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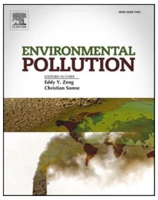

A novel experimental approach to assess the effect of contamination events on the spatial distribution of organisms in lotic-estuarine landscapes

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ABSTRACT

The riverscape concept includes structural connectivity and functional interactions where upstream processes significantly influence downstream conditions. As such, a landscape perspective is necessary to evaluate the impact of contamination, because it can spread far beyond the release area, potentially reaching estuaries. Therefore, we hypothesized that contamination might affect freshwater organisms' habitat selection in a simulated lotic-estuarine (flow-through) landscape, considering that the estuary zone acts as a stress factor by restricting the options of habitats while the non-contaminated upstream areas could serve as shelter zones to alleviate or even prevent the contamination exposure. The aim of this study was to provide a new method to evaluate the habitat selection response of aquatic organisms when exposed to a contamination event in a simulated lotic-estuarine landscape, including upstream and shelter zones as attractive habitats and an estuary zone. The freshwater shrimp *Atyaephyra desmarestii* was used as model test organism, copper was used as the contaminant, and the Heterogeneous Multi-Habitat Assay System (HeMHAS), was employed to simulate the lotic-estuarine landscape with a multiple connectivity setup. The results showed that contaminated conditions led shrimp to avoid the contaminated area and were carried towards the estuary region with the flow. However, in a static experiment (without flow), both estuary and contaminated regions were avoided and a preference for the shelter (clean) regions was observed. This study highlights the plasticity of organisms' habitat selection behavior within a connected ecosystem, where contamination and salinity pressure together might have serious implications for the distribution of freshwater species. Further, the HeMHAS can successfully integrate flow in simulated heterogeneous landscapes to broad the understanding of contamination effects in aquatic lotic ecosystems.

1. Introduction

The landscape concept has been traditionally used for terrestrial ecosystems (Bastian, 2001), where aquatic environments are considered as subunits linked to the terrestrial landscape by cross-ecosystem flows of material and energy (Wiens, 2002). Whereas, the landscape ecology of running waters (the riverscape concept) includes the structural connectivity and functional interactions of lotic landscapes [connected streams, tributaries, river and estuary (Ward, 1998; Lookingbill et al., 2022; Lee et al., 2022)]. These connectivity and interactions imply that upstream processes and biotic and abiotic elements contribute to modifying downstream ecological conditions (Marsh & Fairbridge, 1999; Moore, 2015). Therefore, the constant input of those elements along the riverscape is determinant for downstream processes such as the availability of resources and the distribution of biodiversity (Tumolo et al., 2020; Silva et al., 2020). Running waters can also transport the contamination to areas far beyond the release area (Carlsen et al., 2004), even reaching the estuaries. Therefore, studies on the impact of contamination should consider the entire landscape of the system, including areas that could be directly or indirectly affected.

The studies assessing contamination in rivers and estuaries are commonly limited to the impacted region and some adjacent areas, which is a valid approach if toxic effects at the local scale are the main goal of the study. However, the landscape perspective of lotic

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ecosystems makes it necessary to study contamination on a larger spatial scale (Johnson, 2002) due to the structural and functional connectivity that exists among upstream and downstream regions, tributaries and estuarine areas. This is even more necessary if the contamination risk assessment is based on the spatial distribution dynamics of species and their habitat selection processes. When the connectivity among areas is taken into account, the risk area tends to be extended. Thus, some effects may be expected, such as: alteration of the displacement of organisms among undisturbed habitats, changes in the availability of food, spawning sites, mating areas (Fausch et al., 2002) and isolation of populations due to habitat discontinuity (chemical fragmentation; Taylor et al., 1993). In addition, when contamination spreads along a river, the number of areas favorable to accommodate life might be reduced, making the downstream displacement of freshwater organisms towards estuarine zones a means of escaping. However, although the contamination dilution benefits downstream habitats, the salinity of the estuarine zones could be a limiting factor (Tockner et al., 2010). Therefore, from a lotic-estuarine landscape perspective, the upstream contamination can affect the downstream species distribution (Torgersen et al., 2021).

The experimental approach of the contamination risk from the lotic landscapes' perspective represents considerable challenges due to some factors that are difficult to simulate. This difficulty is how to: expose organisms to a spatially heterogeneous scenario with different and connected habitats, include the estuarine area into the system and, finally, provide the flow that characterizes lotic systems in that scenario. This complex scenario can be simulated experimentally by using non-forced exposure approaches in multi-compartmented systems (Lopes et al., 2004). Promising results regarding the role of contamination in organisms' habitat selection processes in lotic systems have already been reported (Araújo et al., 2018, 2019; Silva et al., 2020, 2024), where multiple simultaneous variables can act as drivers of the selection process of an habitat, as organisms weigh the costs and benefits between stressors (contamination, predation, euryhaline conditions) and attractors (food, shelter, social needs). This process may involve physiological mechanisms (Petitjean et al., 2023) or behavioral responses (Araújo et al., 2020). The Heterogeneous Multi-Habitat Assay System (HeMHAS) is a non-forced exposure system developed to evaluate the effects of contamination on: avoidance and colonization behaviors, habitat selection plasticity, habitat connectivity, among others (Salvatierra et al., 2025a). The HeMHAS makes it possible to compartmentalize the landscape with different connected areas, thereby creating several environmental scenarios to simulate contamination events. A recent review describing the advantages and weaknesses of the HeMHAS has shown its suitability to study the impact of contamination from a landscape perspective, considering contamination as a driver for species' spatial distribution (Salvatierra et al., 2025a). The HeMHAS has been useful to assess the vulnerability of a shrimp population isolated by contamination (Salvatierra et al., 2022), changes in ecological interactions between marine fish and shrimp due to the contamination at a mesocosm scale (Islam et al., 2024) and the foraging activity of fish combining contamination and spatial habitat fragmentation (Salvatierra et al., 2025b).

In order to include the landscape perspective in ecotoxicological tests, the current study brings a novel method to simulate the disturbance provoked by contamination in a lotic-estuarine landscape. It was firstly hypothesized that contamination might condition the spatial distribution of species in this simulated landscape, limiting the extent of favorable areas. Secondly, the estuary habitat could act as an additional factor restricting the options of habitat selection for the freshwater organisms, due to saline stress. Finally, the non-contaminated upstream area and the shelter tributary stream could serve as refuge zones to alleviate or even prevent the organisms' exposure to contamination. In order to test this proof of concept, the present study aimed to evaluate the habitat selection response of the freshwater shrimp *Atyaephyra desmarestii* (Millet, 1831) when exposed to a contamination event in a simulated lotic-estuarine landscape. This landscape was simulated by using the HeMHAS, including four different regions: a clean upstream region and a shelter region serving as attractive habitats simulating clean tributary streams, a contaminated region discharging contaminant into the river and an estuarine region as an additional restriction factor for freshwater organisms in downstream habitats due to the high salinity. The landscape was tested under a flow-through regime and a static (without flow) condition. The freshwater shrimp *A. desmarestii* was used as the test organism, due to its plasticity in the habitat selection process (Araújo et al., 2019, 2020); and copper was selected as a reference contaminant, as it has been shown to condition the habitat selection behavior of aquatic organisms, including *A. desmarestii* (Vera-Vera et al., 2019).

2. Materials and methods

2.1. *Test organism and culture conditions*

The freshwater shrimp *A. desmarestii* (adults, 2 ± 0.5 cm length) were collected in the Guadalete river basin, close to the river' spring (36°47′54.6"N, 5°19′54.1"W) in Zahara de la Sierra, Spain. After sampling, the shrimp were acclimated in the Institute of Marine Sciences of Andalusia (ICMAN-CSIC, Puerto Real, Spain) in dechlorinated tap water renewed daily at a 25 % rate (until reaching 100 % at the 4th day) in 40 L aquariums with continuous aeration at 20 ± 1 °C. Organic debris was carried along with the shrimp as a food source. Before the experiments, a starvation period of one day was established. No mortality was observed during the acclimation period.

2.2. *HeMHAS – Heterogeneous Multi-Habitat Assay System*

The versatile version #2 of the HeMHAS was used as test system (Salvatierra et al., 2025a). It allows the spatial simulation of landscapes through compartments that can be connected at all corners by passages with rotating doors (manually operated) to allow or restrict the organism's free movement among the compartments. Hence, a multi-connected aquatic habitat can be created under a non-forced exposure approach.

2.3. *Experimental design*

A lotic-estuarine macrohabitat with 16 connected compartments was simulated including: an upstream region as a pristine habitat (blue), a contaminated region simulating a discharge event (red), a clean tributary stream serving as a shelter region (green), and an estuary region (light blue; see schematic representation in Fig. 1). The contaminant was used as a stressor that might condition the habitat selection process of freshwater shrimp, while the estuary region represents a restricted habitat (unfavorable salinity). The upstream and the tributary stream were considered undisturbed habitats.

2.3.1. *Upstream habitat*

The hydrological flow simulated in the system established a non-stressed, upstream region, with negligible influence of either contamination or brackish water. This habitat received the same water used to culture the organisms.

2.3.2. *Contaminated habitat*

To simulate a contamination region, a tributary stream was contaminated with a nominal copper concentration of 25 μg L^{-1}, prepared from a stock solution at 100 mg L^{-1} (Standard solution, Merck reference product). This concentration is within the range of natural events of contamination that characterized this metal up to mg L^{-1} close to mining sources (Morariu et al., 2025); and also is a concentration that triggered an avoidance response in the same species (Vera-Vera et al., 2019). For the chemical analyses, samples from each compartment were

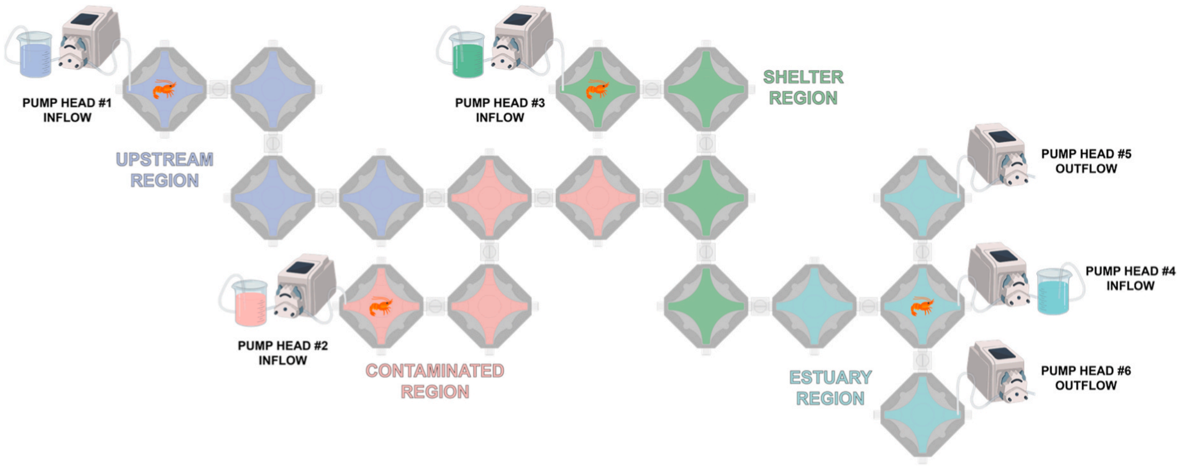

Fig. 1. Spatial setup of the experimental design. Each color represents one region, and a total of 20 organisms were placed at the first compartment of each of the 4 regions (upstream, contaminated, shelter and estuary; colored in blue, red, green and light-blue, respectively). The total number of organisms was 80 for each replicate. The inflow pump heads (#1, 2, 3 and 4) were connected to one peristaltic pump that supplied 70 mL min^{-1} of treated water to each region, while the outflow pump heads (#5 and 6) were connected to other peristaltic pump that removed 140 mL min^{-1} each. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

collected using 45 mL Falcon plastic metal-free tubes and acidified with 65 % HNO_3 at 1 ‰ rate and subsequently stored at 4 °C. The chemical quantification of copper was performed by inductive coupled plasma-mass spectrometry (ICP-MS) with the samples at either 1:5 or 1:20 dilution factor (taking into consideration the proximity to the estuary compartments, given the salinity diffusion of the saltwater). Analyses were carried out at the Institute of Marine Sciences of Andalusia, ICMAN-CSIC (Puerto Real, Spain), following the procedures described by Apha (2007). The calibration curve was matrix match and the range selected was 1–50 μg L^{-1}. The recovery rate was higher than 95 % with a detection limit of 0.015 μg L^{-1}. The concentrations recorded are presented in the Supplementary Material, Fig. S1 and S2.

2.3.3. *Shelter habitat*

The shelter habitat was established as a simulated clean tributary stream where the current flow limited the intrusion of contamination and salinity to this habitat, serving as an undisturbed shelter region. The conductivity values of the freshwater used in this and the previous habitats ranged from 550 ± 25 μS cm^{-1}, while salinity values ranged from 0.3 ± 0.01 ppt.

2.3.4. *Estuary habitat*

Filtered (1 μm filter) saline well water was placed in the most downstream region of the simulated lotic-estuarine landscape. This water is used to culture marine species by the Institute where the experiments were carried out. The conductivity values ranged from 50 ± 2 mS cm^{-1} for the saltwater used in this habitat. The salinity values ranged from 25 ± 0.1 ppt. All conductivity and salinity measurements were performed with a LAQUA EC210 handheld meter.

2.4. *Habitat selection experiments*

A total of 4 experiments were performed: a control with continuous flow, a static control, a treatment test (combining contaminant, saline water and continuous flow), and a static treatment test with contaminant and saline water. The static control experiment and the static treatment tests were performed without the continuous flow, so the water introduced at the beginning of the experiment remained until the end of the experiment without renewal. Each experiment had 3-4 replicates, with 80 shrimp per replicate, being 20 individuals placed in each region, as shown in Fig. 1.

Initially, one tank per region (20-liter volume) was prepared containing the water used in each treatment. From each tank, one peristaltic pump with four inflow pump heads pumped water into the system, such as indicated in Fig. 1: pump head #1 (upstream region with freshwater used for the shrimps' maintenance), pump head #2 (contaminated region with freshwater spiked with copper at 25 μg L^{-1}), pump head #3 (shelter region with maintenance freshwater), and pump head #4 (estuarine region with saline water). These four pump heads provided a flow of 70 mL min^{-1} each, 280 mL min^{-1} for all the system, and connected to the same peristaltic pump. Additionally, to stabilize the water level within the system, two outflow pump heads connected to a second peristaltic pump were placed at the extremities of the estuarine region (pump heads #5 and #6 in Fig. 1) to remove water at a rate of 140 mL min^{-1} each. All the four tanks were continuously aerated. Once the system was filled with fresh/saline water (with the rotatory doors within the passages separating the compartments closed), 20 organisms were introduced as shown in Fig. 1. Finally, all the rotatory doors were opened and both inflow and outflow pumps were simultaneously activated, creating the flow inside the system. Every 30 min and for 4 h, the pumps were switched off, the rotatory doors were closed and the number of shrimp in each compartment was recorded; then, the pumps were switched on. The 4 h experimental time was chosen considering previous studies that used similar exposure times with this species in other habitat selection assays (Vera-Vera et al., 2019; Araújo et al., 2019).

The laboratory conditions during the experiments were of 20 ± 1 °C, with continuous light, and in silence. In addition, to avoid any other effect of the laboratory on the organisms, the spatial orientation of the experimental system in the laboratory was rotated 180° each replicate of each treatment, therefore any external factor influencing the shrimp's habitat selection could be detected and reduced.

2.5. *Data and statistical analysis*

Data of population distribution is represented by counting the number of organisms present in each compartment. Since a Two-way ANOVA test demonstrated that the observations among times were homogeneous, only the data of the 4th hour was used for posterior analyses. The number of organisms in each region was counted and, also, the number of organisms in each area (Fig. 2). In this case, the areas represent the two compartments with no interference from the main river section. With this, the dependent variable for the statistical analysis was the percentage of organisms in each area, while the sources of variation were the different areas and whether the treatment had flow or

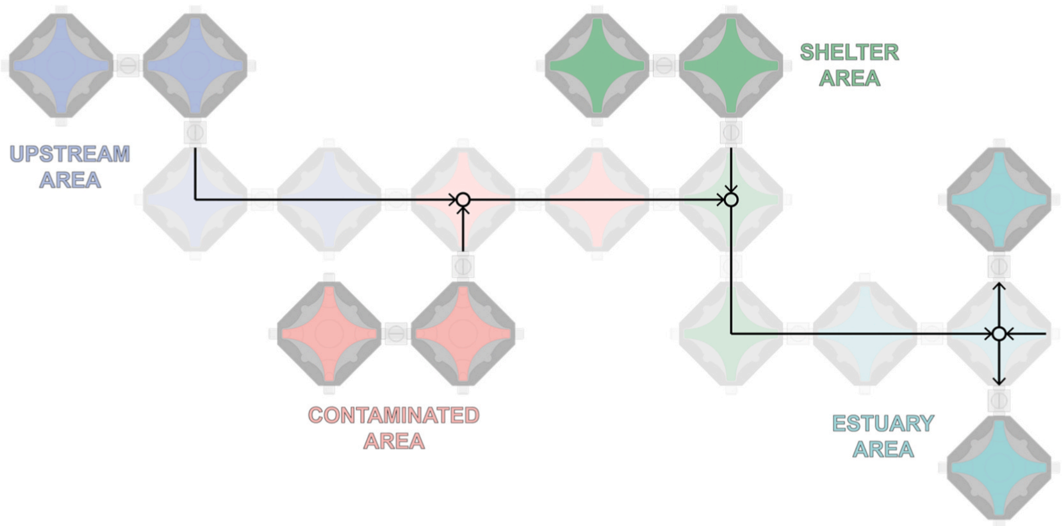

Fig. 2. Spatial clustering of the treatments for statistical analysis. Each area grouped the two compartments located in the top-end of each region (upstream, contaminated, shelter and estuary). The central zone of the system, represented by the 8 Gy compartments, were not considered in the habitat selection analyses as it represents a mixing area among the different regions.

not. For the statistical analysis, after a Kolmogorov-Smirnov normality test and a Brown-Forsythe equal variance test, a Two-Way general linear model ANOVA (for the control experiments) and a Two-Way balanced design ANOVA (for the treatment experiments) were used. The post-hoc Tukey test was used for all pairwise multiple comparison procedures, discriminating between areas and flow treatments. All statistical analyses were performed using Sigmaplot (v.15.0).

3. Results

In the control experiments (all compartments filled with freshwater) the organisms were distributed randomly among the four areas regardless of the flow condition. However, the organisms tended to occupy the area compartments (outside the river's main course) instead of the central compartments (Fig. S3 in Supplementary material). Under both flow and static conditions, a large part (around 40 %) of the shrimp moved to the most downstream region.

In the experiments with stressors (copper and salinity), the organisms' population distribution varied among the areas and it was more noticeable between the shelter and estuary areas with different flow conditions (Fig. 3). In addition, the percentage of occupancy for the treatment experiments was higher in the central compartments than in the area compartments, which was the opposite tendency of the control experiments.

If we consider only the population distribution of the organisms that occupied the areas, the control experiments evidenced no statistical differences in the percentage of organisms, independently of the flow (Fig. S4 in Supplementary Material). In the treatment tests with the contaminant and saltwater, significant differences were found among areas ($F_{3,24} = 3895$; $p = 0{,}021$), but since the interactions between areas and flow was statistically significant ($F_{3,24} = 6432$; $p = 0{,}002$), the differences among areas were discriminated from the flow treatments. Under flow conditions, the post-hoc test indicated that there were no statistical differences in the percentage of organisms among the areas (see uppercase letters in Fig. 4). However, in the absence of flow, the shrimp preferred the shelter area, with statistically significant differences compared to the estuary ($p = 0{,}001$), contaminated ($p = 0{,}002$), and upstream ($p = 0{,}029$) areas (see lowercase letters in Fig. 4). Comparisons of the results between the flow and static conditions, within the same treatment, showed that the population percentage within the shelter and estuary areas significantly differed between the flow conditions ($p = 0.002$ for the shelter area, *0.014* for the estuary area; *

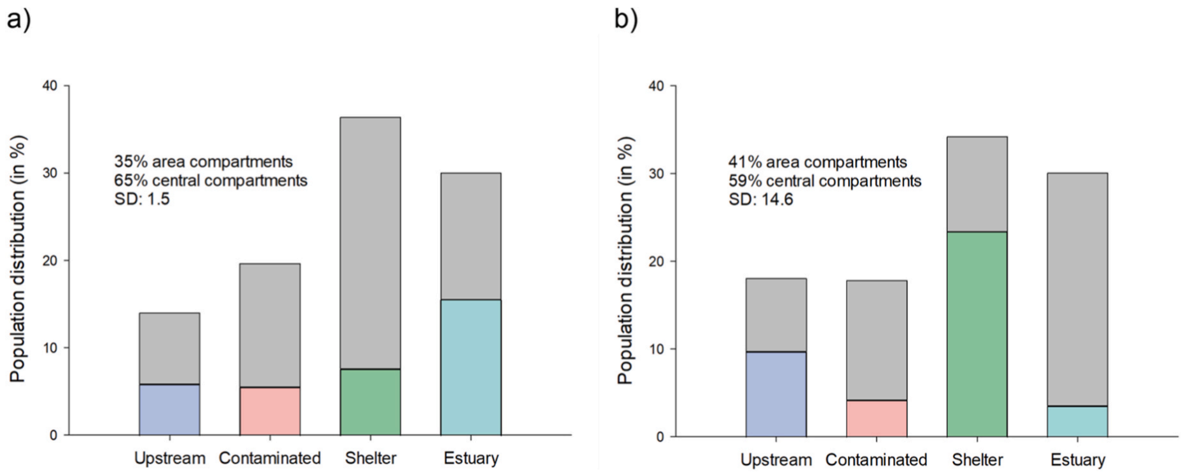

Fig. 3. Percentage of occupancy in the experiments with contamination and saltwater after 4 h within the area compartments (colored portion of the bars) and the central zone of the river (light gray portion of the bars), both with: a) flow conditions, and b) static conditions. For clarity, please examine Fig. 3 with Fig. 2.

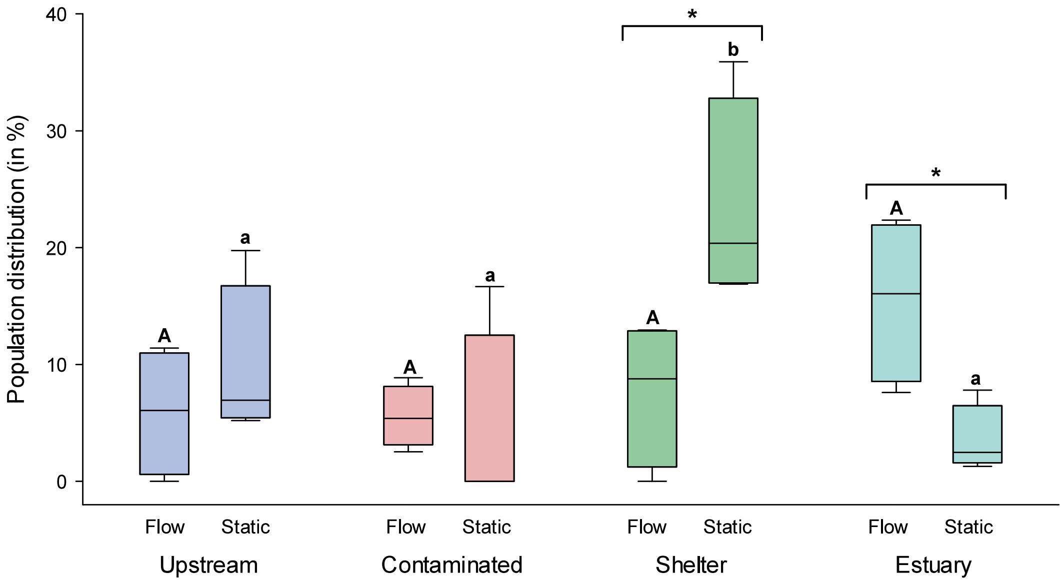

Fig. 4. Median population distribution (% of organisms) and interquartile ranges at the 4th h of the treatment experiment (including contamination and saltwater) under flow (left boxplot of each upstream, contaminated, shelter and estuary area) and static (right boxplot of each upstream, contaminated, shelter and estuary area) conditions. The letters over the boxes represent the statistical differences among the areas in the experiments with flow (upper case) and static (lower case) conditions. The * represent statistical differences between the flow and static conditions within the area.

symbol in Fig. 4). All the statistical analyses are presented in Supplementary Material, section 4.

4. Discussion

The current study brought a novel method to evaluate how the contamination in a lotic-estuarine landscape with different connected habitats can condition the habitat selection of freshwater organisms. In this first proof of concept, the results with the shrimp *A. desmarestii* evidenced that in the control experiments the shrimp distributed randomly among the areas both with flow and without (Fig. 4), with no preference for any area in particular. On the contrary, in the experiments with contamination, the shrimp moved out of the contaminated area, with a slight preference for the estuary region in the experiments with flow, but with a preference for the shelter area in the absence of flow. This change in distribution between the controls and the test experiments highlights the potential of contamination to affect the spatial distribution of organisms along the lotic-estuarine landscape. Copper has been previously studied with this species as a reference contaminant, where static exposure scenarios have evidenced negative effects in the biochemical performance and survival (Quintaneiro et al., 2015, 2016), and also triggered an avoidance behavior influencing the habitat selection pattern (Vera-Vera et al., 2019; Araújo et al., 2020). This trend is also consistent with other contaminants and with other species (see reviews of Araújo et al., 2016; Moreira-Santos et al., 2019; Salvatierra et al., 2025a).

Beyond these antecedents, copper was addressed from a general perspective of the contamination in riverscapes, presenting a complementary approach to study how contamination drives the spatial distribution of organisms in a landscape by providing two perspectives regarding contamination: a) as a habitat selection driver in a chemically heterogeneous landscape, and b) as a non-static element that flows downstream thereby potentially affecting a large extent of the river. The role of contamination driving the spatial dynamics of organisms has been evaluated previously by Schulz and Liess (1999), who evaluated the stream population dynamics of *Gammarus pulex* and *Limnephilus lunatus* in a multi-species field study under contamination scenarios in two agricultural tributaries and the connected headstream. The authors evidenced that during run-off events, *G. pulex* migrated from the potentially contaminated headstream towards the uncontaminated tributary, regarded as a refuge habitat and site of recolonization, a tendency also evidenced by aquatic invertebrate communities in response to pesticides (Knillmann et al., 2018).

Through the use of non-forced exposure systems to simulate different landscape setups, recent studies evaluated the role of a non-contaminated tributary river as a shelter, such as Araújo et al. (2018). These authors observed that fish tended to move downstream, avoiding the most contaminated water from a river and, after reaching the confluence point with another river, the fish moved upstream, selecting the water from the less impacted area. So, within such a landscape, the presence of a tributary river could function as a way to alleviate the stress provoked by contamination. Another two analogous experimental studies were performed with water samples from Amazonian rivers (Silva et al., 2020, 2024), and these studies showed that the organisms tended to select the water with lower levels of contamination. This novel approach to study contamination-driven organisms' spatial distribution from a landscape perspective is facilitated by non-forced exposure experimental systems as such the HeMHAS.

The use of a continuous flow with water with different characteristics going down the river's main course provides a relevant perspective for landscape ecotoxicology studies. Traditionally, studies with a flow-through system use only one concentration for each treatment (Colvin et al., 2016; Don Xavier et al., 2019). In contrast, the current study included four regions with different chemical characteristics. Despite the limited spatial scale, this approach could be considered a very useful mechanism to study the dynamics of contamination in a river by integrating different habitats, including undisturbed pristine habitats, contamination discharges and estuaries. The flow within the system also made it possible to control the dispersion of the contamination, thus maintaining the upstream and shelter habitats clean (Supplementary material, Fig. S1); while in the static experiment the contaminant dispersion affected other regions (Supplementary material, Fig. S2). This could explain the slight aversive response that the shrimp showed to the contaminant in the static experiment (Fig. 4). The heterogeneity of habitats improves our understanding of the impact of contamination in rivers, which is not necessarily only restricted to the discharge point; so, contaminants may also condition the quality of downstream habitats. With this contextualization, the higher percentage of occupancy in the central compartments dominated by the current (Fig. 4) might be a consequence of the confluence of flows, which transported the contaminant downstream. In this scenario, passive avoidance could have been triggered (current-driven drift), carrying organisms downstream (Taylor et al., 1994; Roper et al., 1995; Araújo et al., 2016).

The approach proposed here is critical to understand the plasticity of organisms' habitat selection behavior in lotic connected ecosystems, mainly by including the estuaries as an osmotic restrictor to freshwater species. The hydrological dynamics of estuaries with varying tide fluctuation and riverine contribution make this area very important in the population dispersal of freshwater communities, given the restriction in the habitat use caused by the pressure of the salinity/conductivity levels. Some studies have reported that *A. desmarestii* has a certain tolerance for salinity levels (Janssens de Bisthoven et al., 2006), and it has been sampled within a conductivity range of >3.4 mS cm^{-1} in the Guadalete and Guadiana rivers (Gallardo-Mayenco, 1994), and >5 mS cm^{-1} in Ebro delta (Forés et al., 1986). This gives us an estimated natural slight tolerance range of this species close to estuarine zones related to salinity pressure. In relation to this study, while in the experiments with flow there was a constant freshwater contribution in the estuary area (<3.5 mS cm^{-1} in 75 % of the system, but >10 mS cm^{-1} in the estuary region; Fig. S5 in Supplementary material), the static conditions favored the spread of the saltwater (>3.5 mS cm^{-1} in 80 % of the system; Fig. S6 in Supplementary material). An experimental study with samples from the Guadalete river – Spain (Araújo et al., 2019), found that conductivity levels of up to 2 mS cm^{-1} did not limit the habitat selected by this organism, such as observed in the current study. However, when seawater spread more widely, the salinity became a source of stress for the freshwater shrimp, as they decided to explore upstream for habitats with better conditions (Fig. 4).

The combined effects of contamination and osmotic stress for aquatic species, such as the lotic-estuarine landscape simulated in the current study, are likely to jeopardize the population persistence of species in downstream regions, especially for those that are not regularly adapted to such environmental fluctuations, as that combination might exacerbate the toxicity of contaminants (Lee et al., 2010). This simultaneity of environmental variables is important to provide realistic experimental conditions and hence improving the ecological relevance of the results. In this sense, the connectivity of compartments and the heterogeneity in landscapes that HeMHAS provides makes it possible to customize structural flow-connected habitats and form complex spatial landscape setups. Moreover, the implementation of flow-through in the system has sustained the concentration of contaminated and uncontaminated compartments over time, a long-lasting and challenging issue for the non-forced ecotoxicology approach (Salvatierra et al., 2025a). It is also important to highlight that flow-experimentation scenarios have a limited perspective of the hydrological dynamics of riverscapes (seasonal and predictable periods of low frequency flow disturbances, or short-term events such as sudden floods and droughts; Rolls et al., 2012; Fausch et al., 2002), However, the simulation of lotic conditions in ecotoxicity assays is still scarce, and therefore, the current study can contribute to broad the ecological perspective of the study of contamination effects in lotic ecosystems.

From a critical perspective, the interpretation of this study's results was made cautiously. The flow scenario simulated represent specific conditions: a flow of 70 mL min^{-1} and a continuous discharge of copper, with a concentration fluctuating from 20 μg L^{-1} in the contaminated area and progressively diluting downstream to ~2 μg L^{-1}. Although under natural conditions this scenario is plausible, differences due to seasonal or even daily hydrological variations cannot be discarded. Therefore, considering that the spatio-temporal stochasticity of habitats (dry and rainy periods) and its natural conditions are difficult to accurately replicate in any experimental system, the frequency and intensity of flow should be adjusted according to the needs of the study. According to Tockner et al. (2010), the evaluation of multiple-stressor effects on the habitat selection response of organisms is critical to cope with the upcoming pressures originating from global change; hence, the HeMHAS could help integrate the heterogeneity behind ecosystem processes. Consequently, regulatory managers and stakeholders should consider that, to maintain suitable aquatic ecosystems, it is important to take into account the heterogeneity and the connectivity among habitats in lotic-estuarine landscapes, thereby avoiding reductionism in the study of contamination only from the local area (Johnson, 2002). It is therefore important to ensure a minimum number of areas that organisms can use as shelter zones unthreatened by contamination, not only for aquatic species (Schulz & Liess, 1999), but also for major invertebrates and terrestrial fauna depending of such fragile community structures (Echeverría-Sáenz et al., 2022). This supposes a challenging task considering that protected areas are continually subjected to different pressures from multiple, indirect and external sources (Rodríguez-Jorquera et al., 2017). Finally, apart from the effects of contamination, the spatial configuration of connected habitats, and the choice restriction of downstream estuarine habitats, the current study also presents the integration of simultaneous variables that could complement the ecological relevance of studies by providing a landscape perspective of aquatic lotic-estuarine systems.

5. Conclusion

The proof of concept here tested successfully evidenced the suitability of the HeMHAS to simulate contamination events in lotic-estuarine landscapes to assess its effects on the spatial distribution of organisms. This novel experimental approach was able to study the habitat selection plasticity of the freshwater shrimp *A. desmarestii* in a simulated heterogeneous lotic-estuarine landscape, with contamination as a habitat quality disruptor and an estuary as a habitat use restrictor. Under active flow conditions, the organisms escaped from the contaminant and were carried towards the downstream estuarine habitat. Contrarily, in the absence of flow, the shrimp avoided the contaminated zone and also escaped from the salinity pressure of the estuary region, moving back upstream and towards shelter areas. These findings emphasize the importance of simulating chemically and structurally heterogeneous landscapes integrating an active flow as an important driver in the spatial distribution of chemical signals. Thus, the habitat selection response of populations may be studied with a more integrative approach, where running water might better reduce the uncertainties underlying the population dynamics and persistence in stressed downstream habitats. Lastly, it is important to emphasize the suitability and relevance of the HeMHAS as a system that, despite its limitations in the scope at spatio-temporal scale, it is still a valuable tool to simulate and combine landscape scenarios that could improve our understanding of ecosystems by providing new insights into the risk of contamination from the landscape ecology approach.

CRediT authorship contribution statement

David Salvatierra: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **María Pilar González:** Writing – review & editing, Investigation. **Silvia Echeverría-Sáenz:** Writing – review & editing, Investigation. **Julián Blasco:** Writing – review & editing, Resources, Project administration, Methodology. **Cristiano V.M. Araújo:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David Salvatierra reports financial support, equipment, drugs, or supplies, and writing assistance were provided by Spanish Scientific Research Council. Cristiano V. M. Araujo has patent issued to #P201731426.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.127567.

Data availability

Data will be made available on request.

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