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Are habitable clean areas in heterogeneously contaminated landscapes functioning as escape zones for fish populations to alleviate stress?

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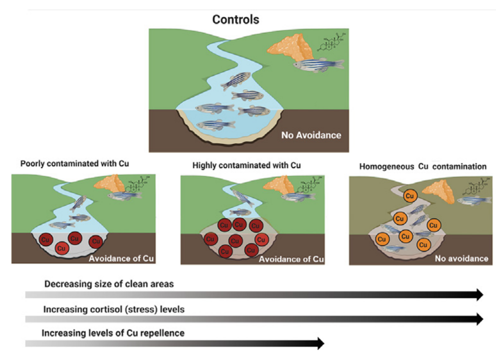
HIGHLIGHTS

- Avoidance behavior and stress were studied in a non-forced exposure approach.
- Both responses were assessed in chemically heterogeneous scenarios.
- Results showed that fish fled from the most copper contaminated areas.
- Higher stress is expected in scenarios with fewer clean areas for fish to flee to.
- Clean areas might alleviate the stress in heterogeneously contaminated landscapes.

GRAPHICAL ABSTRACT

The spatial extension of preserved and uncontaminated areas is crucial to the spatial dynamics of fish populations as escape zones to alleviate stress.



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ABSTRACT

Environmental contamination is a problem that reduces the quality of ecosystems and may make them unsuitable to accommodate life. As many ecosystems are connected, some organisms avoid the stress from continuous exposure to contaminants by moving towards less disturbed areas. However, the landscapes in which organisms move might vary regarding the concentrations of contaminants, in the form of gradients or patches of contamination. Therefore, although it is expected that organisms prefer clean areas, their sporadic contact with contamination should not be ignored, as the greater the probability of being in contact with contaminated areas, the higher the stress. The aim of this study was to assess how the stress (cortisol levels) of zebrafish (*Danio rerio*) varies as a consequence of heterogeneity in the chemical composition of the habitats and the presence of uncontaminated areas in this heterogeneous landscape. Zebrafish were exposed to heterogeneous contamination scenarios containing different concentrations of copper along a free-choice multi-compartmented system, in which they were able to flee from the most contaminated areas. Fish escaped from the most contaminated areas with an avoidance by 50% of population (AC_{50}) at concentrations of 41 (copper gradient scenario), 25 (spatially limited contamination scenario) and 69 (highly contaminated scenario) μg/L. Higher cortisol levels were observed in the populations exposed to homogeneously contaminated and highly contaminated (by copper) scenarios (both with no acceptable clean area to flee to). In summary, the uncontaminated areas might be crucial for the spatial dynamics of fish populations in a chemically heterogeneous landscape due to their role as escape zones to alleviate stress.

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1. Introduction

Although studies about contamination are more commonly related to the damage at the individual level (toxicity) that contaminants can cause to organisms, the loss of the quality of an ecosystem, making it unsuitable to accommodate life, is also of concern (Carlsen et al., 2004; De Lange et al., 2010). An area in which organisms are physiologically stressed and their survival is threatened is not likely to be appropriate for habitation (Doligez and Boulinier, 2008; Moe et al., 2013; Araújo et al., 2020). Contamination also deteriorates the environment in this context, forcing the organisms to pay a physiological cost to counteract toxicity (detoxification) and maintain an equilibrium between fitness and physiological homeostasis (Willmer et al., 2000; Peterson et al., 2017). Therefore, in a heterogeneously contaminated landscape (areas connected with different levels of contamination), if organisms are able to detect contaminants and interpret the chemical signs correctly (Tierney, 2016; Dominoni et al., 2020), they might tend to flee to a more favorable adjacent area (Cairns and Niederlehner, 1996; Tierney et al., 2011; Johnson, 2002). There is here an important shift in the paradigm of ecotoxicity studies: the effects are no longer focused exclusively on the toxicity at the individual level, but also on the spatial distribution of the organisms (Lopes et al., 2004; Vera-Vera et al., 2019); thereby providing an idea of how repellent the contaminated areas are.

One of the necessary conditions that might allow organisms to avoid toxicity is connectivity across chemically heterogeneous habitats (Clements and Rohr, 2009; Moe et al., 2013). In landscapes in which contamination is heterogeneously distributed, the decision to inhabit a contaminated area might impose a physiologically stressful situation for organisms (De Lange et al., 2010; Ribeiro and Lopes, 2013). Therefore, contamination is expected to be tolerable only if the presence of resources or other elements (e.g., lower competition and predation pressure) compensates (Dawidowicz and Wielanier, 2004) for the stress (physiological energy budget) that contaminants produce (Araújo et al., 2020). Additionally, even when organisms select an uncontaminated area to inhabit, if the adjacent areas present some level of contamination, organisms could still be sporadically exposed to contaminants (Spromberg et al., 1998; Fleeger et al., 2003; Moe et al., 2013; Vera-Vera et al., 2019). The question to be addressed here is: What is the importance of habitable clean areas, functioning as escape zones in a heterogeneously contaminated landscape, to alleviate the stress of the entire population?

Based on the hypothesis that the presence of uncontaminated areas within a heterogeneous contamination landscape might reduce the population stress, the aim of this study was to assess how the stress (measured as cortisol levels) in zebrafish (*Danio rerio*) populations (group of individuals exposed to the same contamination scenario) varies as a consequence of the chemical heterogeneity (presence of clean/non-avoidable areas). Initially, we assessed the spatial avoidance response by simulating heterogeneous contamination scenarios through which organisms moved among different levels of contamination. Secondly, we assessed how stressed the populations became by measuring the cortisol levels. Cortisol is a corticosteroid implied in many physiological processes as growth, metabolism, immune function and reproduction (Mommsen et al., 1999), and it is the most commonly measured stress biomarker in fish due to its involvement in the physiological mechanisms to short-term respond to environmental changes (Tellis et al., 2012; Zhang et al., 2015). The levels of cortisol in the fish were not compared considering a given concentration in which organisms stayed (traditional approach based on the forced and continuous exposure to a concentration), but instead we adopted a novel approach from a landscape perspective, integrating the chemical heterogeneity of the exposure scenario. Thus, a single cortisol level was measured for each entire population inhabiting a specific scenario, linking the stress to the whole landscape and considering the contaminated areas with different concentrations and the availability of clean areas to which organisms were expected to flee.

2. Material and methods

2.1. Test organism

The zebrafish (length: 1.5 ± 0.5 cm; weight: 0.170 ± 0.001 g; age: 2–3 months old), obtained from Andalusian Center for Development Biology (CABD; license: #ES 410910008004), were cultured (Ethics Committee; *Junta de Andalucía*, Spain; #2020999001515058) in dechlorinated tap water, with continuous aeration (dissolved oxygen: 6.5 mg/L), temperature of 25 °C, photoperiod of 12:12 and fed (TetraMin Flakes) daily. No food was provided 24 h prior to starting and during the tests. A total of 240 fish were used (see details in Section 2.4).

2.2. Copper

Copper (standard solution from Merck; 1000 mg/L) was used as the contaminant due to its known aversive character that is able to trigger avoidance in many organisms (Moreira-Santos et al., 2019). After each experiment, samples (10 mL) were taken from each compartment, acidified with HNO_3 (μL of acid per mL of sample; pH < 2) and quantified by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo Fisher Scientific; ICAP-Q, Series 03598R), following procedure #3125 described by APHA (1995). The quality control with standard concentrations showed a recovery rate of around 95% and a detection limit of 0.015 μg/L.

2.3. Multi-compartmented exposure system

A six-compartmented, non-forced exposure system was used to simulate gradients of contamination (Fig. 1). Each compartment (50 cm long and with a volume of 1 L) was prepared from two plastic bottles glued at the cut out bases using silicon (Sikaflex-11FC^{+}). The connection between the compartments was made at the mouth of the glued bottles as described by Islam et al. (2019).

2.4. Scenarios and avoidance tests

Five contamination scenarios were simulated (Fig. 1). Two scenarios were homogeneous regarding the levels of contamination in the compartments: clean homogeneous scenario (#1: culture water with no copper – control experiment) and homogeneously contaminated scenario (#2: Cu: 140 μg/L). For the first scenario, no stress was expected, while for the latter, it was expected that the populations should experience stress, as there is no area to escape from the contamination. The other three scenarios were chemically heterogeneous (different copper concentrations in the same landscape) as follows: scenario with a contamination gradient (#3: 0, 0, 100, 100, 500 and 500 μg/L; a smooth heterogeneous scenario with areas to flee to); spatially limited contamination scenario (#4: poorly contaminated: 0, 0, 0, 0, 500 and 500 μg/L; more area to flee to) and a spatially extended contamination scenario (#5: highly contaminated: 0, 0, 500, 500, 500 and 500 μg/L; fewer areas to flee to). All the tests were performed in quadruplicate. The actual copper concentrations measured at the end of the experiments are presented in the Results section. The copper concentrations were selected to include a range from environmentally relevant concentrations to very high, widely repellent concentrations.

In the two homogeneous scenarios, all the six compartments were filled (1 L per compartment) with the respective water (contaminated or not) to confirm the absence of preference by fish for any of the compartments. For tests simulating heterogeneous scenarios, prior to adding the concentrations to the compartments, plasticine plugs wrapped in PVC were introduced, using tweezers, in the space between the connections of each compartment to prevent the concentrations from mixing. Afterwards, the organisms (two fish per compartment; 12 fish per replicate and 48 fish per scenario simulated) were

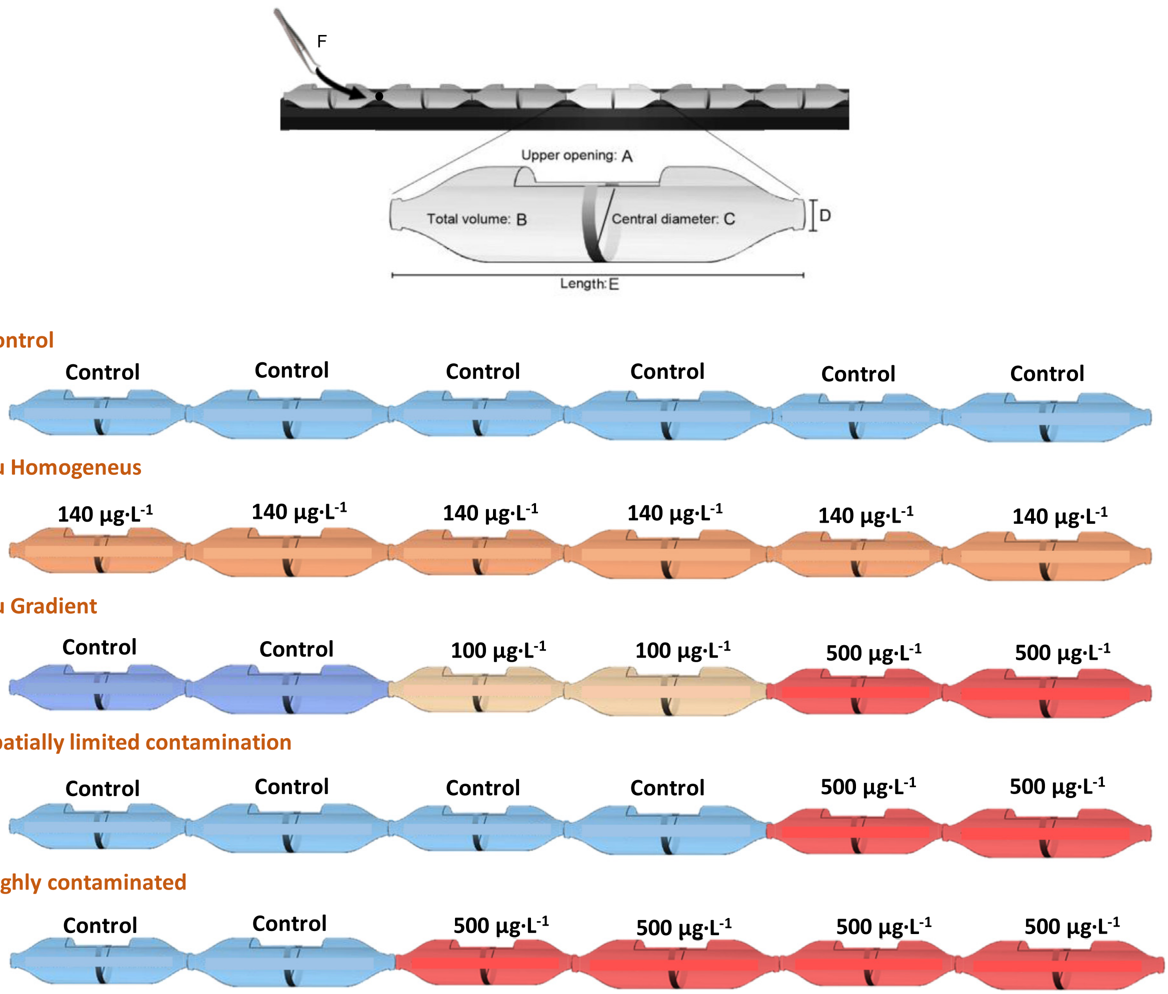


Fig. 1. Multi-compartmented non-forced exposure system (total length: 300 cm; total volume: 6 L) used in the avoidance tests and the different contamination scenarios tested. Compartments: A: 28 × 4 cm (length x width), B: 1 L, C: 8 cm, D: 2.2 cm (diameter), and E: 50 cm. Tweezers (F) were used to introduce the plasticine plugs (black circles) and to close the connections between the compartments.

introduced and the plugs were removed (Fig. 1). The spatial distribution of the fish was recorded at each 30, 60, 90 and 120 min and after 12 h. This recording schedule at each 30 min during the first two hours was established to detect any possible changes in the avoidance response, whereas the limit of 12 h was defined to prevent the mixing of the concentrations and the potential chemical homogeneity caused by the movements of the fish within the system over time. Tests were performed in a dark room at 22 ± 2 °C. A red light was used to prevent observer interference in the fish behavior. At the end, the plugs were again inserted between compartments and samples were taken to determine the final copper concentrations. Experiments started at 9:00 to avoid changes in the cortisol levels due to circadian rhythms. As the goal of this approach was not to provide a concentration-response relationship to assess the stress produced by copper in a typical confined environment (traditional forced exposure), the time spent by each fish in each compartment was not recorded.

2.5. Cortisol extraction and analysis

Fish were sampled for cortisol analyses after 12 h exposure. This time was chosen to allow a stable avoidance response and prevent fluctuations in the cortisol levels due to a very short exposure. After the avoidance experiments, fish from each scenario were sedated and gradually euthanized with 2-phenoxyethanol (around 300 μg/L; ca. 1 min), dried quickly on a paper towel, frozen in liquid nitrogen (−80 °C) and stored at −20 °C. To prevent an additional stress to fish caused by manipulation, the time taken between catching and sedating the organisms was less than 2 min. All the sampled organisms represented a replicate of one scenario. No distinction between the compartments in which the fish was found was made for each scenario, as the relation of stress was made with the scenario instead of the concentration. Before cortisol analysis, the fish were thawed in ice, patted dry (filter paper), weighed (0.5 g; around 6 to 10 fish) and digested following the method described in Sink et al. (2007). All the samples were tested in duplicate (variation was lower than 5%) and using two (100 and 50%) dilution factors. In brief, the fish population from the same scenario and replicate were patted dry (filter paper) and weighed (0.5 g; around 6 to 10 fish). Groups of fish were placed in a tube to which 5 mL of phosphate buffered saline (PBS) was added and homogenized using an Ultra Turrax (VWR Vos 14). Then, the tubes were centrifuged for 5 min at 5000*g* and 6 °C. The supernatant was transferred to another tube, to which 5 mL of ethyl ether was added, and then the extract was vortexed for 30 s. The tubes were again centrifuged at 3500 rpm for 5 min. Next, the supernatant was transferred to another tube and maintained for 24 h in a fume hood until the total evaporation of the ethyl ether. Finally, 1 mL of PBS was added. Analyses of cortisol were performed using the Cortisol ELISA kit (#500360; Cayman Chemical, MI, USA). The assay has a range of quantification of 6.6 to 4000 pg/mL.

2.6. Statistical analyses

The percentage of organisms per compartment in the avoidance tests was analyzed with a mixed-design ANOVA, and Mauchly's test was used to check the sphericity of the data (see Araújo et al., 2020 and Supplementary Material). The Bonferroni post-hoc test was employed to discriminate statistically significant ($p < 0.05$) differences in the percentage of fish along the time and among compartments. In the three avoidance tests with heterogeneous contamination scenarios (scenarios #3, #4 and #5), avoidance (in %) was calculated as described by Moreira-Santos et al. (2008). Firstly, the number of avoiders was determined using the equation: $Avoiders = N_E – N_O$, where N_E is the number of expected organisms and N_O is the number of observed organisms. For the compartment with the highest copper concentration (compartment #6), N_E was determined by the number of organisms introduced at the beginning of the test ($N_E = 2$), since it was not expected that the organisms would move from the lower concentrations to that compartment. For the remaining compartments, N_E was determined considering the organisms introduced initially, plus the organisms introduced into the adjacent compartments with higher concentrations, considering the trend of organisms to avoid the most contaminated areas. The calculation of N_O took into account the organisms recorded in a given concentration and those recorded in the higher concentrations. For instance, for compartment #6, N_O represented the organisms found in that compartment, whereas for the compartment #5, N_O represented the organisms recorded in both the compartments with the two highest concentrations (#5 and #6). This reasoning is based on the fact that the copper did not cause a lethargic effect and, therefore, the organisms able to inhabit higher concentrations (e.g., compartment #6) than that being studied (e.g., compartment #5) were not considered avoiders. As the control compartment contained only culture water or the lowest copper concentration in which the fish could minimize their risk of contamination, avoidance was not expected (N_E - $N_O = 0$). Finally, the avoidance percentage for each compartment was calculated as follows: $(Avoiders/N_E) * 100$. From the avoidance percentages, the AC_X values (copper concentrations causing X% avoidance of the exposed population) and the corresponding confidence intervals (CI) were calculated using PriProbit software (Sakuma, 1998).

The levels of cortisol of each population under different scenarios were compared with the levels of stress observed in the control population, which was considered a basal cortisol level (with no stress). This comparison was made by using the unpaired t-test, since the populations in each scenario are independent, and by applying the Welch correction, as the populations have a different standard deviation.

Linear and exponential regression models between the levels of cortisol and the copper levels present in each scenario were performed. A regression was performed based on the total amount of copper in the system by adding the final copper concentration in the whole system for the different scenarios. The objective of this approach was to integrate all the compartments from a landscape perspective (environmental heterogeneity). The second regression was performed considering the potential of repellency of each compartment in the system. The repellency was established depending on the copper concentrations that could trigger avoidance according to the results of the avoidance tests with the less abrupt scenario: copper gradient (see the table in Fig. 2 in the Results section). For this reason, a classification with three ranges (scale from 1 to 3), based on the AC_{20}, AC_{50} and AC_{80}, respectively, was applied to each compartment depending on the copper concentration: level #1: acceptable area (until 10 μg/L as the avoidance expected is lower than 20%; AC_{20}), level #2: potentially avoidable area (copper levels from >10 until 75 μg/L as it is the range in which the avoidance expected could be considered of concern: more than 20% and less than 80%) and level #3: completely repellent (more than 75 μg/L as the avoidance expected is almost for the entire population; AC_{80} or more). After attributing the levels (1 to 3) to each compartment ($n = 6$) considering the copper concentration measured at the end of the experiment, the values of the levels were added to provide the total value for each scenario (see details in Table S1 – Supplementary Material).

3. Results

3.1. Spatial distribution of zebrafish: avoidance response

The distribution of zebrafish in the two tests with homogeneous scenarios showed that the organisms moved randomly with no preference for any compartment and independently of time; although interaction between time and compartment was observed in the control (homogeneously clean) scenario (Figs. 2.1 and 2.2 and Tables S2 and S3 - Supplementary Material). This behavior validates the results obtained from contamination, in which a preferential response was, in fact, observed.

In the three tests with heterogeneous scenarios, a significant preference for the less contaminated compartments was observed (Figs. 2.3, 2.4 and 2.5). Moreover, the selection of the compartments by zebrafish varied significantly along the time for only two of the three scenarios (the scenario with a contamination gradient and that with spatially extended contamination), but there was interaction between time and compartments in all of them (Tables S4, S5 and S6 – Supplementary Material). If the copper concentrations lower than 50 μg/L are taken as an indicative value, the fish distribution was relatively similar: 68 ± 16% in the gradient scenario, 79 ± 12% in the spatially limited contamination scenario and 62 ± 16% in the spatially extended contamination scenario. Therefore, individuals showed a more limited displacement by not exploring different compartments other than the clean one when there were more contaminated compartments. In fact, the highest percentage of organisms in a clean compartment (statistically different from the adjacent compartments) was recorded in the spatially extended contamination scenario (Fig. 2.5).

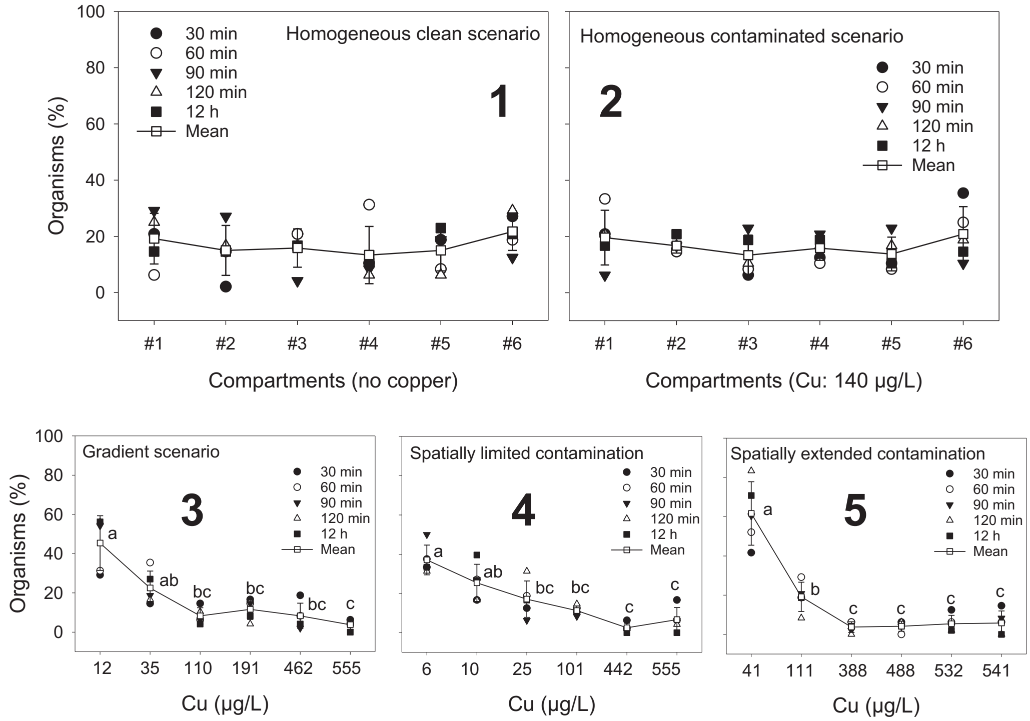
Avoidance was concentration-dependent in all the scenarios (Fig. 2.6), but it varied with the type of contamination scenario. In those scenarios that favored a higher dispersion of organisms (spatially limited contamination scenario) due to the low concentrations in many compartments, the AC_X values were lower (the table in Fig. 2).

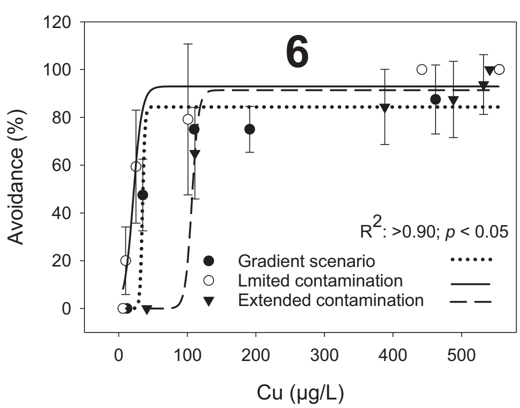
3.2. Population stress: cortisol levels

The cortisol levels of the populations exposed to the two scenarios, highly contaminated (extended contamination) scenario and homogeneously copper contaminated scenario, were statistically different from the control population (homogenous clean scenario) (Fig. 3A and Table S7 in Supplementary Material). The population exposed to the homogenous clean scenario, in which all the six compartments were considered acceptable to inhabit (repellency level #1 - Table S1), showed the lowest stress just like the populations exposed to the copper gradient or the spatially limited contamination scenario. The stressful conditions increased in the populations exposed to the contamination scenario with the biggest number of contaminated compartments, where only one area was classified as clean and the other five were completely repellent (Tables S1). Finally, the population that seemed to show the highest stress level was the one exposed to a scenario in which all the compartments had levels of copper considered as completely repellent with no more favorable area to escape to (Table S1).

3.3. Susceptibility to stress: cortisol levels and availability of areas to flee to

To explain the relationship between the cortisol levels and the copper concentrations, two models based on the total amount of copper in all the system (Fig. 3B) and the scale of repellency of copper (Fig. 3C) are presented. The susceptibility to stress seems not to be linearly related to





Environmental scenario	AC_{20}	AC_{50}	AC_{80}
3: Gradient scenario	9.7 (0.2 - 28)	41 (64 - 78)	173 (95 - 434)
4: Limited contamination	8.7 (3.1 - 15)	25 (14 - 39)	72 (45 - 149)
5: Extended contamination	21 (NC)	69 (0.9 – 144)	220 (65 – 388)

Fig. 2. Spatial distribution (%) at different time periods (30, 60, 90 and 120 min and 12 h and a mean of all the exposure times) of fish populations in the multi-compartmented exposure system in tests with five different scenarios: homogenously clean scenario (1), homogeneously contaminated scenario (2), contamination gradient scenario (3), spatially limited contamination scenario (4) and spatially extended contamination scenario (5). Different letters indicate statistically significant differences in the mean values (Bonferroni post-hoc test; $p < 0.05$). The mean (for 12 h exposure) avoidance (%) and sigmoidal models for the three heterogeneous contamination scenarios are also presented. The table presents the values of AC_{20}, AC_{50} and AC_{80} (μg/L) of copper for zebrafish exposed for 12 h to the three heterogeneous copper contamination scenarios. NC: not calculated.

the total amount of copper in the system (Fig. 3B). A weak linear relationship (Fig. 3C) was observed between the levels of stress and the scale of repellency (see *Statistical analysis* section). However, when an exponential adjustment was used, 70% of the stress seems to be explained by the concentration of copper (Fig. 3C).

4. Discussion

The response triggered by copper was to flee (Fig. 2) and caused stress in zebrafish (Fig. 3). The statistical effect linked to the observation time could be related to the longer period (12*h*) the fish had to explore the whole scenario of each set-up and select an area, as in previous shorter (2−3*h*) studies with the same species and a similar system and contaminant, time was not important (Silva et al., 2018; Islam et al., 2019). Avoidance of copper was expected, as previous studies have shown the repellent character of copper to zebrafish (Moreira-Santos et al., 2008; Araújo et al., 2018; Silva et al., 2018; Islam et al., 2019). In addition, the AC_{50} values here observed (from 25 to 69 μg/L, depending on the scenario; the table in Fig. 1) were similar to those studies of the latter, whose values varied between 16 and 90 μg/L. The AC_{50} values seemed to be dependent on the size of the areas with potentially repellent concentrations: the lowest AC_{50} values were observed in the scenarios with the highest number of areas to flee to, because copper was more gradually dispersed. Comparing the AC_{80} values, it is evident that the avoidance was higher (lower AC_{80} values) in the scenarios with more areas to flee to, which favors the fish trying to avoid the highest concentrations. This effect might be significantly related to the density of fish moving towards the compartments with acceptable levels of copper. For instance, if 40 μg/L was considered an acceptable concentration, in the spatially limited contamination scenario, there were three compartments with concentrations considered acceptable; thus, increasing the avoidance of the high concentrations and alleviating any stress caused by population density in the cleanest compartment. A similar condition was observed in the gradient scenario, in which the fish population could be distributed in two compartments with levels of contamination lower than 40 μg/L.

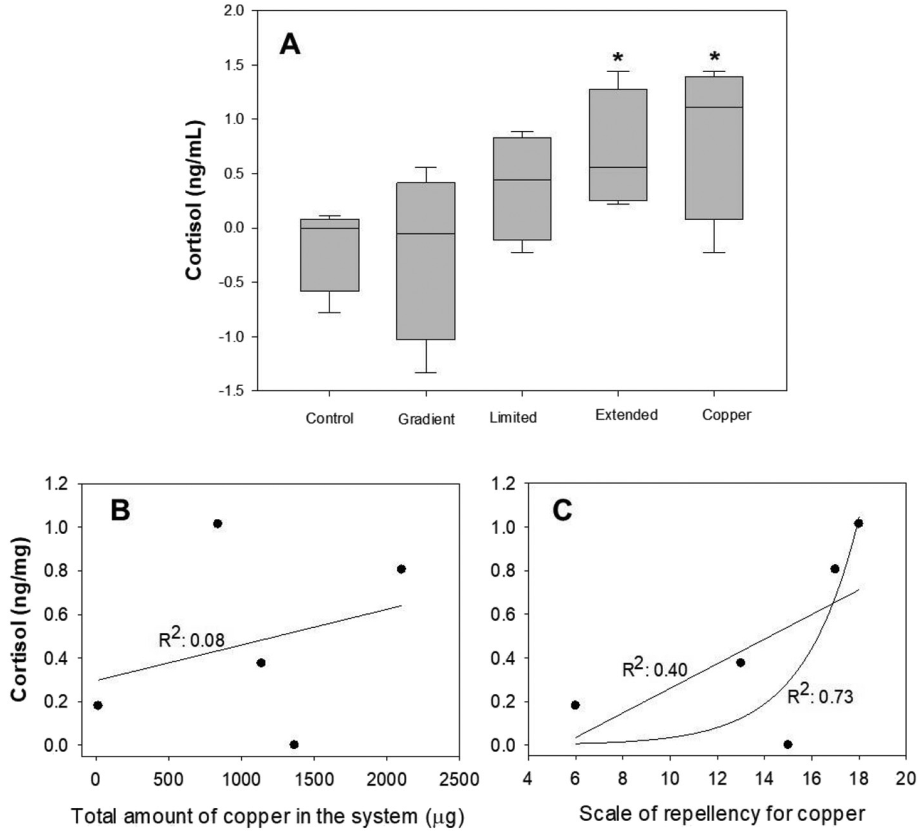


Fig. 3. Boxplot of the levels of whole-body cortisol of zebrafish populations ($n = 4$) exposed to different copper contamination scenarios (A – see details of scenarios in Material and Methods). The asterisks represent statistically significant differences in the cortisol levels of the treatments (populations in different environmental scenarios) compared to the control population according to the unpaired *t*-test with Welch correction. The relationship between stress (cortisol levels) and the copper contamination considering the total amount of copper in the system (B – sum of the copper detected in all the compartments of the system) and the scale of the repellency of copper based on the probability of triggering avoidance (C – here ACs values were taken as the reference – see Materials and Methods for details).

In both scenarios, the AC_{20} values were very similar (9–10 μg/L). However, for the spatially extended contamination scenario, the compartment adjacent to the control presented very high copper levels (around 111 μg/L), forcing the organisms to concentrate in only one compartment. This population density might have induced to a more widespread distribution of the fish to compartments adjacent to the cleanest one with relatively high copper levels. This scenario, conditioned by the chemical heterogeneity with very high concentrations adjacent to the control compartment, seems to have been crucial to defining the stress level of the populations.

The increasing cortisol level observed in the highly contaminated scenario and in the scenario in which copper was homogeneously distributed shows that the fish were under higher stressful conditions (Fig. 3). Possibly, the environmental heterogeneity regarding the copper concentrations and the non-forced character of the exposure might be the main factor that explains the cortisol variability, since in almost all the scenarios the fish were not mandatorily exposed to the stressful factor (copper). The chemical heterogeneity prevented the organisms from being constantly submitted to a stressful environment, which might make the response to stress intermittent (Hontela et al., 1992; Laflamme et al., 2000). Hontela et al. (1992) also observed that a continuous exposure to stress in fish seems to lead to an exhaustion of the cortisol production. Although we did not find a direct and linear relationship between the cortisol production and the amount of copper available in the scenarios, a strong relationship with cortisol production was observed when the probability of triggering avoidance (based on the number of chemically avoidable areas) was considered (Fig. 3). This approach to stress, not linking the stress to one particular concentration, but instead to the environmental quality within a spatially heterogeneous landscape, opens up a new perspective from which contamination can be seen and the role of protected areas in a chemically heterogeneous landscape (Rodríguez-Jorquera et al., 2017; Schiesari et al., 2018). This approach has some similarities with the theories related to the spatial dispersion of populations (MacArthur and Wilson, 1967; Hanski, 1998) and, therefore, it could be applied to landscape ecotoxicology to study the effects of inhabiting chemically heterogeneous landscapes (Angeler and Alvarez-Cobelas, 2005), where the stress to populations would be reduced if more clean areas were available. The reduced number of clean areas to alleviate stress, especially if they are inaccessible, might increase the vulnerability of the ecosystem and migrant populations, including the recipient ecosystems (De Lange et al., 2010; Moe et al., 2013; Ribeiro and Lopes, 2013; Schiesari et al., 2018). In summary, in the current study, we wish to draw attention to the importance of considering the connectivity of habitats and their chemical heterogeneity in environmental risk assessments, and make an appeal to the importance of preserving clean areas in ecosystems due to their potential role as escape zones to alleviate stress. We have used a new ecotoxicological approach to assess the effects of contamination, not only from a perspective of contamination-driven organisms' spatial distribution, but also from the stress response in a heterogeneous landscape scenario.

5. Conclusion

In a heterogeneously contaminated landscape, it is expected that fish avoid the most contaminated areas to reduce the stress caused by the continuous exposure to contaminants. This reduction in the stress (levels of cortisol) seems to be directly related to the availability of areas to which organisms are expected to flee. Therefore, the uncontaminated areas might be crucial for the spatial dynamics of fish populations in a chemically heterogeneous landscape due to their role as escape zones to alleviate stress.

CRediT authorship contribution statement

Contextualization: CVMA, MS, CT, JB
Methodology: CVMA, MS, CT
Investigating: CVMA, MS, JRSP, CT
Resources: CVMA, JB
Writing: CVMA, MS, JSRSP, CT, JB
Funding adquisition: CVMA, JB

Data accessibility

All data are available if requested from the authors.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151713.

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