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A novel approach to assessing environmental disturbance based on habitat selection by zebra fish as a model organism

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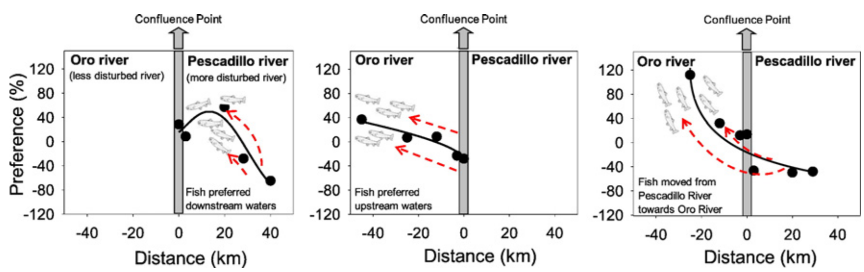
HIGHLIGHTS

- Habitat selection by zebrafish was studied in two connected Ecuadorian rivers.
- A non-forced exposure system simulated the connectivity existent between both rivers.
- Fish preferred downstream (Pescadillo River) and upstream (Oro River) samples.
- Habitat selection-based approach served to assess organisms' spatial distribution.
- Undisturbed habitats can suffer with the arrival of organisms from disturbed habitat.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic ecotoxicity assays used to assess ecological risk assume that organisms living in a contaminated habitat are forcedly exposed to the contamination. This assumption neglects the ability of organisms to detect and avoid contamination by moving towards less disturbed habitats, as long as connectivity exists. In fluvial systems, many environmental parameters vary spatially and thus condition organisms' habitat selection. We assessed the preference of zebra fish (*Danio rerio*) when exposed to water samples from two western Ecuadorian rivers with apparently distinct disturbance levels: Pescadillo River (highly disturbed) and Oro River (moderately disturbed). Using a non-forced exposure system in which water samples from each river were arranged according to their spatial sequence in the field and connected to allow individuals to move freely among samples, we assayed habitat selection by *D. rerio* to assess environmental disturbance in the two rivers. Fish exposed to Pescadillo River samples preferred downstream samples near the confluence zone with the Oro River. Fish exposed to Oro River samples preferred upstream waters. When exposed to samples from both rivers simultaneously, fish exhibited the same pattern of habitat selection by preferring the Oro River samples. Given that the rivers are connected, preference for the Oro River enabled us to predict a depression in fish populations in the Pescadillo River. Although these findings indicate higher disturbance levels in the Pescadillo River, none of the physical-chemical

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variables measured was significantly correlated with the preference pattern towards the Oro River. Non-linear spatial patterns of habitat preference suggest that other environmental parameters like urban or agricultural contaminants play an important role in the model organism's habitat selection in these rivers. The non-forced exposure system represents a habitat selection-based approach that can serve as a valuable tool to unravel the factors that dictate organisms' spatial distribution in connected ecosystems.

1. Introduction

For management and conservation purposes, an ecosystem's "integrity" can be assessed through the simultaneous integration of three lines of evidence (the triad approach) in a tiered fashion, from simple to more complex and relevant experiments and observations (Crane et al., 2007). First, ecological monitoring data clarify whether adverse effects are occurring. Second, chemical and physical measurements help identify potential stressors. Third, the ecotoxicological line of evidence establishes the causal relationship between stressors and adverse effects. In this last line of evidence, the traditional ecotoxicological approach forcedly exposes model organisms to field-collected samples and compares their response with those under optimal or reference conditions. However, many organisms are able to avoid chemical stressors well before physiological damage occurs (Wells et al., 2004; Rosa et al., 2012; Araújo et al., 2016a). Understanding organisms' ability to avoid stressors by moving to a more suitable environment is an important but relatively unexplored aspect of the third line of evidence in ecotoxicological studies.

The ability of an organism to detect and avoid contamination can be decisive for its survival. Avoidance responses limit (albeit over the short term) or even prevent continuous exposure of an organism to contamination, thereby diminishing the risk of suffering lethal or sub-lethal toxic effects (Wells et al., 2004; Tierney et al., 2010). An organism's ability to identify and correctly interpret the risk of exposure to contaminants is beneficial as physiological toxic effects are minimized. However, when organisms emigrate from an area to avoid environmental disturbance, there are potential and immediate consequences in both the avoided area and nearby surroundings, including: (i) loss of individuals (avoiders) in the directly affected habitat and (ii) increased competitive pressure in adjacent areas caused by an influx of the avoiders (Hansen et al., 1999a; Exley, 2000; Fleeger et al., 2003; Wells et al., 2004). The consequences of avoidance can be as detrimental as individual mortality at the population, community and ecosystem levels (Robinson, 2009).

Determining the effect of contaminants on the behavior and spatial distribution of species is an important first step to understanding the role of contamination in shaping community structure and dynamics. This applies to both the immediate disturbed habitat and undisturbed neighboring areas. Although evidence is limited, contaminant-driven spatial distribution has been verified in field studies, for example: adult Atlantic salmon (*Salmo salar*) were hindered from migrating upstream and forced to return downstream prematurely when exposed to high levels of copper and zinc (Saunders and Sprague, 1967); fathead minnows (*Pimephales promelas*) avoided natural streams contaminated with metals (Hartwell et al., 1987); in situ observations showed a preferential distribution of Atlantic salmon and brown trout (*S. trutta*) in uncontaminated habitats (Åtland and Barlaup, 1995); reduced trout (*S. trutta*) populations in Montana creeks (USA) were partially attributed to the avoidance of heavy metals (Woodward et al., 1995); and *S. salar* showed avoidance of wood pulp waste, despite the fact that this substance is considered non-toxic (Thorstad et al., 2005).

Integrating avoidance responses in site-specific ecological risk assessments requires assays that examine the ability of organisms to move away from disturbed habitats (Moreira-Santos et al., 2008; Araújo et al., 2016a). Such assays are usually performed under experimental conditions that allow organisms to move freely along a gradient of field-collected samples diluted with optimal medium (Lopes et al., 2004; Silva et al., 2017). These experiments allow researchers to make detailed observations of organisms' movements and behavior under controlled conditions. However, extrapolating experimental results to real scenarios of habitat disturbance can be problematic because real situations are much more complex and heterogeneous than a linear gradient of a chemical stressor diluted with an optimal medium. For example, connectivity allows organisms to migrate to less disturbed areas to avoid stressors. Moreover, organisms' displacement under natural conditions may be motivated less by avoidance of stressors and more by the active pursuit of more suitable habitats (see review by Araújo et al., 2016a).

Although contaminants are considered an important factor for habitat selection, many other environmental (e.g., water hardness, conductivity, turbidity, depth, dissolved oxygen, etc.) and biological factors (e.g., life stage, presence of food, competitors, predators, etc.) can be as or more important than contamination (Hansen et al., 1999b; Wells et al., 2004; Gerhardt, 2007; Shahadat Hossain et al., 2012; Arimoro et al., 2014; Zhou et al., 2016). Such factors can have synergistic effects under natural environmental conditions, making the task of identifying and quantifying the most aversive or attractive parameter for a particular organism difficult. For example, density, biomass and growth of the estuarine mudsnail *Peringia ulvae* were highest in an area with intermediate contamination along a mercury gradient, most likely due to resource and refuge availability rather than toxicity (Cardoso et al., 2013); when exposed to two selectable conditions (chemical predator cue and toxic cyanobacteria), the fish *Gasterosteus aculeatus* preferred to feed in a potentially toxic environment to reduce predation risk (Engström-Öst et al., 2006); high water temperature was more stressful than metal contamination for trouts (*Oncorhynchus mykiss*, *S. trutta* and *Salvelinus fontinalis*) (Harper et al., 2009); fish larvae were more abundant in contaminated estuaries compared to undisturbed habitat (McKinley et al., 2011), possibly because the fish could associate the stimulus caused by the presence of certain toxicants to food (Tierney, 2016); and tilapia (*Oreochromis* sp.) fry performed intermittent displacements to previously avoided concentrations of fish farming effluent when food availability was increased (Araújo et al., 2016b). Models of species behavior and distribution in contaminated environments need to couple such preference behavior for suitable habitat with the ecotoxicity-related avoidance response discussed previously. However, the mathematical formulae used to quantify avoidance and associated concepts, such as the median avoidance dilution or concentration, are unsuited to model potential migration routes across a complex set of interconnected habitats. Cross-checking empirical data on habitat selection with the physical, chemical and ecological properties of a particular study site would enable researchers to focus on specific areas of concern and potential rather than previously targeted stressors when implementing the second and third tiers of ecological risk assessment. This would save time and money because less effort would need to be devoted to areas identified as undisturbed during the first tier.

Here we develop and apply such an approach by comparing a model organism's habitat selection with environmental parameters to discern potential areas of contamination. The ideal field scenario to be tested would be a network of connected areas that putatively differ with respect to stressor types and intensities. Signaling a paradigm shift in ecotoxicology, this approach broadens the scope from the effects of contamination on individuals to those on the spatial distribution of