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Experimental evidence of contamination driven shrimp population dynamics: Susceptibility of populations to spatial isolation

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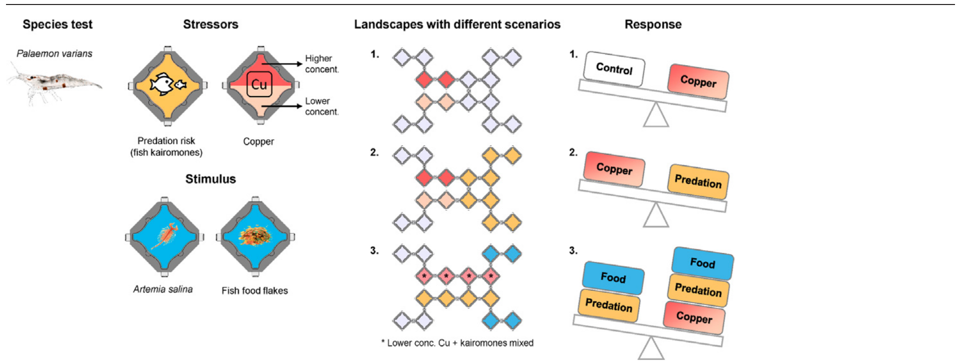
HIGHLIGHTS

- Contamination showed to be a population distribution driver at the landscape scale.
- Anti-predator and foraging behaviors were influenced by contamination.
- Contamination makes it more difficult to colonize new attractive areas.
- The HeMHAS versatile version #2 is suitable to test chemically heterogeneous areas.

GRAPHICAL ABSTRACT



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ABSTRACT

Contamination is likely to affect the composition of an ecological landscape, leading to the rupture of ecological connectivity among habitats (ecological fragmentation), which may impact on the distribution, persistence and abundance of populations. In the current study, different scenarios within a spatially heterogeneous landscape were simulated in the Heterogeneous Multi-Habitat Assay System (HeMHAS) to evaluate the potential effect that contamination (copper at 0.5 and 25 μg/L) might have on habitat selection by the estuarine shrimp *Palaemon varians* in combination with two other ecological factors: predator presence and food availability. As a result, *P. varians* detected and avoided copper; however, in the presence of the predation signal, shrimps shifted their response by moving to previously avoided regions, even if this resulted in a higher exposure to contamination. When encouraged to move towards environments with a high availability of food, a lower connectivity among the shrimp populations isolated by both contamination and predation risk simultaneously was evidenced, when compared to populations isolated only by the risk of predation. These results indicate that contamination might: (i) trigger avoidance in shrimps, (ii) prevent colonization of attractive foraging areas, (iii) enhance populations' isolation and (iv), make populations more susceptible to local extinction.

1. Introduction

Landscape-scale ecology comprises complex structures where a flow of energy, matter and organisms occurs among ecosystems as a consequence of the interactions of multiple factors and their landscape organization (spatial heterogeneity) (Johnson, 2002; Farina, 2006). The complexity of

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simultaneously acting factors that form an ecological landscape is a major challenge in environmental studies. Therefore, most studies have focused on only one dominant driver rather than multiple drivers, although the latter together might actually define the functional and structural patterns of communities (e.g., the use of resources in a spatially heterogeneous environment and how organisms live, reproduce, disperse, and interact) within a landscape mosaic (Turner, 2005).

The effects of landscape composition on populations (which and how many there are and how they are spatially arranged) have become a relevant area to investigate (McGarigal and Cushman, 2002; Heinrichs et al., 2015), since alterations in a landscape's composition affect the persistence of a population (Bond et al., 2015). Changes in an ecological landscape are typically linked to spatial fragmentation, separating a habitat into smaller and more isolated fragments (Haddad et al., 2015). In aquatic systems, fragmentation may be caused by any agent, either natural or anthropogenic, that decreases interspecies and intraspecies' interactions and increases the isolation of populations (Fuller et al., 2015; Selkoe et al., 2016). From an ecotoxicological point of view, contamination-driven habitat fragmentation can generate a population isolation that supposes a rupture in connectivity, which may lead to a total or partial cessation of the displacement of individuals among habitats (Fahrig and Nuttle, 2005). If mobility among habitats is limited, the persistence of populations can be significantly threatened (Holyoak, 2000), as it may impact on population dynamics, including distribution, persistence, and abundance of species (Galic et al., 2013). Although the chemical barrier effect produced by contaminants has hardly been studied in aquatic ecosystems, it has become relevant since some studies have shown evidence of the risk it represents for populations (Noatch and Suski, 2012; Le Pichon et al., 2020) and how the isolation of a population might increase its vulnerability to extinction (Ribeiro and Lopes, 2013; Grilli et al., 2015).

The application of an approach based on landscape ecology with multiple factors integrated in connected habitats has been neglected in the field of ecotoxicology due to the methodological limitations of ecotoxicological tests (Cairns and Niederlehner, 1996; Araújo et al., 2020a). Traditionally, the impact of contaminants on organisms has been assessed through ecotoxicity tests, in which organisms are exposed to contamination in a confined environment and, from the relationship of concentration-response, the potential effects for the organisms are predicted (Schmitt-Jansen et al., 2008). This approach is certainly important, as the information obtained has greatly helped to manage contamination for stakeholders and decision makers (Moermond et al., 2016; Rudén et al., 2016). However, in natural aquatic environments, although isolation may be caused by pollution where chemically heterogeneous areas are connected, here a forced exposure scenario is not always the case (Fahrig and Nuttle, 2005). In the light of this, some studies have applied non-forced multi-compartmented system exposures as complementary methods to evidence the ability of organisms to escape from contaminated habitats (Beitinger and Freeman, 1983; Giattina and Garton, 1983). The creation of multi-compartmented systems has made it possible to simulate aquatic contamination scenarios where organisms can move freely, thus escaping from exposure to stressed environments (Lopes et al., 2004; Araújo et al., 2016a), which provides a new perspective on the risks of contamination for the environment. This ability to detect and avoid contamination might cause a loss of population abundance and biodiversity at the local scale and isolate populations (Islam et al., 2019; Vera-Vera et al., 2019), which may significantly affect the composition and dynamics of the landscape.

The general hypothesis assumes that contamination in aquatic environments may isolate populations, even though mobility is not spatially restricted. In addition, the presence of contamination may change the natural response of organisms to a stressor, such as the risk of predation, and a stimulus like food, thereby changing the spatial dynamics and connectivity among populations distributed in landscapes. Hence, in order to simulate more complex scenarios and test the effect of contamination in a spatially broader and connected landscape, a new and versatile model of the HeMHAS [Heterogenous Multi-Habitat Assay System; (Araújo et al., 2018)] was developed. This new HeMHAS used here allows researchers to create heterogeneous scenarios with different spatial arrangements of the contamination, as is expected to occur in some natural ecosystems. By using this system, it is possible to incorporate a more ecological approach, in which different factors can be included in a landscape made up of different and connected habitats and then the contamination-driven spatial distribution of populations can be studied (Araújo and Blasco, 2019). Therefore, the aim of the present study was to evaluate the effect that contamination may exert in aquatic ecosystems, possibly leading to the isolation of populations in a spatially connected landscape. The use of the new HeMHAS made it possible to simulate environmental scenarios in which two stressors (contamination and presence of predators) and one stimulant factor (food) were integrated simultaneously. Thus, it was possible to study the role of contamination versus the innate role of predation risk and food availability concerning the spatial distribution and connectivity among populations in a heterogeneous landscape. Copper was used as reference chemical as it is a well-known contaminant of global concern and able to trigger avoidance in organisms (Moreira-Santos et al., 2008; Araújo and Blasco, 2019), including the estuarine shrimp *Palaemon varians* (Araújo et al., 2020b). Fish kairomones were used to simulate predation risk, as it reduces the habitat-affinity for organisms (Fuller et al., 2015).

2. Materials and methods

2.1. Test organism and culture conditions

The estuarine shrimp *Palaemon varians* is distributed in brackish and saline waters throughout Europe, from the western Baltic Sea and British Isles southwards to the western Mediterranean (Smaldon, 1993; González-Ortegón and Cuesta, 2006). Its utility as a sensitive organism has been demonstrated in recent ecotoxicology studies (Araújo et al., 2019a, 2020b; Ehiguese et al., 2019), thereby becoming a relevant species for research in estuarine areas.

As described by Araújo et al. (2019a), organisms were collected from the salt pond Salina La Esperanza (Puerto Real, Spain), and transported in local water to the laboratory. The organisms were cultured in 250 L tanks with filtered (1 μm filter) well saline water in a flow-through system, continuously aerated, and fed with either *Artemia salina* or ground Tetramin® flakes. Food was supplied three times per week during a one-week acclimation period before the experiments. The temperature (22 ± 1 °C) and photoperiod (12:12 h, light/dark) were checked daily. The culture was considered suitable for testing when mortality did not exceed 10% of the total population. Only shrimps with a length of 2.5 ± 0.5 cm were used in the experiments to avoid differences in the response due to the life stage.

2.2. HeMHAS – heterogeneous Multi-Habitat Assay System

The test system HeMHAS was constructed from polyoxymethylene (acetal), a highly engineered thermoplastic material. The high density, surface properties, and low chemical reactivity of this material prevent the adsorption of contaminants (Mergler et al., 2004; Araújo et al., 2019a), which makes it a suitable material for use in ecotoxicity tests. This new versatile version of the system (HeMHAS version #2) comprises several independent square habitats (compartments) with a concave inner chamber. These compartments can be connected from all corners by small bridges with rotating doors, which can be operated manually. Hence, a connected multi-habitat scenario can be simulated where organisms can move freely among compartments (see Fig. 1 and Supplementary Material).

2.3. Stressors and attractive stimulus

Two environmentally relevant stressors were used: copper, as a stressor related to contamination, and fish kairomones, as a stressor simulating the presence of predators. To simulate the contamination scenarios, two copper concentrations were used: 0.5 μg/L, representing the environmental levels found in some estuaries and deltas of southwest Spain (González-Ortegón et al., 2019), and 25 μg/L, representing a high contamination level capable

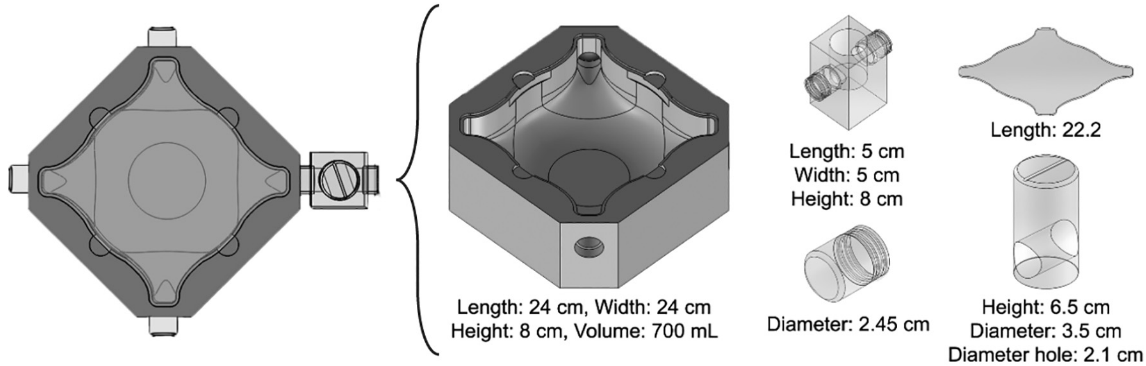

Fig. 1. Characteristics of the HeMHAS used as the test system. See details of the system in Supplementary Material.

of triggering an avoidance response in cladocerans (Lopes et al., 2004; Gutierrez et al., 2012), fish (Araújo et al., 2019b; Islam et al., 2019), and shrimps (Vera-Vera et al., 2019; Araújo et al., 2020b). Both concentrations were prepared from a standard solution (Merck; 1000 mg/L) by dilution with saline water to the two concentrations selected.

Kairomones were obtained from the culture laboratory of the marine fish *Seriola dumerili* and *Sparus aurata*. Both organisms were cultured in a Recirculating Aquaculture System (RAS) with a flow of c.a. 5000 L per hour under regulated temperature (~19 °C), and fed with commercial fish food (Skretting®). The filtering mechanism of the RAS is composed of a sand and a biological filter to retain small particles, ammonium and nitrites, converging in a skimmer where a liquid is obtained and it was used to signal the presence of fish (predator). We used this filtered liquid as a predator signal, because there was no source of contamination in the culture tanks: the water used to culture the fish was the same that was used to culture the shrimps and the amount of food provided for the fish was measured to prevent generating any residues. For the preliminary experiments, a 10% concentration of this filtered liquid was prepared by dilution with the control water to assess the repellency of the kairomones. This water containing kairomones was tested against control water in a non-forced free-choice experiment, in which shrimps had the option to select from two different environments: culture water with or without kairomones. The results demonstrated that the culture water without kairomones was widely preferred (~85% of shrimps moved to zones without kairomones, see Figs. S2b and S2c in the Supplementary Material), which confirmed the hypothesis of kairomones as a repellent factor. For the following experiments with kairomones, the concentration of the filtered liquid was 5% to reduce the simulation of the threat of predation, as an avoidance of 85% is extremely high.

Juveniles (<1.0 cm) of *A. salina* and flakes provided for feeding shrimps were used as a source of attraction to stimulate the colonization response (see description below: Section 2.4.3).

At the end of each assay, samples (around 10 mL per replicate) from each compartment and replicate containing copper were collected using 45 mL Falcon plastic sampling tubes, acidified with 65% HNO_3 (1 μL of acid to 1 mL of sample) and stored at 4 °C for subsequent analyses. The identification and quantification of copper was performed using the commercially available seaFAST preconcentration system working off-line and was analyzed by ICP-MS (Thermofisher) (see details in Poehle et al., 2015). The analysis QC/QA was carried out by running blanks and reference material. Periodically, calibration with standard solutions were performed at the concentration range of 10, 50, 100, 200 and 500 μg/L. The recovery was higher than 95% and there was a detection limit of 0.015 μg/L. Due to the lack of knowledge about the composition of the filtered liquid used as chemical signal of predator, the kairomone levels were not measured.

2.4. Habitat selection experiments

A total number of 11 experiments simulating environmental scenarios involving the presence of contaminant (copper), predators (fish kairomones) and food (*A. salina* and Tetramin® flakes) were conducted. The aim of the experiments was to create heterogeneous environments with different configurations of connectivity among them. To create such heterogeneity, individual HeMHAS compartments were connected and the rotating doors were closed to isolate the compartments from each other and to avoid an early mixing of the concentrations before the experiments began. Then, shrimp populations were placed in four or two different regions (see details for each experiment below). The rotating doors were then opened to create connectivity among the different environments and to allow the free displacement of the shrimps throughout the system.

All the experiments including control were performed in the daytime (regulated temperature of 22 ± 1 °C) in darkness to prevent external interferences in the shrimps' movements, and were conducted in quadruplicate (except for the copper experiments, which were done in triplicate). The location of the organisms was recorded every 30 min during the 4 h exposure period. This time span has been demonstrated to be sufficient to record spatial avoidance in a multi-compartmented system like the HeMHAS (Araújo et al., 2019a, 2020b).

To avoid any possible effect of the laboratory conditions on the shrimps' behavior, the spatial disposal of the experimental system in the laboratory was changed for each replicate of the different experiments. In this way, the region selected by the shrimps could not be influenced by external factors. Control experiments demonstrated a population distribution of the shrimps with no preference for any region in particular (further information in Supplementary Material). Details of each environmental scenario simulated with the different stressors and attractive stimulus are provided below.

2.4.1. Copper experiments

In this scenario, the shrimp populations were distributed in the four compartments in the corners of the system (20 individuals in each region), as indicated in Figure 2a. Two shrimp populations were introduced in regions with a low (0.5 μg/L; LCC – low copper concentration; colored in light pink) and high (25 μg/L; HCC – high copper concentration; colored in red) levels of copper, while another two populations were placed in regions with no copper (UHC – undisturbed area close to an area with copper level – and ULC – undisturbed are close to an area with low copper level; Fig. 2a; legends in Fig. 2b). Although two shrimp populations were allocated to the contaminated regions (with four compartments each), organisms from those regions were introduced into the compartments without copper (see Fig. 2a). All the adjacent regions were connected, except those contaminated by copper, to discriminate the effect that different levels of contamination could produce on populations. To assess the distribution of the organisms, compartments were grouped in two ways: regions (grouping 4 compartments) as a global and more extended scale, and areas (grouping 2 compartments) as a specific and more local perspective (Fig. 2b).

2.4.2. Copper and fish kairomone experiments

The experiments with copper and kairomones followed an experimental design similar to the previous one, but in the two uncontaminated regions

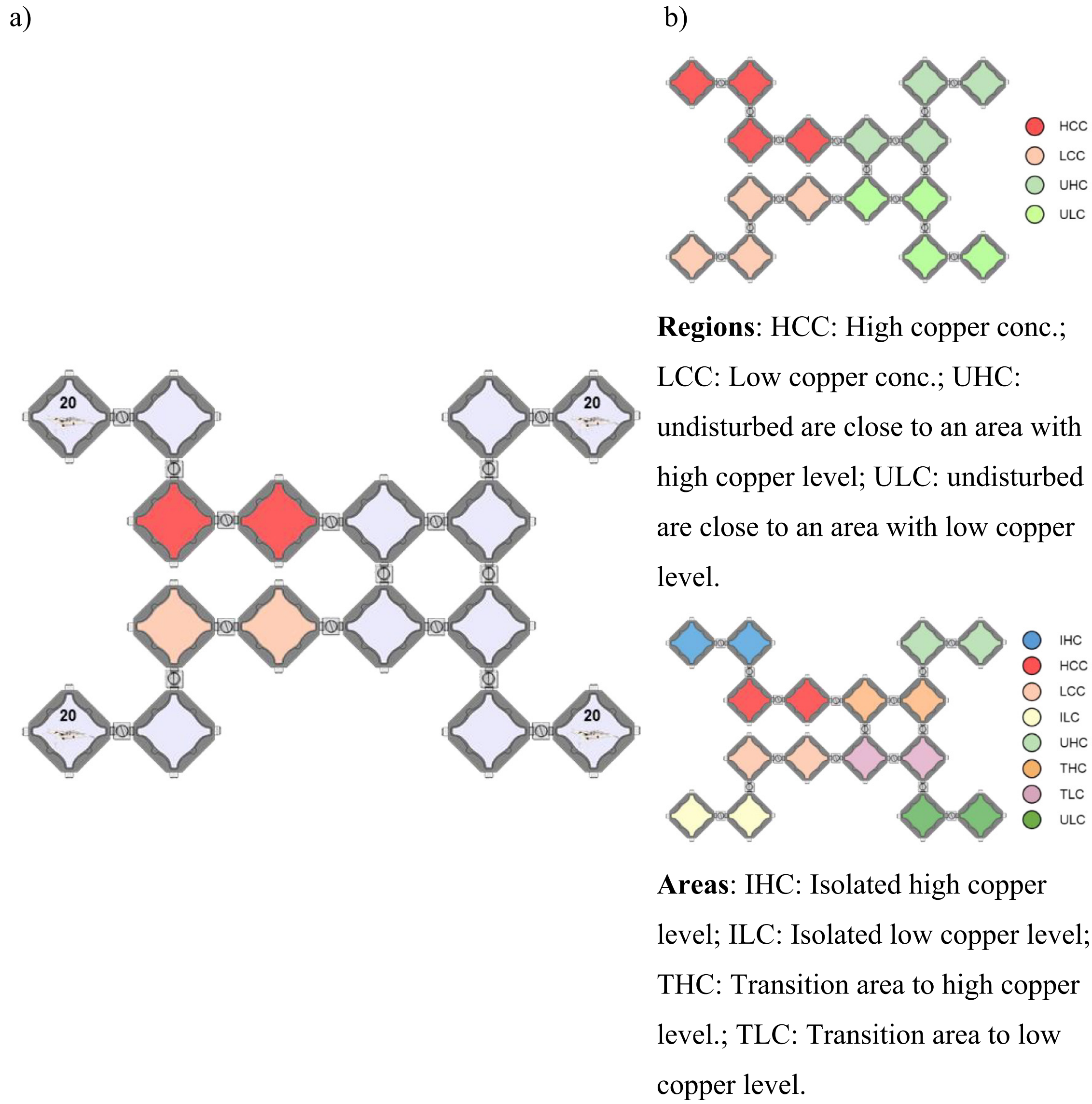

Fig. 2. Scheme of the a) experimental design for copper experiments and b) spatial arrangement limiting regions (upper) and areas (lower) for data analysis. The number 20 indicates the number of shrimps that were initially introduced into each compartment. In the identification of regions and areas, "conc." means concentration of copper.

(8 compartments colored in ocher) the organisms were exposed to the predator chemical signal (fish kairomones diluted at 5%) (Fig. 3a). Similarly to the copper experiments, the compartments were grouped in regions and areas (Fig. 3b).

2.4.3. Colonization experiments

A more complex scenario with two simultaneous stressors (kairomones and copper) and two attractive areas (with food) was simulated (Fig. 4a). The experimental design consisted of two regions with no copper, kairomones nor food (colored as white), in which the organisms were initially introduced ($n = 40$ in each). At the other extreme of the landscape, areas (colored in blue) were free of contamination and predator signal, but with food (0.5 g of grounded Tetramin® flakes in the left compartment and 20 individuals of *A. salina* in the right compartment), and were therefore considered areas attractive for colonization. Between the two initial areas, and those to be colonized, the populations were separated by two regions: one region (colored in light red) was contaminated with kairomones (at 5%) and low copper levels (0.5 μg/L) and another region (colored in ocher) was only contaminated with kairomones (at 5%). To evaluate the distribution of organisms in this scenario, the compartments were evaluated from three perspectives: (i) populations isolated (initial areas with two compartments) by low or high stressor pressure, (ii) populations inhabiting two less favorable regions (with four compartments each) with stressors (copper + kairomones or only kairomones), and (iii) populations in the two colonized areas (two compartments in each) (Fig. 4b).

2.5. Data and statistical analysis

The distribution of organisms (in %) for each compartment and observation time was calculated, considering regions (four compartments) or areas (two compartments). Then, the median and the standard error among the replicates and for all the time observations were calculated. Comparisons were made among areas and among regions, which are composed of the same number of compartments (four for regions and two for areas). For the colonization experiments, different calculations were used for each approach proposed (see details in 2.4.3 section). For the undisturbed clean areas and the areas with food, the difference of the final population with respect to the initial population was calculated as the percentage of the sum of organisms in the grouped compartments divided by the quotient among the total number of organisms in the experiment and the number of grouped compartments. For the disturbed regions, considering that half of the compartments TCK (Transit-copper-kairomones)

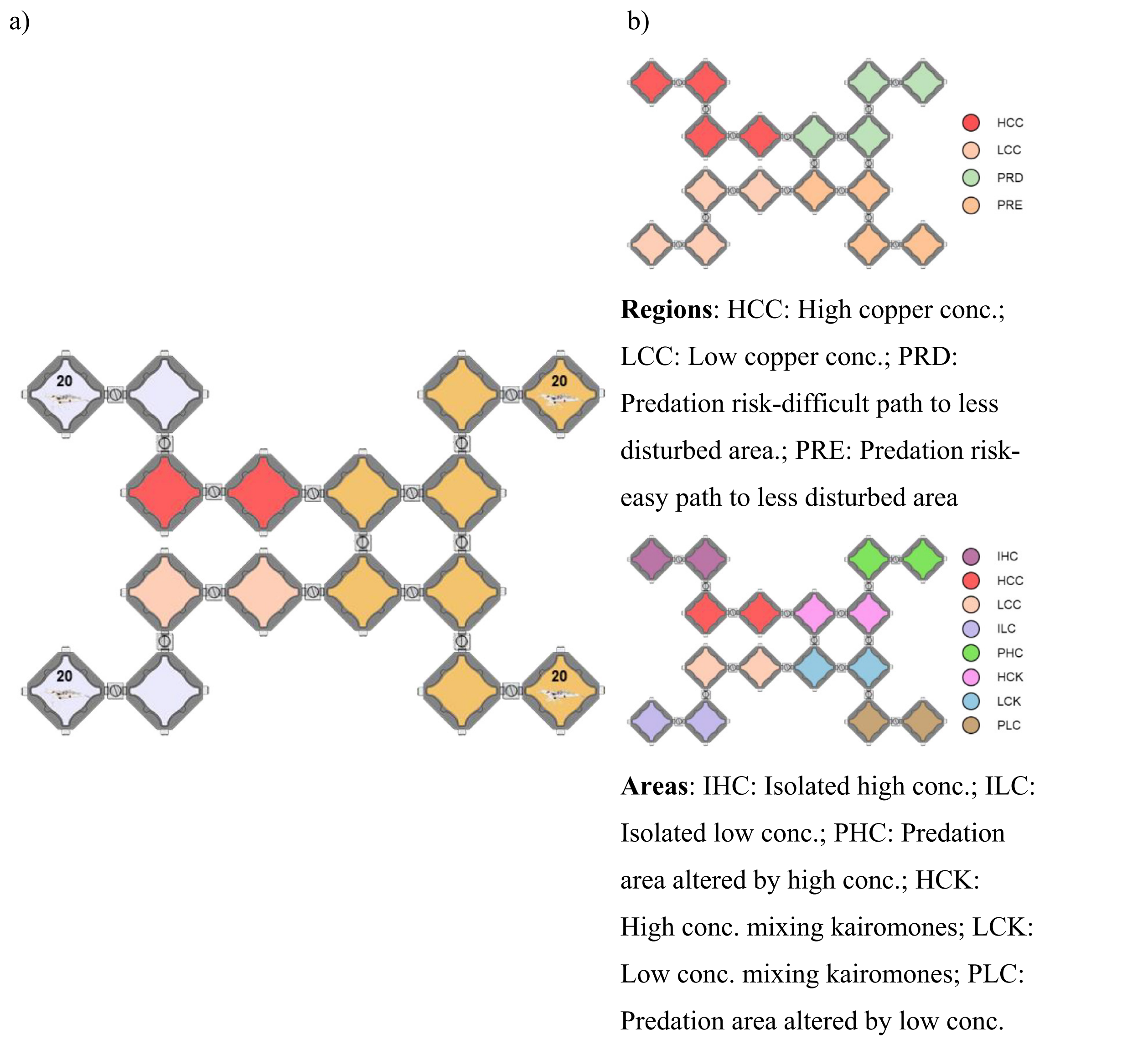

Fig. 3. Scheme of the a) experimental design for the copper and kairomone experiments and b) spatial arrangement limiting regions (upper) and areas (lower) for data analysis. The number 20 indicates the number of shrimps that were initially introduced in each compartment.

and TOK (Transit-only kairomones) were not connected, the calculation was performed by dividing the total population (80 shrimps) by half; then, the percentage of organisms in those compartments was calculated considering the total number of organisms (40).

Statistically significant differences in the distribution of shrimps (%) among regions/areas were analyzed using a mixed design ANOVA (repeated measures), where time was treated as a within-subjects factor (repeated measure) and regions/areas were treated as a between-subjects factor. The sphericity of the repeated measures was evaluated using Mauchly's test and the Greenhouse-Geisser correction for degrees of freedom was applied if the variances of the differences were not equal ($p < 0.05$). If the mixed design ANOVA detected a difference among times or regions/areas, the Bonferroni post-hoc test was applied to discriminate pairwise differences ($p < 0.05$). If time was not a significant factor ($p > 0.05$), replicates within the same observation time were merged and a one-way ANOVA was used, treating each observation time as a replicate. Afterwards, to discriminate statistical differences among regions/areas, Tukey's multi-comparison tests were performed. For the colonization experiments, the repeated measures ANOVA test was not performed since, due to the nature of the experiments, several regions and areas started with zero organisms, it was expected that the arrival of organisms would depend on time. Since comparisons were made between two regions or areas in the colonization experiments, the Student-Newman multiple comparisons test was applied.

3. Results

3.1. Spatial distribution of shrimps in the different scenarios

The mixed-design ANOVA (repeated measures) indicated that the shrimps' distribution did not vary with time ($F < 0.0001$; $p = 1{,}000$) in the experiments with copper and copper + kairomones. Since time was not relevant, the one-way ANOVA test was used to find statistical differences among regions and areas (See details in Supplementary Material).

3.1.1. Copper experiments

The distribution of the organisms in the experiments with copper was statistically different when regions ($F = 29.63$; $p < 0.0001$; Fig. 5a) or areas ($F = 15.66$; $p < 0.0001$; Fig. 5b) were compared. Among regions (see Fig. 2 and section 3.1 in Supplementary Material for details), the undisturbed region (ULC) located farther away from the most contaminated region (HCC) had the highest population of shrimps. Although the number

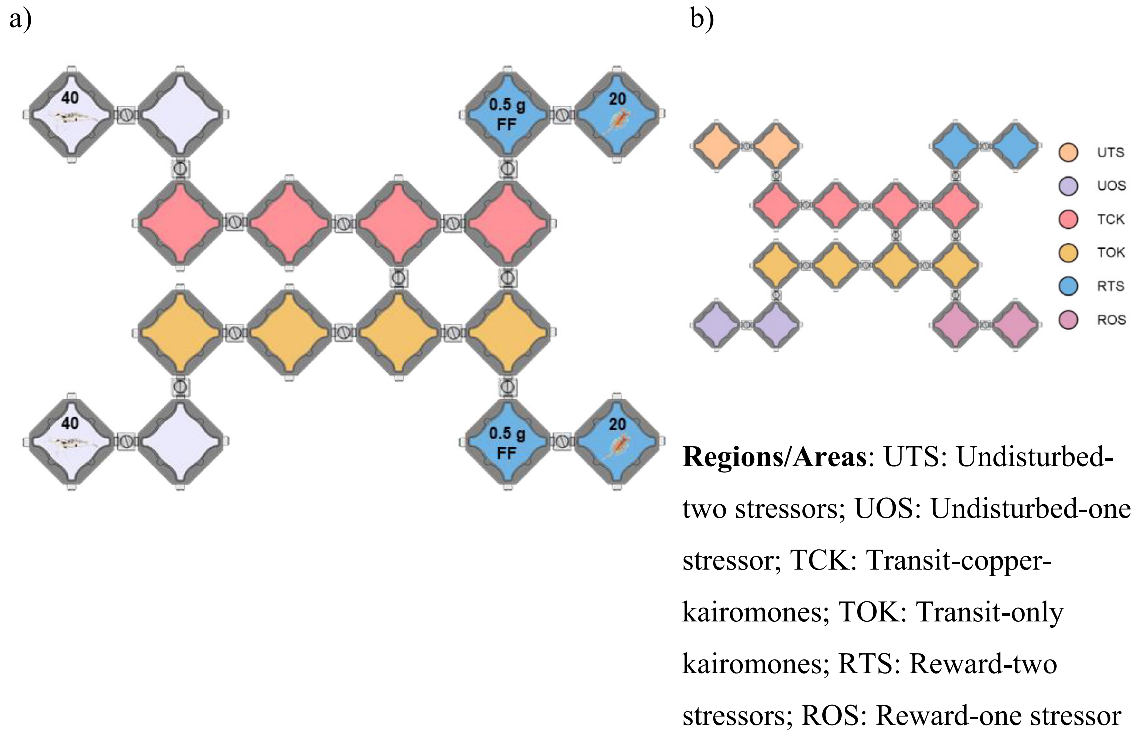

Fig. 4. Scheme of the a) experimental design for the colonization experiments with copper, kairomones and food (FF: fishfood flakes); and b) spatial arrangement in regions and areas for data analysis. The numbers 40 and 20 indicate the number of shrimps and artemias that were introduced in each compartment, respectively.

of shrimps in other regions was statistically similar, a clear tendency to avoid the highly contaminated region can be observed (Fig. 5a). When the landscape is analyzed per areas (see Fig. 2 and section 3.2 in Supplementary Material for details), this tendency to avoid the areas contaminated with copper is even more evident, with less than 5% of shrimps occupying the most contaminated areas. In addition, two potential effects of this highly contaminated area can be envisaged (Fig. 5b): (i) the population in IHC seems to be isolated and (ii) the clean zones connected to a higher contamination level (UHC region and THC area) support fewer shrimps than the zones connected with a lower contamination level (ULC region and TLC area).

3.1.2. Copper and fish kairomone experiments

In the double-stressor experiments, the shrimp's avoidance response was stronger for the two regions with kairomones, mainly those (PRD region) adjacent to the region with the highest copper concentration (Fig. 5c). Contrary to the results observed only with copper, the contaminated regions (HCC and LCC) without a predator signal were preferred when compared to the uncontaminated areas but with fish kairomones (Fig. 5c). However, the arrangement of areas showed that the clean areas within copper regions (IHC and ILC) were significantly ($p < 0.0001$) preferred (See Fig. 5d and sections 4.1 and 4.2 in Supplementary Material for details).

3.1.3. Colonization experiments

For the colonization tests, the shrimp populations showed a clear trend to move from the uncontaminated areas to other uncontaminated areas with food (Fig. 6a). Regardless of the zone they had to cross, the reduction in those populations was statistically similar over time ($F = 1.52$; $p = 0.238$). The population dynamics within the disturbed regions (Fig. 6b) showed a slight trend to increase with time. When the observations over time were considered as replicates, the two transient regions were statistically different (unpaired *t*-test; $t = 2.198$; df: 14; $p = 0.045$): a higher percentage of shrimps was observed in the region with only kairomones. However, when comparisons between both regions were made individually for each time, no statistical difference was detected (Student-Newman test; $p > 0.05$ – see all the comparisons in section 5 in Supplementary Material). The shrimp populations in the areas with food and considered likely to be colonized (Fig. 6c) experienced a trend to increase over time as expected; however, although the area with food adjacent to the region with only kairomones showed a regular population increase, the area with food adjacent to the region with two stressors (copper and kairomones) showed an irregular and intermittent population increase. The only statistic dissimilarity found by the Student-Newman test over the course of time between both areas was found at 4 h ($p = 0.035$).

4. Discussion

The present study provides a new approach to assess how contamination interferes with the dynamics of spatially connected populations of the estuarine shrimps *P. varians* in a heterogeneous landscape. It seems that it may lead to the isolation of populations. In order to increase the ecological relevance of the approach, the effects of contamination on the spatial patterns of a population of shrimps were studied in combination with another stressor (predation risk) and a stimulus factor (food).

When exposed to a landscape with copper, the shrimps were able to detect it and flee from it, as described by Araújo et al. (2020b). The area with the highest copper concentration was strongly avoided when compared to the other areas. The repellent effect of copper at a relatively high concentration (25 μg/L) seemed to have the potential to cause the isolation of the populations, or at least to decrease the connectivity among populations. Although to a lesser extent, this repellency of contamination had some effect on the populations inhabiting the clean areas immediately adjacent to the most contaminated compartments, where the number of individuals was slightly lower. Contamination seems to create a barrier that leads to a chemical fragmentation of the landscape (Noatch and Suski, 2012), in spite of spatial connectivity. Some studies using multi-compartmented exposure systems have shown the potential of contamination to fragment habitats. For instance, Islam et al. (2019) found that copper acted as a chemical barrier for the freshwater fish *Danio rerio* resulting in a rupture in the

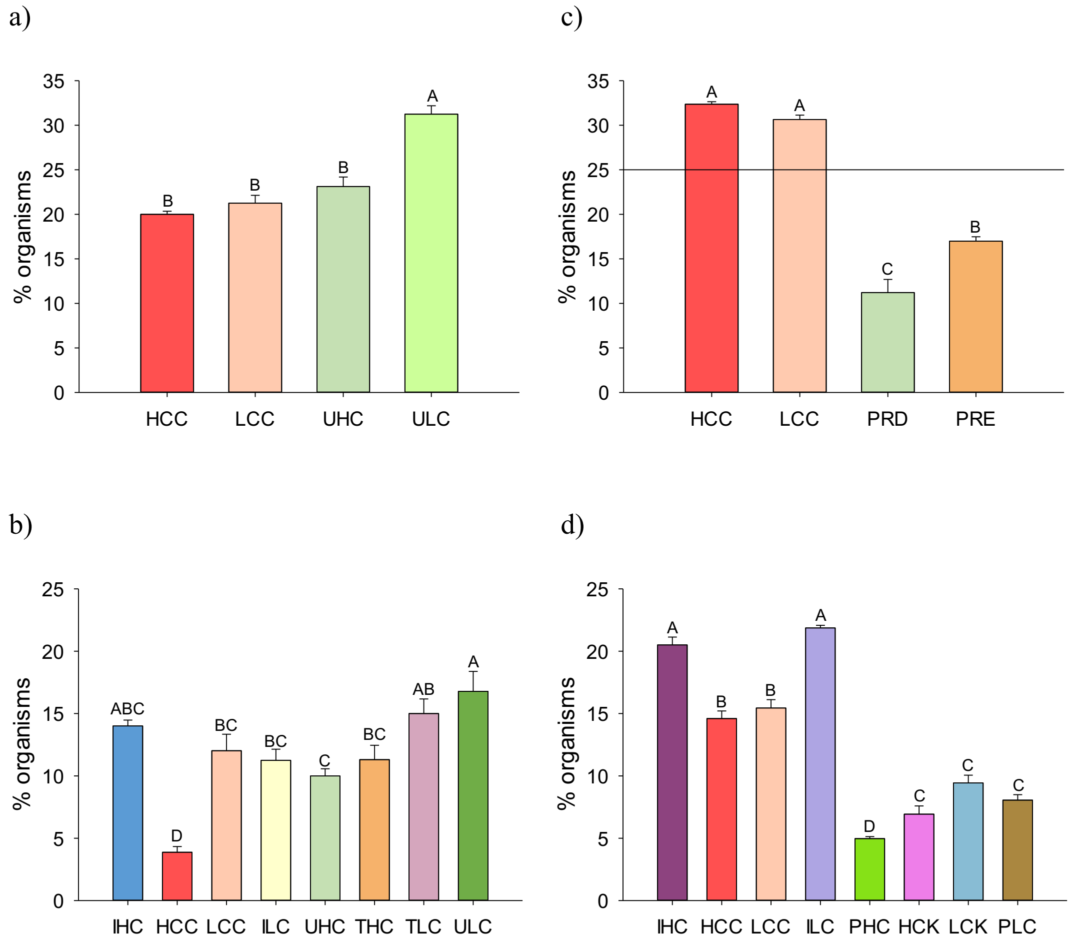

Fig. 5. Distribution (in %; median and standard error) of the shrimps in the different regions and areas in the experiments with copper (a and b) and with copper + kairomones (c and d). Legends for the regions and areas are described in Figs. 2 and 3. The horizontal line indicates the percentage of shrimps expected in a homogeneous distribution among compartments. Abbreviations: HCC: **H**igh **C**opper **C**oncentration; LCC: **L**ow **C**opper **C**oncentration; UHC: **U**ndisturbed along to **H**igh **C**oncentration; ULC: **U**ndisturbed along to **L**ow **C**oncentration; PRD: **P**redation **R**isk-**D**ifficult path to less disturbed area; PRE: **P**redation **R**isk-**E**asy path to less disturbed area; IHC: **I**solated along to **H**igh **C**oncentration; ILC: **I**solated along to **L**ow **C**oncentration; UHC: **U**ndisturbed along to **H**igh **C**oncentration; THC: **T**ransition to **H**igh **C**oncentration; TLC: **T**ransition to **L**ow **C**oncentration; ULC: **U**ndisturbed along to **L**ow **C**oncentration; PHC: **P**redation area altered by **H**igh **C**oncentration; HCK: **H**igh **C**oncentration mixing **K**airomones; LCK: **L**ow **C**oncentration mixing **K**airomones; PLC: **P**redation area altered by **L**ow **C**oncentration.

connection between habitats separated by contamination; and this effect could cause a serious connectivity problem among populations, as shown by Araújo et al. (2019c) who found that discharges from a waste water treatment plant chemically fragmented the landscape and caused isolation among the populations of the freshwater shrimp *Atyaephyra desmarestii*.

Taking into consideration that organisms' habitat selection process might not be based exclusively on contamination, the risk of predation was also included in the second experiment. The experimental design tried to simulate (using kairomones as the predator signal) a situation in which potential shrimp predators move to clean regions. When fish kairomones were added to previously preferred regions, the behavior of the populations changed: the shrimps fled from the predator signal, moving towards the contaminated regions, but with a clear preference for the less contaminated areas. This response makes it evident that having mechanisms protecting against predation is an important ecological factor that influences many aspects of the biology of prey species, including, in this case, the shrimps' physiology and behavior (Dodson, 1989; Ocasio-Torres et al., 2015), but up to what point does it remain in the presence of contamination? In a study performed by Sovová et al. (2014), fish forcedly exposed to copper decreased their anti-predator response; and even at low concentrations, copper influenced ecological decisions and survival with respect to predators (Gosavi et al., 2020). Furthermore, Araújo et al. (2020c) found that the freshwater shrimp *A. desmarestii* showed behavioral plasticity in habitat selection when simultaneously exposed to contamination (copper), predation signal and shelters: in the presence of predation risk and no shelter, the shrimps moved towards contaminated areas with no predation signal, making a balance between a minimal risk of predation with an acceptable level of contamination. Evolutionarily, aquatic species have developed an innate and immediate sensory system for predator recognition (Ferrari et al., 2010). However, although contamination can cause quick avoidance responses (Araújo et al., 2020a), the need to respond to this risk seems to be less immediate compared to the threat of predation. This cost-benefit balance in the presence of several stressors generates a functional compensation for stressor effects (Fischer et al., 2013; Heuschele et al., 2020), but it could be time-dependent if the exposure lasts longer. In this case, the act of inhabiting a contaminated area may bring undesirable consequences related to toxicity and irreversible damage to the chemosensory system responsible for the detection and escape from contamination (Azizishirazi and Pyle, 2015). In addition, if areas providing shelter against predators are exposed to contamination, the organisms' susceptibility to the toxic effects of the contaminant might be increased, as they do not have areas to alleviate both the pressures of contamination and predators.

The last experiment, in which colonization behavior was assessed, confronted the organisms with a challenging landscape scenario: either to stay in a clean area with no food or move through disturbed regions to colonize a clean region with food. The shrimp populations reached the areas with food, which could be considered a priority for the organisms. The

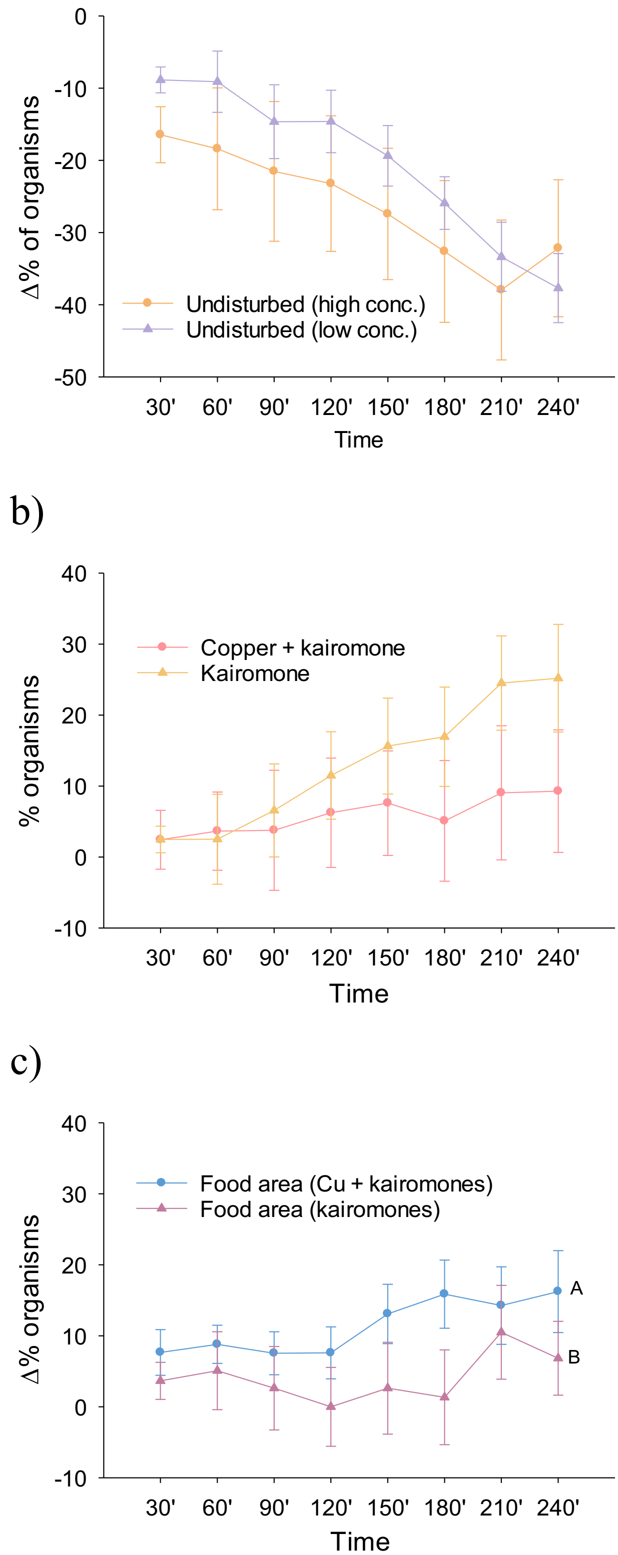

Fig. 6. Median and standard error of the shrimp populations in the colonization experiments over time: *a)* the population decline from the initial areas; *b)* the population dynamics within the disturbed areas, and *c)* the percentage of the population that colonized the clean areas with food and no predator signal.

displacement of organisms seemed to have been more regular throughout the region where there was no copper, while the region contaminated with copper combined with predation signal had a lower population dynamic. Certainly, the contamination level is a relevant factor to consider regarding foraging activities. For instance, Araújo et al. (2016b) found that tilapia fry might colonize previously avoided contaminated areas to feed if the availability of food is greater. However, Islam et al. (2019) found that zebra fish were prevented from colonizing areas with food if they had to cross a highly contaminated chemical barrier. Thus, it is evidenced that even though food is indisputably an attractive factor, contamination can condition the choice of organisms to forage, and thus they are likely underuse the food resources of the habitat.

Human-modified landscapes are increasingly fragmenting habitats and varying their spatial complexity and connectivity (Fuller et al., 2015; Gilarranz et al., 2017; Yeager et al., 2020). This unfavorable scenario supposes a susceptibility to population decline and dispersion as habitat is lost and disturbances such as contamination restrict habitat use. Isolated subpopulations formed due to contamination reduce the number of individuals and limit the connectivity, which affects the interspecific trade-off and might lead to a loss of genetic variability, triggering a likely long-term extinction (Ribeiro and Lopes, 2013). To address this, collaboration between ecotoxicologists and decision makers is crucial to minimize the risk of contaminants in ecosystems. Measures should be developed and then taken to maintain suitable ecosystems for organisms, either by restricting human activities, contamination sources or protecting the areas where organisms can meet their nutritional requirements and the predator-prey relationship remains unthreatened by contamination (Depledge and Galloway, 2005; Gilarranz et al., 2017). However, it should be taken into consideration that contamination can reach these protected areas from multiple, indirect and external sources (Rodríguez-Jorquera et al., 2016). Therefore, to prevent habitat fragmentation and reduce the ecological imbalance at the landscape level to protect the biodiversity and function of ecosystems, it is necessary to identify potential sources of contamination and to monitor their effects; not only from an individual perspective (toxicity), but also from a landscape perspective (spatial distribution of organisms and connectivity among populations).

Finally, it is important to highlight that assay systems such as HeMHAS seem to be useful to simulate the connectivity between habitats by including a multi-factorial approach to chemically heterogeneous landscape scenarios. More important than the effects of the copper at the specific concentrations used in the current study (the levels of contamination were arbitrarily selected to simulate low and high contamination loads) and the levels and composition of kairomones, our results bring this new version of HeMHAS as a complementary tool to be applied to environmental risk assessments that could complement the traditional ecotoxicological approaches by providing a landscape view for stress ecology.

5. Conclusions

The present study shows that contamination might interfere with the spatial dynamics of shrimp populations fleeing from contaminated areas. It was observed that contamination might also prevent the colonization of attractive areas if they are influenced by, or connected to, sources of contamination, which might act as a driver for the isolation of populations, making them more susceptible to local extinction. Furthermore, contamination exerts a stress that might exacerbate the effects produced by predation, jeopardizing a population's persistence. Thus, shrimps' habitat choice in complex scenarios when confronted by contamination, predation risk and food availability should be taken into account in environmental risk assessments, since the maintenance of the population might depend to some extent (but not exclusively) on the spatial arrangement of these factors. The approach applied in the current study (using the versatile HeMHAS version) brings a wider view of how contamination could be studied from a landscape scale and it provides ecotoxicology with a complementary methodological and conceptual tool to reach a higher level of ecological relevance.

CRediT authorship contribution statement

David Salvatierra: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Ángela Rodríguez-Ruiz**:

Investigation. **Andrea Cordero**: Investigation. **Julio López-Doval**: Methodology, Analysis, Writing - review & editing. **Francisco Baldó**: Methodology, Analysis, Writing - review & editing. **Julián Blasco**: Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition. **Cristiano V.M. Araújo**: Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition.

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.2022.153225.

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