantly affect subjects' visual learning and reversal learning performance (Wilcoxon rank sum test: $W = 97.5, P = 0.137$; $W = 48.0, P = 0.278$, respectively). In each trial, the experimenter inserted the support with the stimulus in the water on one of the short sides of the apparatus, which was counterbalanced between trials. The stimulus was presented only when the subject was in the main sector opposite to that predetermined for the trial.

When the subject approached the stimulus (i.e. swam closer than one body length facing the stimulus), the experimenter administered a small food reward, approximately three or four brine shrimp nauplii suspended in water, using a Pasteur pipette. After the administration of the reward, the stimulus was left in the tank for 5 min in an attempt to strengthen the association between the colour and reward. Each trial lasted for a maximum of 15 min. If the subject did not approach the stimulus within this period, the trial was considered null and repeated later. On the second day of the habituation (i.e. on day 8 after moving the subjects into the apparatus), the experimenter conducted 12 trials presenting both colour stimuli simultaneously, one on the right and one on the left corner of the short wall of the predetermined main sector (Fig. 1a). The right—left side of the correct stimulus was randomized between trials. If the subject approached the correct stimulus, the experimenter administered the reward in front of the correct stimulus. If the subject approached the incorrect stimulus, the trial continued until the subject chose the correct stimulus and obtained the reward or up to a maximum of 15 min. During this period, the subject could potentially choose the incorrect stimulus multiple times with no consequences. If the subject did not make a choice within 15 min, the trial was considered invalid and repeated later. After administering the reward, the experimenter removed the incorrect stimulus and left the correct stimulus in the apparatus for 5 min.

On the following day (on day 9 after moving the subject into the apparatus), the learning performance assessment began. The procedure of each trial resembled that of the previous day (i.e. the second habituation day). However, if the subject chose the incorrect stimulus, the experimenter removed both stimuli and did not administer the food reward. Each trial was considered completed after the subject's first choice and no correction was allowed. We performed 12 trials per day and recorded the subject's first choices (i.e. correct or incorrect colour). The subject underwent the same procedure until it reached the significant learning criterion of 17 correct choices out of 24 trials over the course of 2 consecutive days (chi-square test: $X^2 = 4.167, P = 0.041$). We recorded the day on which the subject reached the learning criterion as an additional measure of performance. The maximum period for attaining the
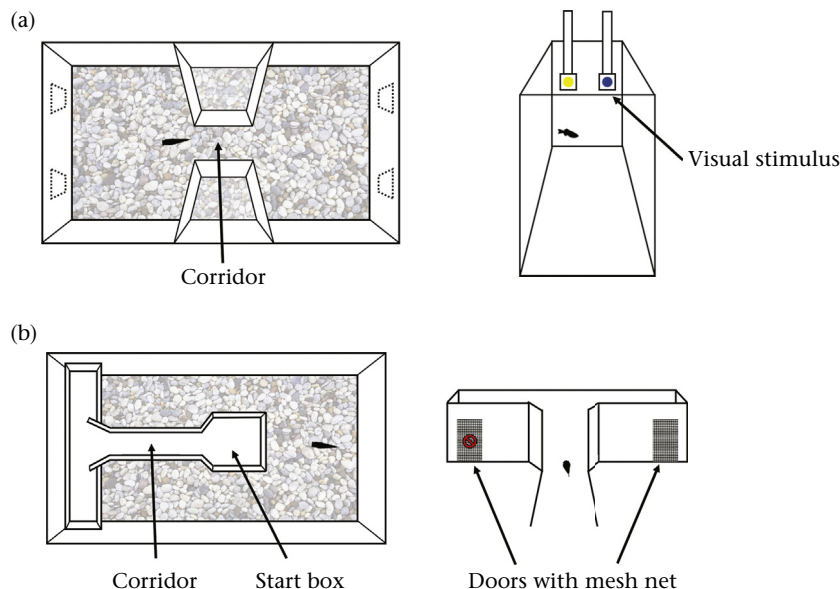

Figure 1. Diagrams of the experimental apparatuses. (a) Aquarium used to train the fish in the visual discrimination learning and visual reversal learning task. (b) Aquarium with the T-maze apparatus used in the spatial learning and spatial reversal learning task.

criterion was set at 30 days. Subjects that failed to meet the criterion were not used in the following tasks.

Procedure of the Visual Reversal Learning Task

After a subject met the learning criterion of the visual learning task, we administered the reversal learning task, following Montalbano et al. (2022). The experimenter performed 12 trials per day with the same modalities of the learning experiment, but with a reversed colour—reward association. Therefore, the subject had to learn to choose the colour that was previously not rewarded. For this visual reversal learning task, we adopted the same criterion and maximum number of testing days used in the previous visual learning task and collected the same variables (number of days to reach criterion and correct or incorrect choices in each day).

Procedure of the Spatial Discrimination Learning Task

The visual reversal learning test was followed by a day without testing and then we commenced the spatial learning task. We followed a paradigm previously exploited in different studies in teleost species (Lucon-Xiccato et al., 2022; Wang et al., 2020). First, we removed the trapezoidal sectors used for the visual tasks. Then, we inserted a white plastic T-maze into the apparatus (Fig. 1b) that was suspended 1.5 cm below the surface of the water. The T-maze consisted of a 9 × 6.5 cm start box leading to a 12 × 3 cm corridor and a choice sector from which the subject could either turn left or right to enter one of the two identical arms (9.5 × 4 cm). The choice sector was larger than the corridor to minimize the risk that a subject performed a left—right choice by swimming close to one of the walls. Both arms of the maze ended with a door. As the two doors were positioned inward, the subject was unable to see them when choosing between the two corridors (Fig. 1b). The door of one arm, pseudorandomly predetermined for each subject, was blocked by a mesh net. For each subject, the blocked door was the same for the entire learning task (but it was switched in the reversal task, see below). The other door allowed the subject to return to the main area of the apparatus. It was provided with a mesh net that was cut into 0.5 cm strips so that the fish could swim through them. The rewarded arm assigned to each subject did not significantly affect its spatial learning and spatial reversal learning performance (Wilcoxon rank sum test: $W = 79.5$, $P = 0.375$; $W = 72.0$, $P = 0.152$, respectively). Between sessions and during the night, the two doors were blocked with a plastic panel to avoid the subject swimming through them.

From the day after inserting the maze, the experimenter administered 12 spatial learning trials per day, which were divided into two sessions as explained for the visual tasks. To start each trial, the experimenter collected the zebrafish with a net and placed it into the start box. The subject was then free to swim until it reached the open door and exited the maze. In this procedure, the motivation to exit the maze was the opportunity to return to the main sector of the apparatus, which was familiar to the subject, had significantly deeper water and environmental enrichments, and was the place where the food was administered (Lucon-Xiccato & Bisazza, 2017; Miletto Petrazzini et al., 2017). Using the subject's head as reference, we recorded the first arm of the maze entered as correct or incorrect choice. In each trial, the subject could correct an initial wrong choice within 15 min. This was done to ensure that the subject returned to the main compartment. It was not possible to prevent the second choice (as in the visual learning task), because this would require the experimenter to manually move the subject back to the main compartment, potentially causing it unnecessary stress. The learning procedure continued until the subject reached the learning criterion of 17 correct choices out of 24 trials in 2 days. We also recorded the number of days to reach the criterion of each individual. The maximum period for attaining the criterion was set at 30 days. Subjects that failed to meet the criterion were not used in the following task.

Procedure of the Spatial Reversal Learning Task

After a subject met the learning criterion of the spatial learning task, we administered the spatial reversal learning task following the protocol designed by Miletto Petrazzini et al., (2017). By switching the mesh net, we switched the open door between the two arms of the T-maze. Therefore, the subject had to learn to select the arm that was previously unrewarded to return to the main sector of the apparatus. The criterion for the completion of the spatial reversal learning task and maximum number of testing days allowed were the same as the previous spatial learning task, as were the performance metrics collected (number of correct choices on each day and number of days to reach the criterion).

Statistical Analyses

Analyses were performed in RStudio (version 2023.03.1+446), with two-tailed tests and threshold for significance at $P = 0.05$. We first conducted a preliminary test with a simulation approach to ensure that the number of subjects reaching the learning criterion in each task was not due to chance. For each task, we generated 'simulated subjects' as sets of random binomial trials. The simulated subjects in each task corresponded to the number of subjects involved in the real experiment. The sets of random binomial trials of each simulated subject were clustered in groups of 12 sequential trials (corresponding to the number of daily trials administered to the fish in the experiment). The number of clusters (i.e. the simulated days) corresponded to the observed maximum number of days to reach the criterion in the task. We then replicated the simulation 10 000 times per each task and counted the simulated subjects that reached the learning criterion in each simulation. We then computed a P value indicating the probability of obtaining the observed number of subjects reaching the learning criterion due to chance as: (number of simulations with a number of successful simulated subjects > number of observed successful subjects)/ number of simulations. A significant P value would indicate that the results obtained in the study were unlikely to be due to subjects reaching the learning criterion because of random performance fluctuations.

Thereafter, we analysed the data collected in each task using a similar two-step approach. As the first step, we compared the number of days taken by the subjects from the two treatments to reach the learning criterion using Wilcoxon rank sum tests. Then, we performed an analysis that also considered the increase in performance across days of testing, which was considered an indication of learning. Because the choice of the subject followed a binomial distribution, as the dependent variable we used a matrix with the number of errors and the number of correct choices performed on each day of testing. We analysed this variable with a generalized linear mixed-effects model (GLMM) with binomial error distribution ('glmer' function of the 'lme' R package). Treatment and day of testing were fitted as the fixed effects, and subject ID as the random effect. We expected to find a significant treatment*day of testing interaction if the two experimental groups of fish had different learning curves.

We additionally performed an analysis focusing on the presence of individual cognitive differences across tasks. For this, we ran four analysis of covariance (ANCOVA) models fitted with the individuals' performance on one task (number of days to criterion) as the

dependent variable and performance in another task as the predictor. In each model, the performance in the task administered later during the study was fitted as the dependent variable and performance in the earlier task as the independent variable. The combination of tasks contrasted was as follows: (1) visual reversal versus visual learning; (2) spatial reversal versus spatial learning; (3) spatial learning versus visual learning; and (4) spatial reversal versus visual reversal. These combinations allowed us to understand whether some individuals were generally more efficient in (1) the visual tasks, (2) the spatial tasks, (3) the learning tasks or (4) the reversal tasks. In each of these models, we also fitted the

treatment as a factor to study whether it modulated the relationship between the performances in the different tasks.

RESULTS

Visual Discrimination Learning

Considering both treatments, all 24 subjects reached the criterion of the colour discrimination learning task. The entire sample of subjects required 5.50 ± 4.40 days (mean ± SD), with a maximum of 15 days to reach the criterion. The simulation
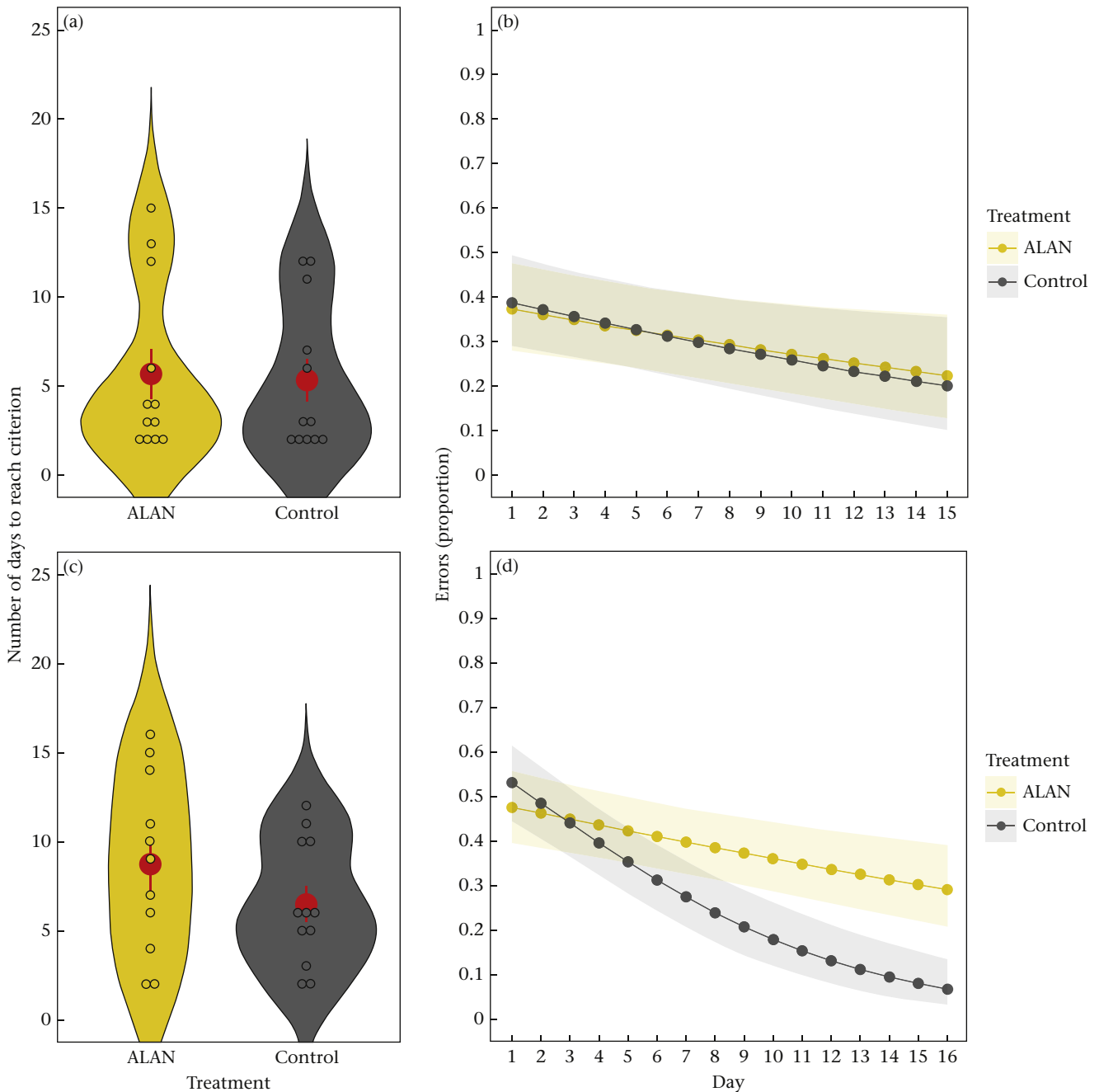

Figure 2. Performance of subjects from the artificial light at night (ALAN) and control treatments in the visual tasks. (a) Number of days to reach the criterion in the visual discrimination learning task; (b) number of errors in each day of training in the visual discrimination learning task; (c) number of days to reach the criterion in the visual reversal learning task; (d) number of errors in each day of training in the visual reversal learning task. In (a) and (c), empty dots represent individual subjects, filled red dots represent observed means and error bars represent standard errors. In (b) and (d), points and shaded areas represent means and 95% confidence intervals predicted by the generalized linear mixed-effects models, respectively.

demonstrated that this occurrence of subjects reaching the learning criterion could not be achieved by chance (percentage of simulated subjects reaching the criterion within 15 days: $31.99 \pm 9.65\%$; $P < 0.001$). There was no significant effect of the treatment on the number of days necessary to reach the criterion (Wilcoxon rank sum test: $W = 78.5, P = 0.721$; Fig. 2a).

The repeated measures model revealed a significant decrease in the number of errors across the days of testing (GLMM: $X^2_1 = 9.830, P = 0.002$), showing that fish progressively learned the colour discrimination (Fig. 2b). The main effect of treatment and the interaction between treatment and day

of testing were not significant ($X^2_1 = 0.008, P = 0.929$; $X^2_1 = 0.169, P = 0.681$, respectively; Fig. 2b).

Visual Reversal Learning

All the subjects except one from the ALAN treatment (23 of 24; 95.83%) reached the criterion of the colour reversal learning task. The remaining subject stopped participating in the trials and was excluded from the following analyses. Considering both treatments, the remaining subjects required 7.83 ± 4.43 days to reach the criterion, with a maximum of 16 days. The simulation
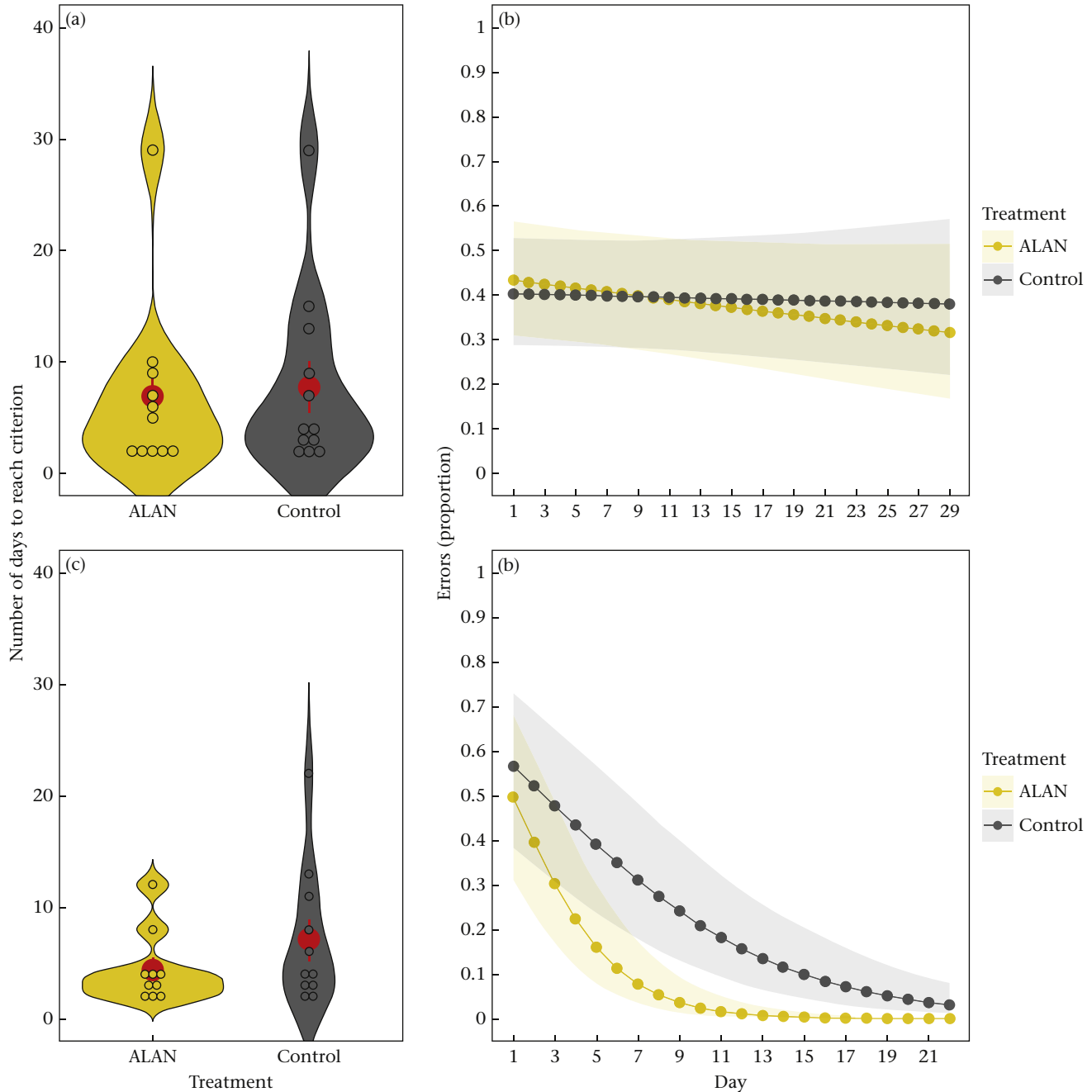

Figure 3. Performance of subjects from the artificial light at night (ALAN) and control treatments in the spatial tasks. (a) Number of days to reach the criterion in the spatial learning task; (b) number of errors in each day of training in the spatial learning task; (c) number of days to reach the criterion in the spatial reversal learning task; (d) number of errors in each day of training in the spatial reversal learning task. In (a) and (c), empty dots represent individual subjects, filled red dots represent observed means and error bars represent standard errors. In (b) and (d), points and shaded areas represent means and 95% confidence intervals predicted by the generalized linear mixed-effects models, respectively.

demonstrated that this occurrence of subjects passing the learning criterion could not be achieved by chance (percentage of simulated subjects reaching the creation within 16 days: 33.79 $\pm$ 9.60%; $P < 0.001$). There was no significant effect of the treatment on the number of days to reach the criterion ($W = 95.0$, $P = 0.191$; Fig. 2c).

The repeated measures model revealed a significant decrease in the number of errors across the days of testing (GLMM: $X^2_1 = 33.311$, $P < 0.001$), showing that fish progressively learned the reversal task (Fig. 2d). The main effect of treatment was not significant ($X^2_1 = 0.666$, $P = 0.415$). The interaction between treatment and day of testing was significant ($X^2_1 = 16.663$, $P < 0.001$), indicating that the number of errors decreased more rapidly in the zebrafish from the control treatment (Fig. 2d).

Spatial Learning

Considering both treatments, all 23 subjects that completed the previous task and were used in the spatial learning task reached the criterion within 7.35 $\pm$ 7.80 days (maximum = 29 days). The simulation demonstrated that this rate of success could not be achieved by chance (percentage of simulated subjects reaching the criterion within 29 days: 53.28 $\pm$ 10.47%; $P < 0.001$). There was no significant effect of the treatment on the number of days to reach the criterion ($W = 58.0$, $P = 0.637$; Fig. 3a).

The repeated measures model revealed that the decrease in the number of errors across the days of testing due to learning was not statistically significant ($X^2_1 = 1.245$, $P = 0.265$; Fig. 3b). The main effect of treatment and the interaction between treatment
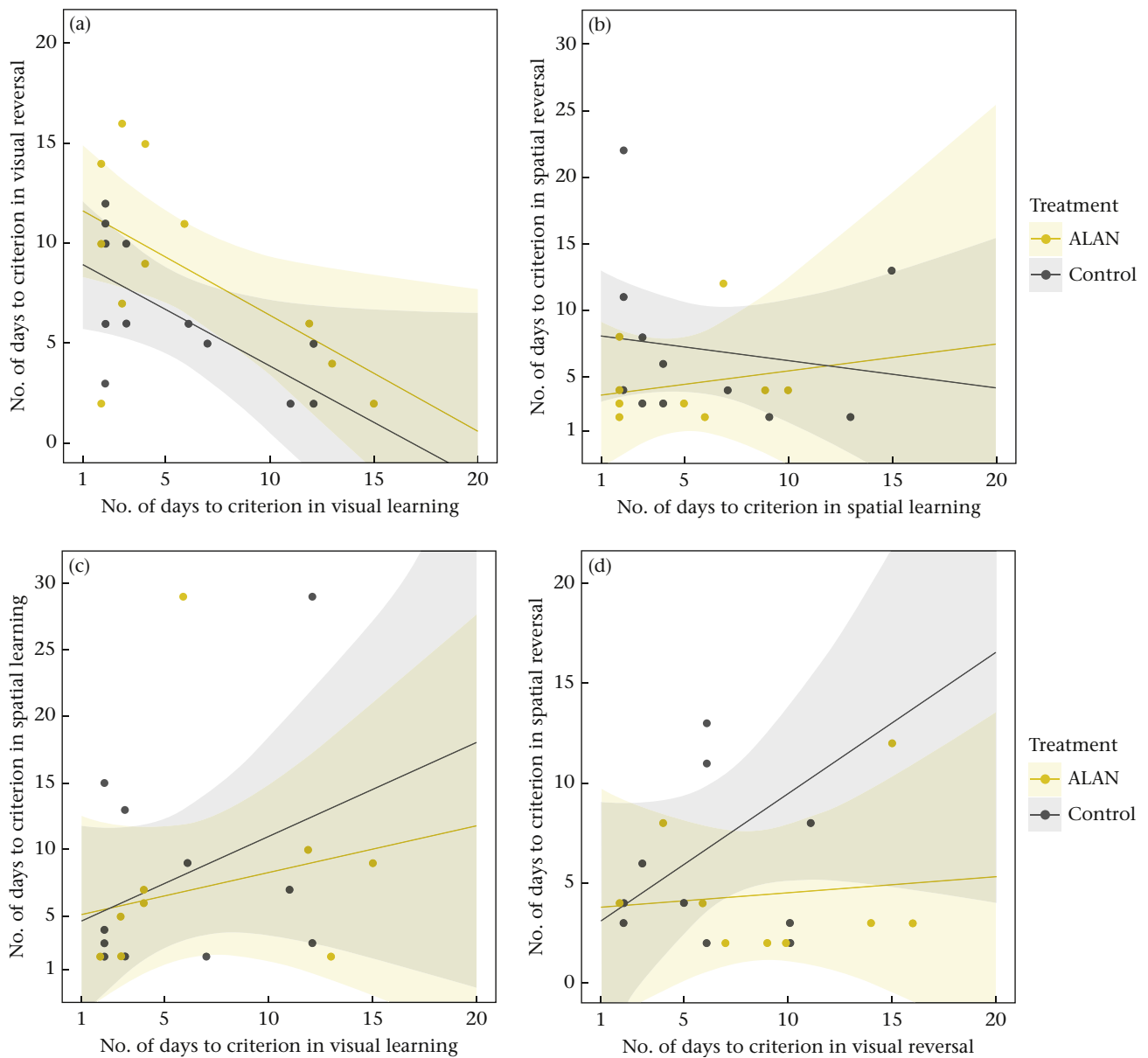

Figure 4. Covariations between cognitive performance in the different tasks for subjects of the artificial light at night (ALAN) and control treatments. Scatterplots of the number of days to reach the criterion in (a) visual discrimination learning task versus visual reversal learning task, (b) spatial discrimination learning task versus spatial reversal learning task, (c) visual discrimination learning task versus spatial learning task and (d) visual reversal learning task versus spatial reversal learning task. Points represent individual subjects; lines and shaded areas represent estimates and 95% confidence intervals predicted by the ANCOVAs, respectively.

and day of testing were not significant ($X^2_1 = 0.039$, $P = 0.843$; $X^2_1 = 0.674$, $P = 0.412$, respectively; Fig. 3b).

Spatial Reversal Learning

All but two subjects (one in the control treatment and one in the ALAN treatment which stopped participating in the trials) reached the criterion of the spatial reversal learning task (21 of 23 successful subjects; 91.30%). Considering both treatments, the subjects required 5.81 ± 5.05 days to reach the criterion, with a maximum of 22 days. The simulation showed that this occurrence of subjects passing the learning criterion could not be achieved by chance (percentage of simulated subjects reaching the creation within 22 days: 43.57 ± 10.34%; $P < 0.001$). There was no significant effect of the treatment on the number of days necessary to reach the criterion ($W = 40.5$, $P = 0.316$; Fig. 3c).

The repeated measures model revealed a significant decrease in the number of errors across the days of testing (GLMM: $X^2_1 = 107.948$, $P < 0.001$), showing that fish progressively learned the reversal task (Fig. 3d). The main effect of treatment was not significant ($X^2_1 = 1.805$, $P = 0.179$). The interaction between treatment and day of testing was significant ($X^2_1 = 16.325$, $P < 0.001$), indicating that the number of errors decreased more rapidly in the fish from the ALAN treatment (Fig. 3d).

Covariations Between Different Tasks

The results indicate that an individual's performance in the visual learning task was negatively correlated with its performance in the visual reversal learning task (ANCOVA: $F_{1,19} = 10.194$, $P = 0.005$; Fig. 4a). This relationship was not significantly modulated by the treatment ($F_{1,19} = 0.003$, $P = 0.959$). A control model with the addition of the effect of colour used as a reward indicated that such a negative relationship was not due to this factor ($F_{1,15} = 0.486$, $P = 0.496$). The remaining ANCOVA analyses (Fig. 4b–d) revealed no significant relationship between the performance in the two different tasks (spatial reversal versus spatial learning: $F_{1,17} = 0.012$, $P = 0.916$; visual learning versus spatial learning; $F_{1,19} = 1.673$, $P = 0.211$; visual reversal versus spatial reversal: $F_{1,17} = 0.793$, $P = 0.386$). In none of these models was there a significant interaction between the predictor and the treatment (spatial reversal versus spatial learning: $F_{1,17} = 0.389$, $P = 0.541$; visual learning versus spatial learning; $F_{1,19} = 0.208$, $P = 0.654$; visual reversal versus spatial reversal: $F_{1,17} = 1.401$, $P = 0.253$; Fig. 4b–d).

Discussion

In this study, we investigated the impact of ALAN on the cognitive abilities of adult zebrafish using a battery of four tests. Our findings revealed discernible ALAN-induced alterations in cognitive flexibility, evident in two reversal learning tasks conducted in different contextual settings: one involving visual discrimination and other spatial discrimination. No significant effects were observed in the two learning tasks assessed. In particular, our results demonstrated that control subjects consistently outperformed ALAN-exposed subjects in the visual reversal learning task. Conversely, an opposing trend was observed in the spatial reversal learning task, where ALAN-exposed subjects exhibited a performance advantage compared with their control counterparts. These intriguing results suggest that the impact of ALAN on zebrafish cognition is persistent, although task dependent.

The observation of an impact of ALAN on cognitive flexibility, but not on learning, aligns with findings of function-specific plasticity identified in previous fish studies (Montalbano et al., 2022; Queller et al., 2023; Vila Pouca et al., 2021). For example, Montalbano et al. (2022) reported that guppies, *Poecilia reticulata*, exposed to environmental factors such as live prey, social companions and structural enrichments exhibited enhanced learning abilities compared to guppies from barren environments. However, the treatment did not affect cognitive flexibility and inhibitory control, another executive function. The fact that two reversal learning tasks produced divergent effects differentiates our results from previous findings. This was not expected because according to the most common view, executive functions are not task specific, i.e. the same executive function is involved in many different tasks (Diamond, 2013; Packwood et al., 2011). Therefore, our two reversal learning tasks were supposedly assessing the same function (i.e. cognitive flexibility) albeit in different contexts (visual versus spatial). Moreover, irrespective of the level of specificity of executive functions, our results do not meet our initial expectation of a general negative impact of ALAN on cognitive functioning as a consequence of generalized physiological alterations (Maille & Schradin, 2017).

We propose three potential explanations for the outcomes of the reversal learning tasks. First, the umbrella term of 'cognitive flexibility' might actually encompass distinct functions that activate contextually, such as in visual versus spatial contexts, at least in zebrafish. Under this scenario, ALAN, and possibly light conditions in general, could differentially affect these cognitive flexibility functions. To accept or reject such an explanation is challenging, given the current ambiguity surrounding the structure of fish cognition (Aellen et al., 2022). Second, performance in the reversal learning tasks might be influenced by other cognitive or perceptual functions, and ALAN may impact these additional functions. For instance, in cane toads, *Rhinella marina*, ALAN decreases the pupil light reflex (Secondi et al., 2023). We are not aware of the same effect of ALAN in fish, but another study reported that the guppy's eye is affected by environmental light conditions (Kimbell et al., 2019). An impairment of the visual system similar to that described in cane toads might explain the reduced performance of ALAN-exposed zebrafish in the visual reversal learning task; however, it is not in line with the absence of ALAN effects on the visual discrimination learning phase. Notably, our spatial task could be solved by exploiting different strategies (e.g. allocentric versus egocentric; McAroe et al., 2016), and strategy preference by the subjects might affect their reversal performance. Our experiment was not designed to disentangle the spatial strategies used by the subjects. Therefore, we cannot rule out that the ALAN treatment affected fish preference for a specific spatial strategy, and this ultimately determined the effect on the reversal task. The third explanation revolves around noncognitive factors. Motivation, boldness and anxiety are known to impact cognitive performance (Rowe & Healy, 2014). Several studies indicate that ALAN alters behaviours, potentially contributing to cognitive performance, such as personality (Velasque et al., 2023) and boldness (Kurvers et al., 2018). Considering that our tasks involved different motivators, such as food reward in the visual task and escaping an unpleasant environment in the spatial task, the effect of ALAN on boldness or foraging motivation could indirectly shape the observed results in the reversal learning tasks. A similar effect could be mediated by the use of different perceptive mechanisms (Halfwerk & Slabbekoorn, 2015) to find the reward in the two tasks, considering, for instance, that in the unpleasant environment of our spatial task, shier fish might be more attuned to the use of olfaction (Mikheev et al., 2006; but see Hall & Suboski, 1995). However, if this explanation was true, we should have found the opposite effect of ALAN in the learning phases of the visual and spatial tasks. Therefore, our data do not fully support this third explanation.

Despite the unclear mechanisms underlying reversal learning alterations, our study aligns with early reports on the potential effects of ALAN on animals' cognitive abilities (Buniyaadi et al., 2022; Meijdam et al., 2023). This could have ecological consequences for fish exposed to ALAN in nature. The reduced cognitive flexibility in the visual task due to ALAN might lead to fitness reduction; although, to the best of our knowledge, a relationship between reversal learning abilities and fitness has not been demonstrated. With the same speculative reasoning, one may, however, expect that the observed increase in spatial flexibility in the ALAN treatment could counterbalance this fitness reduction. Owing to this uncertainty, while our study raises the concern of potential impairment of fish cognition by ALAN, it is imperative to carry out studies in natural environments focusing on at least two goals. The first goal is confirming the presence of ALAN impacts on cognition by comparing the cognitive performance of populations collected from habitats with and without light pollution, an approach that has been successfully applied for other traits (e.g. activity: Martín et al., 2018; circadian activity patterns: Duarte et al., 2019; sleep: Raap et al., 2017). The second goal is quantifying the fitness consequences of alterations in reversal learning abilities, which can potentially be achieved, for instance, with the approach of relating individuals' cognitive performance to their survival or reproductive success (Cole et al., 2012; Huebner et al., 2018). Recently, attempts have been made to apply cognitive paradigms for fish to field settings (Jungwirth et al., 2024), potentially paving the way for the studies proposed here.

Notably, in an earlier study, we found that a 3-day exposure to ALAN during embryonic development impaired zebrafish habituation learning (Lucon-Xiccato, De Russi, et al., 2023). The absence of effects on adult learning, even after extended exposure to ALAN in the current study, suggests that the cognitive system might be particularly sensitive to this form of pollution during development. Previous studies have demonstrated significant impacts of light conditions on the vertebrate nervous system during development (Dadda & Bisazza, 2012; Rogers & Deng, 1999), and pollution during early development is known to have critical effects on animals' biology (Gauthier & Vijayan, 2020; Merola et al., 2021; Santos et al., 2020). We suggest that future studies should focus on the impact of ALAN during early development, when it could be particularly detrimental. Moreover, it is important to conduct research to understand whether early life ALAN impacts can be mitigated if the natural light/dark alternation is restored. In various species, the impact of early life stress on cognition appears relatively stable and produces long-term consequences (Alves et al., 2022; Saleh et al., 2017). However, in fish, there is evidence that light-mediated cognitive plasticity might be related to seasonality and, hence, is reversible (López-Olmeda et al., 2021). This type of study will provide useful information to policymakers for environmental restoration projects.

Author Contributions

Gaia De Russi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giulia Montalbano:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Elia Gatto:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Elena Maggi:** Writing – review & editing, Conceptualization. **Stefano Cannicci:** Writing – review & editing, Conceptualization. **Cristiano Bertolucci:** Writing – review & editing, Supervision, Conceptualization. **Tyrone Lucon-Xiccato:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Data Availability

The data set supporting this article is available in the Supplementary material.

Declaration of Interest

The authors declare no conflict of interest.

Acknowledgments

Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2024.10.008.

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