Different housing conditions for zebrafish: what are the effects?

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Abstract

Zebrafish is a popular experimental model in several research areas but little is known about the effects of using different strains or housing conditions. Poor control of genetic background and housing conditions could affect experimental results and data reproducibility. Here we investigated the effects of two possible sources of variation on zebrafish behaviour: fish origin and environmental parameters (light intensity, water temperature and noise). Zebrafish behaviour was then examined using the 'novel tank test', one of the most common paradigms used to assess anxiety-like behaviours in zebrafish. Our results show that an increase in light intensity alters fish behaviour, particularly freezing duration and distance from the bottom of the tank, indicating increased anxiety. Swimming activity increased at the lowest temperature (25°C). However, different levels of background noise did not cause any significant changes in behaviour. Differences were also found between zebrafish strains and populations: while the AB strain from laboratory 1 was minimally influenced by variation in holding conditions, the AB strain from laboratory 2 was highly affected by changes in temperature, light, and background noise. Our study shows that variation in strains and holding conditions can significantly influence the results of behavioural testing and should be carefully considered in the experimental design and properly reported to improve data interpretation and reproducibility.

Keywords: zebrafish strain, anxiety-like behaviour, replicability, welfare

1. Introduction

Zebrafish is one of the most popular animal models in scientific research. It is a valuable tool in behavioural neuroscience (Stewart et al., 2014; Abreu et al., 2021), drug discovery (Trigueiro et al., 2020), toxicology (MacRae et al. 2023), human pathologies (Adhish and Manjubala 2023), genetics (Rafferty and Quinn, 2018), and ecology (Li et al., 2023). Recently, much debate exists on the causes for the low reproducibility and replicability in scientific research, including zebrafish research. One potential source of variation that may lead to low reproducibility is insufficient knowledge on the influence of environmental factors and variation in genetic background (Gerlai, 2019).

Many environmental factors can affect zebrafish physiology and behaviour. Some of these are tightly controlled in zebrafish facilities, as their control is essential for maintaining good health and high welfare, such as dissolved oxygen, osmolarity or pH (Lawrence, 2007). However, other parameters are rarely measured or controlled, but may still represent sources of variation, such as light intensity and background noise. Zebrafish are usually maintained in large-scale facilities that accommodate several multi-shelved rack systems. This design can result in light intensity variation depending on the racks' position and height (Gerlai, 2019). Evidence shows that light conditions can affect zebrafish growth and development (Villamizar et al., 2014), reproductive performance (Adato et al., 2016; Abdollahpour et al., 2020), circadian rhythms (Di Rosa et al., 2015), learning and memory (You et al., 2020). However, to what extent variation in lighting conditions can affect other aspects of zebrafish behaviour is not known. Background noise is another common feature of fish facilities caused by the operation of water pumps, aerator, chillers and other equipment. The negative effects of noise have been demonstrated in many fish species (Celi et al., 2016; Vazzana et al., 2017), including zebrafish (Lara and Vasconcelos, 2019; 2021). Nevertheless, there are no recommendations regarding noise conditions for zebrafish husbandry.

Water temperature is one of the most important parameters influencing behaviour and physiology of poikilothermic fish, such as zebrafish (Haesemeyer, 2020). In their natural

environment, zebrafish can face a wide temperature variation (Spence et al., 2008). For instance, critical thermal minima and maxima of 6.2°C to 41.7°C respectively have been reported for zebrafish (Cortimeglia and Betinger, 2005). However, while the effects of extreme temperatures on behaviour (Toni et al., 2019; Angiulli et al., 2020) are well studied, much less is known about the consequences of small and short-term temperature changes on zebrafish behaviour, common in laboratory practices.

Most zebrafish behavioural research does not report noise and light intensity conditions. At the same time, temperature is usually described for the holding facility but much less frequently for the experimental conditions.

Most studies report the strain used, frequently the AB strain (also referred to as wild-type AB) and the outbred wild-type (WT) zebrafish (Audira et al., 2020). The AB strain was the first established zebrafish strain, developed from two lineages, A and B, acquired from pet shops. The AB is bred worldwide and is now the strain most frequently used in research (Crim and Lawrence, 2021).

The WT refers to outbred fish acquired from pet shops, fish farms, or collected from their natural environment. These fish are also used as experimental subjects or to establish a local population in the laboratory. WT zebrafish are expected to present higher genetic variation and differ more between laboratories than inbred strains (Gerlai, 2019; Crim and Lawrence, 2021).

Behavioural differences between zebrafish strains have been reported—for instance, wild-caught zebrafish tend to display higher anxiety levels than laboratory-inbred zebrafish (Kalueff et al., 2015). Nevertheless, due to their independent origins, zebrafish strains are expected to differ from wild populations and also between each other.

The notion that variation in genetic background and in housing conditions may affect behavioural outcomes is perhaps intuitive, and many studies with zebrafish corroborate this idea. However, fully inbred zebrafish strains do not exist (Gerlai, 2019), and the use of different wild or laboratory-derived subpopulations, whose genetic make-up is typically unknown, may increase unwanted variation and contribute to low reproducibility. Likewise, because the effects

of some environmental parameters are still poorly understood, they may not be controlled (or measured), adding confounding factors to the experimental design. For example, whether zebrafish are visually isolated or can see their neighbours may affect their behaviour (Fernandes et al 2019).

To address these issues, we evaluated the effects of fish origin and environmental parameters. AB zebrafish from two different origins and one population of wild-type were exposed to different light intensity conditions, background noise, and temperature. After seven days, fish behaviour was evaluated using the novel tank test. We observed marked differences in behaviour depending on zebrafish origin, environmental parameters, and their interaction.

2. Material and Methods

2.1 Fish housing

Adult zebrafish from two AB populations and one farmed population (+6 months-old, mixed sex, 3.5 ± 0.2 cm) were used in this study. One AB population originated from the breeding stock (first generation) at the Centre of Sustainable Aquaculture Research (CSAR) – Swansea University (Wales-UK – Laboratory 1). The second AB population was acquired from the breeding stock at the Pontifícia Universidade do Rio Grande do Sul (PUCRS) (Porto Alegre - Brazil). Fish eggs were obtained from a fifth generation of zebrafish kept and bred in the laboratory. The eggs were transferred to the Fish Lab at Federal University of Rio Grande do Norte (UFRN, Natal – Brazil – Laboratory 2), and reared until maturation.

The farmed population used in this study was acquired from a fish farm (Natal-Brazil) at three months old and named FR (farm-reared). All populations were housed in standard 2L (4 fish/L) tanks housed in zebrafish racks in closed water recirculating systems with mechanical, biological and chemical filtration and a controlled photoperiod of 12h light/12h dark,light intensity of 222lux (fluorescent tubes), water temperature of 28°C and lab noise of 39dB.
. Fish were fed twice a day with commercial feed (Sparos or Alcon Basic, 44% protein, 5% total fat).

Data from the AB population from Location 1 were collected at CSAR (Swansea University) from April to May 2019. This was also used to establish baseline values for tested parameters (light intensity, temperature and noise) at the zebrafish facility. Data from Location 2 for AB and FR were sampled in Brazil from October to November 2019 at the Fish Lab (UFRN), where we replicated the environmental conditions defined at location 1.

All the experiments in this study were approved by the Animal Ethics Committees of Swansea university (permit number 060318/54) and UFRN (CEUA, permit number 226.009).

2.2 Experimental conditions

From the stock tanks, fish were transferred to 8L acrylic tanks in groups of 5 fish, 3 tank replicas per treatment, and maintained at different conditions of light intensity, temperature, and background noise during seven days (Fig. 1).

To evaluate the effect of different light intensities, we measured the light intensity in the tanks at the water surface and at the stock rack's intermediate shelf (222 Lx) (HighMed digital Luxmeter HM-832). We established values for low intensity (74 Lx) and high intensity (445 Lx) treatments. Two fluorescent lamps (Led tube light, power: 25W (4000K)) above and in front of the rack were used as light sources. Light intensity differed depending on the rack shelves' tank position (height and depth). For these tanks, the sound level was kept at 39 dB and water temperature at 28°C.

To evaluate the effects of background noise, we measured the noise level using the Moto G9 smartphone app (https://play.google.com/store/apps/details?id=com.gamebasic.decibel) at different points inside the zebrafish room and established a low (39 dB) and high values (62 dB) of ambient noise. The highest level of background noise was found close to the aeration system. Experimental tanks were distributed in the room according to the level of noise present: 3 tanks were located in the rack near the aeration system and 3 tanks were located in the most distant rack. Water temperature was maintained at 28°C and light intensity at 222 Lux.

To test the effects of water temperature, tanks were maintained at 25°C or 28°C by means of thermostats, and were monitored with individual digital thermometers in each tank. The noise level was kept at 39 dB and light at 222 Lux.

After seven days, the zebrafish locomotor activity and anxiety-like behaviour were assessed using the Novel tank test (NTT) (Egan et al., 2009). Fish were individually transferred to a 2L test tank in which conditions were fixed at 26°C water, 222 Lux of light intensity and 39 dB of ambient noise. Fish behaviour was recorded during 15 minutes with a camera placed in front of the tank and videos were analysed using the Zebtrack software (Pinheiro-da-Silva et al., 2017). The behavioural parameters evaluated were average and maximum swimming speed, total distance travelled, distance from the bottom of the tank and time spent freezing. An increase in time spent freezing and a decrease in activity and in the distance to the bottom of the tank characterize anxiety-like behaviour in zebrafish (Kalueff et al., 2013; Silva et al., 2019).

2.3 Statistical analysis

Initially, data were evaluated for the presence of outliers (Cleveland dotplot) and collinearity (VIF) (Zuur et al., 2010). Multidimensional scaling is a multivariate statistical method that presents the variables in a spatially (graphically) matrix, facilitating the understanding of the data (Ding, 2018). A pair of objects in a data set is presented as distances between points in a multidimensional space (2 or 3 dimensions) (Borg and Groenen, 2005). However, this procedure presents some problems related to the choice of the best method for normalization and distance measurements of multidimensional scaling (Walesiak and Dudek, 2017). To solve this problem, we used the optSmacofSym_NMDS function from the mdsOpt R package (Walesiak and Dudek, 2018). A selection of the best data normalization process and distance measurement was based on two criteria defined by Walesiak and Dudek (2017): Kruskal Stress-1 adjustment measure and the Hirschman-Herfindahl HHI index, time based on Stress values by point. We chose the normalization methods n1, n2, n3, n5, and n5a and distance measurements Manhattan, Euclidean, Squared Euclidean, Maximum, GDM1, in the

selection function. The procedure was performed for the Light, Temperature and Noise data. We determine the best normalization methods and distance measurements. The following types of normalization and distance definition for the data set were selected: Temperature (norm. N2 and d. M. Manhattan), Noise (norm. N2 and d. M. GDM1), and Light (norm. N5 and d. M. Euclidean), environmental variables together (norm. N2 and d. M. Maximum), and fish strain (norm. N2 and d. M. Maximum).

Then, we applied the smacofSim function to perform multidimensional scaling in a symmetric dissimilarity matrix using SMACOF - Stress Minimization using Majorization (de Leeuw & Mair, 2009; De Leeuw & Mair, 2011). A permutation procedure was used to obtain the significance value of the SMACOF model, using the Permtest function. The jackmds (De Leeuw and Meulman, 1986) function was used to verify a measure of stability of the mds. Then, we produced a graph of the VMU (Vector Model of Unfolding) of the smacof package. According to Tucker (1960), VMU is a biplot graph, similar to Principal Component Analysis (PCA), obtained from the decomposition of the singular values of a transposed similarity matrix. The biplot relates the sampling units with the variables investigated. Initially, each sampling unit could be a studied individual. The individual is represented by a vector that starts from the centre of a two-dimensional (orthogonal) plane. The vector has a direction in space, explaining the relationship of each individual to the variables investigated (Borg, 2020). In our case, these vectors are replaced by points. From these points (that are the fish), we create centroids that represent the averages of the coordinates of the points. Thus, we created centroids for each studied group (environmental variables). In addition, we insert vectors that represent the studied behavioural variables. The graph aided to verify the relationship between the centroid of the environmental variables and the behavioural variables. Graphics were created using the ggplot2 package (Wickham, 2016). We tested the difference between the response variable matrix (behavioural parameters) and the explanatory factor: fish population at each treatment and respective levels that were temperature (25° and 28°C), light intensity (222 lx, 445 lx, 74 lx) and background noise (39 and 62 dB) using permutational multivariate analysis of variance (1000 permutations). We applied the test for each environmental variable. The function

adonis.pairwise, from the EcolUtils package (Salazar, 2015), was used to compare fish populations at each treatment level. We chose this function because the data entry argument, "dist.mat" accepts any dissimilarity matrix. After that, all the data were included in a single matrix. Then, we performed the same analyses to verify the dissimilarity between fish populations and between environmental variables.

3. Results

For light intensity, the stress value of NMDS analysis between populations was 0.116, with $p<0.001$ (permutation from the permtest function of the smacof package). The stability of the NMDS solution, based on the jacknife resampling calculation, was 0.913, with a dispersion rate of 0.14 (from the jackmds command in the smacof package). First, we will present the VMU figures that represents a descriptive analysis. In this graph we compared vectors and centroids position in relation to the axis, centroids that are in same position and direction of the vectors are positively correlated to them, whilst when they are in opposite directions it represents a negative correlation. Vectors length depicts its explanatory power. Following the descriptive analysis, we presented the bar plot and respective permanova test that indicates statistical significance between groups. In this graph, each bar represents the behavioural responses for each treatment level and population. The bars above zero indicate that the mean value of the group is higher than the general mean value of all groups, and bars below zero show lower mean values than the general mean value of all groups. Line thickness is proportional to mean values

Figure 2a presents the graph of the vector model of unfolding (VMU) for light intensity treatments (445 lx, 222 lx and 74 lx). The percentage of explained variance of VMU was 74%. Swansea fish centroids are negatively related to the locomotor parameters (maximum speed, average speed and distance travelled) and freezing, while they are positively related to distance from the bottom. FR groups (222 lx and 74 lx) are negatively related to the locomotor parameters and distance from the bottom, but positively related to freezing. FR 445 lx centroid are positively related to locomotor and freezing response but negatively related to distance from

the tank bottom. The AB Natal population showed a positive relationship with locomotor parameters and distance from the tank bottom, and negatively related to freezing. The permanova test showed that populations differed in their response to light intensity (Permanova: $F_{8, 105} = 14.191$; $p < 0.001$) (Fig. 2b). Post hoc tests showed that for the AB Natal population, the swimming parameters differed between high (445 lx) and intermediate (222 lx) light intensity but that 445lx and 222lx did not differ from 74 lx. For FR Natal population, post hoc tests showed that 74 lx differed from 445 lx, but it did not differ from 222 lx. There was no effect of light intensity on swimming behaviour for the AB Swansea.

Considering the effects of background noise, the stress value of the NMDS analysis was 0.099, with a value of $p < 0.001$. The jacknife stability of the NMDS solution was 1.043, with a dispersion rate of 0.14. The percentage of explained variance of VMU was 88.3%. Figure 3a shows that the centroids of the AB. AB Swansea centroids are negatively related to locomotor parameters (maximum speed, average speed and total distance travelled) and freezing, and positively related to distance from the bottom. AB Natal population, the 62 dB group showed a positive relation with distance travelled, average speed and maximum speed, while the 39 dB group had a weaker relationship. The FR group showed a positive relationship for freezing and a negative relationship for distance from bottom. The 62 dB group still showed a positive relation to maximum speed, average speed and distance travelled, while for the 39 dB group, the relation was practically null. The permanova analysis showed statistical differences between the populations (Permanova: $F_{5, 66} = 13.28$; $p < 0.001$), but not between treatments (Fig. 3b). Post hoc test shows that AB Natal presents the higher mean values while the main difference between AB Swansea and FR are related to the anxiety indicators: freezing and distance from the tank bottom.

The analysis of temperature showed that the stress value of the NMDS analysis was 0.085 ($p < 0.001$). The jacknife stability of the NMDS solution was 0.918, with a dispersion rate of 0.13. The percentage of explained variance of VMU was 93%. The VMU plot (Fig. 4a) evidenced that the AB Swansea centroids show low relation to all the parameters as they are close to zero. For the AB Natal, the 28°C group was positively related to distance from bottom

and negatively related to average speed, distance travelled and maximum speed. The opposite was observed for AB Natal 25°C. The FR Natal followed a positive relation to freezing and negative for distance from bottom. The permanova test showed that temperature effects differ between populations, (Permanova: F5, 72 = 17.35; p <0.001) (Fig 4b). In the AB Swansea treatments did not affect the behavioural responses. For AB Natal, 25°C increased the mean values of the locomotor parameters compared to 28°C. For the FR, 28°C decreased locomotor parameters.

The NMDS analysis of environmental variables x behavioural variables presented a stress value of 0.1 (p <0.001). The jacknife stability of the NMDS solution was 0.948, with a dispersion rate of 0.15. The VMU (Fig. S1a) demonstrates the centroids of light and background noise show null relation to locomotor parameters, a negative relation to distance from the bottom and a positive relation to freezing. Temperature had a positive relation to freezing and locomotor parameters and a negative relation to distance to the bottom. The bar plot (Fig. S1b) corroborates the VMU showing that the effects of light and noise were similar on fish behaviour and different from the temperature effects. The permanova test showed statistical significance between the experimental groups (Permanova: F2, 261 = 52.15; p <0.001). Light and noise had lower mean values for distance travelled, average speed and maximum speed. Freezing and distance from bottom presented different responses (higher and lower mean values for light and noise, respectively). The temperature treatment showed effects on average and maximum speed, distance travelled and distance from the bottom, which were all increased in the higher temperature.

For the three population comparisons (Fig. S2a) the position of the centroids shows that the AB Swansea population was the least affected by the variables (the centroid is close to zero). AB Natal centroid is positively related to locomotor parameters, the anxiety indicator (freezing and distance from the bottom) show null relation. For FR Natal the centroid is positively related to freezing and negatively related to distance from bottom and the relation to locomotor parameters is null. Statistically, populations were different from each other (Permanova: F2, 261 = 10.09; p <0.001) (Fig. S2b). For AB Swansea, freezing, distance

travelled, maximum speed and average speed presented lower mean values, while distance from the bottom presented higher mean values. AB Natal presented higher mean values for distance from bottom, distance travelled, average speed and maximum speed, freezing presented lower mean values. For FR Natal, the mean values were higher for freezing and average speed and lower for distance from bottom distance travelled and maximum speed.

4. Discussion

We evaluated the effects of three environmental variables (light intensity, background noise, and water temperature) on the behavioural response of different zebrafish strain populations in a novel tank test. We found that increased light intensity altered FR and AB Natal populations' behaviour, increasing freezing duration and reducing locomotion in AB natal, indicating increased anxiety-like behaviour. Reducing the water temperature impacted AB Natal and FR populations, increasing all locomotor parameters. Increased noise intensity induced increased locomotion in AB Natal and FR fish. Thus, we observed that zebrafish strain populations presented different responses: while AB fish from Swansea was the least altered by holding conditions, FR fish was highly affected by temperature, light, and noise variation.

The controlled and stable conditions in the laboratory reduce the range of sensory stimuli fish are exposed to, decreasing behavioural flexibility (Salvanes et al., 2013). The behavioural responses result from complex interactions between genes and the environment (Charney, 2017; Anreiter et al., 2017), and inbred populations with limited genetic diversity reared in highly controlled conditions may be expected to show less behavioural variation than outbreed populations (Schluter, 2000). Although AB strains are not fully inbred and differ genetically (Gerlai, 2019), they are expected to vary less than farmed (FR) zebrafish.

In the novel tank test, locomotor and anxiety-like responses in zebrafish are characterized by an initial decrease in locomotion, increase in freezing and movement towards the bottom of the tank (Kalueff et al., 2013). This behaviour gradually eases as fish become acclimatized to the new environment (Egan et al., 2009; Cachat et al., 2010; Maximino et al., 2010), and begin to be more explorative behaviour (Cachat et al., 2010). The anxiety-like

behaviour caused by the novel tank procedure was not the same in all populations and holding conditions. The 12% increase in temperature (from 25 to 28°C) affected fish behaviour much more than the 500% increase in light intensity or the 59% increase in background noise (see centroids far from zero in VMU graphs).

The temperatures used in this study were not extreme and were inside the recommended range for zebrafish (Aleström et al., 2020). Temperature is one of the main abiotic factors influencing the physiology and behaviour of ectothermic species, such as zebrafish (Haesemeyer, 2020). Typically, basal metabolic rate and swimming activity are affected by water temperature (López-Olmeda and Sánchez-Vázquez, 2011; Abozaid et al., 2020), depending on acclimatization temperature and duration of exposure (Angiulli et al., 2020; Nonnis et al. 2021). Abrupt changes in water temperature were shown to decrease swimming speed in zebrafish larvae (Abozaid et al., 2020) and affect gene expression in the brain, which interfere with cognition, synaptic function, and neurotransmitter release (Nonnis et al., 2021). The behaviour differences we observed in this study may have resulted from changes in brain function and basal metabolic rate. While in natural conditions water temperature changes gradually, in laboratory situations fish are moved from tanks and may face hasty differences in temperature that affect their responses. Moreover, it seems that fish from hatchery (as the FR fish tested here) are more sensitive to temperature variation (Fig. 4) than AB populations. Similar results were obtained by Salvanes et al. (2007), who observed that wild cod present more variation in behavioural responses when placed in a novel environment than cod from a hatchery.

Light variations mainly affected FR and AB Natal locomotor response (Fig. 2). Zebrafish is a diurnal species (Moura et al., 2017) adapted to a wide range of light intensities presenting a duplex retina with rod and cone photoreceptors (Fleisch and Neuhauss, 2006). For diurnal species, increased light intensity during daytime increases arousal and activity levels (Deep et al., 2012; Soler et al., 2019), consistent with what we found for zebrafish. Brighter illumination was also shown to reduce anxiety-like and depressive-like behaviour and improve spatial memory in diurnal animals (Marcus et al., 2001; Ikeno and Yan, 2018; Yan et al., 2019).

Given the importance of zebrafish as a translational model for affective disorders, our study indicates that more attention should be paid to report light intensity in experimental work involving zebrafish. Although we tested light intensity variation, other parameters of light such as duration of the light phase and wavelength composition of light are shown to affect behaviour and cognition in rodents (Steinman et al., 2011; Itzhacki et al., 2018) and should be considered in future studies with zebrafish. It is known that wavelength composition and properties of the visible light affect zebrafish behaviour (Thornberri et al. 2008; Guggiana-Nilo and Engert 2016).

Regarding noise, there is a lack of studies that attained to understand background noise effects on behaviour. In this study, the increase in background noise caused changes in locomotor parameters in AB natal population and affected both locomotion and freezing in FR population (Fig. 3). Noise, both anthropogenic and natural, are usually considered in wild areas and treated as sound pollution, which was shown to cause damage to the auditory epithelium (McCauley et al., 2003, Dahl et al., 2020), increase cortisol levels (Wysocki et al., 2006) and affect intraspecific communication patterns (Andrew et al., 2014, Zhao et al., 2021). On the contrary, low intensity classical music was shown to reduce cortisol and anxiety-like behaviour in zebrafish (Barcellos et al., 2018). Here, we tested noise generated by aeration pumps used in fish laboratories to supply air to the tank. This type of noise is ignored by researchers, as it is not acoustic pollution and is usually overlooked in fish studies. However, as we observed here, background noise affects behaviour and should be considered an interfering variable in behaviour studies.

Detailed protocols exist for the use of behavioural tests in zebrafish research (Bencan et al., 2009; Cachat et al., 2010), including guidance on acclimatization (Egan et al., 2009; Cachat et al., 2010; Parker et al., 2012), methods for breeding zebrafish (Tsang et al., 2017) and a long review on experimental issues and solutions regarding zebrafish research reproducibility and replicability (Gerlai 2018). Regarding the effects of housing conditions, our study adds information to avoid unwanted variation that affect the reproducibility of results. Our results demonstrated that the behavioural response of the tested populations differed from each other

(Fig S2), probably reflecting differences in their history of domestication. The AB Swansea population presented similar behavioural responses in all holding conditions, suggesting that this population is less phenotypically flexible than the others in terms of behavioural response. Bhat et al. (2015) also found low levels of behavioural variation in a lab-reared zebrafish population and more variable behaviours in two wild populations.

5. Conclusion

Our study adds to the growing literature on the roles of rearing environment and genetic background on fish behaviour. More importantly, we showed how changes from holding to testing conditions might influence the results of behavioural tests and recommend that these are carefully considered and detailed in experimental design to increase reproducibility and facilitate data interpretation. The zebrafish strains used should be reported. Whenever possible these should come from a single origin, as even within the AB strain, different origins can generate significant differences in behavioural response.

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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References

Abdollahpour, H., Falahatkar, B., & Lawrence, C. 2020. The effect of photoperiod on growth and spawning performance of zebrafish, Danio rerio. Aquac. Rep. 17, 100295. doi: 10.1016/j.aqrep.2020.100295

Abozaid, A., Tsang, B., Gerlai, R., 2020. The effects of small but abrupt change in temperature on the behavior of larval zebrafish. Physiol. Behav. 227, 113-169. doi: 10.1016/j.physbeh.2020.113169

Adatto I., Krug L., Zon, L.I., 2016. The Red Light District and Its Effects on Zebrafish Reproduction. Zebrafish. 13(3), 226-9. doi: 10.1089/zeb.2015.1228. Adhish, M., & Manjubala, I. (2023). Effectiveness of zebrafish models in understanding human diseases—A review of models. Heliyon (9) e14557. doi:10.1016/j.heliyon.2023.e14557

Angiulli, E., Pagliara, V., Cioni, C., Frabetti, F., Pizzetti, F., Alleva, E., Toni, M., 2020. Increase in environmental temperature affects exploratory behaviour, anxiety and social preference in Danio rerio. Sci. Rep. 10 (1), 1-12. doi: 10.1038/s41598-020-62331-1.

Anreiter, I., Sokolowski, H.M., Sokolowski, M.B., 2017. Gene–Environment Interplay and Individual Differences in Behavior. Mind Brain Educ. 12 **(**4), 200-211. doi:10.1111/mbe.12158

Audira, G., Siregar, P., Strungaru, S.A., Huang, J. C., & Hsiao, C. D. 2020. Which zebrafish strains are more suitable to perform behavioral studies? A comprehensive comparison by phenomic approach. Biology. 9 (8), 200. doi: 10.3390/biology9080200

Barcellos, H.H., Koakoski, G., Chaulet, F., Kirsten, K.S., Kreutz, L.C., Kalueff, A.V., Barcellos, L.J. 2018. The effects of auditory enrichment on zebrafish behavior and physiology. Peer J. 6, e5162. doi: 10.7717/peerj.5162. eCollection 2018

Bencan, Z., Sledge D., Levin, E. 2009. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol. Biochem. Behav. 94, 75–80. doi: 10.1016/j.pbb.2009.07.009

Borg, I. 2020. Unfolding persons' personal values in ideal-point and vector models. Pers. Individ. Differ. 166, 110-206. doi:10.1016/j.paid.2020.110206

Celi, M., Filiciotto, F., Maricchiolo, G., Genovese, L., Quinci, E. M., Maccarrone, V., Mazzola, S., Vazzana, M., Buscaino, G. 2016. Vessel noise pollution as a human threat to fish: assessment of the stress response in gilthead sea bream (Sparus aurata, Linnaeus 1758). Fish Physiol. Biochem. 42 (2), 631-641. doi:10.1007/s10695-015-0165-3

Charney, E. 2017. Genes, behavior, and behavior genetics. Wiley Interdisciplinary Reviews: Cogn. Sci. 8 (1-2). e1405–doi:10.1002/wcs.1405

Cortemeglia, C., Beitinger, T. L. 2005. Temperature tolerances of wild-type and red transgenic zebra danios. Trans. Am. Fish. Soc. 134 (6), 1431-1437.

Dahl, P.H., Keith Jenkins, A., Casper, B., Kotecki, S.E., Bowman, V., Boerger, C., Dall'Osto, D.R., Babina, M.A., Popper, A.N. 2020. Physical effects of sound exposure from underwater explosions on Pacific sardines (*Sardinops sagax*). J. Acoust. Soc. Am. 147 (4), 2383-2395. doi:10.1121/10.0001064

de Abreu, M.S., Kalueff, A.V. 2021. Of mice and zebrafish: The impact of the experimenter identity on animal behavior. Lab. Anim. 50 (1), 7-7. doi: 10.1038/s41684-020-00685-9.

de Almeida Moura, C., da Silva Lima, J.P., Silveira, V.A. M., Miguel, M.A.L., Luchiari, A.C. 2017. Time place learning and activity profile under constant light and constant dark in zebrafish (Danio rerio). Behav. Processes. 138, 49-57. doi: 10.1016/j.beproc.2017.02.015

De Jong, K., Amorim, M.C.P., Fonseca, P.J., Fox, C. J., Heubel, K.U. 2018. Noise can affect acoustic communication and subsequent spawning success in fish. Environ. Pollut. 237, 814-823. doi:10.1016/j.envpol.2017.11.003

Di Rosa, V., Frigato, E., López-Olmeda, J.F., Sánchez-Vázquez, F.J., Bertolucci, C. 2015. The light wavelength affects the ontogeny of clock gene expression and activity rhythms in zebrafish larvae. PloS one, 10 (7), e0132235. doi: 10.1371/journal.pone.0132235

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., ... & Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205 (1), 38-44. doi: 10.1016/j.bbr.2009.06.022

Fleisch, V.C., Neuhauss, S.C.F. 2006. Visual Behavior in Zebrafish. Zebrafish. 3 (2), 191–201. doi:10.1089/zeb.2006.3.191

Gerlai, R. (2019). Reproducibility and replicability in zebrafish behavioral neuroscience research. Pharmacol. Biochem. Behav. 178, 30-38. doi: 10.1016/j.pbb.2018.02.005.

Haesemeyer, M. 2020. Thermoregulation in fish. Mol. Cell. Endocrinol. 518, 110986. doi: 10.1016/j.mce.2020.110986.

Ikeno, T., Yan, L. 2018. A comparison of the orexin receptor distribution in the brain between diurnal Nile grass rats (Arvicanthis niloticus) and nocturnal mice (Mus musculus). Brain res. 1690, 89-95. doi: 10.1016/j.brainres.2018.04.002

Itzhacki, J., Clesse, D., Goumon, Y., Van Someren, E.J., Mendoza, J. 2018. Light rescues circadian behavior and brain dopamine abnormalities in diurnal rodents exposed to a winter-like photoperiod. Brain Struct. Funct. 223 (6), 2641-2652. doi: 10.1007/s00429-018-1655-8.

Kalueff, A.V., Echevarria, D.J., Homechaudhuri, S., Stewart, A.M., Collier, A.D., Kaluyeva, A. A., ... & Song, C. 2016. Zebrafish neurobehavioral phenomics for aquatic neuropharmacology and toxicology research. Aquat. Toxicol. 170, 297-309. doi: 10.1016/j.aquatox.2015.08.007

Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., ... & Schneider, and the Zebrafish Neuroscience Research Consortium, H. 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish. 10 (1), 70-86. doi: 10.1089/zeb.2012.0861

Koolhaas, J.M., De Boer, S.F., Coppens, C.M., Buwalda, B. 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front. Neuroendocrinol. 31 (3), 307-321. doi: 10.1016/j.yfrne.2010.04.001

Lara, R.A., Vasconcelos, R.O. 2019. Characterization of the natural soundscape of zebrafish and comparison with the captive noise conditions. Zebrafish. 16 (2), 152-164. doi: 10.1089/zeb.2018.1654

Lara, R.A., Vasconcelos, R.O. 2021. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. Sci. Rep. 11 (1), 1-14. doi: 10.1038/s41598-021-85296-1

Lawrence, C. 2007. The husbandry of zebrafish (Danio rerio): a review. Aquaculture. 269 (1-4), 1-20. doi: 10.1016/j.aquaculture.2007.04.077

Li, H., Liu, Y., Chen, Q., Jin, L., & Peng, R. 2023. Research Progress of Zebrafish Model in Aquatic Ecotoxicology. Water. 15(9), 1735. doi: 10.3390/w15091735

López-Olmeda, J.F., Sánchez-Vázquez, F.J. 2011. Thermal biology of zebrafish (Danio rerio). J. Therm. Biol. 36 (2), 91–104. doi: 10.1016/j.jtherbio.2010.12.005

Marcus, J.N., Aschkenasi, C.J., Lee, C.E., Chemelli, R.M., Saper, C.B., Yanagisawa, M., & Elmquist, J.K. 2001. Differential expression of orexin receptors 1 and 2 in the rat brain. J. Comp. Neurol. 435 (1), 6-25. doi:10.1002/cne.1190

Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., & Gouveia Jr, A. 2010. Measuring anxiety in zebrafish: a critical review. Behav. Brain Res. 214 (2), 157-171. doi:10.1016/j.bbr.2010.05.031

McCauley, R.D., Fewtrell, J., Popper, A.N. 2003. High intensity anthropogenic sound damages fish ears. J. Acoust. Soc. Am. 113 (1), 638-642. doi:10.1121/1.1527962

MacRae, C. A., & Peterson, R. T. 2023. Zebrafish as a mainstream model for in vivo systems pharmacology and toxicology. Annu. Rev. Pharmacol. Toxicol. 63, 43-64.

Nonnis, S., Angiulli, E., Maffioli, E., Frabetti, F., Negri, A., Cioni, C., ... & Toni, M. 2021. Acute environmental temperature variation affects brain protein expression, anxiety and explorative behaviour in adult zebrafish. Sci. Rep. 11 (1), 1-21. doi: 10.1038/s41598-021-81804-5.

Parichy, D.M., Postlethwait, J.H. 2020. The biotic and abiotic environment of zebrafish. Behav. Neural Genet. Zebrafish. 3–16. doi:10.1016/b978-0-12-817528-6.00001-2

Parker, M.O., Millington, M.E., Combe, F.J., Brennan, C.H. 2012. Housing conditions differentially affect physiological and behavioural stress responses of zebrafish, as well as the response to anxiolytics. PloS One. 7 (4), e34992. doi: 10.1371/journal.pone.0034992

Radford, A.N., Kerridge, E., Simpson, S.D. 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav. Ecol. 25 (5), 1022-1030. doi:10.1093/beheco/aru029

Rafferty, S.A., Quinn, T.A. 2018. A beginner's guide to understanding and implementing the genetic modification of zebrafish. Prog. Biophys. Mol. Biol. 138, 3–19. doi: 10.1016/j.pbiomolbio.2018.07.005

Salvanes, A.G., Moberg, O., Braithwaite, V.A. 2007. Effects of early experience on group behaviour in fish. Anim. Behav. 74 (4), 805-811. doi: 10.1016/j.anbehav.2007.02.007

Salvanes, A.G.V., Moberg, O., Ebbesson, L.O., Nilsen, T.O., Jensen, K.H., Braithwaite, V.A. 2013. Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proc. Roy. Soc. Ser. B Biol Sci. 280, 2013-1331. doi: 10.1098/rspb.2013.1331

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford: Oxford University Press

Sih, A., Bell, A.M. 2008. Insights for behavioral ecology from behavioral syndromes. Adv. Study Behav. 38, 227-281. doi:10.1093/beheco/arx032

Silva, P., Garcia de Leaniz, C., Luchiari, A.C. 2019. Fear contagion in zebrafish: a behaviour affected by familiarity. Anim. Behav. 153, 95-103. doi: 10.1101/521187

Steinman, M.Q., Crean, K.K., Trainor, B.C. 2011. Photoperiod interacts with food restriction in performance in the Barnes maze in female California mice. Eur J Neurosci. 33 (2), 361-70. doi: 10.1111/j.1460-9568.2010.07528

Stewart, A. M., Braubach, O., Spitsbergen, J., Gerlai, R., Kalueff, A.V. 2014. Zebrafish models for translational neuroscience research: from tank to bedside. Trends Neurosci. 37 (5), 264-278. doi: 10.1016/j.tins.2014.02.011

Toni, M., Angiulli, E., Miccoli, G., Cioni, C., Alleva, E., Frabetti, F., ... & Maffioli, E. 2019. Environmental temperature variation affects brain protein expression and cognitive abilities in adult zebrafish (Danio rerio): A proteomic and behavioural study. J. Proteom. 204, 103396. doi:10.1016/j.jprot.2019.103396

Trigueiro, N.S.S., Canedo, A., Braga, D.L.S., Luchiari, A.C., Rocha, T.L. 2020. Zebrafish as an Emerging Model System in the Global South: Two Decades of Research in Brazil. Zebrafish. 17(6):412-425. doi: 10.1089/zeb.2020.1930.

Vazzana, M., Celi, M., Arizza, V., Calandra, G., Buscaino, G., Ferrantelli, V., ... & Sarà, G. 2017. Noise elicits hematological stress parameters in Mediterranean damselfish (Chromis chromis, perciformes): a mesocosm study. Fish Shellfish Immunol. 62, 147-152.

Villamizar, N., Vera, L.M., Foulkes, N.S., Sánchez-Vázquez, F.J. 2014. Effect of lighting conditions on zebrafish growth and development. Zebrafish. 11 (2), 173-181. doi: 10.1089/zeb.2013.0926.

Wysocki, L.E., Dittami, J.P., & Ladich, F. 2006. Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128 (4), 501-508. doi:10.1016/j.biocon.2005.10.020

Wong, R.Y., French, J., Russ, J.B. 2019. Differences in stress reactivity between zebrafish with alternative stress coping styles. R. Soci. Open Sci. 6 (5), 181797. doi:10.1098/rsos.181797

Yan, L., Lonstein, J.S., Nunez, A.A. 2019. Light as a modulator of emotion and cognition: lessons learned from studying a diurnal rodent. Horm. Behav. 111, 78-86. doi: 10.1016/j.yhbeh.2018.09.003

You, M.S., Yang, W.B., Cheng, C.H., Yu, S., Chang, H.C., Yu, H.S. 2020. Red LED light treatment promotes cognitive learning through up-regulation of trpm4 in zebrafish. J. Photochem. Photobiol. B: Biol. 213, 112073. doi: 10.1016/j.jphotobiol.2020.112073

Figure captions

Fig 1. Experimental design. Zebrafish from 3 different populations (AB Swansea, AB Natal and Farmed fish (FR)) were exposed during 7 days to different treatments regarding light intensity (74lx, 222lx, 445lx), background noise (39dB and 62dB), and temperature (25°C and 28°C). Each treatment had 3 tank replicas (five fish per tank), totalizing 15 fish tested per treatment level. After exposure, fish were recorded for 15 min in the Novel Tank Test. Videos were analysed for locomotor and anxiety-like behaviours.

Fig 2. Graphical representation of light treatments' effects on behaviour per population evaluated. (a) Vector model of unfolding (VMU) of light intensity. Coloured circles represent the centroids (mean values) for each combination of treatment and population. Centroids that are in the same position and direction of the vectors are positively correlated to them, while opposite directions indicate a negative correlation. Vector's length indicates the explanatory power of each variable analysed. (b) Bar plot represents the results of the Permanova test between treatment levels and populations. The bars above zero indicate that the mean value of the group is higher than the general mean value of all groups, and bars below zero show lower mean values than the general mean value of all groups. Thick bars represent higher mean values whilst thinner bars represent lower mean values. Different letters indicate significant statistical differences.

Fig 3. Graphical representation of background noise effects on behaviour per population evaluated. (a) In the VMU (Vector model of unfolding), coloured circles represent the centroids (mean values) for each combination of treatment and population. Centroids that are in the same position and direction of the vectors are positively correlated to them, while opposite directions indicate a negative correlation. Vector's length indicates the explanatory power of each variable analysed. (b) Bar plot represents the results of the Permanova test between treatment levels

and populations. The bars above zero indicate that the mean value of the group is higher than the general mean value of all groups, and bars below zero show lower mean values than the general mean value of all groups. Thick bars represent higher mean values whilst thinner bars represent lower mean values. Different letters indicate significant statistical differences.

Fig 4**.** Graphical representation of temperature treatments' effects on behaviour per population evaluated. (a) In the VMU (Vector model of unfolding), coloured circles represent the centroids (mean values) for each combination of treatment and population. Centroids that are in the same position and direction of the vectors are positively correlated to them, while opposite directions indicate a negative correlation. Vector's length indicates the explanatory power of each variable analysed. (b) Bar plot represents the results of the Permanova test between treatment levels and populations. The bars above zero indicate that the mean value of the group is higher than the general mean value of all groups, and bars below zero show lower mean values than the general mean value of all groups. Thick bars represent higher mean values while thinner bars represent lower mean values. Different letters indicate significant statistical differences.

Fig S1. Distribution of behavioural variables (distance from bottom, total distance travelled, maximum speed, average speed, and freezing) according to environmental variables: light, background noise and temperature. (a) Vector model of unfolding (VMU) represents a descriptive analysis. (b) Bar plot represents the results of the Permanova test between treatment levels and populations. Different letters indicate statistical differences.

Fig S2. Distribution of behavioural variables (distance from bottom, total distance travelled, maximum speed, average speed, freezing) according to populations: AB Natal, AB Swansea, FR Natal. (a) Vector model of unfolding (VMU) represents a descriptive analysis. (b) Bar plot represents the results of the Permanova test between treatment levels and populations. Different letters indicate statistical differences.