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Influence of interspecific interactions on avoidance response to contamination

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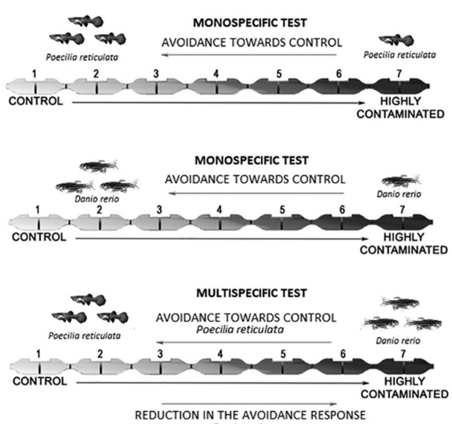
HIGHLIGHTS

- The avoidance response of guppy and zebrafish to a copper gradient was evaluated.
- Contamination gradients were simulated using a non-forced exposure system.
- The effects of interspecific interactions on avoidance response was studied.
- Guppy and zebrafish avoided potentially toxic copper concentrations.
- The magnitude of avoidance by zebrafish was affected by the presence of guppy

GRAPHICAL ABSTRACT



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ABSTRACT

An increasing number of studies have shown the ability of organisms to escape from toxic effects due to contamination, by moving spatially towards less contaminated habitats. However, this issue has been investigated in monospecific scenarios, without considering possible interactions between species during the contamination avoidance process. It is widely known that the spatial distribution of one species can be affected by another one, in different ways. Therefore, the main question addressed in the present study was as follows: Might interspecific interaction between the freshwater fish *Danio rerio* (zebrafish) and *Poecilia reticulata* (guppy) change their behavior patterns in terms of avoidance in the presence of a copper gradient? Zebrafish and guppies exposed to a copper gradient were tested for avoidance responses in a free-choice, non-forced, static, multi-compartmented exposure system, using two distinct approaches: (1) monospecific tests, in which only one species was exposed to the copper gradient, at two different population densities; and (2) multispecific tests, in which both species were tested simultaneously. In the control (with no copper) monospecific tests, both species were randomly distributed; however, in the control multispecific test, *P. reticulata* tended to aggregate. In the

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monospecific tests with a copper gradient, both species avoided copper in a similar way, with AC_{50} (concentration triggering avoidance in 50% of the exposed population) values between 15 and 18 $\mu g \cdot L^{-1}$, irrespective of the population density. However, in the multispecific tests, *P. reticulata* displaced *D. rerio* to previously avoided copper levels, consequently increasing the AC_{50} of *D. rerio* to 75 $\mu g \cdot L^{-1}$. This study shows the importance of understanding the interactions among species in contaminated areas, and the way that one species can prevent the avoidance behavior of another.

1. Introduction

The risks to organisms resulting from the exposure to contaminants are commonly related to damage caused to individuals, including lethal and sublethal effects (Martinez-Haro et al., 2015). Such damage is generally studied by means of ecotoxicological tests with forced exposure, involving the direct and continuous exposure of organisms to contaminants. Although less frequent, some earlier studies have indicated that organisms are able to detect and avoid contamination, thereby preventing them from suffering toxic effects (see review by Araújo et al., 2016). Evidence of avoidance to contamination was reported by Folmar (1976), who exposed the fish *Salmo gairdneri* (rainbow trout) to nine herbicides in a bi-compartmentalized system and observed how the fish preferred the clean region. Using a similar system, Gunn and Noakes (1986) evaluated the avoidance behavior of the fish *Salvelinius fontinalis* caused by low pH and addition of aluminum. In both studies, toxic effects at the individual level resulting from continuous exposure were prevented by the avoidance response to contamination.

Recently, a new approach using a free-choice, multi-compartmented, non-forced system was proposed for use in ecotoxicological studies to assess how the spatial distribution of organisms is driven by contaminants (Lopes et al., 2004). The aim of this non-forced exposure approach is to simulate contamination gradients or patches, so that the effects are no longer measured using individuals, but instead using their spatial distribution. This approach expands the concept of environmental disturbance beyond the traditional one based exclusively on the toxicity (and bioaccumulation) at the organism level. Evidence of contamination-driven spatial displacement (avoidance response) in non-forced exposure systems has been described for many organisms including fish (Moreira-Santos et al., 2008; Araújo et al., 2014, 2018; Silva et al., 2017, 2018), amphibians (Araújo et al., 2014; Vasconcelos et al., 2016), and invertebrate species (Lopes et al., 2004; Araújo et al., 2016) exposed to different contaminants.

Although evidence of avoidance responses to contamination has been widely reported for many fish species (see review by Araújo et al., 2016), it is recognized that habitat selection processes may be conditioned by several factors other than contamination, such as temperature (Stehfest et al., 2017), pH (Fost and Ferreri, 2015), presence of predators (Scherer and Smee, 2016) and competitors (Dunlop et al., 2006), among others. In the natural environment, organisms are simultaneously exposed to several factors, so it is necessary to understand the extent to which contamination influences the habitat selection process, and the ways that these factors affect the avoidance response. For instance, Scherer and Mcnicol (1998) observed that *Coregonus clupeaformis* avoided exposure to Cu, Zn, and Pb, but that the avoidance response was suppressed when the environment was shaded. Dunlop et al. (2006) found that the escape responses of *Carassius auratus* and *Oncorhynchus mykiss* to a harmful acute stimulus (electric shock) changed if the two species were present together. Araújo et al. (2016) observed that the habitat selection by tilapia (*Oreochromis* sp.) exposed to a contamination gradient in a non-forced exposure system was changed when food was offered, with the organisms moving to previously avoided areas in order to feed. These results indicate that while fish can avoid toxic effects by moving to less contaminated habitats, the presence of a more attractive factor (or one of immediate importance) in the potentially avoidable habitat can change the avoidance pattern.

Interspecific interaction might also affect the spatial distribution of organisms due to competition for food, space, or other factors intrinsic to each population (Begon et al., 2007). Studies have clearly demonstrated that the structure and functioning of communities can be greatly altered when different populations cohabit. For instance, the presence of *Salmo gairdneri* (rainbow trout) in a stream flume influenced the individual fitness of *Luxilus coccogenis* (warpaint shiner), in terms of prey capture success and feeding efficiency (Elkins and Grossman, 2014). The presence of the fish *Carassius auratus* forced the amphibian *Ichthyosaura alpestris* to remain longer in the terrestrial environment (Laurane et al., 2017). In a study performed in the North-Western Mediterranean Gulf, despite the overlap of three small pelagic fish (*Sardina pilchardus*, *Engraulis encrasicolus*, and *Sprattus sprattus*), the biomass of each species differed locally due to the influence of one over the others (Saraux et al., 2014).

In the presence of contaminants, changes in the patterns of interaction (such as competition and aggressiveness) among fish populations (Lingaraja et al., 1979; Clotfelter and Rodriguez, 2006) are expected to occur. Therefore, considering that (i) fish can avoid contamination by moving spatially towards less contaminated habitats, and (ii) contamination can affect the behavior of fish in terms of aggressiveness, competition, and spatial displacement, the main question addressed in the present study was: Might interspecific interaction between the freshwater fish *Danio rerio* (zebrafish) and *Poecilia reticulata* (guppy) change their patterns of avoidance to contamination? The goal of the present study was to assess if the avoidance responses of these two fish populations exposed to a contamination gradient might be affected by interspecific interactions. It was expected that the less aggressive species would be displaced towards previously avoided contaminated habitats. Copper (Cu^{2+}) was selected as a test contaminant as it is an avoidable pollutant for fish (Moreira-Santos et al., 2008; Araújo et al., 2015) and produces toxic effects such as chemosensory deprivation (McIntyre et al., 2008), changes in gene expression (Craig et al., 2010), feeding inhibition (Abdel-Moneim et al., 2015), effects on the swimming performance of larvae (Acosta et al., 2016), morphological and metabolic alterations (Chatterjee et al., 2016), and changes in the surfaces of the gills (Fu et al., 2016). *P. reticulata* and *D. rerio* were selected as test organisms because they (i) are easy to cultivate, (ii) are widely used as model organisms in the fields of ecology, evolution, and ethology, and (iii) have already been shown to spatially avoid chemical contamination (Moreira-Santos et al., 2008; Araújo et al., 2014, 2014; Silva et al., 2017).

2. Materials and methods

2.1. Test organisms

Individuals of the fish *P. reticulata* and *D. rerio* (2 to 3 months old and with total length between 1.0 and 1.5 cm) were obtained from the São Paulo Agency of Agribusiness and Technology (Pindamonhangaba, Brazil) and the company Power Fish (Rio de Janeiro, Brazil), respectively. The use of the fish was approved by the Ethics Committee on the Use of Animals (Institute of Biosciences, University of São Paulo; protocol #236/2015). The organisms were acclimated under laboratory conditions (at the Ecotoxicology Laboratory of the Lorena School of